Research Article

Curcumin Mitigates Accelerated Aging after Irradiation in *Drosophila* by Reducing Oxidative Stress

Ki Moon Seong,[1] Mira Yu,[2] Kyu-Sun Lee,[3] Sunhoo Park,[1] Young Woo Jin,[1] and Kyung-Jin Min[2]

[1] *National Radiation Emergency Medical Center, Korea Institute of Radiological & Medical Sciences, Seoul 139-706, Republic of Korea*
[2] *Department of Biological Sciences, Inha University, 100 Inha Street, Incheon 402-751, Republic of Korea*
[3] *Bionanotechnology Research Center, Korean Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea*

Correspondence should be addressed to Kyung-Jin Min; minkj@inha.ac.kr

Curcumin, belonging to a class of natural phenol compounds, has been extensively studied due to its antioxidative, anticancer, anti-inflammatory, and antineurodegenerative effects. Recently, it has been shown to exert dual activities after irradiation, radioprotection, and radiosensitization. Here, we investigated the protective effect of curcumin against radiation damage using *D. melanogaster*. Pretreatment with curcumin (100 μM) recovered the shortened lifespan caused by irradiation and increased eclosion rate. Flies subjected to high-dose irradiation showed a mutant phenotype of outstretched wings, whereas curcumin pretreatment reduced incidence of the mutant phenotype. Protein carbonylation and formation of γH2Ax foci both increased following high-dose irradiation most likely due to generation of reactive oxygen species. Curcumin pretreatment reduced the amount of protein carbonylation as well as formation of γH2Ax foci. Therefore, we suggest that curcumin acts as an oxidative stress reducer as well as an effective protective agent against radiation damage.

1. Introduction

Out of several hundred aging theories, the most popular aging theory is oxidative stress theory of aging. It claims that aging is caused by oxidative damage to macromolecules. Oxidative stress is the result of an imbalance between generation of reactive oxygen species by essential life systems and detoxification of reactive radicals by defense mechanisms within organisms [1]. Disruption of the normal redox state of cells induces cytotoxic effects through production of reactive intermediates, which inflict damage to all cellular components, including proteins, lipids, and DNA [2]. Reactive oxygen species such as superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), and hydroperoxyl ($HO_2^{\bullet}$) are generated by natural respiration in animals and environmental stresses such as radiation, chemicals, and heat [3, 4].

Although the biological effects of low-dose radiation less than 100 mSv have not been fully established, exposure to high-dose radiation caused by unexpected accidents related to artificial sources has many deleterious consequences in humans, including organ malfunction, malignant cancer development, genetic mutagenesis, and developmental abnormalities [5–7]. Moreover, ionizing radiation has long been used as a standard medical treatment to kill cancer cells and shrink tumors [8]. Cancer radiotherapy destroys chromosomes by making it impossible for them to proliferate. Normal cells are also damaged by this therapy, which is the main drawback of this medical procedure. Several antioxidative natural extracts have been combined together in order to reduce radiation injury and protect normal cells [9]. For example, melatonin has been shown to imbue significant radiation protection against chromosomal aberrations and micronuclei formation when administered to mice prior to radiation exposure [10]. Further, several flavonoid compounds such as quercetin, myricetin, and orientin have been reported as potent antioxidants with radioprotective ability [11]. Resveratrol, a polyphenolic plant product, was

also shown to attenuate radiation damage in *C. elegans* by scavenging ROS [12].

Curcumin derived from turmeric is a representative plant phenolic compound possessing therapeutic properties [13, 14]. It is known to eliminate oxygen free radicals, inhibit lipid peroxidation, and protect cellular macromolecules such as DNA from oxidative stress [15, 16]. Curcumin has been shown to reduce chromosomal aberrations in models of human breast cancer, probably due to its antioxidative activity [17]. Fruit flies fed a curcumin diet have shown an extended lifespan, improved health, and modulated expression of aging-associated genes [18, 19].

Due to its antioxidative activity, curcumin has been proposed as a radiation protector. Pretreatment with curcumin has been shown to protect lymphocytes against γ-radiation-induced cellular damage [15]. Curcumin also was found to protect against cutaneous radiation-induced damage in mice [20]. However, several previous studies showed that curcumin has no protective effect against the clastogenicity of γ-radiation [21–23]. Therefore, it remains unclear whether or not curcumin indeed acts as a radiation protector. Moreover, most studies on the radioprotective effects of curcumin have been performed at the cellular level [24–26]. Studies using model animals would more strongly support the conclusion that curcumin protects against radiation damage. Therefore, we evaluated the protective effect of curcumin against ionizing radiation using *D. melanogaster* and found that curcumin may be effective as a radiation protector.

2. Materials and Methods

2.1. Fly Husbandry. We performed all experiments using wild-type Canton-S flies. Larvae of the Canton-S strain were grown on standard cornmeal-sugar-yeast (CSY) medium (5.2 g of cornmeal, 11 g of sucrose, 11 g of yeast [MP Biomedicals, Solon, OH], 1.1 mL of 20% tegosept, and 0.79 g of agar per 100 mL of water) supplemented with several grains of live yeast. The rearing room was maintained at 25°C with 45% humidity on a 12 h : 12 h light-dark cycle.

2.2. Curcumin Pretreatment. Stock solution of curcumin (5 mM) was prepared dissolving curcumin (218580100, Acros Organics) in 99% ethanol and was supplemented to sucrose-yeast (SY) food at a concentration of 100 μM. Same amount of ethanol was supplemented to food without curcumin. Collected eggs were reared in the SY food containing curcumin before irradiation at the 3rd instar larvae stage.

2.3. γ-Irradiation Exposure. Eggs were collected from young female flies over 12–14 h and reared on SY medium. 3rd instar larvae were irradiated in a γ-irradiation machine (^{137}Cs, IBL 437N; CIS Bio International, Gifsur-Yvette, France) at a dose rate of 0.8 Gy/min. Following irradiation, nonirradiated and irradiated flies were maintained contemporaneously in the same incubator at 25°C.

2.4. Pupation and Eclosion Rates. Irradiated larvae were checked daily to determine pupation and eclosion rates.

Pupation rate was calculated based on the total number of pupae divided by the number of larvae, whereas eclosion frequency was calculated based on the total number of eclosed flies divided by the number of larvae.

2.5. Lifespan. When irradiated larvae were eclosed, adult flies were collected over 48 h and randomly assigned to 500 mL demography cages to achieve a final density of 100 females and 100 males per cage. SY diets were prepared with 10 g of sucrose, 10 g of yeast, 1.1 mL of 20% tegosept (w/v in ethanol), and 0.79 g of agar per 100 mL of water. The vials containing SY diets were changed every 2 days, and all mortalities were recorded. Three replicates were established for each dose level.

2.6. Detection of Protein Oxidation (Protein Carbonylation). Protein carbonylation was measured using an OxyBlot protein oxidation detection kit according to the manufacturer's instructions (Millipore). Briefly, radiation-exposed larvae under each condition were homogenized in lysis buffer (50 mM Tris-HCL pH 7.4, 150 mM NaCl with protease inhibitor cocktail). For the positive control, protein sample was prepared from larvae fed 20 mM paraquat for 16 h. Protein samples were then treated with 2,4-dinitrophenylhydrazine (DNPH). Reaction of DNPH with carbonylated proteins allows the formation of 2-4-dinitrophenylhydrazone (DNP), which can be detected with anti-DNP antibody. Samples were subjected to 10% SDS-PAGE and transferred onto a PVDF membrane (Roche). DNP groups were then immunodetected with rabbit anti-DNP antibody, followed by secondary anti-HRP antibody and ECL revelation. To normalize protein loading, the transferred SDS-PAGE gel was stained with Coomassie blue.

2.7. γH2Ax Foci Staining. To detect double-strand breaks, irradiated larvae were dissected in cold PBS and fixed for 20 min at room temperature in PBS containing 4% paraformaldehyde. After washing and blocking with PBS containing 0.1% Triton and 2% BSA, wing imaginal discs were incubated with antiphosphorylated H2Ax (γH2Ax, Upstate Biotechnology). For visualization, samples were mounted in VECTASHIELD Mounting Media (Vector Lab), and fluorescence images were acquired using a FluoView confocal microscope (Olympus).

2.8. Statistical Analyses. All demographic data were presented as the mean $\pm$ SEM and analyzed with a one-way analysis of variance (ANOVA) on ranked data using standard survival models in the JMP statistical package and Prism software (GraphPad, La Jolla, CA). Asterisk indicates significant difference from the control (**$P < 0.001$ and *$P < 0.05$). The tests used and sample sizes for each experiment are indicated.

3. Results

3.1. Effect of Curcumin Pretreatment on Drosophila Lifespan after Radiation Exposure. Previous studies have reported
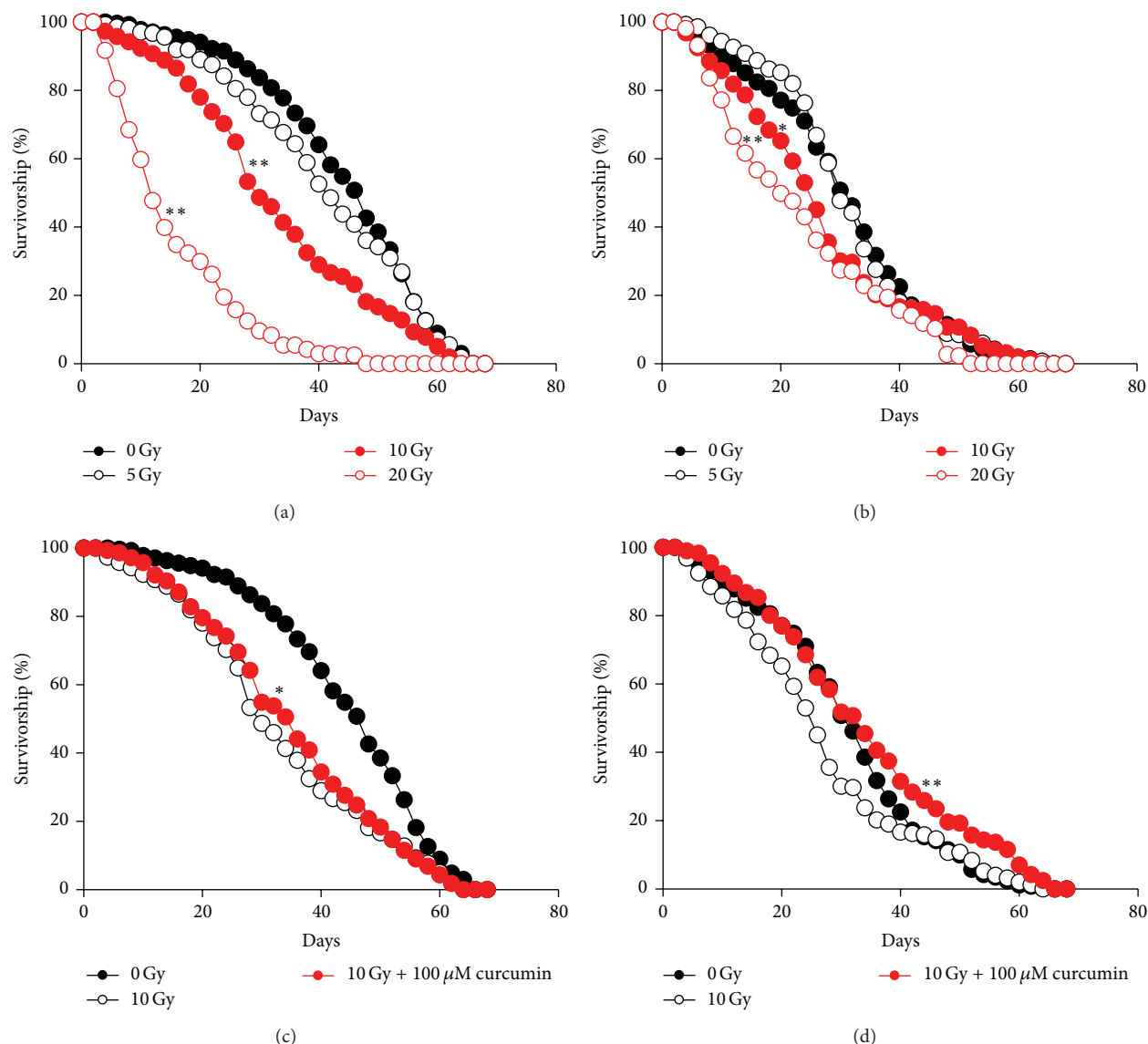
FIGURE 1: Curcumin pretreatment recovers shortened fly lifespan by ionizing radiation. Several doses of ionizing radiation were administered at the 3rd larval stage, and the lifespans of adult males (a) and females (b) were measured. Larvae were fed 100 μM curcumin from egg hatching before 10 Gy of irradiation at the 3rd larval stage, and the lifespans of adult males (c) and females (d) were measured ($^*P < 0.05$, $^{**}P < 0.01$).

that ionizing radiation reduces the lifespan of *Drosophila* to various degrees depending on the irradiation dosage and strain genetic background [27, 28]. Here, we first subjected larvae of fruit flies to irradiation at several doses and then recorded lifespans of adults in order to determine the optimal dose to analyze the effects of curcumin (Figures 1(a) and 1(b)). The effect of curcumin pretreatment was evaluated in flies irradiated at 10 Gy, which showed a mean lifespan of approximately 30 days in both males and females (Table 1). We reared Canton-S flies after egg hatching with fly medium containing 100 μM curcumin, and ionizing radiation was administered at the 3rd instar larva stage. 100 μM curcumin was chosen as the most effective dose based on preliminary experiment. Flies pretreated with curcumin showed significant extension of their mean lifespan—5.5% for males ($P < 0.01$) and 26.5%

for females ($P < 0.01$) (Table 1, Figures 1(c), 1(d)). These data indicate that curcumin pretreatment extended the lifespan of irradiated flies by mitigating the harmful effects of ionizing radiation.

3.2. Effect of Curcumin Pretreatment on Drosophila Development after Radiation Exposure. All insects, including *Drosophila*, undergo marked morphological changes during their development to adult stage known as metamorphosis, which is an excellent parameter to detect physiological effects following environmental fluctuation. Here, we measured pupation and eclosion rates of flies pretreated with curcumin after irradiation. The pupation rate of curcumin-pretreated flies was not significantly different after irradiation ($P > 0.07$) (Figure 2(a)). However, the eclosion rate of flies was
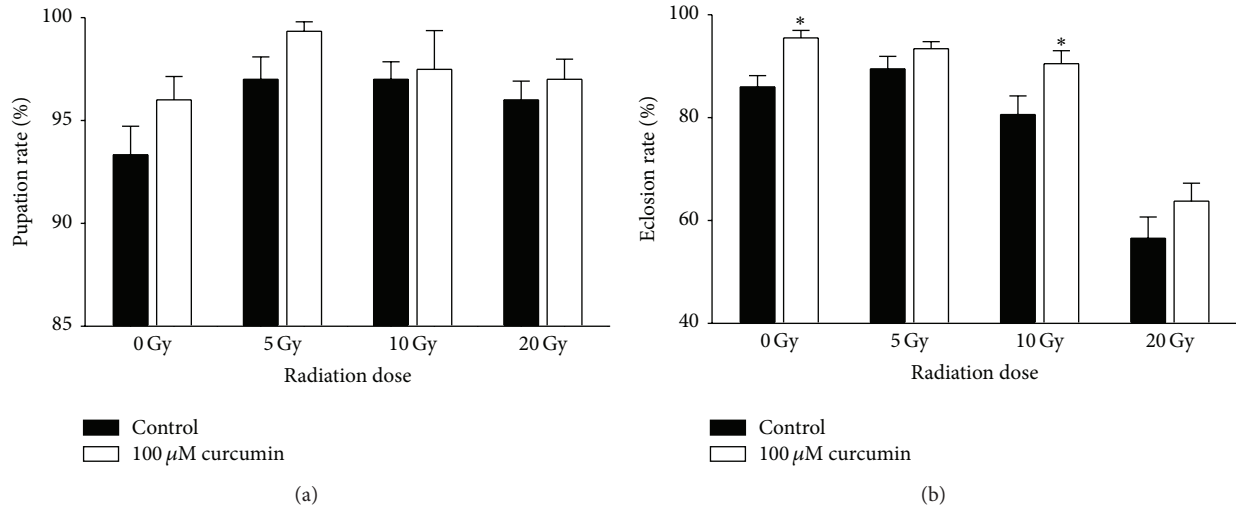
(a)

(b)

FIGURE 2: Curcumin pretreatment increases the eclosion rate of fruit flies. Pupation rate (a) and eclosion rate (b) of irradiated flies were recorded as described in the materials and methods section. Pretreatment with curcumin improved the eclosion rate in some treatments ($^*P < 0.05$).

TABLE 1: Mean longevity recovery by pretreatment of curcumin.

Treatment	Flies numbers	Mean longevity	SD	P value
Male				
Control	270	44.6889	0.82040	—
IR[a]	259	33.2819	0.96298	<0.0001[*]
IR + curcumin[b]	279	35.1183	0.89536	<0.0001[**]
Female				
Control	262	31.1908	0.84744	—
IR[a]	253	27.0119	0.93228	0.0151[*]
IR + curcumin[b]	286	34.1748	0.95931	<0.0001[**]

[a]Radiation at 10 Gy was irradiated at 3rd instar larval stage.
[b]Flies were cultivated in the medium containing 100 μM of curcumin until irradiation.
[*]Compared with control, [**]compared with IR group.

reduced as the radiation dose increased. The eclosion rate of control flies was 83%, whereas that of flies irradiated at 20 Gy was reduced to 58%. Curcumin pretreatment distinctly augmented the eclosion rate in irradiated flies with statistical significance ($P < 0.05$) (Figure 2(b)). Curcumin pretreatment without irradiation also increased the eclosion rate ($P < 0.05$).

3.3. Effect of Curcumin Pretreatment on Drosophila Phenotype after Radiation Exposure.

High-dose irradiation has been shown to induce chromosomal mutations and malformation of external organs [29–31]. Here, we analyzed the specific phonotype caused by irradiation to determine whether or not curcumin reduces the mutagenic effects of ionizing radiation. Irradiation with 20 Gy at the 3rd instar larval stage resulted in outstretched wings on bodies of adult flies (Figure 3(a)), and the frequency of the mutant phenotype increased as

the radiation dose increased (Figure 3(b)). Specifically, no mutant phenotype was observed at 0 Gy of irradiation, whereas about 60% of flies showed the mutant phenotype at 20 Gy of irradiation. Although curcumin pretreatment did not significantly reduce the frequency of mutation, lower frequency of the mutant phenotype was a tendency in all curcumin-pretreated groups (Figure 3(b)).

3.4. Effect of Curcumin Pretreatment on ROS Generation after Radiation Exposure.

The phenotypic data acquired in this study indicate that curcumin reduced the various stresses caused by ionizing radiation. Since it is well known that radiation induces oxidative stress and curcumin is an excellent antioxidant, we examined whether or not curcumin detoxifies radiation-induced oxidative damage. Protein carbonylation is known to be a key biomarker of oxidative stress generated by carbonyl (CO) groups (aldehydes and ketones), which are produced on protein side chains, especially in proline, arginine, lysine, and threonine, following their oxidation [32]. Here, we extracted protein lysates from flies and performed protein carbonylation assay as described in Section 2. Protein carbonylation increased upon irradiation, whereas curcumin pretreatment obviously reversed this in irradiated flies. Paraquat, known to be a chemical inducing cellular protein carbonylation, was used as a positive control (Figure 4).

3.5. Effect of Curcumin Pretreatment on DNA Damage after Radiation Exposure.

Reduced oxidative stress by curcumin could diminish the damage inflicted by ionizing radiation. DNA double-strand breaks caused by radiation-induced ROS impair normal cellular survival. In mammal, phosphorylated H2Ax (γH2Ax) foci, an indicator of DNA double-strand breaks, are found in the nucleosomes near radiolytic damaged region [33]. Since antibody to mammalian γH2Ax can recognize Drosophila γH2Av based on sequence homology [34], we monitored the radiation-mediated DNA damage
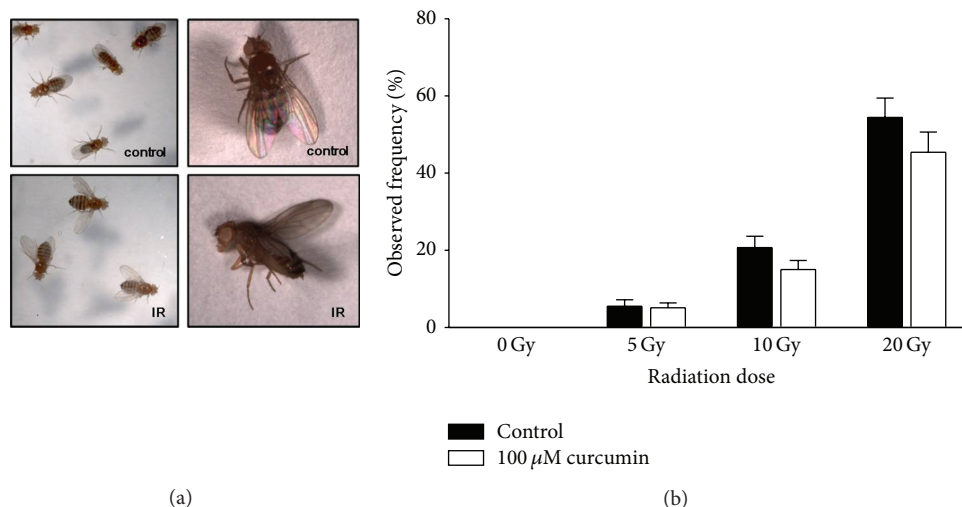
(a)

(b)

Figure 3: Irradiation increases incidence of flies with outstretched wings. Some irradiated flies emerged with outstretched wings (a). The incidence of flies with outstretched wings increased as the radiation dose increased. Curcumin pretreatment tended to reduce incidence, but the difference was not significant in all treatments.
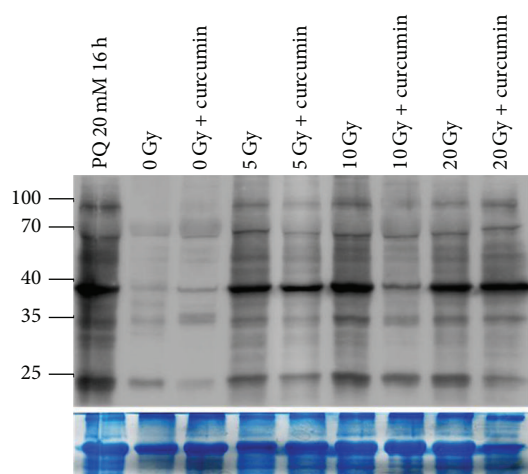

Figure 4: Curcumin pretreatment reduces radiation-induced protein carbonylation. Both paraquat (positive control) and irradiation increased protein carbonylation, whereas curcumin pretreatment decreased protein carbonylation. A gel image stained with Coomassie blue was used as an internal control of protein loading amount in SDS-PAGE.

with γH2Ax foci in larval wing disc. Here, ionizing radiation induced DNA breaks in a dose-dependent manner, whereas curcumin pretreatment significantly reduced formation of γH2Ax foci in the larval wing disc of *Drosophila* (Figure 5). These data indicate that curcumin reduced radiation induced the genome instability in *Drosophila* by increasing resistance to oxidative stress.

4. Discussion

In this paper, we presented data showing that curcumin reversed the shortened lifespan of irradiated flies as well as increased the eclosion rate. Curcumin also attenuated oxidative stress and DNA alterations caused by ionizing radiation. Interestingly, irradiation caused a larger reduction in lifespan in males than in females, whereas curcumin pretreatment was more effective in females than in males (Figure 1). This sexually dimorphic difference may be due to differential hormonal regulation of male and female fecundity [35–37]. It may also be due to gender differences in susceptibility to oxidative stress between males and females. Some parameters of free radical processes are different between male and female *Drosophila*. For example, a previous study showed differences in oxygen consumption of extracted mitochondria and mitochondrial DNA copy number between male and female *Drosophila* [37].

To our knowledge, this is the first report showing that high-dose irradiation of larvae results in an abnormal outstretched wing phenotype (Figure 3). Generally, *Drosophila* adults are quite resistant to irradiation. Even 500 Gy of radiation has been shown to have little effect on adult survival following irradiation at the adult stage (unpublished data), which may be due to the cuticular exoskeleton of flies. Unlike adults, *Drosophila* larvae are susceptible to irradiation due to their soft cuticular structure. As mentioned above, in this study, lifespans were greatly reduced and incidence of the outstretched wing phenotype increased as the radiation dose increased. Actually, half of the flies emerged with the outstretched wing phenotype when 20 Gy of radiation was administered to 3rd instar larvae. It remains unknown which signaling pathway is involved in the formation of the outstretched wing phenotype, but we suspect the JAK/STAT signaling pathway since it participates in the formation of the imaginal wing disc [38, 39]. Further investigation is necessary to determine the molecular mechanisms of outstretched wing formation after irradiation.

Eclosion rates of nonirradiated or irradiated flies were improved by curcumin pretreatment (Figure 2). As an explanation, curcumin has the potential to remove ROS generated
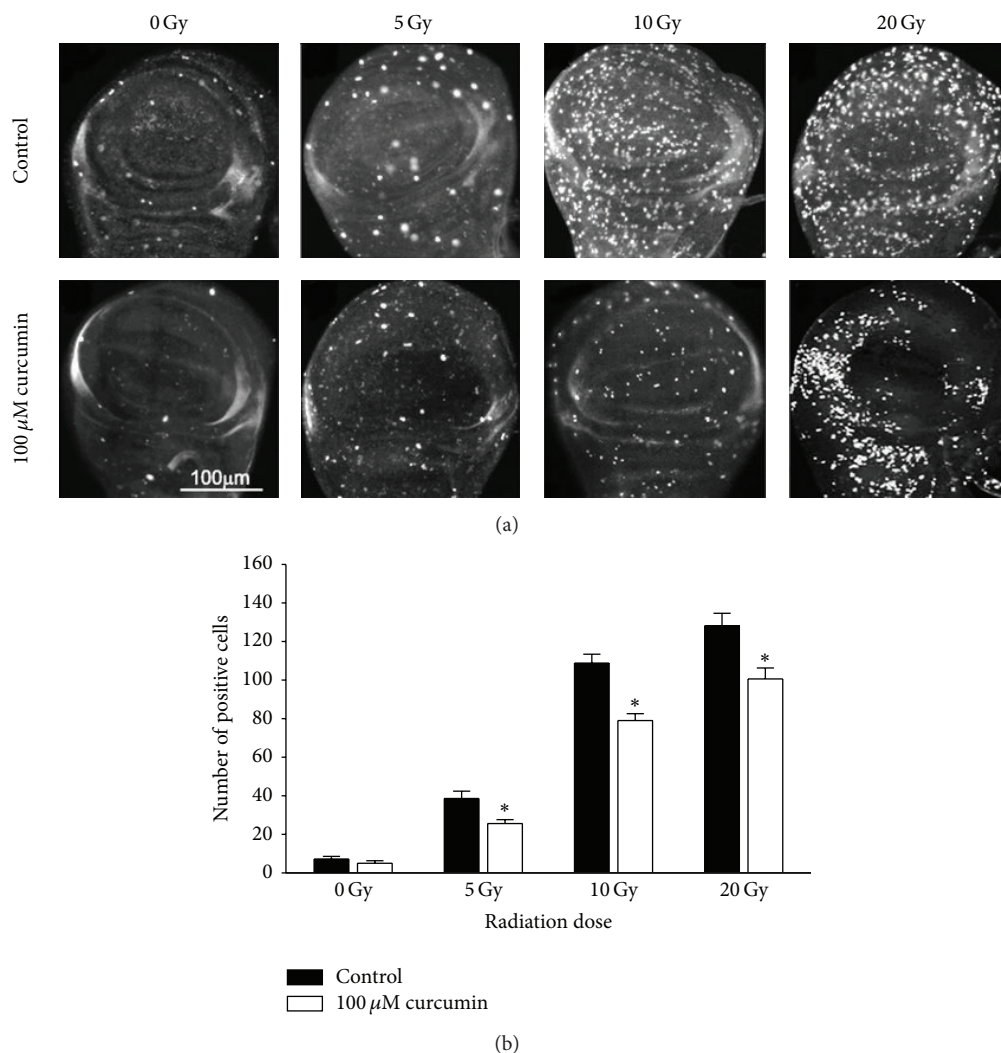
(a)

(b)

FIGURE 5: Curcumin reduces formation of radiation-induced γH2Ax foci. Phosphorylated H2Ax was used as a marker of DNA double-strand breaks. Foci on wing discs were detected by immunostaining with specific antibodies for γH2Ax (a). The incidence was measured by counting spots (b) and analyzed statistically ($^*P < 0.05$).

during development and/or radiation exposure. A previous report of delayed aging upon curcumin treatment supports our observations since aging is tightly coupled with ROS generation [18]. Both 20 mM paraquat and irradiation increased protein carbonylation, which itself was reduced by curcumin treatment (Figure 4). Similarly, pretreatment with curcumin to irradiated lymphocytes reduced lipid peroxidation and increased antioxidative properties, thereby preventing injury to lymphocytes [15]. Overall, curcumin provided *Drosophila* with augmented resistance to overcome radiation-induced oxidative stresses.

Collectively, these effects of curcumin may be due to its scavenging activity and distinct structural characteristics. First, curcumin has a hydrophobic structure that allows it to easily pass through the plasma membrane into the cytoplasm, where it can scavenge ROS more easily than hydrophobic molecules [40]. Second, curcumin has electron-donating groups such as phenolic hydroxyl groups and a β-diketone structure responsible for removing free radicals from cells

[15]. Increased resistance to oxidative stress by curcumin could be attributed to its transcriptional regulation; namely, curcumin can activate transcriptional factors and increase the expression of genes involved in oxidative defense [41, 42].

However, some scientists have remarked that curcumin could be a "double-edged sword," similar to other herbal antioxidants in tumorigenesis [43, 44]. The carcinogenic or prooxidant effects of curcumin have been shown to be mediated by mechanisms such as iron depletion, inhibition of cytochrome p450, and interference with the p53 tumor suppressor pathway [40, 45, 46]. Moreover, other lines of evidence raise concerns about the safety of curcumin for cancer treatment. Specifically, curcumin shows diverse clinical effects depending on its concentration [47]. To develop curcumin into a preventive or therapeutic drug, the optimal dose that elicits only desirable effects should be determined.

The development of radioprotectors is an area of great significance due to its wide applications in planned radiotherapy as well as unexpected radiation exposure. Although

some conflicting behaviors of curcumin on radioprotective function were reported, there are number of studies showing that curcumin offers protection to normal cells from radiation [15, 20, 48]. Our present data using *Drosophila* prove that curcumin improved radioresistance by relieving oxidative stress, thereby consolidating the radioprotective effects of curcumin.

5. Conclusions

In this paper, we have presented data showing that that curcumin relieves the oxidative stress and DNA damage caused by high-dose radiation in *Drosophila*. Curcumin pretreatment extends lifespan and decreases the frequency of mutagenic phenotype caused by ionizing radiation. Given antiaging benefits of curcumin from antioxidative properties, it will be of interest to determine whether curcumin can be used as a radioprotective agent in mammalian models.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Ki Moon Seong and Mira Yu contributed equally.

Acknowledgments

References

[1] T. Finkel and N. J. Holbrook, "Oxidants, oxidative stress and the biology of ageing," *Nature*, vol. 408, no. 6809, pp. 239–247, 2000.

[2] K. S. Fritz and D. R. Petersen, "Exploring the biology of lipid peroxidation-derived protein carbonylation," *Chemical Research in Toxicology*, vol. 24, no. 9, pp. 1411–1419, 2011.

[3] P. J. Hansen, "Effects of heat stress on mammalian reproduction," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1534, pp. 3341–3350, 2009.

[4] F. Caputo, R. Vegliante, and L. Ghibelli, "Redox modulation of the DNA damage response," *Biochemical Pharmacology*, vol. 84, no. 10, pp. 1292–1306, 2012.

[5] K. M. Seong, C. S. Kim, B.-S. Lee et al., "Low-dose radiation induces drosophila innate immunity through toll pathway activation," *Journal of Radiation Research*, vol. 53, no. 2, pp. 242–249, 2012.

[6] S. A. Lorimore, P. J. Coates, and E. G. Wright, "Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation," *Oncogene*, vol. 22, no. 45, pp. 7058–7069, 2003.

[7] K. B. Moysich, R. J. Menezes, and A. M. Michalek, "Chernobyl-related ionising radiation exposure and cancer risk: an epidemiological review," *The Lancet Oncology*, vol. 3, no. 5, pp. 269–279, 2002.

[8] N. J. Curtin, "DNA repair dysregulation from cancer driver to therapeutic target," *Nature Reviews Cancer*, vol. 12, no. 12, pp. 801–817, 2012.

[9] F. E. Koehn and G. T. Carter, "The evolving role of natural products in drug discovery," *Nature Reviews Drug Discovery*, vol. 4, no. 3, pp. 206–220, 2005.

[10] F. M. Badr, O. H. M. El Habit, and M. M. Harraz, "Radioprotective effect of melatonin assessed by measuring chromosomal damage in mitotic and meiotic cells," *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*, vol. 444, no. 2, pp. 367–372, 1999.

[11] V. Benkovic, A. Horvat Knezevic, D. Dikic et al., "Radioprotective effects of propolis and quercetin in γ-irradiated mice evaluated by the alkaline comet assay," *Phytomedicine*, vol. 15, no. 10, pp. 851–858, 2008.

[12] K. Ye, C.-B. Ji, X.-W. Lu et al., "Resveratrol attenuates radiation damage in Caenorhabditis elegansby preventing oxidative stress," *Journal of Radiation Research*, vol. 51, no. 4, pp. 473–479, 2010.

[13] L. Pari, D. Tewas, and J. Eckel, "Role of curcumin in health and disease," *Archives of Physiology and Biochemistry*, vol. 114, no. 2, pp. 127–149, 2008.

[14] B. B. Aggarwal and B. Sung, "Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets," *Trends in Pharmacological Sciences*, vol. 30, no. 2, pp. 85–94, 2009.

[15] M. Srinivasan, N. R. Prasad, and V. P. Menon, "Protective effect of curcumin on γ-radiation induced DNA damage and lipid peroxidation in cultured human lymphocytes," *Mutation Research: Genetic Toxicology and Environmental Mutagenesis*, vol. 611, no. 1-2, pp. 96–103, 2006.

[16] Q.-Y. Wei, W.-F. Chen, B. Zhou, L. Yang, and Z.-L. Liu, "Inhibition of lipid peroxidation and protein oxidation in rat liver mitochondria by curcumin and its analogues," *Biochimica et Biophysica Acta—General Subjects*, vol. 1760, no. 1, pp. 70–77, 2006.

[17] S. Somasundaram, N. A. Edmund, D. T. Moore, G. W. Small, Y. Y. Shi, and R. Z. Orlowski, "Dietary curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer," *Cancer Research*, vol. 62, no. 13, pp. 3868–3875, 2002.

[18] K. S. Lee, B. S. Lee, S. Semnani et al., "Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in drosophila melanogaster," *Rejuvenation Research*, vol. 13, no. 5, pp. 561–570, 2010.

[19] L.-R. Shen, F. Xiao, P. Yuan et al., "Curcumin-supplemented diets increase superoxide dismutase activity and mean lifespan in Drosophila," *Age*, vol. 35, no. 4, pp. 1133–1142, 2013.

[20] P. Okunieff, J. Xu, D. Hu et al., "Curcumin protects against radiation-induced acute and chronic cutaneous toxicity in mice and decreases mRNA expression of inflammatory and fibrogenic cytokines," *International Journal of Radiation Oncology Biology Physics*, vol. 65, no. 3, pp. 890–898, 2006.

[21] N. Aravindan, J. Veeraraghavan, R. Madhusoodhanan, T. S. Herman, and M. Natarajan, "Curcumin regulates low-linear energy transfer γ-radiation-induced NFκB-dependent telomerase activity in human neuroblastoma cells," *International Journal of Radiation Oncology Biology Physics*, vol. 79, no. 4, pp. 1206–1215, 2011.

[22] J. Veeraraghavan, M. Natarajan, P. Lagisetty, V. Awasthi, T. S. Herman, and N. Aravindan, "Impact of curcumin, raspberry extract, and neem leaf extract on rel protein-regulated cell death/radiosensitization in pancreatic cancer cells," *Pancreas*, vol. 40, no. 7, pp. 1107–1119, 2011.

[23] P. Javvadi, A. T. Segan, S. W. Tuttle, and C. Koumenis, "The chemopreventive agent curcumin is a potent radiosensitizer of human cervical tumor cells via increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase pathway," *Molecular Pharmacology*, vol. 73, no. 5, pp. 1491–1501, 2008.

[24] R. Parshad, K. K. Sanford, F. M. Price et al., "Protective action of plant polyphenols on radiation-induced chromatid breaks in cultured human cells," *Anticancer Research A*, vol. 18, no. 5, pp. 3263–3266, 1998.

[25] S. M. Khopde, K. I. Priyadarsini, S. N. Guha, J. G. Satav, P. Venkatesan, and M. N. Aswathanarana Rao, "Inhibition of radiation-induced lipid peroxidation by tetrahydrocurcumin: possible mechanisms by pulse radiolysis," *Bioscience, Biotechnology and Biochemistry*, vol. 64, no. 3, pp. 503–509, 2000.

[26] S. Kapoor and K. I. Priyadarsini, "Protection of radiation-induced protein damage by curcumin," *Biophysical Chemistry*, vol. 92, no. 1-2, pp. 119–126, 2001.

[27] K. M. Seong, C. S. Kim, S.-W. Seo et al., "Genome-wide analysis of low-dose irradiated male *Drosophila melanogaster* with extended longevity," *Biogerontology*, vol. 12, no. 2, pp. 93–107, 2011.

[28] A. A. Moskalev, A. S. Iatskiv, and V. G. Zaĭnullin, "Effect of low-dose irradiation on the lifespan in various strains of Drosophila melanogaster," *Genetika.*, vol. 42, no. 6, pp. 773–782, 2006.

[29] D. R. Boreham, J. A. Dolling, C. Somers, J. Quinn, and R. E. Mitchel, "The adaptive response and protection against heritable mutations and fetal malformation," *Dose-Response*, vol. 4, no. 4, pp. 317–326, 2006.

[30] S. J. Garte and F. J. Burns, "Oncogenes and radiation carcinogenesis," *Environmental Health Perspectives*, vol. 93, pp. 45–49, 1991.

[31] O. Vos, "Effects and consequences of prenatal irradiation," *Bollettino della Società Italiana di Biologia Sperimentale*, vol. 65, no. 6, pp. 481–500, 1989.

[32] I. Dalle-Donne, R. Rossi, D. Giustarini, A. Milzani, and R. Colombo, "Protein carbonyl groups as biomarkers of oxidative stress," *Clinica Chimica Acta*, vol. 329, no. 1-2, pp. 23–38, 2003.

[33] H. V. Goutham, K. D. Mumbrekar, B. M. Vadhiraja et al., "DNA double-strand break analysis by γ-H2AX foci: a useful method for determining the overreactors to radiation-induced acute reactions among head-and-neck cancer patients," *International Journal of Radiation Oncology Biology Physics*, vol. 84, no. 5, pp. e607–e612, 2012.

[34] J. P. Madigan, H. L. Chotkowski, and R. L. Glaser, "DNA double-strand break-induced phosphorylation of *Drosophila* histone variant H2Av helps prevent radiation-induced apoptosis," *Nucleic Acids Research*, vol. 30, no. 17, pp. 3698–3705, 2002.

[35] T. O. Hansen, P. Sarup, V. Loeschcke, and S. I. S. Rattan, "Age-related and sex-specific differences in proteasome activity in individual Drosophila flies from wild type, longevity-selected and stress resistant strains," *Biogerontology*, vol. 13, no. 4, pp. 429–438, 2012.

[36] K. G. Iliadi, N. N. Iliadi, and G. L. Boulianne, "Regulation of drosophila life-span: effect of genetic background, sex, mating and social status," *Experimental Gerontology*, vol. 44, no. 8, pp. 546–553, 2009.

[37] J. W. O. Ballard, R. G. Melvin, J. T. Miller, and S. D. Katewa, "Sex differences in survival and mitochondrial bioenergetics during aging in Drosophila," *Aging Cell*, vol. 6, no. 5, pp. 699–708, 2007.

[38] B. A. Callus and B. Mathey-Prevot, "SOCS36E, a novel Drosophila SOCS protein, suppresses JAK/STAT and EGF-R signalling in the imaginal wing disc," *Oncogene*, vol. 21, no. 31, pp. 4812–4821, 2002.

[39] L. Sefton, J. R. Timmer, Y. Zhang, F. Béranger, and T. W. Cline, "An extracellular activator of the Drosophila JAK/STAT pathway is a sex- determination signal element," *Nature*, vol. 405, no. 6789, pp. 970–971, 2000.

[40] S. Oetari, M. Sudibyo, J. N. M. Commandeur, R. Samhoedi, and N. P. E. Vermeulen, "Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver," *Biochemical Pharmacology*, vol. 51, no. 1, pp. 39–45, 1996.

[41] T.-S. Huang, S.-C. Lee, and J.-K. Lin, "Suppression of c-Jun/AP-1 activation by an inhibitor of tumor promotion in mouse fibroblast cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 12, pp. 5292–5296, 1991.

[42] L. Korutla and R. Kumar, "Inhibitory effect of curcumin on epidermal growth factor receptor kinase activity in A431 cells," *Biochimica et Biophysica Acta*, vol. 1224, no. 3, pp. 597–600, 1994.

[43] S. A. Marathe, I. Dasgupta, D. P. Gnanadhas, and D. Chakravortty, "Multifaceted roles of curcumin: two sides of a coin!," *Expert Opinion on Biological Therapy*, vol. 11, no. 11, pp. 1485–1499, 2011.

[44] W. Kim, K. M. Seong, and B. Youn, "Phenylpropanoids in radioregulation: double edged sword," *Experimental and Molecular Medicine*, vol. 43, no. 6, pp. 323–333, 2011.

[45] P. Tsvetkov, G. Asher, V. Reiss, Y. Shaul, L. Sachs, and J. Lotem, "Inhibition of NAD(P)H:quinone oxidoreductase 1 activity and induction of p53 degradation by the natural phenolic compound curcumin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 15, pp. 5535–5540, 2005.

[46] Y. Jiao, J. Wilkinson IV, X. Di et al., "Curcumin, a cancer chemopreventive and chemotherapeutic agent, is a biologically active iron chelator," *Blood*, vol. 113, no. 2, pp. 462–469, 2009.

[47] E. Burgos-Morón, J. M. Calderón-Montaño, J. Salvador, A. Robles, and M. López-Lázaro, "The dark side of curcumin," *International Journal of Cancer*, vol. 126, no. 7, pp. 1771–1775, 2010.

[48] G. C. Jagetia, "Radioprotection and radiosensitization by curcumin," *Advances in Experimental Medicine and Biology*, vol. 595, pp. 301–320, 2007.