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Antioxidant Blend of Curcumin and Broccoli Seed Extract Exhibits Protective Effect on Neurodegeneration and Promotes *Drosophila* Lifespan

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Abstract

Antioxidants and related compounds are anti-inflammatory and exhibit great potential in promoting human health. They are also often considered to be important elements in the process of neurodegeneration. Here we describe a antioxidant blend of Curcumin and Broccoli Seed Extract (BSE). Flies treated with the blend exhibit extended lifespan. RNA-seq analysis of samples from adult fly brains reveals a wide array of new genes with differential expression upon treatment with the blend. Interestingly, abolishing expression of some of the identified genes in dopaminergic (DA) neurons does not affect DA neuron number. Taken together, our findings reveal an antioxidant blend that promotes fly longevity and exhibits protective effect over neurodegeneration, demonstrating the importance of antioxidants in health and pathology.

Dear Editor:

Antioxidants have long been intriguing substances due to their potential in improving human health and preventing disease. For instance, Curcumin, the active ingredient in turmeric, is a polyphenolic compound that exerts potent anti-inflammatory and anti-oxidative effects (Panchal et al., 2008; Lestari and Indrayanto, 2014; Nahar et al., 2015; Liu et al., 2019; Cao et al., 2020). In addition, Sulforaphane, one of the active compounds in Broccoli seed extract (BSE), is a potent activator of Nrf2 which induces a host of antioxidant gene expression (Yanaka, 2017; Russo et al., 2018; Bao et al., 2019; Huang et al., 2019). These compounds have been tested separately, but the effect of a mixture of some, such as Curcumin and BSE, has not been tested. Basic research and clinical studies have been done to elucidate the positive effects of these compounds, however, studies providing conclusive evidence for their efficacy against human diseases are still lacking (Pulido-Moran et al., 2016; Kim and Clifton, 2018; Russo et al., 2018;

Huang et al., 2019). In particular, the impact of these compounds on aging and the cellular pathways underlying the action and mechanism of these compounds remain unexplained.

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Drosophila melanogaster (Fruit flies) is a highly trackable genetic model system for aging research. These animals exhibit sophisticated genetics, short lifespan, and low maintenance requirements. In addition, *Drosophila* have been used for modeling different human diseases including Alzheimer's disease and Parkinson's disease (PD). They greatly contribute to the anti-aging mechanism under pathological conditions, thus providing an excellent platform for investigating the effects of antioxidant-containing compounds.

To analyze the effect of our proprietary blend, a mixture of Curcumin and BSE, we first tested the lifespan of *Drosophila* treated with Curcumin or BSE. Interestingly, both male and female flies treated with Curcumin alone at a concentration of 2 g/L exhibited extended lifespan compared to the non-treated control flies (Figure 1A and D). Both male and female flies treated with BSE alone at a concentration of 1.2 g/L also exhibited extended

lifespan compared to the non-treated control flies, whereas male flies treated in a concentration of 0.6 g/L exhibited the greatest extension (Figure 1B and E). Furthermore, flies were treated with the blend as a mixture of Curcumin and BSE in a ratio 3 to 5 (Figure 1C and F). Interestingly, both male and female flies treated with the mixture in a concentration of 0.8 g/L exhibited the greatest extended lifespan (Figure 1C and F). Lifespan mean showing similar trends were also plotted accordingly (Figure 1G to J). Taken together, these results indicate that our tested blend promotes *Drosophila* longevity and extends fly lifespan.

In order to reveal the mechanism of how the blend treatment affects fly lifespan, RNA sequencing (RNA-seq) analysis was conducted using adult fly brains collected from animals in the presence or absence of the tested blend (Methods in Supplementary Materials). More than 70 differentially expressed genes, including
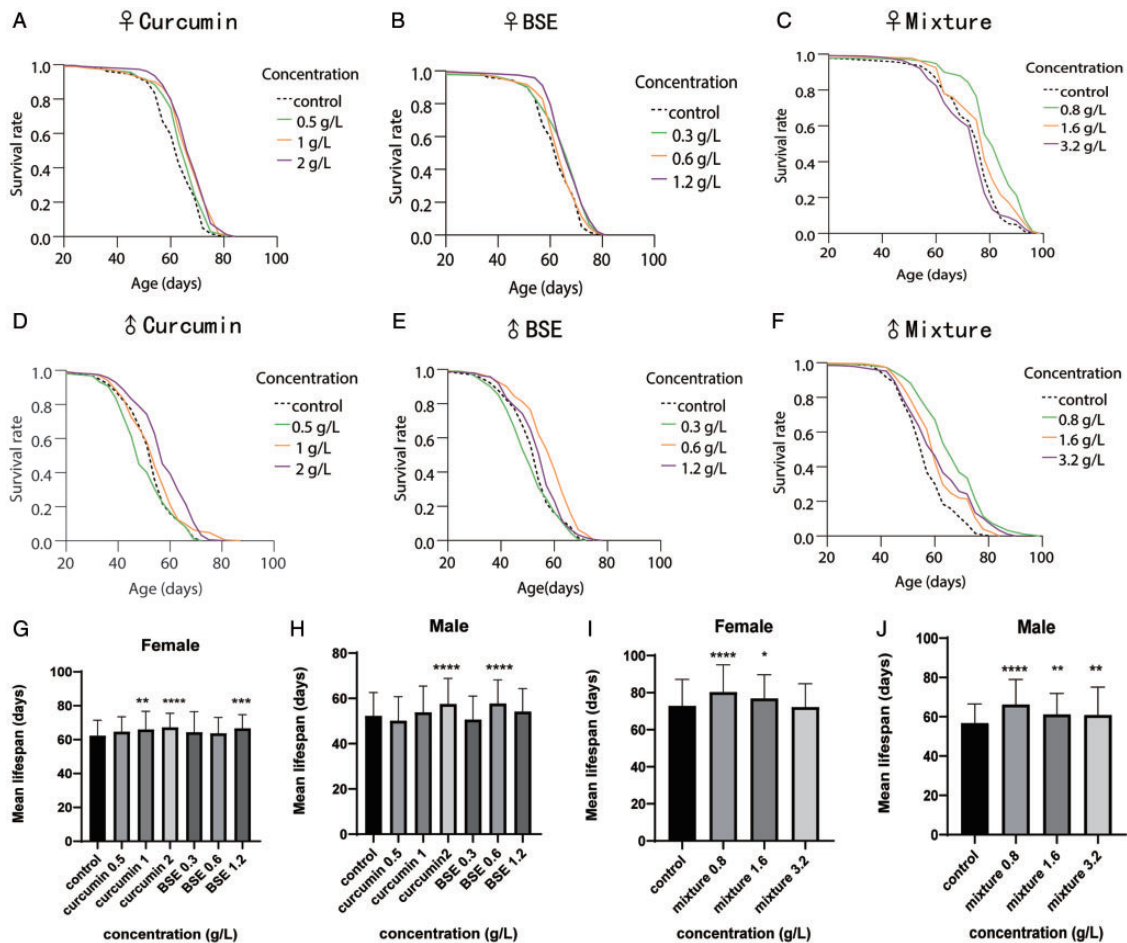

Figure 1. The Effect of the Tested Blend on *Drosophila* Lifespan. A–F: Survival curve of both female and male flies treated with the following: Curcumin at 0.5, 1, and 2 g/L (A,D), BSE at 0.3, 0.6, and 1.2 g/L (B,E) mixture of Curcumin and BSE at 0.8, 1.6, and 3.2 g/L (C,F). G–J: Mean lifespan of flies treated with Curcumin, BSE, or the blend. Average 200 flies were analyzed for each group. Data were shown as mean ± SEM. P-values of significance (indicated with asterisks, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) were calculated using one-way ANOVA with Bonferroni multiple comparison test among three groups or above. Prism and SPSS software were used to complete the statistical analysis.
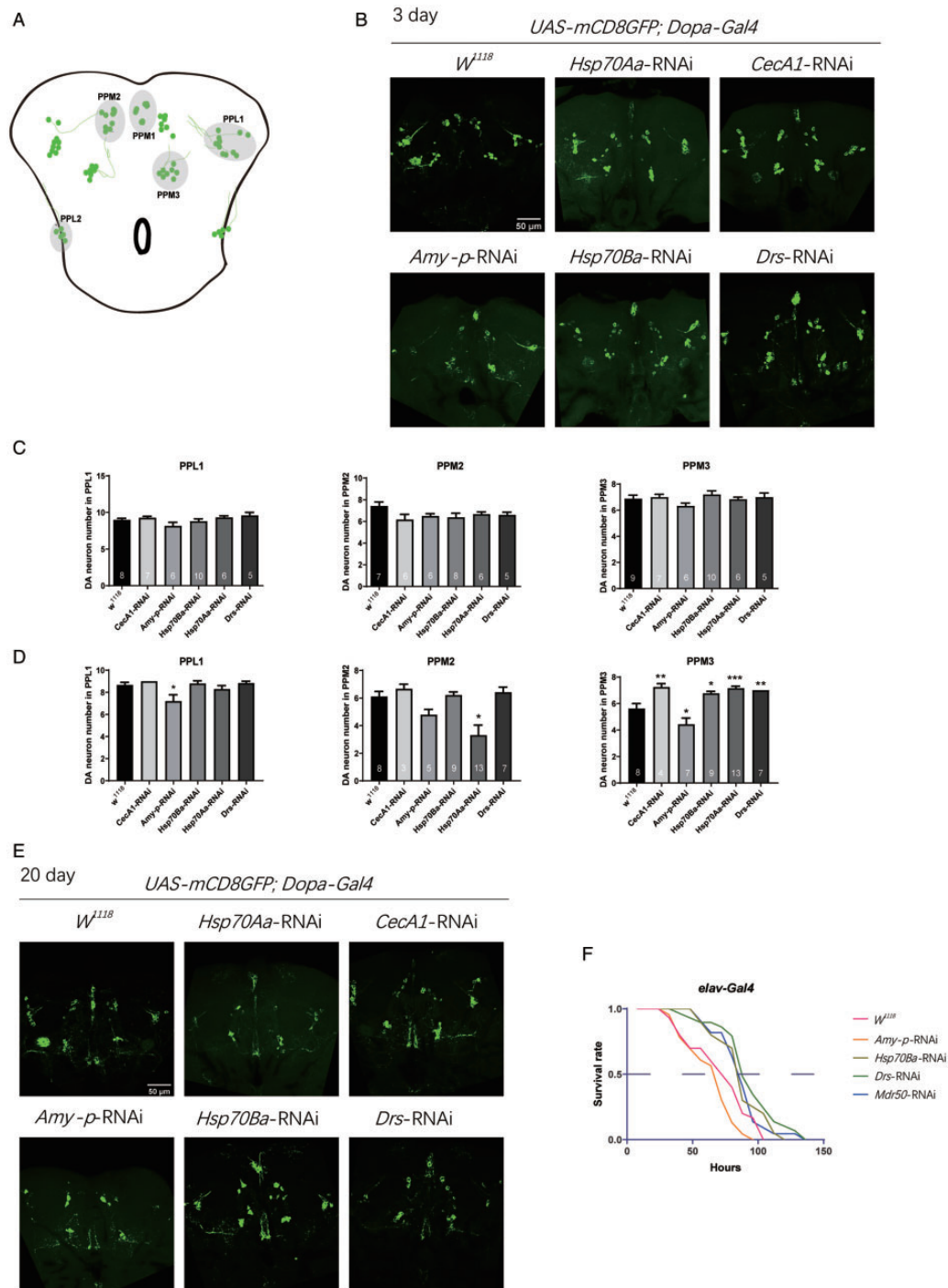
Figure 2. Treatment With the Tested Blend Exhibit Protective Effect on DA Neurodegeneration. A: A schematic illustration of the posterior DA neuron clusters (PPL1, PPL2, PPM1/2, and PPM3, green) in adult fly brains. Representative confocal images (B) and quantifications (C) of DA neuron number and fluorescent intensities in brains of 3-day-old flies with the indicated genotypes. Transgenic flies expressing RNAi for candidate genes like *Hsp70Ba*, *Hsp70Aa*, *CecA1*, *Amy-p*, and *Drs* in DA neurons were analyzed. Note that no significant difference is detected in the DA neuron number between control and most RNAi groups. Representative confocal images (D) and quantifications (E) of DA neuron number in brains of 20-day-old flies with the indicated genotypes. Transgenic flies expressing RNAi for candidate genes like *Hsp70Ba*, *Hsp70Aa*, *CecA1*, *Amy-p*, and *Drs* in DA neurons were analyzed. Note that no significant difference is detected in the DA neuron number between control and most RNAi groups except *Amy-p*-RNAi in PPL1 and *Hsp70Aa*-RNAi in PPM2. DA neuron number in PPM3 cluster for all RNAi groups except *Amy-p* increases. F: Transgenic flies expressing the RNAi for candidate genes like *Hsp70Ba*, *Drs*, and *Mdr50* were exposed to paraquat. Note that the RNAi expression increases fly survival rate. Average 10-15 brains were analyzed for each genotype. Data were shown as mean ± SEM. P-values of significance (indicated with asterisks, * p<0.05, ** p<0.01, *** p<0.001) were calculated using one-way ANOVA with Bonferroni multiple comparison test among three groups or above. Prism and SPSS software were used to complete the statistical analysis.

upregulated and downregulated ones, were identified (Supplementary Table 1). Principal component analysis (PCA) of RNA-seq results indicates differential gene expressions between samples from control and blend treated animals (Supplementary Figure S1). These results indicate that most of the genes were downregulated upon treatment with the tested blend. These genes were then further categorized by GO and KEGG analysis and cellular pathways mediated by these genes were identified (Supplementary Table S1). Some of the pathways are related to longevity and metabolism, further supporting the notion that our tested blend regulates *Drosophila* lifespan.

Based on the fact that antioxidants are important factors in neurodegenerative diseases (Mattson et al., 2008; Niedzielska et al., 2016; Umeno et al., 2017; Pinho et al., 2019), we took advantage of the *Drosophila* PD model established in the lab and analyzed the effects of the compound on dopaminergic (DA) neurodegeneration. DA neurons are located in clusters and named upon their relative positions in adult fly brains (Budnik and White, 1988; Nassel and Elekes, 1992). These clusters include protocerebral posterior lateral (PPL)1, PPL2, protocerebral posterior medial (PPM)1, PPM2, and PPM3 (Figure 2A). We first selected the candidate genes identified in the RNA-seq analysis with greater difference in expression levels for further analysis. Downregulation in expression levels of these genes were confirmed by qPCR (Supplementary Figure S2A). Selective knock-down of candidate gene expression was mediated by transgenic flies expressing RNAi in the DA neurons, with the RNAi knock-down efficiency verified by RT-PCR (Supplementary Figure S2B). Interestingly, as flies aged, the number of DA neurons in clusters PPL1, PPM2, and PPM3 remains similar in flies from 3- to 20-day-old, indicating that silencing expressions of these candidate genes does not cause severe DA neurodegeneration over time (Figures 2B to E). Moreover, the number of DA neurons in cluster PPM3 increases significantly over time when expressing most of the RNAi (Figure 2E). These findings implicate a protective effect of these candidate gene expressions on DA neurodegeneration. Given that these candidate gene expressions were reduced when flies treated with the tested blend, it is conceivable that the test blend exhibit protective effect on DA neurodegeneration via the regulation of these candidate gene expressions. Finally, exposure to paraquat, an organic compound that links to PD, causes fly death in a time frame of 40-60 hours. Whereas our control flies died rather early, transgenic flies expressing RNAi against different candidate genes did not accelerate the fly death; rather, the RNAi expression protected the flies from early death (Figure 2F). Taken together, our results indicate that these candidate genes differentially expressed upon treatment with our tested blend exhibit protective effects towards DA neurodegeneration and promotes fly longevity. Our findings open up the opportunity to study a wide array of new genes underlying the mechanism of aging and provide insights into the anti-aging effect of antioxidant compounds using our established fly model.

Author Contributions

J.C, H.W, M.S.H, and Y.R conceived and designed the study. J.C and H.W performed the experiments. J.C, H.W, M.B, D.S, Y.P, M.S.H, and Y.R analyzed the data. M.S.H and Y.R wrote the paper with the input of J.C and H.W. All authors read and approved the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplementary material for this article is available online.

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