Current Research in Food Science 5 (2022) 1640–1648

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Antiaging effect of anthocyanin extracts from bilberry on natural or UV-treated male *Drosophila melanogaster*

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ARTICLE INFO

Handling editor: Professor A.G. Marangoni

Keywords:
Anthocyanin
Drosophila melanogaster
Ultraviolet radiation
Antioxidant

ABSTRACT

Anthocyanins from bilberry (*Vaccinium vitis-idaea*) are one of the most abundant sources of polyphenols and are widely used in the food, medicine and cosmetics industries due to their antioxidation properties, but few studies have investigated their antiaging properties. Based on our previous examination, the effect of anthocyanin extracts from bilberry (BANCs) on several characteristics of natural and UV-treated male *Drosophila melanogaster*, including their lifespan, fecundity, and antioxidant capacity, was studied, and the related mechanisms were preliminary explored. The results indicated that BANCs can effectively prolong the average and maximum lifespan and improve the reproductive capacity and antioxidant capacity of natural and UV-treated flies. In particular, BANCs significantly changed the growth cycle, sex ratio and content of ROS in the fat bodies of the offspring and decreased the expression levels of antioxidant- and autophagy-related genes in UV-treated flies. Collectively, the results demonstrate that BANC supplementation in the medium effectively alleviated the aging process, and this effect was not directly correlated with the antioxidant and autophagy signaling pathways in the body of *D. melanogaster*.

1. Introduction

Bilberry (*Vaccinium vitis-idaea*) is a common dietary fruit and one of the most abundant sources of polyphenols, particularly anthocyanins (ANCs), which are water-soluble natural pigments that exist widely in plants, are flavonoids (Fang, 2014), and contain more than 20 types of known components, such as pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (Filippini et al., 2003). Under natural conditions, ANCs bind to glucose, rhamnose, galactose, arabinose and other glucosides to form anthocyanidins (Mattioli and Francioso, 2020). In recent years, ANCs have attracted increasing attention from researchers because of their role in the prevention and treatment of inflammation and cancer due to their excellent antioxidant characteristics and ability to scavenge free radicals (Zhao et al., 2021). Additionally, many studies have proven that ANCs exert a protective effect against liver damage caused by many heavy metal ions and harmful chemicals (Fan et al., 2019) and can strongly improve both memory and eyesight. However, few studies have investigated the anti-UV radiation and anti-aging activities of ANCs and their relevant mechanisms.

Aging is a normal process that occurs in various tissues and organs, and the biological functions of the body degenerate with age. In addition, the effects of aging are reflected in various characteristics and behavioral aspects, such as the lifespan, exercise vitality, and reproductive ability (Gunes et al., 2016), and are related to free radicals, which are a product of oxidation (Harman, 1995). Many studies have shown that oxidative stress, a type of oxidative damage caused by an imbalance between oxidation and antioxidant mechanisms, is a crucial contributor to the aging process (Zhang et al., 2018; Åhlberg, 2021).

Ultraviolet (UV) radiation, which is part of the sunlight spectrum and a type of electromagnetic radiation, is divided into three different bands, UVA (320–400 nm), UVB (280–320 nm) and UVC (100–280 nm)(Silván et al., 2016). Exposure to moderate UV light can promote vitamin D synthesis and prevent rickets (Holick, 2017), but excessive exposure can harm human health and cause DNA damage in skin cells that leads to dark spots and even skin cancer (Bagde et al., 2018). In addition, UV radiation could induce oxidative stress by the production of reactive oxygen species (ROS), which accelerates aging (Hsieh et al., 2018).

The antioxidant signaling pathway of NRF2 transcription factor (nuclear factor erythroid 2-like 2) is considered the best defense system against oxidative stress and toxicants (Chen et al., 2015). NRF2, a transcription factor with high sensitivity to oxidative stress, can bind to ARE in the nucleus, promote the transcription of a wide variety of

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https://doi.org/10.1016/j.crfs.2022.09.015
Received 6 May 2022; Received in revised form 25 August 2022; Accepted 12 September 2022
Available online 19 September 2022

antioxidant genes, such as superoxide dismutase (SOD)(Wang et al., 2019), glutathione peroxidase (GPx)(Margis et al., 2008), and catalase (CAT)(Sepasi Tehrani and Moosavi-Movahedi, 2018), and effectively remove excessive ROS. Macroautophagy is a lysosomal degradation pathway involved in protein and organelle degradation, which maintains cell homeostasis and provides energy to cells by degrading misfolded proteins and damaged organelles into small molecules. Recent studies have shown that ROS can initiate autophagosome formation and autophagic degradation by acting as cellular signaling molecules (Chen et al., 2009). The level of autophagy in cells can be measured by detecting the expression of autophagy-related (ATG) genes (Levine and Kroemer, 2019). Numerous studies have shown that oxidative stress is closely related to inflammatory responses and autophagy (Filomeni et al., 2015, Tian and Wang, 2017). In brief, autophagy and oxidative stress are important mechanisms for the maintenance of homeostasis in all animal tissues and are extremely relevant to aging.

Drosophila melanogaster (*D. melanogaster*) has become a model organism due to its short life cycle, and a large number of its metabolic pathways, physiological functions and developmental stages are similar to those of mammals (Oboh et al., 2022). Therefore, studying antiaging in *D. melanogaster* would have important theoretical and practical significance in this field (Nakajima et al., 2020). Based on our previous examination (Zhang et al., 2022), we used male *D. melanogaster* to explore the effects of bilberry ANC extracts (BANCs) on delaying the natural or UV-treated aging process and preliminarily examined the underlying mechanism.

2. Materials and methods

2.1. Experimental materials and reagents

The BANCs were prepared by extraction with ethanol after enzymatic hydrolysis by Zhejiang Huiyuan Pharmaceutical Co., Ltd. The relative ingredient values were analyzed using HPLC–MS methods by Qingdao Kechuang Quality Testing Co., Ltd. The ingredients included delphinidin 3-O-glucoside (14178.80 μg/g), cyanidin 3-O- arabinoside (100.69 μg/g), peonidin 3-O-glucoside (313.82 μg/g), cyanidin (1877.14 μg/g) and pelargonidin (25.97 μg/g). 4′,6-Diamidino-2-phenylindole (DAPI, from Beijing Solarbio Science & Technology Co., Ltd), dihydroethidium (DHE, from Shanghai 4 A Biotech Co., Ltd), erioglaucine disodium salt (Sigma Co., Ltd, USA), anhydrous ethanol (Shanghai Cloud Chemical Co., Ltd.), propionate and glacial acetic acid (Hangzhou Gaojing Fine Chemical Co., Ltd.) of analytical grade were used in this study. Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and total protein assay kits were purchased from Nanjing Jiancheng Bioengineering Co., Ltd. Sucrose (commercial), maize flour (commercial), AGAR powder (commercial) and yeast powder (commercial) were purchased from Hangzhou Best Biological Technology Co., Ltd.

2.2. Animal culture and lifespan examination

D. melanogaster Cation S lines were obtained from the *Drosophila* Stock Center at the Shanghai Academy of Life Sciences, Chinese Academy of Sciences. The flies were raised on basal medium (20.4 g of corn flour, 15.6 g of sucrose, 1.68 g of yeast powder, 1.8 g of agar powder, 1.2 mL of propionic acid and 190 mL of distilled water). The fruit flies were maintained in a chamber with controlled temperature and humidity (25 °C, 65% humidity) and a 12-h light cycle. Newly unmated *D. melanogaster* were anesthetized with CO_2 and divided into a control group that was cultured in basic media and experimental groups that were cultured in media with different concentrations of BANCs (0, 2.5, 5, and 10 mg/mL) according to preliminary results. Twenty male flies from each tube (10 tubes in each group, 200 flies of each group) were transferred into a culture tube with the corresponding medium. The food was replaced every 3 d, and the natural death time of each tube was counted until all *D. melanogaster* died. The mean life and maximum life were calculated for each experimental group.

2.3. UV irradiation treatment and lifespan examination

Unmated adults were selected 8–12 h after emergence. After mild anesthesia was administered with CO_2, the male flies were selected under a microscope and collected in a common Petri dish, which was covered with a net to prevent the flies from flying away. The Petri dishes containing fruit flies were directly placed on a sterile operating table with a UV lamp (30 W, 220 V, 50 Hz) at a distance of 30 cm and irradiated for 30 min (Pimentel et al., 2003). After irradiation, the wall of the tube was covered with a black cloth to prevent resurrection of light. In the UV radiation experiment, male fruit flies were randomly divided into the following groups: the blank control group, which was (CTRL-0) cultured in basal medium without radiation, the positive control group (CTRL-30) was cultured in basal medium and subjected to UV radiation for 30 min, and the experimental group (5.0–30) was cultured in medium with 5 mg/mL BANC and subjected to UV radiation for 30 min. The lifespan of UV-treated male *D. melanogaster* was examined using the same protocol as that used for flies that aged naturally.

2.4. Assay of the climbing ability of natural and UV-treated male D. melanogaster

The tube containing 20 flies was tapped until all the flies reached the bottom of the tube. Ten seconds later, the number of fruit flies at the top and bottom was recorded. The number of fruit flies on the top was subtracted from the number of flies on the bottom, and the percentage was calculated; this experiment was repeated three times. This analysis of exercise activity was performed 7 days after the flies were subjected or not subjected to UV irradiation.

2.5. Examination of the fecundity of natural and UV-treated male D. melanogaster

After flies were administered or not administered UV irradiation, the male flies of the control and irradiated groups were collected, and 10 male flies from each group and virgin flies of the same age without irradiation were randomly divided into pairs of male and female flies in each tube. After 3 days, the parents were released, and the timing of each growth stage from egg laying to feathering into flies was recorded. The progeny of *D. melanogaster* were collected. After feeding for 40 days, the number of flying *D. melanogaster* was recorded, and the flying sex ratio of each group was calculated according to a previous study (Ma et al., 2021).

2.6. Examination of the antioxidant capacity of natural and UV-treated male D. melanogaster

Male fruit flies were screened and divided into 4 groups (3 tubes per group), and each tube contained 30 fruit flies. On the 2nd (only UV-treated flies) and 7th days of culture, *D. melanogaster* were starved for 2 h and anesthetized with CO_2. The weight of the fruit flies in each tube was recorded. In proportion to the total weight of the weighed fruit fly (1:9), 0.9% normal saline was added to homogenize the samples under ice bath conditions, and centrifugation was performed at 4 °C and 2500 r/min for 20 min. The supernatant was used to determine the protein content, MDA content, and activities of SOD and CAT in whole flies according to the manufacturer's instructions.

2.7. ROS content in the fat body of *D.* melanogaster after UV irradiation

DHE can enter cells freely through living cell membranes and is oxidized by intracellular ROS to form ethylene oxide, and ethylene oxide can be incorporated into chromosomal DNA to produce red

fluorescence, which can be measured to reflect the ROS content in living cells (Issa et al., 2018). Unmated male adults were selected 8–10 h after emergence, and *D. melanogaster* were randomly divided into three groups after UV irradiation. *Drosophila* fat bodies were dissected and extracted in a frozen PBS solution. The separated adipocytes were combined with DHE in the dark for 30 min and then washed with PBS for 15 min. The adipocytes were fixed with 4% paraformaldehyde for 30 min in the dark and then washed with PBS for another 15 min. The adipocytes were combined with DAPI in the dark for 5 min, added to PBS and washed for 15 min. The tablets were sealed with 70% glycerol, observed by fluorescence microscopy and photographed. The ROS content in the fat body was indicated by the fluorescence intensity of DHE measured using ImageJ software.

2.8. Examination of gene expression in D. melanogaster after UV irradiation

After irradiation, male *D. melanogaster* were randomly divided into 3 groups (3 tubes per group, 25 flies in each tube), cultured, and collected until the 7th day of culture. Quantitative real-time PCR was used to quantitatively analyze the expression of antioxidant stress and cell autophagy-related genes. Primers were designed and synthesized by Wcgene Biotech (Shanghai, China). The mRNA expression levels were determined using *Rp49* as an internal reference, and each relative expression level of mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table 1.

2.9. Data analysis

All the tests were repeated three times. GraphPad Prism 6.0 (GraphPad Software, San Diego, USA) was used for the statistical analyses, and ImageJ (National Institutes of Health, Bethesda, USA) was used for image analyses. Differences among treatments were identified by one-way analysis of variance followed by Tukey's multiple comparisons test. Difference with $p < 0.05$ were deemed statistically significant.

3. Results

3.1. Lifespan of naturally aging D. melanogaster

As shown in Fig. 1A, the survival rate of *D. melanogaster* in the experimental group administered BANCs differed from that of the control group. Before 20 d of aging, the survival rate of both the control group and the experimental group was higher than 90%, and the difference between the two groups was not significant. From 30 to 40 days, the survival rate of *D. melanogaster* in the various groups decreased significantly. The survival rate of *D. melanogaster* administered 5.0 and 10.0 mg/mL BANCs was significantly higher than that of the control group, and no significant difference was detected between the experimental group administered 2.5 mg/mL BANCs and the control group. As shown in Fig. 1B, the maximum lifespans of *D. melanogaster* in the 5.0 and 10.0 mg/mL BANC-treated groups were prolonged by 3.87% ($p < 0.01$) and 3.34% ($p < 0.05$), respectively, compared with that of the control group. The average lifespans of the 2.5, 5.0, and 10.0 mg/mL BANC-treated groups were prolonged by 9.16% ($p < 0.01$), 11.90% ($p < 0.001$) and 6.88% ($p < 0.05$), respectively, compared with that of the control group.

Table 1
Real-time PCR primers of antioxidant stress and cell autophagy-related genes.

Gene name	Sequence 5′–3′	Annealing temp
AMPK	F: AGAGGTCTGCACCAAGTTCG, R: GTTTATTTGGTTGGCCGCGT	60 °C
Atg1	F: AAGGGCAGACAAGAGTCCAT, R: GTTCTCCCGCTTCCTCCTTT	60 °C
Atg5	F: ATATGCTTCCAGGCGGATCG, R: AACCACACAGCTCCATCCTG	60 °C
Atg8a	F: TCTAGCCACAGCAGTTAGCG, R: TTGTGTAGAGTGACCGTGCG	60 °C
GCL	F: GACACCGATACGCATTGCAC, R: CTCACCACGGAATCCTGCTT	60 °C
GSTS	F: CAGACCGTCAAGGACAACGA, R: TCGCGCTTGACCATGTAGTT	60 °C
NRF2	F: AGCTTCTGTCGCATGGTTGA, R: AGCCGTTGCTAACATGTCCA	60 °C
RP-49	F: AGGGTATCGACAACAGAGTG, R: CACCAGGAACTTCTTGAATC	60 °C

3.2. *Climbing ability*, reproductive ability and antioxidant capacity of *natural aging* D. melanogaster

As shown in Fig. 2A, BANCs had no significant effect on the climbing ability of fruit flies compared with that of the control group at the age of 7 d. As illustrated in Fig. 2B, the reproductive ability of *D. melanogaster* in the experimental groups treated with 2.5 mg/mL and 5.0 mg/mL BANCs was 311.13% ($p < 0.01$) and 265.64% ($p < 0.05$) higher than that of the control flies at the age of 7 d, respectively. The number of feathered offspring produced by the flies treated with 10 mg/mL groups BANCS was higher than that obtained with the control group, but the difference was not significant.

As shown in Fig. 2C, the SOD activity of *D. melanogaster* in the 2.5, 5.0 and 10.0 mg/mL experimental groups was increased by 56.32% ($p < 0.001$), 29.81% ($p < 0.05$) and 22.41% ($p < 0.01$), respectively, compared with that of the control group at the age of 7 d. In addition, CAT activity of *Drosophila* was increased by 60.69% ($p < 0.01$) and 89.45% ($p < 0.01$) in the 2.5 and 10.0 mg/mL experimental groups, respectively, compared with that of the control group, and the MDA content of 2.5, 5.0 and 10.0 mg/mL BANC-treated *D. melanogaster* was decreased by 72.38% ($p < 0.01$), 51.70% ($p < 0.01$) and 48.43% ($p < 0.01$), respectively, compared with the control level.

According to the results from the natural aging experiments, 5 mg/mL BANCs exerted a better antioxidant effect on and extended the lifespan of *D. melanogaster*, and a concentration of BANCs of 5 mg/mL was selected for UV treatment.

3. 3Lifespan of UV-treated D. melanogaster

As shown in Fig. 3A, before the age of 20 d, the survival rate of *D. melanogaster* in both the control group and the experimental group was higher than 90%. Starting at 20 d, the survival rate began to decrease significantly, and the 5.0–30 group exhibited improved survival rates compared with the CTRL-30 group. The mean lifespan of the CTRL-30 group was 13.27% lower than that of the CTRL-0 group ($p < 0.05$), and the mean lifespan of the 5.0–30 group was 10.16% higher than that of the CTRL-0 group ($p < 0.05$), as shown in Fig. 3B. Similarly, the maximum lifespan of the CTRL-30 group was 10.80% lower than that of the CTRL-0 group ($p < 0.05$), and the maximum lifespan of the 5.0–30 group was 15.33% higher than that of the CTRL-0 group ($p < 0.05$), as shown in Fig. 3B. The results showed that UV radiation reduced the lifespan of *D. melanogaster*, whereas BANCs reduced the effect of radiation on the lifespan.

3. 4Developmental cycles, fecundity and sex ratio of UV-treated D. melanogaster offspring

As shown in Fig. 4A, compared with those of the CTRL-0 group, the larval stage of fruit fly offspring in the CTRL-30 group was prolonged by 5.7 days, with an elongation rate of 186.57% ($p < 0.01$); the pupal stage was prolonged by 2.5 days, with an elongation rate of 66.41% ($p < 0.01$); and the whole development cycle was prolonged by 8.4 days, with an elongation rate of 85.05% ($p < 0.01$). Compared with that of the CTRL-30 group, the growth cycle of fruit fly offspring in the 5.0–30

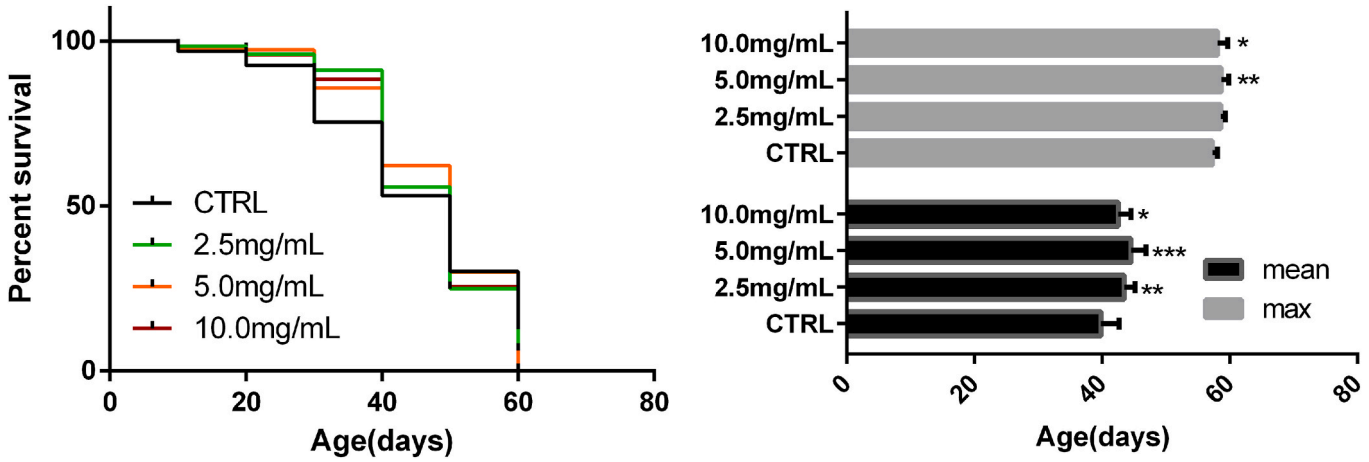

Fig. 1. Survival curve and lifespan of naturally aging male *D. melanogaster* fed a medium containing various BANC doses (0, 2.5, 5, and 10 mg/mL). Lifespan (A) and mean and maximal lifespan (B) of the various experimental groups administered BANCs (2.5, 5, and 10 mg/mL) or the control group not administered BANCs (0 mg/mL). Data from triplicate experiments are expressed as the means±SEMs (n = 3), and $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ indicate significant differences.

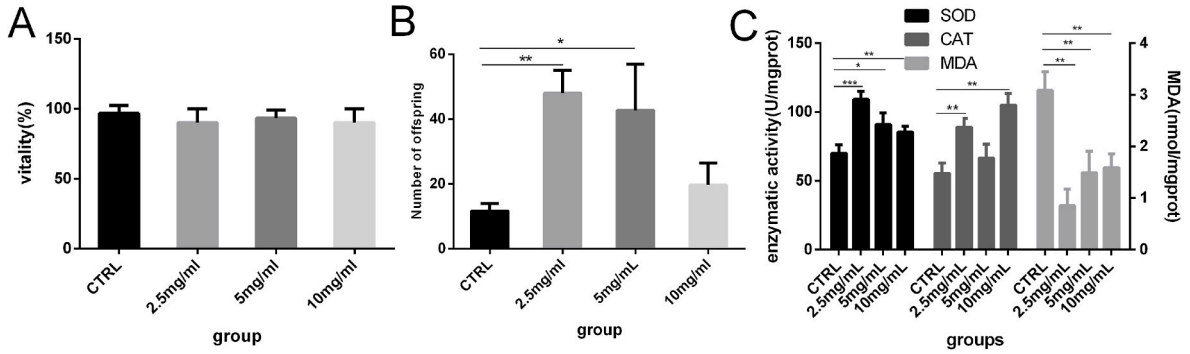

Fig. 2. Climbing ability (A), reproductive capacity (B), antioxidant enzyme activity (C) and MDA contents (C) of male *D. melanogaster* fed medium containing various BANC doses (0, 2.5, 5, and 10 mg/mL) on the 7th day. Data from triplicate experiments are expressed as the means±SEMs (n = 3), and $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ indicate significant differences.

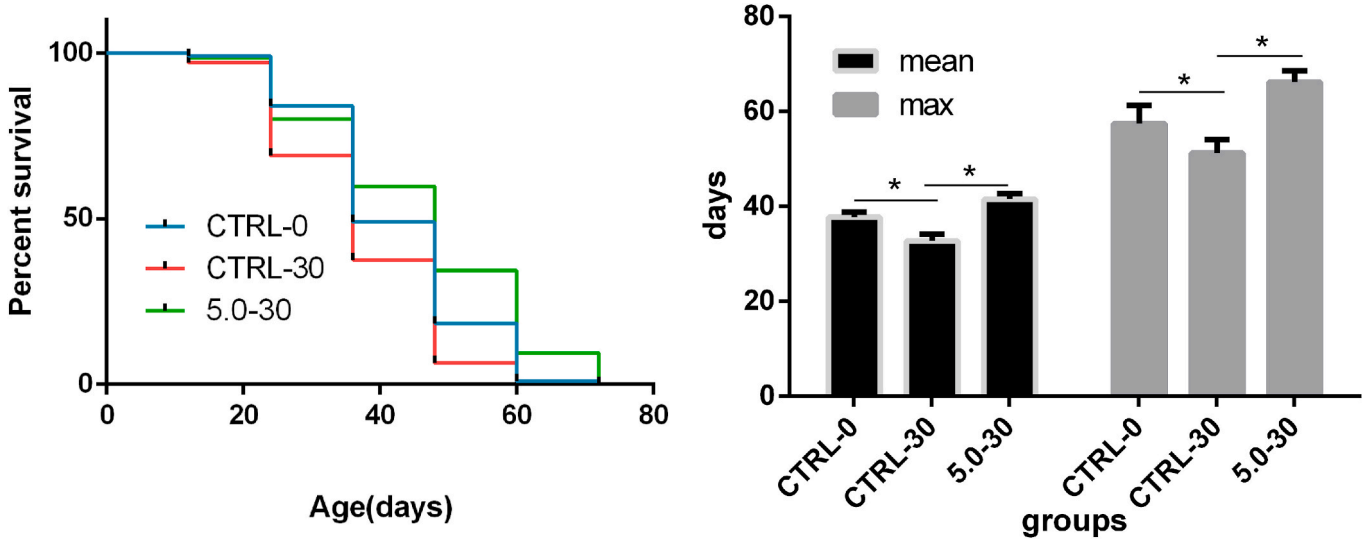

Fig. 3. Lifespan (A) and mean and maximal lifespan (B) of male UV-treated *D. melanogaster* administered BANCs. CTRL-0 is the control group, CTRL-30 is the UV-treated group, and 5.0–30 is the UV-treated group administered BANCs. Data from triplicate experiments are expressed as the means±SEMs (n = 3), and $*p < 0.05$ indicates significant differences.

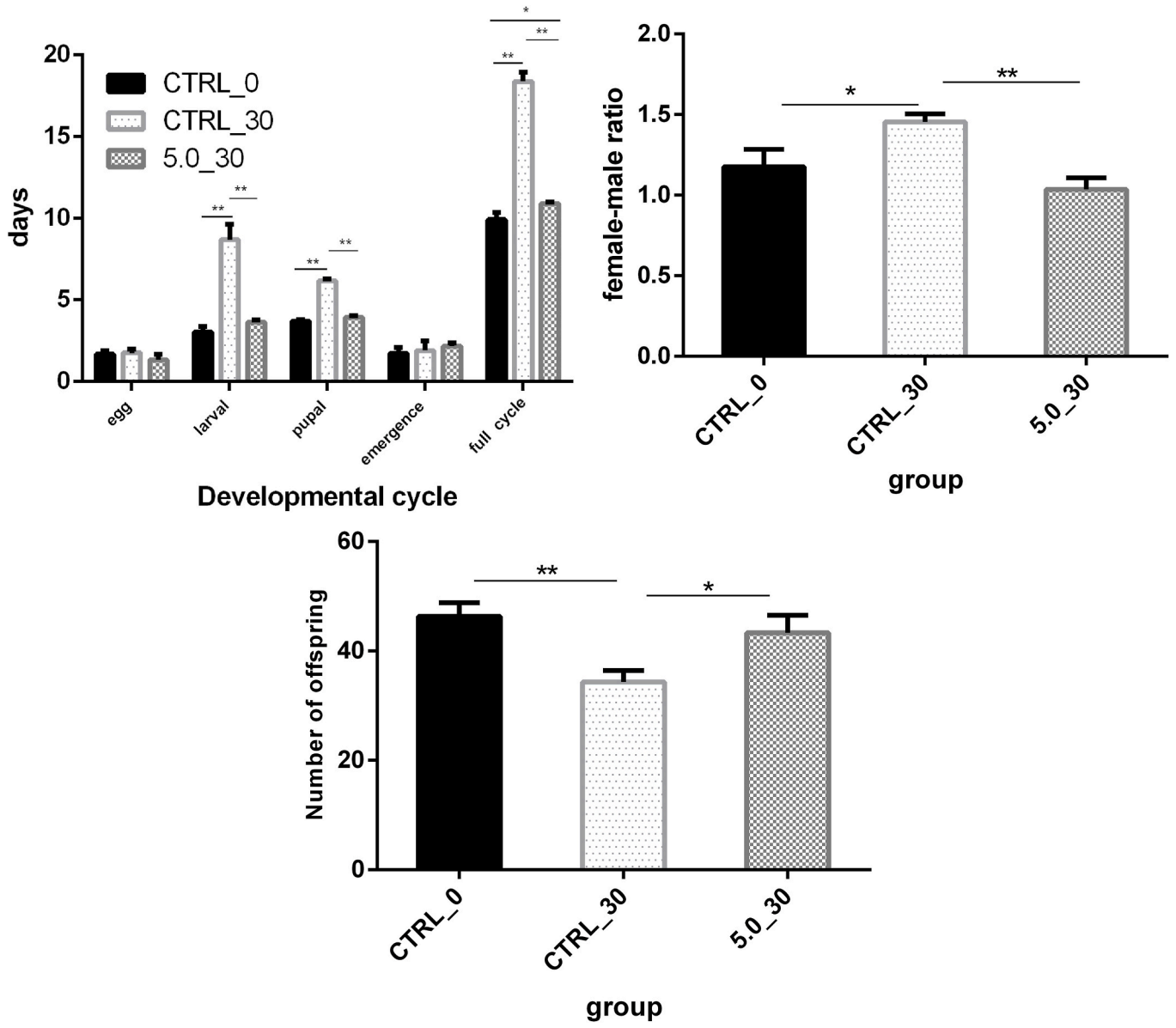

Fig. 4. Offspring growth cycle (A), sex ratio (B) and fecundity (C) of male UV-treated *D. melanogaster*. CTRL-0 is the control group, CTRL-30 is the UV-treated group, and 5.0–30 is the UV-treated group administered BANCs. Data from triplicate experiments are expressed as the means±SEMs (n = 3), and *$p < 0.05$ and **$p < 0.01$ indicate significant differences.

group supplemented with BANCs was significantly reduced after 30 min UV irradiation; specifically, the larval stage was reduced by 5.1 days, with a reduction rate of 58.31% ($p < 0.01$), the pupal stage was reduced by 2.2 days, with a reduction rate of 36.12% ($p < 0.01$), and the whole development cycle was reduced by 7.5 days, with a reduction rate of 40.66% ($p < 0.01$), compared with those of the CTRL-30 group. The whole development cycle of the 5.0–30 group was prolonged by 7.5 days, with an elongation rate of 10.10% ($p < 0.05$), compared with that of the CTRL-0 group.

As shown in Fig. 4B, the ratio of females to males in the offspring of the CTRL-30 group was 23.74% ($p < 0.05$) higher than that of the CTRL-0 group. No significant difference in the ratio of females to males in the offspring was found between the 5.0–30 group and the CTRL-0 group. The ratio of females to males in the offspring of the CTRL-30 group was increased by 11.39% compared with that of the 5.0–30 group ($p < 0.01$). As shown in Fig. 4C, the number of generations in the CTRL-30 group was decreased by 25.9% ($p < 0.01$) compared with that of the CTRL-0 group, and the number of fruit fly offspring in the 5.0–30 group was increased by 26.21% ($p < 0.05$) compared with that of the CTRL-30 group. No significant difference in the number of fruit flies was found between the CTRL-0 group and the 5.0–30 group. The results showed that UV radiation increased the proportion and decreased the number of females in *D. melanogaster* offspring and that BANCs could alleviate the effect of UV radiation on the reproductive ability of *D. melanogaster*.

3. 5Antioxidant enzyme activity and MDA content of UV-treated *D. melanogaster*

Fig. 5A shows that at the age of 2 d, SOD activity of *D. melanogaster* in the CTRL-30 group was decreased by 19.14% ($p < 0.01$) compared with that of the CTRL-0 group, and SOD activity of the 5.0–30 group was increased by 14.87% ($p < 0.05$) compared with that of the CTRL-30 group. At the age of 7 d, SOD activity of *D. melanogaster* in the CTRL-30 group was decreased by 23.49% ($p < 0.01$) compared with that of the CTRL-0 group, and SOD activity of the 5.0–30 group was increased by 23.53% ($p < 0.01$) compared with that of the CTRL-30 group. On the

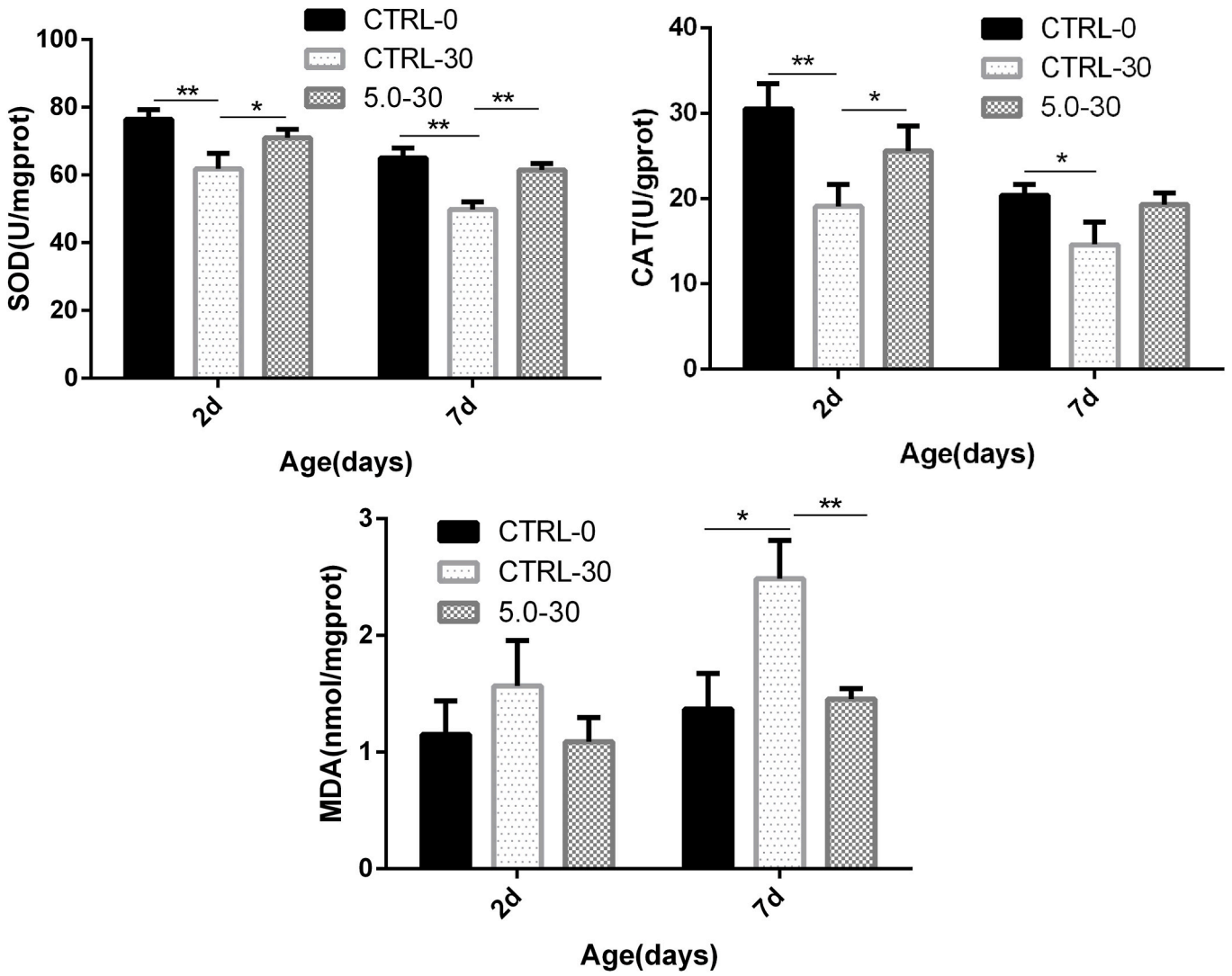

Fig. 5. Effect of BANCs on the antioxidant enzyme activity (A, B) and MDA contents (C) in whole male UV-treated *D. melanogaster* on the 2nd and 7th days. CTRL-0 is the control group, CTRL-30 is the UV-treated group, and 5.0–30 is the UV-treated group administered BANCs. Data from triplicate experiments are expressed as the means±SEMs (n = 3), and $*p < 0.05$ and $**p < 0.01$ indicate significant differences.

2nd day, CAT activity of *D. melanogaster* in the CTRL-30 group was decreased by 37.38% ($p < 0.01$) compared with that of the CTRL-0 group, and CAT activity of the 5.0–30 group was increased by 33.77% ($p < 0.05$) compared with that of the CTRL-30 group. At the age of 7 d, CAT activity of *D. melanogaster* belonging to the CTRL-30 group was decreased by 28.32% ($p < 0.01$) compared with that of the CTRL-0 group, as shown in Fig. 5B. At the age of 7 d, the MDA content of the CTRL-30 group was increased by 81.53% ($p < 0.05$) compared with that of the CTRL-0 group, and the MDA content of the 5.0–30 group was decreased significantly by 41.58% ($p < 0.01$) compared with that of the CTRL-30 group, as shown in Fig. 5C.

3. 6ROS content in the fat body of UV-treated D. melanogaster

UV irradiation significantly increased the level of ROS in *D. melanogaster* fat bodies, and as shown in Fig. 6A, a significant difference was detected between the CTRL-30 group and the 5.0–30 group. The average fluorescence intensity of the *D. melanogaster* fat body of the CTRL-30 group was increased by 171.28% ($p < 0.01$) compared with that of the blank control group. The average fluorescence intensity of the *D. melanogaster* fat body of the 5.0–30 group was decreased by 58.39% ($p < 0.01$) compared with that of the CTRL-30 group, but no significant difference was detected between the CTRL-0 group and the 5.0–30 group, as shown in Fig. 6B. The results showed that UV irradiation could significantly increase the content of ROS in *D. melanogaster* fat bodies and that BANCs could reduce the effect of UV irradiation on *D. melanogaster*.

3. 7Gene expression levels of **antioxidant and autophagy signaling** pathways in UV-treated D. melanogaster

The expression levels of AMPK, ATG1, ATG5, and ATG8a in *D. melanogaster* belonging to the CTRL-30 group were significantly increased by 65.61% ($p < 0.001$), 134.07% ($p < 0.001$), 42.91% ($p < 0.0001$), and 88.75% ($p < 0.0001$), respectively, compared with those in the CTRL-0 group, as shown in Fig. 7A. The expression levels of AMPK, ATG5, and ATG8a in the 5.0–30 group were decreased by 26.31% ($p < 0.05$), 53.17% ($p < 0.0001$), 25.58% ($p < 0.0001$), respectively, compared with those in the CTRL-30 group, as illustrated in Fig. 7A. The expression levels of NRF2, GCL, GSTs, and SOD in *D. melanogaster* in the CTRL-30 group were increased by 108.56% ($p < 0.0001$), 128.94% ($p < 0.0001$), 86.81% ($p < 0.001$), and 73.71% ($p < 0.0001$), respectively, compared with those in the CTRL-0 group, and the expression levels of NRF2, GCL, GSTs, SOD in *D. melanogaster* in the 5.0–30 group were

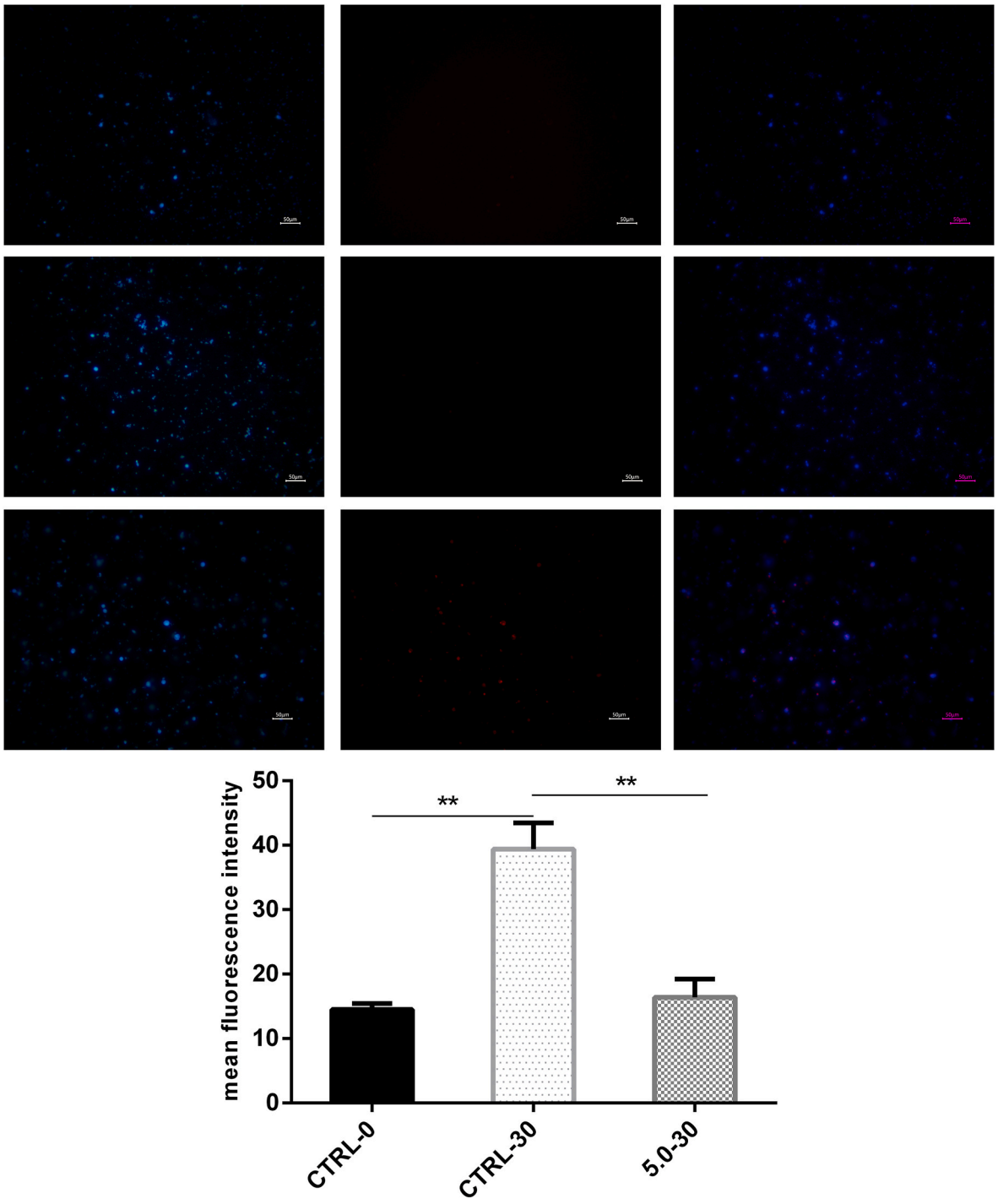

Fig. 6. DHE (red) and DAPI (blue) staining of the fat body of UV-treated *D. melanogaster*. (A) Fluorescence image of the fat body. Scale bar: 50 μm. (B) Average fluorescence intensity of each group. CTRL-0 is the control group, CTRL-30 is the UV-treated group, and 5.0–30 is the UV-treated group administered BANCs. Data from triplicate experiments are expressed as the means±SEMs (n = 3), and $^{**}p < 0.01$ indicates significant differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

decreased by 29.53% ($p < 0.001$), 18.02% ($p < 0.001$), 61.81% ($p < 0.0001$), and 34.61% ($p < 0.0001$), respectively, compared with those in the CTRL-30 group, as shown in Fig. 7B.

4. Discussion

According to the free radical theory, the aging process is thought to cause an imbalance between oxidative damage and antioxidative defense (Ashok and Ali, 1999). Therefore, researchers have suggested that preventing oxidative damage by enhancing the antioxidative defense status may counteract aging and age-associated disorders, such as cancer (Staats et al., 2018), cardiovascular disorders (Reis, et al., 2016) and some neurodegenerative disorders (Winter et al., 2019). The UVA component of sunlight is considered the main cause of the prominent changes that occur in the dermal extracellular matrix (ECM) and potent DNA mutagenic lesions (Hayakawa et al., 1995). UVB radiation is primarily a DNA-damaging agent because it is directly absorbed by DNA and is known to cause cyclobutane pyrimidine dimers (CPDs) and 6-4

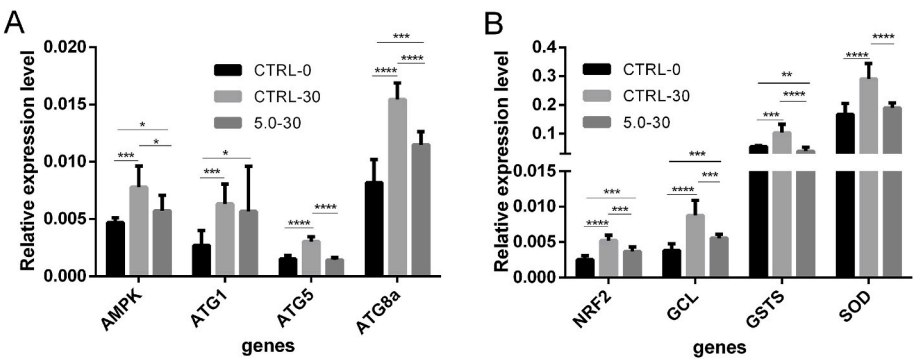

Fig. 7. Effects of BANCs on the gene expression levels of NRF2 and autophagy signaling pathways in UV-treated *D. melanogaster*. (A) Autophagy signaling pathway genes and (B) NRF2 signaling pathway genes. CTRL-0 is the control group, CTRL-30 is the UV-treated group, and 5.0–30 is the UV-treated group administered BANCs, and the relative gene expression levels were normalized to the Rp49 level. Data from triplicate experiments are expressed as the means ±SEMs (n = 3), and $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$ indicate significant differences.

pyrimidine-pyrimidone dimers (6–4 PP) by ROS generation (Schuch et al., 2017). It has been reported that various radiation sources can not only change the lifespan but also significantly affect the fertility of *D. melanogaster*. Consequently, to delay the aging process by attenuating the oxidative stress induced by excessive ROS, ANC agents, which are well known as antioxidants, have attracted much attention.

According to previous studies, the lifespan is the most intuitive indicator of the degree of aging (Levine et al., 2018), and exercise vitality is negatively correlated with aging (Gilbert et al., 2014). Additionally, some studies have shown that reproduction, which involves physical exertion, exhibits a trade-off with aging, and many species can increase their lifespan by reducing their fertility. In the natural aging study, BANCs, particularly the dose of 5.0 mg/mL, significantly improved the average life expectancy and maximal lifespan, and the reproductive ability was improved in all the experimental groups, but the climbing ability of male flies fed medium containing BANCs was not significantly improved. After UV radiation for 30 min, the survival rate, including the mean lifespan and maximum lifespan, of the group administered BANCs was higher than that of the group not administered BANCs. Moreover, the ratio of females to males in the experimental group not administered BANCs was significantly increased, whereas that in the experimental group administered BANCs was decreased. Therefore, we can conclude that BANCs can delay the aging process in natural and UV-treated male *D. melanogaster*.

Additionally, we found that BANCs could effectively increase SOD and CAT activity and decrease the MDA content in natural and UV-treated male *D. melanogaster*. According to the DHE dyeing test, BANCs can significantly reduce the ROS content in the UV-treated *D. melanogaster* fat body, which is an important metabolic organ that is equivalent to the liver and adipose tissue in mammals (Géminard et al., 2009; Staats et al., 2018), and restore the ROS content to the level observed without irradiation. Excess ROS production by UV radiation can overwhelm the endogenous antioxidant capacity and thus accelerate the aging of the organism (Au et al., 2002). Because oxidative stress usually causes damage to cells and results in the production of corresponding harmful substances, such as autophagosomes, we wondered whether BANCs could activate the NRF2 and autophagy signaling pathways, which could regulate peroxide and waste removal in cells (Maruzs et al., 2019). In the experiment, the results obtained for four genes (GCL, GSTS, NRF2, and SOD) in the NRF2 signaling pathway showed that oxidative stress induced by UV irradiation resulted in increased expression of these genes in flies, but the expression was significantly downregulated in the flies administered BANCs. The expression levels of genes in the autophagy signaling pathway (AMPK, ATG5, and ATG8a) showed the same results as those obtained from the analysis of the NRF2 signaling pathway. Therefore, a certain amount of BANCs can effectively delay the senescence process in male fruit flies through antioxidant effects in the body. However, in this experiment, BANCs did not regulate autophagy or the antioxidant signaling pathway to decrease free radical production in UV-treated male *D. melanogaster*. The results were not consistent with other studies of ANCs, including the result that bilberry ANCs induce autophagy via the AMPK−mTOR signaling pathways (Li et al., 2019), and might be attributed to BANCs having a direct effect on antioxidant enzymes and peroxide. According to the literature, freeze-dried ANCs can directly bind to free radicals to directly induce antioxidant effects and can also achieve indirect antioxidant effects by reducing other oxidants that have been oxidized (Leopoldini et al., 2010). Additionally, ANCs can directly activate the antioxidant enzyme system through various methods, such as stimulating the production and activity of SOD and CAT and strengthening the endogenous antioxidant system (Bártíková et al., 2013), and ANCs can directly bind to MDA and reduce the MDA content (Wei et al., 2017).

In conclusion, BANCs can obviously alleviate the aging progress of natural and UV-treated male *D. melanogaster*, and these results are reflected by an extended lifespan and improved fecundity. The preliminary analysis showed that the mechanism involves direct removal of peroxide and activation of the antioxidant enzyme system in the *D. melanogaster* body without activation of the NRF2 and autophagy signaling pathways.

Funding

This research was financially supported by a grant (2019C02064) from the Science and Technology Development Project of Zhejiang Province, China.

CRediT authorship contribution statement

Guocai Zhang: Methodology. **Xianjun Dai:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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