

Berberis vulgaris L. extract supplementation exerts regulatory effects on the lifespan and healthspan of *Drosophila* through its antioxidant activity

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Abstract

Worldwide aging population continues to increase, so the concept of healthy longevity medicine has become increasingly significant in modern society. *Berberis vulgaris* L. fruits serve as a functional food supplement with a high concentration of bioactive compounds, which offer numerous health-promoting benefits. The goal of this study was to investigate the geroprotective effect of *Berberis vulgaris* L. extract. Here we show that extract of *Berberis vulgaris* L. can, depending on concentrate, increase lifespan up to 6%, promote healthspan (stress resistance up to 35%, locomotor activity up to 25%, integrity of the intestinal barrier up to 12%, metabolic rate up to 5%) of *Drosophila melanogaster* (*in vitro*) and exhibits antioxidant (using red blood cell tests) and antiglycation activity (using glycation of bovine serum albumin) (*in vitro*). In addition to this, the extract does not exhibit cytotoxic properties *in vitro*, unlike the well-known polyphenolic compound quercetin. qRT-PCR revealed the involvement of metabolic, heat shock response and lipid metabolism genes in the observed effects.

Introduction

Aging represents the biological process of progressive decline in the functionality of living organisms over time (Rose et al. 2012). As the aging progresses our cells, tissues, and organs gradually experience functional decline, leading to increased susceptibility to diseases and reduces the overall resistance to physiological stress (Ucar and Benayoun 2018). The use of nutritional supplements is one of the methods to fight against aging (Gao et al. 2023a). Dietary supplements enriched with bioactive compounds represent a promising approach to influencing physiological processes, thereby contributing to overall health and longevity. Within the kingdom of dietary supplements, anthocyanins have attracted considerable attention. These natural compounds are abundant in fruits, vegetables, grains, and berries (Rahaman et al. 2023). Anthocyanins have been reported to combat oxidative stress, one of the significant contributors to aging and age-related diseases (Wang et al. 2022). By increasing the body's resistance to physiological stress and potentially delaying cellular aging, anthocyanins present can help with health promotion and lifespan extension (Kalt 2019; Wallace and Giusti 2019). Anthocyanins have been shown to possess potent antioxidant properties and have been linked to a reduced risk of age-related diseases such as cardiovascular disease, cancer, and neurodegenerative disorders (Chhoden et al. 2023; Khoo et al. 2017; Salehi et al. 2020). Anthocyanidins have the ability to suppress the reaction of non-enzymatic glycosylation (glycation), playing a certain role in the entire reaction process (Gao et al. 2023b). Recent studies have also shown that anthocyanins can extend the lifespan and healthspan of model organisms such as *Drosophila melanogaster*, *Caenorhabditis elegans* and mice highlighting their potential as natural supplements for promoting healthy aging (Butelli et al. 2008; Chen et al. 2013; Pandey et al. 2022; Xu et al. 2021). One of the sources of these compounds is berries of barberry.

Berberis vulgaris L, commonly known as the European barberry, is a deciduous shrub that produces small, red berries. The plant is native to Europe and parts of Asia, and its berries have been used for medicinal purposes for centuries due to their high content of bioactive compounds (Eroğlu et al. 2020). Particular interest in recent years are the anthocyanins which can be found in *Berberis vulgaris* L. berries. The fruit

of this plant contain high levels of anthocyanins, including cyanidin-3-O-glucoside and delphinidin-3-O-glucoside (Eroğlu et al. 2020).

The consumption of berries has gained increasing interest in recent years due to their potential health benefits (Alam et al. 2021). In particular, the role of berries in promoting healthy aging has become a focus of research. This study showed the potential lifespan and healthspan (locomotor activity, stress resistance, metabolism, intestinal integrity) effects of *Berberis vulgaris* L. extract (BE) supplementation at different concentrations, as well as elucidated the possible mechanisms of action through investigation of antioxidant and antiglycation activities. As model systems, we used *Drosophila melanogaster*, mammalian cells and tissue homogenates.

Methods

Berry Picking, Extraction and Chemical Analysis

The berries of *Berberis vulgaris* L. were obtained from the "Scientific collection of living plants" (N 507428) of the Botanical Garden of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences (61°37'08.3 'N 50°45'42.0 E) in July of 2020. Berries of *Berberis vulgaris* L. were processed to extract anthocyanins using an ethanolic extraction method. The extraction focused on isolating anthocyanins due to their solubility in the given conditions, while other phenols remained largely unextracted. The extraction was performed according to the laboratory schemes described in the previous work (Golubev et al. 2022).

Fly stock and maintenance conditions

Drosophila melanogaster wild-type *Canton-S* strain was acquired from the Bloomington *Drosophila* Stock Center at Indiana University (#64349, BDSC, USA). Flies were maintained at 25°C with a 12-hour light cycle in a Binder KBF720-ICH constant climate chamber (Binder, Germany). The fly medium was prepared by dissolving the following components per 1 L of water: 92 g corn flour, 32.1 g dry yeast, 5.2 g agar, 136.9 g glucose, 8 ml propionic acid (Sigma-Aldrich, USA), and 5 ml methyl 4-hydroxybenzoate (Sigma-Aldrich, USA).

Lifespan assay

Within 24 hours after imago eclosion the flies were sorted by sex (mated males and non-virgin females) using CO₂ anesthesia and randomly distributed into control and experimental vials (5 vials per group with 30 flies per vial for each sex). The number of dead flies was recorded daily, surviving flies were moved to new vials twice weekly for the duration of their lives. The median and maximum (age of 90% mortality) lifespans were determined. This assay was replicated 3 times for control, 0.01, 0.1, 1, 2.5, 5 mg/ml and 2 times for 50 mg/ml.

Analysis of Stress Resistance

To explore the effects of BE on stress resistance, flies were exposed to 0.1 mg/ml and 50 mg/ml BE under high-stress conditions from the 10th day after imago eclosion and treatment with investigated compounds. The DAM2 *Drosophila* Activity Monitor (TriKinetics Inc., USA) was used to assess stress resistance. The time of death was determined by measuring locomotor activity at 60-minute intervals. The flies were housed in small 5 × 65 mm glass tubes throughout the experiment. Resistance to oxidative stress was assessed by exposing the flies to a dietary medium containing 2% agar, 5% sucrose, and 20 mM paraquat (Sigma-Aldrich, USA). Hyperthermia was induced by exposing the flies to a constant temperature of 35°C with the same medium but without paraquat. Stressful conditions were maintained until the flies died, without transferring them to fresh tubes. The assay was replicated twice, with 32 flies per replicate.

Locomotor activity analysis

The spontaneous locomotor activity variations with age were assessed utilizing the LAM25 Locomotor Activity Monitor (TriKinetics Inc., USA) under a 12-hour light/dark cycle. Data from 10 flies were accumulated over a 24-hour period and presented as the mean total daily locomotor activity. Mated male and non-virgin female flies were examined independently. Weekly assessments were performed on the same group of flies, beginning at week 1 and continuing through week 9 of their lifespan. During the experiment, flies were maintained on a nutrient medium containing BE at concentrations of 0.1 and 50 mg/ml, while 96% ethanol was administered to the control vials. The experiment was performed in five replicates, with each replicate consisting of 10 flies.

Respirometry

The metabolic rate of the flies was evaluated by measuring CO₂ production using flow-through respirometry with the Multiple Animal Versatile Energetics (MAVEN) platform (Sable Systems International, USA). A basal respiratory mixture, consisting of 20% oxygen and 80% nitrogen with zero CO₂ content, was supplied from a cylinder at a flow rate of approximately 20 ml/min through each of the 16 respirometric chambers simultaneously. Respirometry was performed on groups of 10 adult flies in each chamber. The CO₂ production was normalized to the insect wet body mass. Automatic switching of the respiratory mixture flow between the chambers and the MAVEN baseline occurred at a 16:1 alternating ratio, with a 3-minute dwell time per chamber. The differential concentration of carbon dioxide was measured using a Licor 850 infrared gas analyzer (Licor, USA). Three chambers with 10 flies each were used for each experimental condition.

Analysis of intestinal integrity

The Smurf assay was employed to assess intestinal barrier permeability (Rera et al. 2012). Both the control and experimental groups of flies aged 9 weeks were included in the study. The experimental flies were maintained on a nutrient medium containing BE at a concentration of 0.1 and 50 mg/ml. Flies were exposed to a food medium containing 2.5% (w/v) Brilliant Blue FCF dye for a period of 16 hours. Flies exhibiting increased intestinal permeability, as indicated by blue coloration outside of the digestive tract, were counted. The assay was performed in duplicate, with each replicate consisting of 70–100 flies.

Quantitative real time PCR

Gene expression was measured by quantitative real-time polymerase chain reaction (qRT-PCR) with a reverse transcription step. RNA was isolated using an Aurum Total RNA Mini kit (Bio-Rad, USA) according to the manufacturer's instructions. RNA concentration was measured using a Quant-iT RNA Assay Kit (Invitrogen, USA) according to the manufacturer's instructions. cDNA was synthesized according to the iScript cDNA Synthesis Kit (Bio-Rad, USA) from the resulting RNA solution. The reaction mixture for the PCR reaction was prepared based on qPCRMix-HS SYBR (Evrogen) and primers (Table S1). The primer design or selection was performed using QuantPrime online tool (Arvidsson et al. 2008) and FlyPrimerBank: an online database for *Drosophila melanogaster* gene expression analysis (Hu et al. 2013), respectively. The polymerase chain reaction was carried out in a CFX96 amplifier (Bio-Rad, USA) using the following program: 1) 95°C for 30 s, 2) 95°C for 10 s, 3) 60°C for 30 s, 4) steps 2–3 were repeated 49 times 5) DNA melting step.

The expression level of the studied genes was calculated relative to the expression of the housekeeping genes *β-Tubulin at 56D* and *Ribosomal protein L32* using the CFX Manager 3.1 software (Bio-Rad, USA). Differences were considered significant changes (increase or decrease) in expression: $p < 0.05$ according to the Student's t-test and crossing the Regulation Threshold. The level of mRNA expression was determined using qRT-PCR in the experimental flies, which were previously contained at nutrient medium with *Berberis vulgaris* extract at the same concentrations as in the lifespan experiments (0.1 and 50 mg/ml) at the ages of 10 days. Flies were transferred to vials with fresh medium twice a week. For each experimental variant, 20 males or 10 females were used. The experiments were carried out in 3 biological replicates and 3 technical replicates. The delta–delta CT method was used for quantification (Livak and Schmittgen 2001).

Antioxidant activity

Commercially available phosphate buffered saline (PBS, pH 7.4), AAPH (Sigma-Aldrich), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), 2-thiobarbituric acid (TBA), trichloroacetic acid (Alfa Aesar), ascorbic acid (ICN Biomedical Inc.), FeSO₄ (reagent grade) were used for the work. Erythrocyte and tissue substrate incubation of laboratory animals were performed in a thermostatically controlled Biosan ES-20 shaker. Absorption was measured using a Thermo Spectronic Genesys 20 spectrophotometer. Absorption and fluorescence spectra were analyzed

using the BMG LABTECH CLARIOstar Plus multiplate reader. Radical scavenging (RSA) was assessed by the ability to interact with DPPH (Sevgi et al. 2015) and with ABTS using standard methods (Boulebd et al. 2021; Celik et al. 2010; Re et al. 1999; Tabart et al. 2009). Total antioxidant activity (TAA) was assessed by the ability to reduce Mo(VI) to Mo(V) in acidic conditions, with the formation of a colored phosphomolybdenum complex (Cheurfa et al. 2019; Demirci et al. 2018). The antioxidant activity (AOA) of compounds was assessed by their ability to inhibit lipid peroxidation (LPO) processes in substrates (emulsions) obtained from homogenates of the brain and testes of laboratory mice (Acker et al. 2009; Buravlev et al. 2018; Chawla et al. 2005; Kim 2013; Stefanello et al. 2013).

To evaluate erythrotoxicity, antioxidant, and membrane-protective activity of compounds, a 0.5% (v/v) suspension of erythrocytes from laboratory mice in phosphate-buffered saline (PBS, pH 7.4) was used. Erythrotoxicity of compounds was assessed (in vitro) by their ability to induce hemolysis. Membrane-protective and antioxidant activity were determined by the degree of inhibition of oxidative hemolysis initiated by AAPH or H₂O₂ (Takebayashi et al. 2010), inhibition of oxidation of oxyhemoglobin in erythrocytes (Van den Berg, 1992), as well as inhibition of hemolysis and oxidation and degradation of heme (Nagababu et al. 2008). Each experiment was performed in 4–10 replicates.

Glycation of albumin by glucose

The study was carried out on the model of glycation of bovine serum albumin (BSA, fraction V) with glucose. The reaction medium contained 0.36 M glucose and 1 mg/mL BSA dissolved in phosphate buffer solution (pH 7.4, 0.05 M). The test samples were diluted with 30% aqueous ethyl alcohol to concentrations of 1.5, 0.5 0.15 or 0.015 mg/ml. The final concentration of extract after addition to the reaction medium was 48.4, 16.1 4.84 or 1.61 mkg/ml (the final concentration of ethyl alcohol was ~ 0.97%). Aminoguanidine, a well-known antiglycation agent, was used as a reference compound. Control samples contained an equivalent volume of solvent. The samples were incubated for 24 h at 60°C. Fluorescence was measured at 335 nm excitation and 385 nm emission (spectrofluorimeter M 200 PRO, TECAN).

Isolation of peritoneal macrophages

Peritoneal macrophages (PM) were isolated from the peritoneal exudate of C57bl/6j mice. To accumulate PM, 1 ml of 3% peptone solution was injected intraperitoneally. To obtain macrophages after 3 days the mice were euthanized by cervical dislocation. Cells of peritoneal exudate were obtained by aseptically washing the abdominal cavity with 5 ml of sterile Hanks's solution (+ 4–6°C) without calcium and magnesium ions. The total number of cells was counted and their viability was assessed in a Goryaev counting chamber (Russia) with 0.4% trypan blue staining (Sigma-Aldrich, USA). The proportion of living cells exceeded 95%. The cell concentration was adjusted to 1.0×10⁶ cells/ml in complete nutrient medium DMEM (Gibco) supplemented with 2 mM L-glutamine (Gibco), 10% heat-inactivated fetal bovine serum (BioClot, Germany), supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin (Gibco)

and plated 200 µl/well in 96-well transparent plates (SPL Life Sciences Co., Ltd., Korea). It was left for 2 h at 37°C in a humidified atmosphere with 5% CO₂, after which the wells were washed to remove non-adherent cells. After 24 hours of incubation, 20 µl of the supernatant was taken and 20 µl of solutions of the test substances were added.

Lactate dehydrogenase assay

An increase in the content of the enzyme lactate dehydrogenase (LDH) in the culture medium is a marker of an increase in the permeability of the cell membrane and cell death. To determine its content in supernatants, a method based on spectrophotometric detection of NADH loss in a mixture containing pyruvate was used. 10 µl of supernatants taken 24 hours after incubation of PM with barberry extract or quercetin as a control compound (10 or 100 µg/ml, end concentration) was mixed with 250 µl of 0.194 nM/L NADH solution dissolved in 54 mM phosphate buffer solution, pH 7.5. Then, 25 µl of a 6.48 mM pyruvate solution was added to the mixture. The optical density was determined at a wavelength of 340 nm for 20 minutes. Conversion of optical density into cell viability was carried out according to a standard curve (intact cells – 100%, Triton X-100–0%).

MTT assay

The effects of the extract on cell viability was determined using the MTT colourimetric test. All examined cells were diluted with the growth medium to 3.5×10^4 cells per mL and the aliquots (7×10^3 cells per 200 µL) were placed in individual wells in 96-multiplates (Eppendorf, Germany) and incubated for 24 h. The next day the cells were treated with barberry extract or quercetin as a control compound at the concentrations of 10 or 100 µg/ml and incubated for 24 h at 37°C in a 5% CO₂ atmosphere. After incubation, the cells were then treated with 20 µL MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg mL⁻¹ in PBS) and incubated for 4 h. After an additional 4 h incubation, the medium with MTT was removed and DMSO (150 µL) was added to dissolve the formazan crystals. The plates were shaken for 10 min. The optical density of each well was determined at 560 nm.

Morphological Analysis of Cellular Cytotoxicity

After 24 hours of incubation with the examined extract, the culture medium was removed from the wells. The wells were rinsed twice with Hank's solution. Then, cells were fixed by introducing 150 µL of May-Grünwald's dye (MiniMed, Russia) into each well. After 5 minutes, the fixative was removed and the cells were washed with buffered water (pH 6.8 – 7.2), for 5 cycles. For staining fixed cells, 150 µL of working solution of azur-eosin in a modification of Romanowsky-Giemsa solution (ABRIS, 1 part dye + 9 parts buffered water) was injected into the wells of the plate (after air-drying). After staining the cells for 30 minutes at room temperature, the plate was washed with buffered water (ABRIS, pH 6.8 – 7.2; 5 cycles), air-dried, and analyzed using a Mikmed-6 microscope (LOMO, Russia) at magnification (objective × 20,

eyepiece × 10). The effect of two concentrations of the investigated extract on macrophages was assessed by identifying morphological signs of cytotoxicity (apoptosis, necrosis) compared with intact cells, cells treated with Triton-X-100 and cells treated with quercetin at two concentrations.

Statistical analysis

To evaluate the statistical significance of differences in gene expression levels, we will employ a multi-factorial analysis of variance (ANOVA) complemented by a post hoc test using Tukey's HSD test. The log-rank test will be applied to assess the statistical significance of differences between survival curves. The significance of differences in median maximum lifespan will be determined using Fisher's exact test. To account for multiple comparisons, we will adjust the p-values using the Bonferroni correction. All statistical analyses will be conducted using TIBCO Statistica (version 13.3, TIBCO Software, Palo Alto, CA, USA) and the OASIS 2 online application for survival analysis. This rigorous statistical approach will ensure the validity and reliability of our findings. This is the way we plan to approach our experimental design and statistical analysis to ensure the validity and reliability of our findings. The statistical significance of differences in the parameters characterizing the antioxidant activity of the extract was assessed by the Mann-Whitney U-test. Data were analyzed using Microsoft Office Excel 2007 (Microsoft, USA) and Statistica 13.3 (StatSoft Inc, USA) software packages. Experimental data are presented as mean values with standard error of the mean (SEM). Mathematical processing of the data for antiglycation and cytotoxic properties was carried out using Microsoft Excel 2012 (Microsoft, USA) software. Statistical analysis and graphical representation of the results were conducted using GraphPad Prism 8.0 (GraphPad Software, Inc., USA). Experimental data are presented as mean values with standard error of the mean (SEM).

Results

Chemical Composition of Berberis vulgaris L. berries.

The BE chromatogram was acquired using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) methodology. The resulting chromatogram (Fig. 1) revealed the presence of cyanidin (CAS Number: 13306-05-3), pelargonidin (CAS Number: 134-04-3), and malvidin (CAS Number: 643-84-5).

Lifespan effects

To examine the effect of BE on fly longevity, we assessed the survival of flies exposed to continuous extract throughout their lives. Mortality curves, compared using the modified Kolmogorov–Smirnov test, demonstrated that the survival of wild-type *Canton-S* female flies exposed to BE was significantly increased compared to the control flies. In females BE at concentrations of 0.01, 0.1, 1 and 5 mg/ml increased median lifespan up to 6% ($p < 0.05$) (Fig. 2A; Table 1) and the maximum lifespan up to 3% ($p < 0.05$) (Fig. 2A; Table 1) at concentrations of 0.1 and 1 mg/ml. However BE treatment did not induce

almost any significant effects in males. Except the BE at concentrations of 50 mg/ml showed decreased effect on the maximum lifespan by 5% ($p < 0.01$) (Fig. 2B; Table 1) of *D. melanogaster* males and females.

Table 1
The effect of BE on longevity of *Drosophila melanogaster*.

Variant, mg/ml	Sex	M (day)	dM (%)	Fisher's Exact Test		90% (day)	90% (%)	Fisher's Exact Test		n
				p	p ^{bonf}			p	p ^{bonf}	
Control		57	n/a	n/a	n/a	67	n/a	n/a	n/a	636
0.01 mg/ml		57	0	0.658	1	68	2	0.508	1	415
0.1 mg/ml		57	0	0.747	1	68	2	0.6	1	387
1 mg/ml		58	1	0.310	1	67	0	0.960	1	410
2.5 mg/ml		58	2	0.077	0.46	67	0	0.666	1	413
5 mg/ml		56	-2	0.312	1	67	0	0.177	1	414
50 mg/ml		55	-4	0.158	0.95	64	-5	0.008	0.048	222
Control		64	n/a	n/a	n/a	74	n/a	n/a	n/a	625
0.01 mg/ml		67	5	0.002	0.017	75	1	0.118	0.590	361
0.1 mg/ml		68	6	0.001	0.006	76	3	00	0	397
1 mg/ml		67	5	0	0	76	3	0.001	0.005	353
2.5 mg/ml		64	0	0.398	1	74	0	0.243	1	381
5 mg/ml		67	5	0	0.001	75	1	0.001	0.005	361
50 mg/ml		63	-2	0.054	1	70	-5	0	0	231
M – median lifespan, 90% – age of 90% mortality (maximum lifespan), dM – difference median lifespan, p ^{bonf} – Holm-Bonferroni sequential correction, d90% – difference age of 90% mortality, N – number of flies, – males, – females. The significance of differences in median and maximum lifespan was calculated by the Fisher's Exact Test.										

Using Cox regression analysis, a method that estimates the hazard ratio (HR) of mortality associated with a specific risk factor, we further analyzed the effects of BE. For females, the risk of mortality decreased at BE concentrations of 0.1 and 1 mg/ml, as indicated by hazard ratios of 0.52 ($p < 0.001$) and 0.56 ($p < 0.001$) (Fig. 2C; Table S2), respectively. At 5 mg/ml, the risk increased with a hazard ratio of 1.4 ($p < 0.001$) (Fig. 2C; Table S2). For males, the risk decreased at a concentration of 2.5 mg/ml (hazard ratio of 0.69 ($p < 0.05$)) (Fig. 2D; Table S2) but increased at 50 mg/ml (hazard ratio of 1.23 ($p < 0.001$)) (Fig. 2D; Table S2). The study underscores the gender-specific impacts of BE on *Drosophila melanogaster*.

lifespan. While female flies demonstrated increased lifespan at certain concentrations, the effects on males were less consistent, with some concentrations even decreasing their lifespan. This suggests interaction between BE dose and gender in lifespan which waits for further investigation into the underlying mechanisms. For further investigation, two concentrations, 0.1 and 50 mg/ml, were selected. These concentrations were chosen in order to explore the underlying mechanisms of the dose-dependent effects observed in our study.

Stress resistance

The interplay between aging and stress resistance forms a critical aspect of the aging process (Ukraitseva et al. 2021). As an organism ages, its resistance to stressors, including heat shock and oxidative stress, typically decreases. This reduction in stress tolerance could, in turn, affect the overall lifespan, underscoring the complex link between aging, stress resilience, and longevity (Soo et al. 2023). As previously mentioned, we used two concentrations (0.1 and 50 mg/ml) that exhibited the most favorable and the most adverse effects, respectively, to examine their influence on stress resistance to two types of stresses (heat shock and oxidative stress). The Bonferroni-corrected log-rank test revealed the impact ($p < 0.05$) of BE on the resistance to both forms of stress in *Drosophila*. Further pairwise comparisons employing the Fisher test revealed a significant 36% ($p < 0.01$) (Fig. 3A; Table S3) increase in median survival for females under oxidative stress at a concentration of 0.1 mg/ml. Additionally, BE notably improved female survival times during exposure to heat shock at both concentrations, with a median and maximum increase of 57 and 27% ($p < 0.001$) (Fig. 3C; Table S4), respectively. At the same time, a high concentration of 50 mg/ml decreased median and maximum male survival by 42 and 41% ($p < 0.01$) (Fig. 3D; Table S4) respectively at heat shock. No significant differences were observed in all other experimental conditions. The results highlight the dose-dependent and gender-specific effects of BE on stress resistance in *Drosophila*, especially under conditions of oxidative stress and heat shock. Interesting that while lower BE concentrations increased female stress resistance, higher concentrations negatively impacted male resistance, emphasizing the complex dynamics of dose and gender in stress response modulation.

Locomotor activity

Physical activity has been linked to the aging process in various organisms, including *Drosophila*, other model animals and humans. A decline in locomotor activity is often observed as these organisms age (Jones and Grotewiel 2011). To investigate the potential influence of barberry extract on motor activity, we employed a two-way analysis of variance (ANOVA) (age $\times$ concentration) to determine its effects.

The analysis identified a significant interaction between BE concentrations and locomotor activity. However, a post-hoc test revealed that, at a concentration of 0.1 mg/ml, BE did not have a significant impact on the locomotor activity of either male or female flies. In contrast, at a concentration of 50 mg/ml, the extract led to a 25% reduction in female locomotor activity at week 4 ($p < 0.001$) (Fig. 4A; Table S5) while increasing male motor activity by 83% ($p < 0.001$) (Fig. 4B; Table S5). The differential effects of barberry extract on locomotor activity, especially at the 50 mg/ml concentration, highlight the

complex interplay between dietary compounds and organisms. The observed 25% reduction in female activity is consistent with our previously reported declines in locomotor functions in females related to aging and might suggest an effect of the extract on this age-associated decline (Golubev et al. 2022). Conversely, the significant increase in male locomotor activity aligns with some studies (Filaferro et al. 2022) suggesting gender-specific responses to certain bioactive compounds. Future studies would benefit the exact mechanisms and potential gender-specific pathways influenced by barberry extract.

The Smurf assay

Aging has been associated with decrease of intestinal barrier integrity, as the function of the intestinal barrier tends to deteriorate over time (Wilms et al. 2020). This decline in intestinal barrier function can have negative consequences on overall health and lifespan. Our investigation of the influence of BE on intestinal barrier integrity in flies aged 9 weeks revealed that, at a concentration of 50 mg/ml, the extract had no significant ($p > 0.05$) effect on either male or female flies. However, at a concentration of 0.1 mg/ml, the extract reduced the permeability of the female intestinal barrier by 12% ($p < 0.01$) (Table S6), indicating a potential protective effect on intestinal function. These data suggest that barberry extract may exert a concentration-dependent influence on the intestinal barrier function, with potential protective properties observed at lower concentrations. The concentration-dependent effects of BE, with notable benefits observed at lower concentrations, resonates with the pharmacological principle that bioactive compounds often exert different effects based on dosage (Martirosyan and Sanchez 2022). Additionally, the gender-specific protective effect needed further investigation, possibly suggesting unique interplays between nature compounds and gender-associated physiological pathways.

Respirometry

Understanding the metabolic processes and energy expenditure is essential for deciphering the complex mechanisms underlying aging and longevity (Catic 2018). The respiratory rate, a critical indicator of cellular metabolic activity, is closely related to the aging process and the overall health of an organism (Bratic and Trifunovic 2010). As aging progresses, alterations in energy metabolism and mitochondrial function have been shown to contribute to the decline in cellular function and which have an effect on organismal healthspan and lifespan (Sun et al. 2016). To assess the potential effects of BE on metabolism we used a one-way ANOVA to compare the metabolic rate between treatment groups and control groups in both sexes. Our analysis revealed a significant ($p < 0.001$) effect of BE on the metabolism of both male and female subjects, suggesting a potential role in modulating age-related changes in energy metabolism. More specifically, post hoc test revealed that BE treated female flies exhibited decreased metabolic levels ($p < 0.001$) (Fig. 5A; Table S7) compared to flies maintained on the control medium. In males, BE showed a completely opposite effect, post hoc revealed ($p < 0.001$) (Fig. 5B; Table S7) elevated metabolic rate compared to control. Our analysis revealed a significant effect of barberry extract on the metabolism of both male and female flies. However, the impact of the extract was found to be sex-specific; it reduced the metabolic rate in females, while increasing it in males. Metabolism

and aging are closely intertwined, and any changes in metabolic activity can significantly influence the aging process. The sex-specific effects of BE on metabolism in our study add valuable information.

Antioxidant activity

It has been demonstrated that barberry berry extract exhibits high radical scavenging activity (RSA) in both the stable radical DPPH test and the more active cation-radical ABTS+ test (Fig. 6; Table 2). The mentioned extract possesses the ability to reduce Mo(VI) to Mo(V) (Fig. 6; Table 2), indicating its overall antioxidant activity (OAA).

Table 2

Sample	RSA (DPPH), %	RSA (ABTS), %	TAA, %	TBARS*/nmol/mL (brain)	TBARS*/nmol/mL (testes)
Control	-	-	-	40.3 ± 0.2	15.6 ± 0.1
0.5 mg/ml					
BE	70.2 ± 0.7	93.0 ± 0.1	12.9 ± 0.1	10.6 ± 0.3*	9.3 ± 0.4*
Trolox	95.1 ± 0.0	98.9 ± 0.0	85.2 ± 0.9	8.7 ± 0.4*	4.9 ± 0.2*
0.05 mg/ml					
BE	37.4 ± 0.4	84.7 ± 1.2	2.5 ± 0.0	11.4 ± 0.3**	-
Trolox	94,9 ± 0.0	98.9 ± 0.0	9.0 ± 0.2	9.3 ± 0.3*	-
*The ability of the samples to inhibit the accumulation of TBARS in the substrate obtained from the brain of mice (1 h after Fe2+/ascorbate LPO initiation) or testicular tissues (3 h after H2O2 LPO initiation) was assessed. Control - samples without test samples. The values are presented as mean ± SEM (n = 4–8). *p < 0.05, **p < 0.01, ***p < 0.001 Mann–Whitney test.					

At a concentration of 0.5 mg/ml, barberry extract significantly inhibits ($p = 0.021$) H₂O₂-induced oxidation of polyunsaturated fatty acids (PUFAs) in lipids of laboratory animal testes. At concentrations of 0.05 and 0.5 mg/ml, it prevents the accumulation of secondary products (TBARS) in Fe²⁺/ascorbate-induced lipid peroxidation (LPO) in a substrate containing brain lipids of laboratory animals, slightly inferior to Trolox ($p = 0.008–0.021$) (Fig. 6; Table S8).

For barberry extract at a concentration of 0.05 mg/ml, slight erythrotoxicity was observed. The level of hemolysis in the control after 5 hours of cell incubation was 4.1 ± 0.1%, while in the presence of barberry extract, it was 6.5 ± 0.3%. Previously, exceptionally high hemolytic activity at the mentioned concentration was identified by us for the acidic fractions of extracts from pine and larch foliage, which is explained by the presence of phenolic acids with surface-active properties (Nikonova et al. 2022).

The minor erythrotoxicity of the barberry berry extract did not hinder further investigation of its biological activity using erythrocytes as test subjects. Oxidative hemolysis of mammalian blood erythrocytes is one of the methods widely used in global practice for evaluating the antioxidant properties of various plant extracts. This approach is based on the ability of antioxidants to increase erythrocyte survival and prevent oxidative degradation of membrane structures and hemoglobin under oxidative stress conditions initiated by various chemical compounds, including AAPH and H_2O_2 . These initiators differ in their ability to penetrate the cell, primary targets of action, mechanisms, and dynamics of oxidative stress development.

It was shown (Fig. 6; Table S8) that barberry extract possesses high statistically significant membrane-protective activity under H_2O_2 -induced stress throughout the entire incubation period, as evidenced by the reduced intensity of oxidative hemolysis in its presence ($p = 0.009$). Barberry extract at a concentration of 0.05 mg/mL not only increased cell survival but also inhibited hemoglobin oxidation, accompanied by the formation of methemoglobin and ferrylhemoglobin ($p = 0.002$) (Fig. 6; Table S8). It is worth noting that although Trolox at the mentioned concentration more actively increased erythrocyte survival, it exhibited pro-oxidant activity in relation to hemoglobin ($p = 0.002$) (Fig. 6; Table S8). A high membrane-protective activity of the extract of barberry berries, which is not inferior to that of Trolox, was also revealed under conditions of AAPH-induced hemolysis (Fig. 6; Table S8). In the presence of both the berry extract and Trolox, a significant increase ($p = 0.014$), in the survival of erythrocytes under the influence of peroxy radicals generated during the thermal decomposition of AAPH was observed during the entire period of the experiment (5 h). The presence of a high antioxidant activity in the barberry extract is also evidenced by a statistically significant decrease in the metHb/oxyHb and ferrylHb/oxyHb ratios in erythrocytes in its presence ($p = 0.0004$, Table S8). In this regard, barberry extract was significantly superior to Trolox ($p = 0.0004$). In conclusion, using various test systems, including mammalian blood erythrocytes, we evaluated the antioxidant activity of the alcoholic extract of barberry berries. It was shown that the barberry extract exhibits high RSA in tests with DPPH and ABTS, as well as overall antioxidant activity assessed by the phosphomolybdenum method. The investigated extract effectively inhibited oxidation of PUFAs in heterogeneous substrates containing tissue lipids (brain and testes) of laboratory animals, initiated by various methods ($\text{Fe}^{2+}/\text{ascorbate}$ and H_2O_2) (Table 2).

Despite the slight erythrotoxicity caused by the presence of phenolic acids, barberry extract demonstrated high membrane-protective and antioxidant activity in the oxidative hemolysis model, initiated by H_2O_2 and AAPH (Fig. 6; Tables S8), increasing cell survival and preventing hemoglobin oxidation within the cells. The obtained results indicate the potential of using barberry berry extract as an effective inhibitor of free-radical processes.

qt-PCR shows correlation between aging and age-related gene

To test whether mechanisms are responsible for effects of lifespan and healthspan, we measured transcript levels of 11 genes involved in age-dependent pathways including the oxidative stress

resistance genes *hif1*, *sod1*, and *sod2*, which act as cellular shields against harmful free radicals. We also investigated the heat shock proteins genes *hsp27* and *hsp68*, known for safeguarding cells from stress and aiding protein folding. The gene 'target of brain insulin' or *tobi*, which has a prominent role in carbohydrate metabolism, and genes like *ilp5* that code for insulin-like peptides, influencing growth, metabolism, and lifespan; immune response genes *AttA* and *DptA*, integral for defense against pathogens, the metabolic read-out gene *bmm* that is involved in the breakdown of fats during energy scarcity, and the lipid metabolism gene *Lip3* which oversees lipid breakdown and synthesis. The majority of the genes examined did not show any changes in regulation when exposed to BE supplementation. However several of them were found affected by BE. One-way ANOVA showed statistically significant effects on the expression level of genes after BE treatment in females (*hsp27*, *hsp68*, *lip3*, *bmm* and *ilp5*; $p < 0.05$) (Fig. 7A; Table S9) and males (*sod1* and *tobi*; $p < 0.05$) (Fig. 7B; Table S9). Follow-up post hoc multiple pairwise comparisons using Tukey's HSD test revealed that BE decreased expression three of eleven genes (*hsp68*, *lip3* and *lip5*; $p < 0.05$) and up-regulated two of eleven genes (*hsp27* and *bmm*; $p < 0.01$) (Fig. 7A) in females. Supplementation of BE also increased activity level of *sod1* and *tobi* in males ($p < 0.05$) (Fig. 7B). In summary, these findings suggest that BE supplementation has a sex-specific effect on the regulation of certain genes involved in age-dependent pathways, which may play a role in influencing lifespan and healthspan.

Antiglycation properties

Exploring the impact of various plant extracts on stress resilience and longevity, our attention was drawn particularly to the potential of barberry extract. The initial analysis for antiglycation activity revealed a promising profile for barberry (Table 3) with IC50 45.4 ug/ml (Fig. 8). The activity of the barberry extract surpassed that of the widely known antiglycation agent aminoguanidine.

Table 3
Antiglycation Activity Values and IC50 Values of Extract Compared to Aminoguanidine (M ± SEM)

Plant № / compound	Concentration, mkg/ml	Activity, %	IC50, mkg/ml
Barberry extract	48.4	51.3 ± 0.9***	45.4
	16.1	29.0 ± 0.5***	
	4.84	20.9 ± 0.6***	
	1.61	15.4 ± 0.4***	
Aminoguanidine	48.4	28.6 ± 1.2***	-
	16.1	16.4 ± 1.0***	

Cytotoxicity evaluation

The subsequent cytotoxicity investigation unveiled a fascinating aspect of barberry extract's behavior in primary macrophage cells. As indicated in Tables 4 and 5, barberry extract, instead of exhibiting cytotoxic properties, demonstrated signs of cyto-vitality in the MTT assay, potentially indicating enhanced cell viability. Moreover, the LDG assay findings corroborated the non-cytotoxic nature of barberry extract. This substantial increase in cell vitality, possibly through the activation of mitochondria, implies a potential effect on stress resilience and, therefore, longevity. While the precise mechanisms are yet to be elucidated, these initial findings underscore the potential of barberry extract as a candidate for further research into lifespan extension. At the same time, quercetin, a widely spread polyphenolic compound used as a reference, had a cytotoxic effect in both tests.

Table 4
Cytotoxic properties of plant extract in primary macrophages (positive value is the sign of cytotoxicity, negative value is the sign of cyto-vitality) in MTT-assay (M ± SEM)

Extract № / compound	Concentration, ug/ml	Toxicity, %
Barberry extract	100	-77.4 ± 5.2***
	10	-28.0 ± 4.9
Quercetin	100	50.1 ± 4.1*
	10	11.8 ± 15.9

Morphological Analysis of Cellular Cytotoxicity

The results of the morphological analysis are presented in Fig. 8 Morphological assessment of stained cell preparations using light microscopy revealed the absence of signs of apoptosis, disintegration of cellular nuclei with chromatin condensation, and "blebbing" phenomena related to cellular cytoskeleton disruption. The population of peritoneal macrophages exposed to various concentrations of the studied barberry extract is morphologically indistinguishable from peritoneal macrophages cultivated without the addition of the studied extract. Microscopic observations noted the presence of a large number of cells, comparable in morphology and quantity to the cells of the intact group, unlike the cells exposed to Quercetin at a concentration of 100 µg (marked cytotoxic effect) and the control toxicant Triton-x-100 (100% cell death, necrosis). Thus, the studied extract at concentrations of 100 and 10 µg/mL does not exert a cytotoxic effect on murine peritoneal macrophages.

Discussion

The current study aimed to investigate the potential health benefits of BE with a focus on promoting healthy aging. We demonstrate the concentrate-dependent effect of BE on lifespan in *D. melanogaster* (Fig. 2; Table 1). As far as we are aware, this is the first study conducted on the effects of barberry extract on the lifespan and healthspan of *D. melanogaster*. However, there is a study that demonstrates the effect of berberine, isolated from barberry, on lifespan of *Drosophila*. Berberine extended the mean, median, and maximum lifespan of *Drosophila melanogaster* by 27, 57, and 78% respectively. The potential

mechanism could be its ability to inhibit the conversion of tryptophan (TRP) into kynurenine (KYN), a pathway associated with aging and aging-associated disorders (Navrotskaya et al. 2012). There are extracts from other plants rich in anthocyanins that have been shown to increase lifespan in various model organisms. In a study conducted by Xiang Li et al. (2019), the black rice anthocyanin extract (BRAE) was found to extend the lifespan of fruit flies by 20% and slow down the loss of locomotor function (Li et al. 2019). Water extracts of *Lycium barbarum* berry (LBE), which are rich in polysaccharides and other components such as anthocyanins, extended the lifespan of *Caenorhabditis elegans* (Zhou et al. 2022). Interestingly, the lifespan extension effect was more prominent in worms with shorter mean lifespan as compared to those with longer mean lifespan. The beneficial effects were more pronounced in organisms with a shorter average lifespan. These studies suggest that anthocyanin-rich extracts from different plants could potentially be used to extend lifespan and improve healthspan in various model organisms, possibly through their antioxidant activity and their ability to modulate various aging-related pathways such as the coenzyme nicotinamide adenine dinucleotide (NAD⁺) through *sir-2.1*.

Anthocyanins are known to have potential effects on metabolic processes and lipid levels according to studies on the *Drosophila* model (Danielewski et al. 2021; Esposito et al. 2015; Valenza et al. 2018; Wang et al. 2016). These studies suggest that anthocyanins can exert hypolipidemic and anti-obesity effects, improve glucose metabolism and insulin sensitivity, suppress lipid accumulation in adipocytes, and reduce LDL cholesterol levels. In our study we link a positive effect of BE with lifespan through increased level activity of *bmm* (Fig. 6; Table S9). The brummer (*bmm*) gene in *Drosophila melanogaster* is known to be the major triglyceride hydrolase and plays a crucial role in lipid catabolism, energy production, regulation of fat storage and physiological fitness (Blumrich et al. 2021; Grönke et al. 2005; Nazario-Yepiz et al. 2021). One study indicates that in flies with excessive fat storage, function of *bmm* is absent which leads to reduction in median lifespan by 10%. (Grönke et al. 2005). Additionally, when *bmm* is suppressed across all tissues or metabolic specific tissues through RNA interference, it results in a short lifespan of *Drosophila* (Nazario-Yepiz et al. 2021). On the other hand, overexpression of *bmm* leads to a reduction in the size of lipid droplets, whereas a deficiency in *bmm* increases triacylglycerol (TAG) storage, leading to obesity and a reduction in lifespan (Shang et al. 2022). At first sight it is challenging to judge how the activity of *bmm* could influence lifespan, but there exists a possible scheme for its action. Aging and dysregulation of lipid metabolism are associated with changes in mitochondrial function (Nowinski et al. 2020). The process of mitochondrial respiration, which generates CO₂ as a byproduct, is central to this relationship. Therefore, age-related changes in lipid metabolism and mitochondrial function could potentially affect the efficiency of mitochondrial respiration and subsequently, CO₂ output. Lipid metabolism involves the degradation of fatty acids, which can act as substrates for mitochondrial respiration (Fussell et al. 2016). In its turn, increased lipid oxidation could stimulate mitochondrial respiration, possibly leading to an increase in CO₂ output. In our study, we observe a decreased CO₂ production level (Fig. 5) and increased antioxidant activity *in vitro* (Fig. 6) thereby supporting the notion that regulation of *bmm* activity can play a role in aging. These findings align with previous research that has demonstrated the potent antioxidant properties of anthocyanins, which are thought to play a critical role in reducing oxidative damage and promoting healthy aging. For example, the antioxidant potential of

barberry extracts varies depending on the part of the plant used (Och et al. 2023). Their research found that the leaf extracts exhibited the highest antioxidant activity, with results ranging from 63.9 mgTE/g (TE - trolox) to 65.2 mgTE/g when assessed against the DPPH radical. The antioxidant activity was also evaluated using the ABTS test, with the highest level found for the barberry leaves (140.5 mgTE/g). This suggests that the leaves of the barberry plant, rich in polyphenols, could be a valuable source of natural antioxidants (Och et al. 2023). This is not the only study demonstrating the antioxidant properties of barberry (Dimitrijević et al. 2020).

Taken together, our findings suggest that barberry extract may modulate lifespan by influencing lipid metabolism, particularly through the regulation of the *bmm* gene. Certainly, further research is required to understand what level of expression has a positive effect, as based on our and other scientific data, the effect can be bidirectional. Some studies have observed extended lifespan in certain organisms but with compromised locomotor activity (Avanesian et al. 2010). Thus, the goal of geroprotective interventions should not only be to increase lifespan, but also to prolong period of good health in organisms

In addition to the direct antioxidant properties of anthocyanins, our findings suggest that BE may also have indirect effects on health indicators. The observed improvements in locomotor activity, stress resistance, and intestinal integrity (Fig. 3, 4; Table S6) indicate that BE could modulate various physiological processes that are associated with aging. The healthspan-promoting effects of berry extract, as observed in our study correspond with other scientific data. Previous studies (Golovinskaia and Wang 2021; Golubev et al. 2022; Kowalska and Olejnik 2016) have documented the beneficial impact of various berry extracts on health and longevity. Our findings corroborate these observations, suggesting that BE also has a substantial role in influencing health indicators.

We also investigated antiglycation properties of BE, as it plays a role in the physiological processes of aging (Table 3). Glycation, a non-enzymatic reaction between reducing sugars and proteins, nucleic acids, or lipids. Over time, glycation can lead to the formation of advanced glycation end-products (AGEs) (Singh et al. 2001). AGEs can accumulate in tissues and exert detrimental effects on health, including the potential development of various diseases. Previously, it was observed strong correlation between antiglycation activity and radical scavenging activity, as well as total phenolic content in wild berries (Harris et al. 2014). Moreover, the same study found that the antiglycation activity of the berries was associated with a reduction in the formation of AGEs, which are known to induce oxidative stress and molecular cross-linkages that can impair protein activity and cause cellular and tissue damage. The antiglycation activity of BE along with its potential influence on AGE formation give us a “key” to understanding of the extract's health-promoting properties.

Conclusion

It is important to note that our study focused on the effects of *Berberis vulgaris* L. extract in different models using *in vitro* and *in vivo* assays, and further research is needed to determine the extent to which these findings can be translated to humans. Additionally, the optimal dosage, formulation, and duration

of treatment with *Berberis vulgaris* L. extract for promoting healthy aging remain to be determined. Future studies should also investigate potential side effects and safety concerns associated with long-term consumption of *Berberis vulgaris* L. extract or its bioactive components.

In conclusion, our study demonstrates the potential health benefits of *Berberis vulgaris* L. extract, particularly its anthocyanin content, in promoting healthy aging. The observed effects on lifespan, locomotor activity, stress resistance, metabolism, intestinal integrity, and gene expression changes highlight the potential of *Berberis vulgaris* L. extract as a natural supplement for combating age-related diseases and promoting overall health. Further research is needed to translate these findings into practical applications and to fully understand the molecular mechanisms underlying the observed effects.

Declarations

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Author Contributions

Conceptualization, M.S. and A.M.; Methodology, D.G., O.S., R.L., M.S. and N.Z.; Software, D.G., O.S., R.L., M.S., N.Z., and S.P.; Investigation, D.G., E.P., N.Z., O.S., P.N., S.P., U.I., N.V., A.B., X.Z., and S.S.; Data Curation, D.G., O.S., M.S., N.Z., and R.L.; Writing – Original Draft Preparation, D.G., E.P., N.Z., O.S., and R.L.; Writing – Review & Editing, A.A.M.; Visualization, D.G. and R.L.; Supervision, M.S., R.L., and A.M.; Project Administration, M.S.; Funding Acquisition, A.M. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

All studies were performed *in vitro* on intact laboratory mouse tissues, without using animals as experimental subjects.

Conflict of 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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Figures

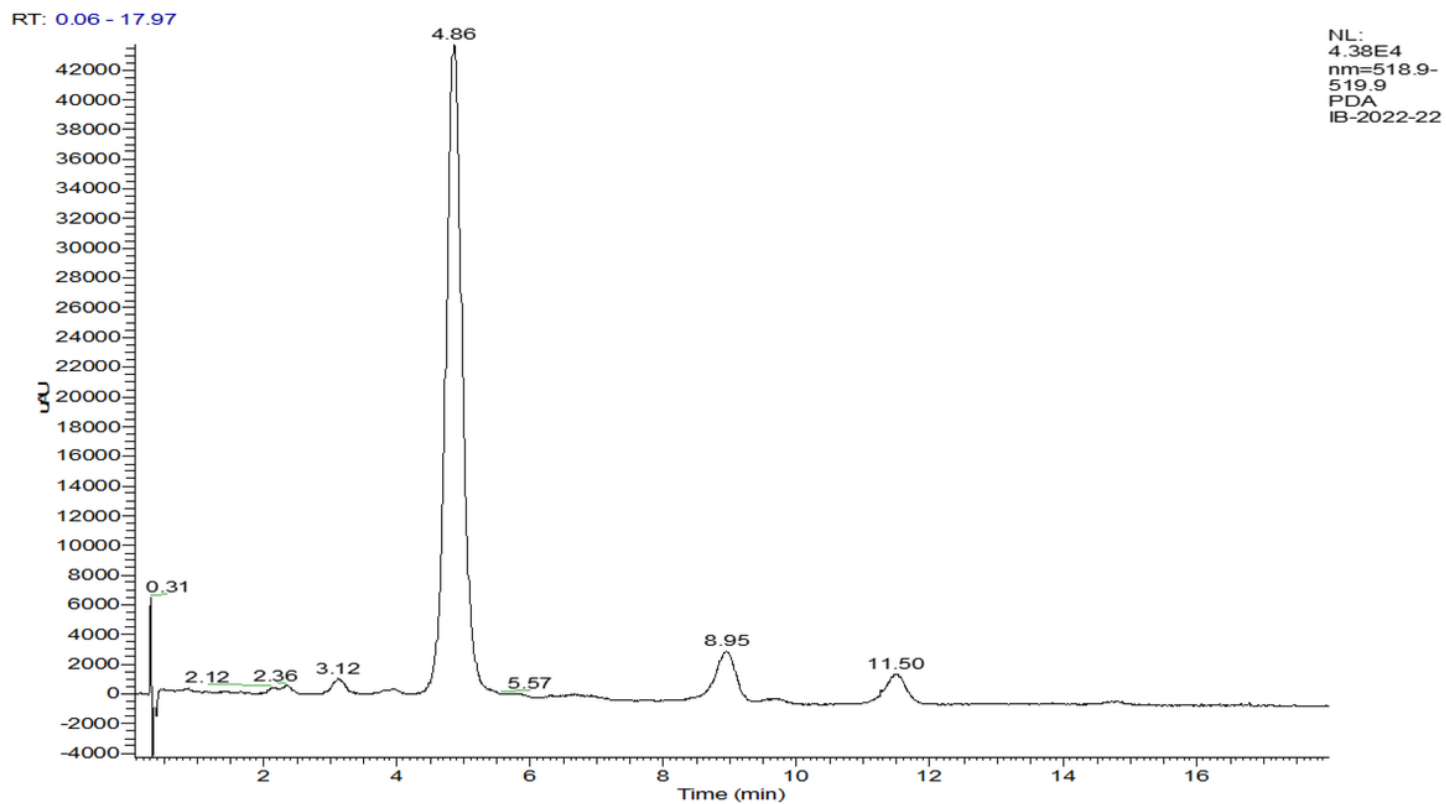


Figure 1

High performance liquid chromatography sample of *Berberis vulgaris* L. berry extract: (1) 3 min – cyanidin; (2) 4 min – pelargonidin ; (3) 11 min – malvidin.

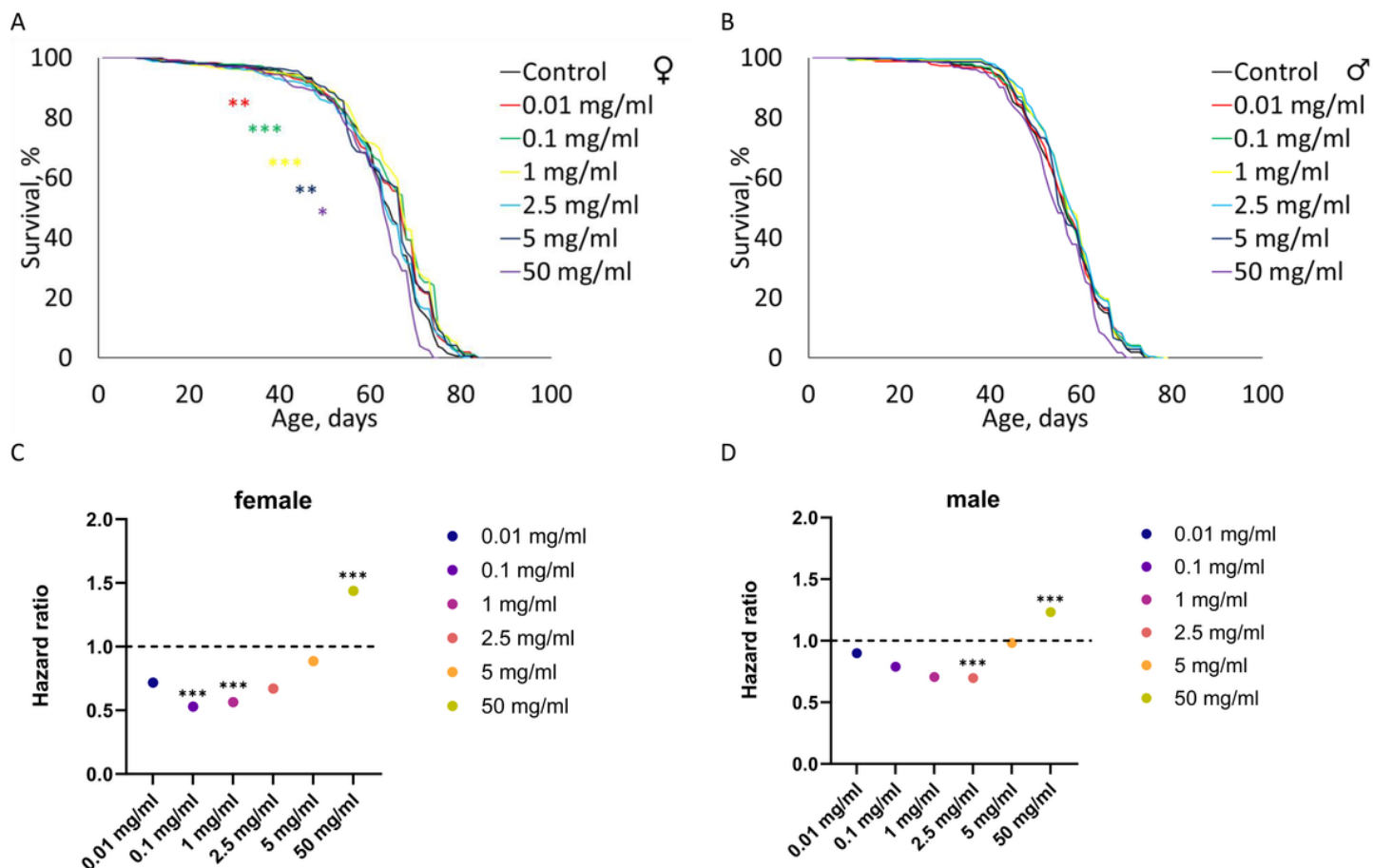


Figure 2

The effect of BE on the lifespan of *D. melanogaster* females (A) and males (B). Survival curves were compared by log-rank test. All data were subjected to Bonferroni's multiple comparisons test. * $p < 0.05$. The ratio between the hazard rates in BE treated and control groups of females (C) and males (D). Dotted lines indicate a hazard ratio of 1, which corresponds to control. Depicted are hazard ratios for different treatments (dots). female, male

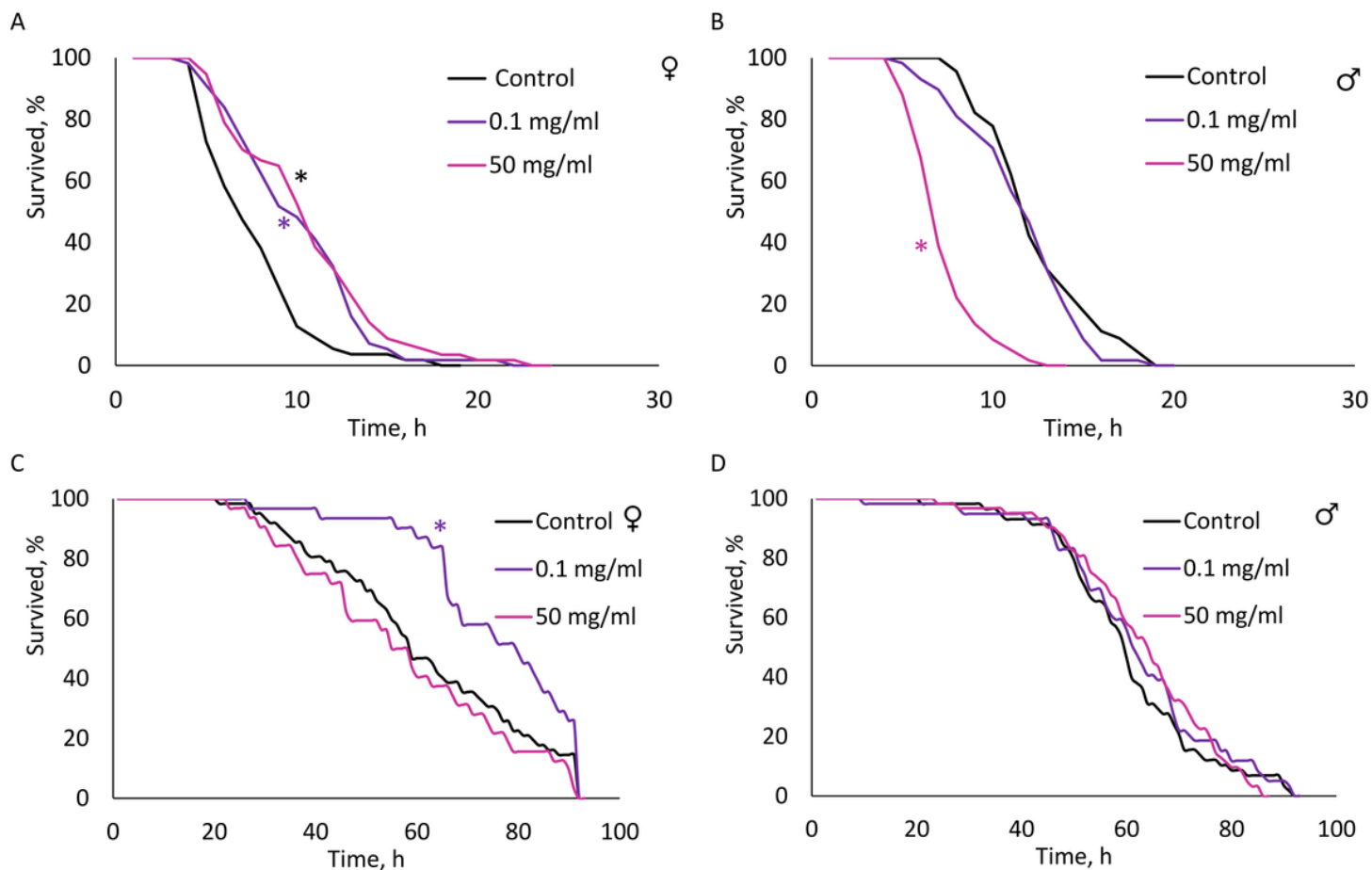


Figure 3

The effect of BE on the stress resistance to oxidative stress (A,B) and heat shock (C,D) of *D. melanogaster* females (A,C) and males (B,D). Survival curves were compared by log-rank test. All data were subjected to Bonferroni's multiple comparisons test. * $p < 0.05$.

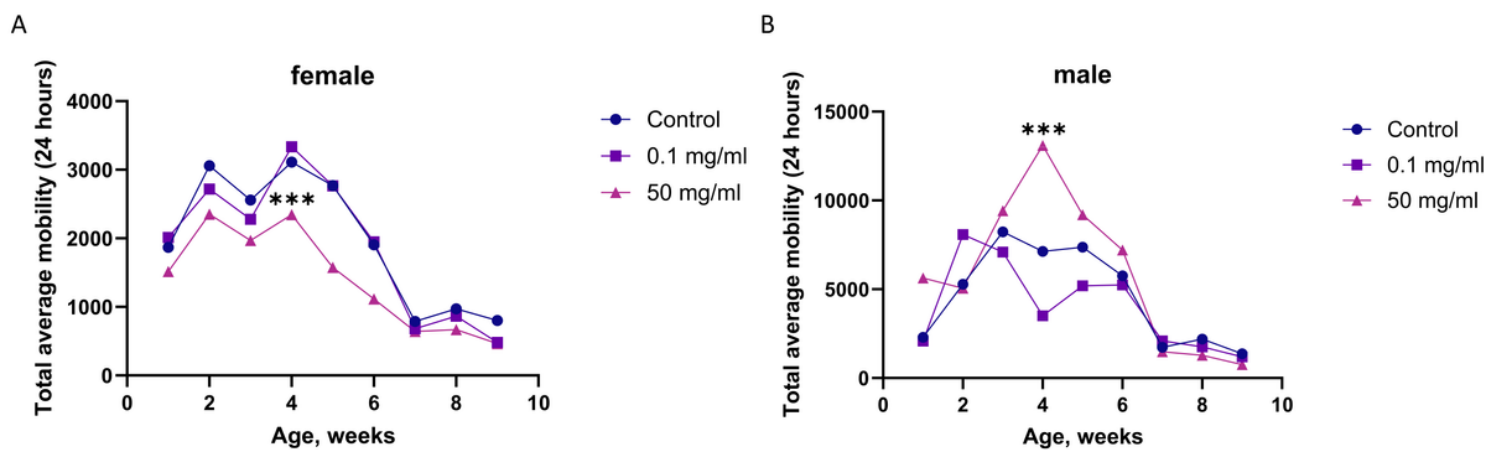


Figure 4

Effects of BE on the age-related changes of average daily locomotor activity in *Drosophila* males (A) and females (B). * $p < 0.05$. Two-way ANOVA (age $\times$ concentration) followed by post hoc Tukey's HSD test was used to indicate the level of statistical significance of differences

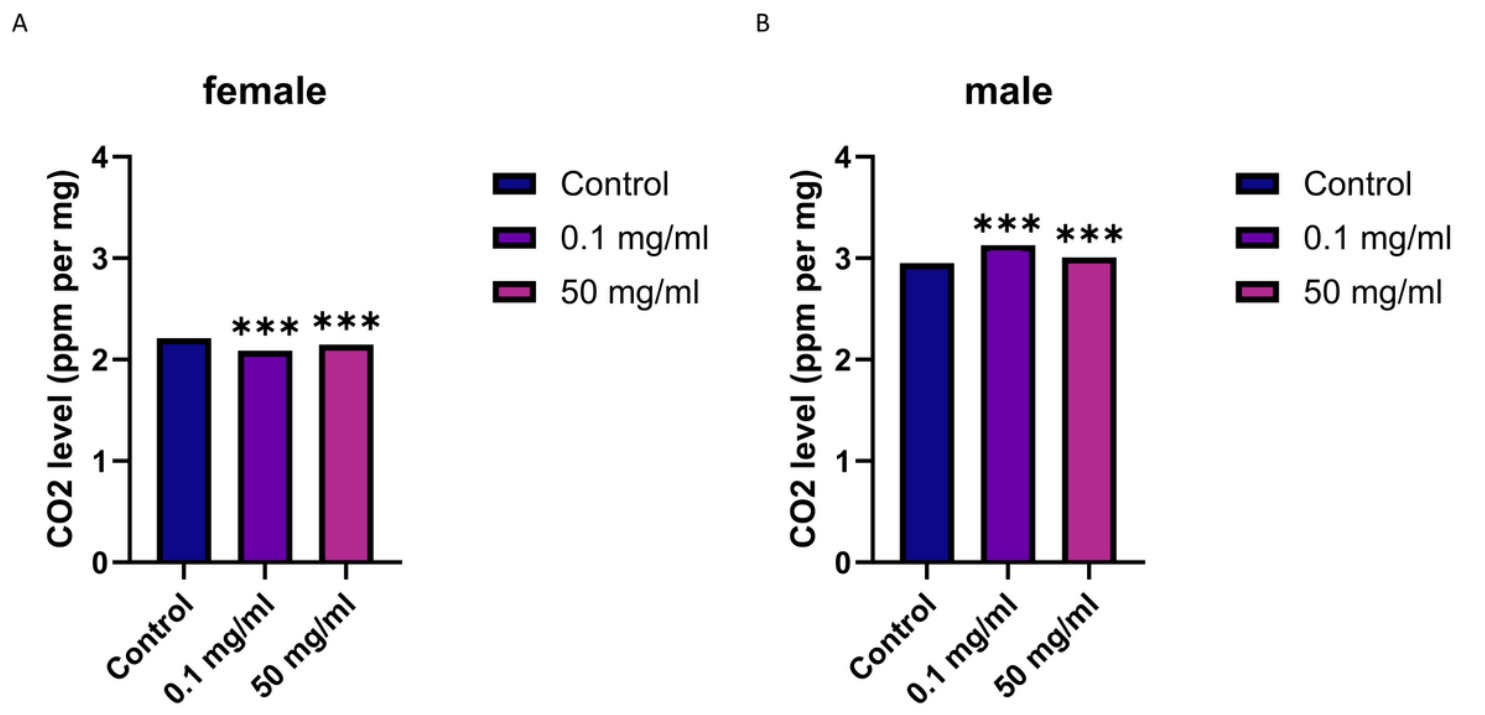


Figure 5

Effects of BE on CO₂ production levels on females (A) and males (B) of varying ages. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – two-factor ANOVA (Analysis of Variance) assessing the effects of media type and age, followed by pairwise comparisons using Tukey's post-hoc test.

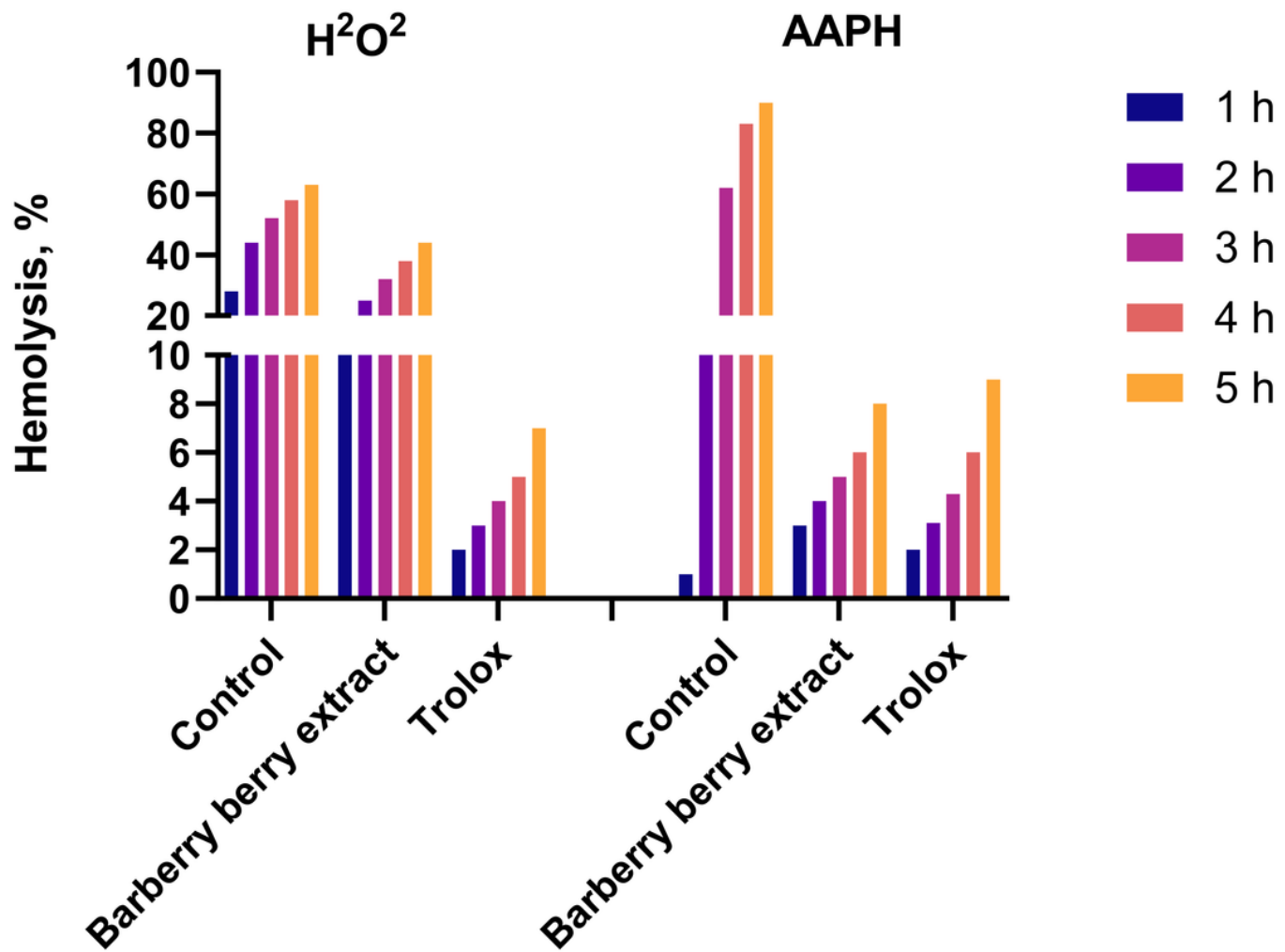


Figure 6

Comparative evaluation of membrane-protective and antioxidant activity of BE solution at a concentration of 10 μ M (model of RBCs oxidative hemolysis; 1–5 h after injection of H_2O_2 (A) or AAPH (B)). The values are presented as mean ($n = 6-7$) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ —Mann–Whitney test

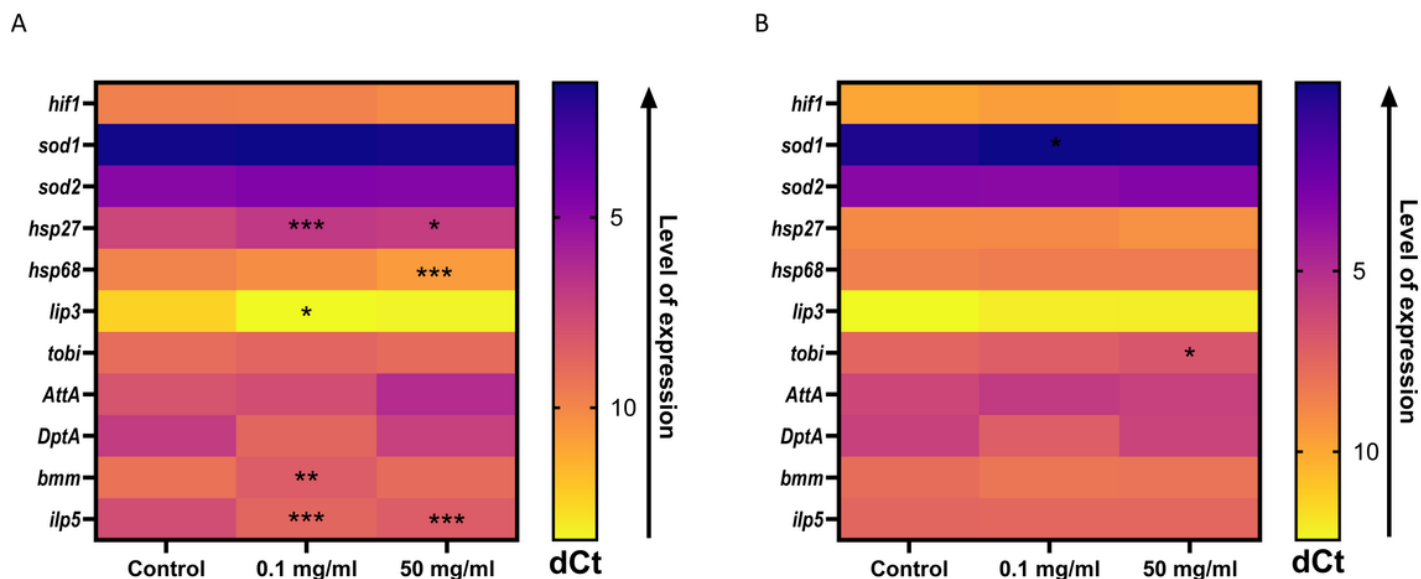


Figure 7

Effects of BE treatment on the relative expression level of stress response genes in female (A) and male (B) flies. One-way ANOVA followed by post hoc Tukey's HSD test was used to compare differences in expression levels of aging-associated stress response genes between control and treated flies, * $p < 0.05$.

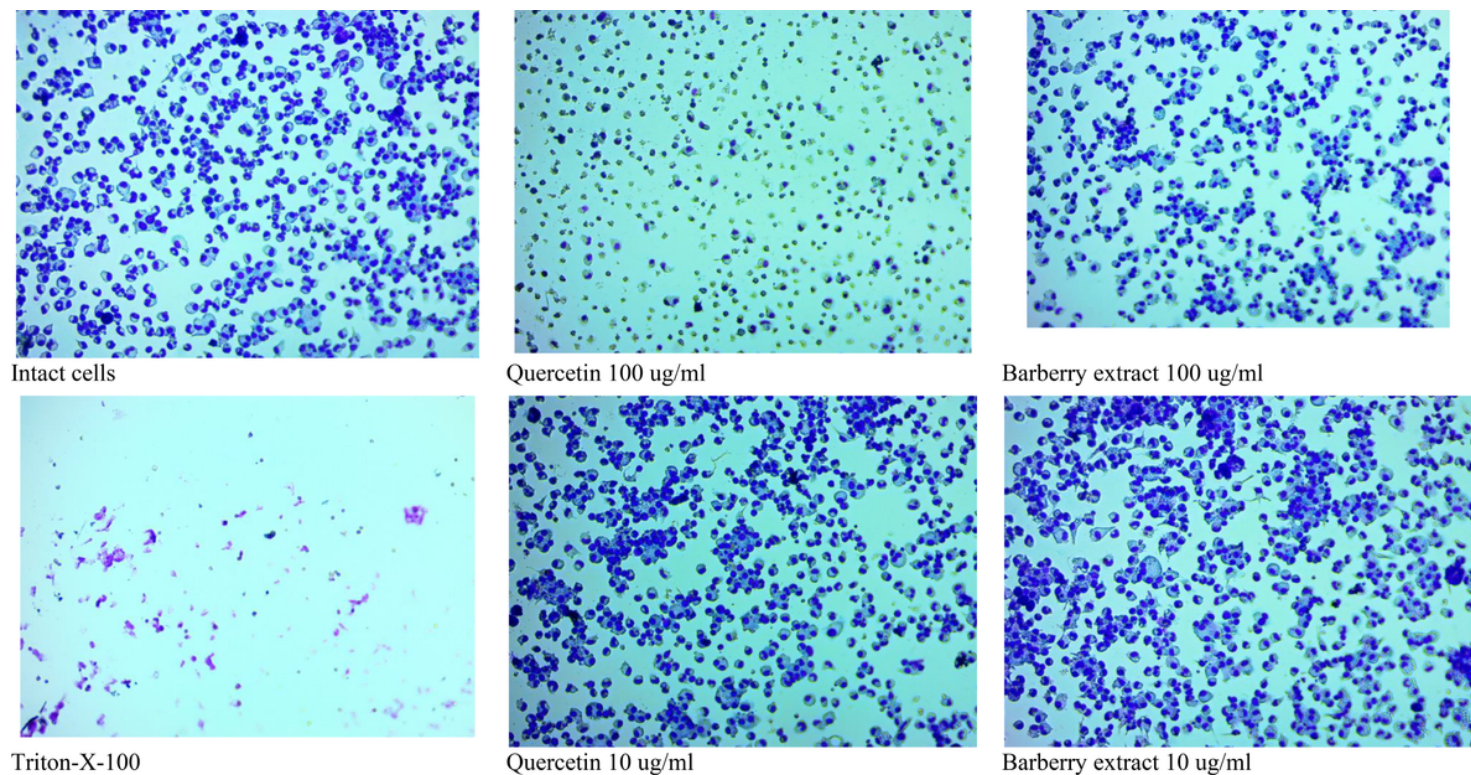


Figure 8

Morphology of murine peritoneal macrophages after 24-hour incubation with the barberry extract or Quercetin at 2 concentrations. Romanowsky-Giemsa azure-eosin staining. Total magnification ×200.

Supplementary Files

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