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Purple Sweet Potato Extract extends lifespan by activating autophagy pathway in male *Drosophila melanogaster*

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ABSTRACT

Introduction: Purple sweet potato is a nutritive food rich in anthocyanins that possess antioxidant effects. *Drosophila melanogaster* owns short growth cycle, fast reproduction, less chromosomes, more mutants, small individuals, therefore, which is an appropriate genetic model organism.
Objective: To investigate the anti-aging activity of Purple Sweet Potato Extract (PSPE) in male *Drosophila melanogaster* and explore the underlying mechanism.
Results: PSPE-induced longevity was associated with improvements in climbing ability and tolerance to stressors such as paraquat and hydrogen peroxide (H_2O_2). Furthermore, PSPE supplementation increased the activity of superoxide dismutase (SOD) and catalase (CAT), as well as expression of SOD and CAT genes, but decreased malondialdehyde (MDA) content. Meanwhile, PSPE decreased the intestinal stem cells (ISCs) proliferation and improved intestinal homeostasis, which was measured by Smurf assay and colony-forming units (CFUs) measurement in aging flies. Additionally, PSPE markedly inhibited the expression of upstream genes AKT-1, PI3K and mTOR and elevated the downstream gene 4E-BP, which further activated the expression of autophagy-related genes (Atg1, Atg5, Atg8a and Atg8b). Moreover, the production of lysosomes increased, indicating that the autophagy pathway was activated.
Conclusion: The results provided direct evidence of PSPE anti-aging effects on an organism level, indicating PSPE could be developed for use in effective anti-aging products.

1. Introduction

Aging impacts various bodily functions, such as stress tolerance and homeostasis; however, its effects can be mitigated via the autophagy signaling pathway to prolong an organism's lifespan (Liu et al., 2020). Intestinal barrier defects could shorten an organism's lifespan, therefore, intestinal barrier dysfunction is a good marker of aging (Rera et al., 2013). In recent studies, maintenance of intestinal homeostasis in *Drosophila melanogaster* has been a hot topic (Woodcock et al., 2015). Many efforts have been made to develop strategies that delay senescence by preserving intestinal homeostasis.

Purple sweet potato (*Ipomoea batatas* L) is a member of the sweet potato family, it is a kind of natural functional food with high edible and medicinal value (Kano et al., 2014). In recent years, purple sweet potato has received enormous attention due to its cheap source, role in health care and range of colors. It contains plentiful anthocyanins which have been reported to show high pH stability, thermostability and antioxidant activity (Chen et al., 2019). Moreover, purple sweet potato anthocyanins are more stable than other plants such as blueberries, cranberries and strawberries, therefore, the purple sweet potato has been suggested as a good source of stable anthocyanins (Hwang et al., 2011). In recent study, purple sweet potato anthocyanins ameliorated oxidative Stress, inflammation among chronic constriction injury-induced neuropathic pain in rats (Widyadharma et al., 2020). Therefore, PSPE may be an potential functional food to delay aging.

Drosophila melanogaster was an ideal model for short life cycle, rapid reproduction and low cost, moreover, it was used as a research model due to its strong genetics, conserved disease pathways and 75%

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pathogenic genes of flies are homologous to humans (Brown et al., 2014). As female flies produced more female hormone which effected lifespan in flies, while the hormone of male flies was at a low level, thus, we used male flies as model organism in the study (Li et al., 2019).

The mammalian Target of Rapamycin (mTOR) signaling pathway regulates organismal growth and homeostasis by sensing changes in the environment. It is involved in many human ailments, including diabetes and Alzheimer's Disease premature senility. A recent study found evidence suggesting that a physical association between mTOR and autophagy plays a key role in the activation of the mTOR pathway (Nazio et al., 2013). Recently, it was reported that purple sweet potato color improved cognitive deficits in high-fat-diet feeding mice by enhancing autophagy (Zhuang et al., 2019). However, it is still unclear whether PSPE extends the lifespan of flies by activating autophagy pathway.

To test the hypothesis, the present study used male *Drosophila melanogaster* to explore the mechanism of anti-aging effects of PSPE supplementation. The results showed that PSPE extended the lifespan, improved the climbing ability and stress tolerance, and dramatically improved intestinal homeostasis and integrity in flies. Moreover, PSPE increased the antioxidant enzyme activity and gene expressions, inhibited the mTOR pathway, and activated the autophagy pathway, thus, extending the lifespan in flies.

2. Material and methods

2.1. Materials and reagents

PSPE was purchased from Tianjin Jianfeng Natural Product R&D Co., Ltd., (Tianjin, China). The SOD, CAT and MDA assay kits used were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TRIzol Reagent, High Capacity cDNA Reverse Transcription Kit, and SYBR Green Kit were purchased from Takara Biological Engineering Co., Ltd. (Takara). FD&C Blue No. 1 and LysoTracker Red were purchased from Sigma Chemical (St Louis, MO, USA). Analytical grade agar, glucose, alcohol, tryptone, ethyl-p-hydroxybenzoate, and other chemical reagents were obtained from a local source.

2.2. Quantitative and qualitative analysis of anthocyanins by liquid chromatography-mass spectrometry (LC-MS)

The anthocyanins of PSPE were measured using a Thermo Finnigan Surveyor LC-MS/MS system, equipped with a photodiode array detector (PDA) and Venusil XBP C18 column (2.1 × 150 mm). The flow rate was 0.2 mL/min at a detection wavelength of 280 nm and the oven temperature was 30 °C. For the mobile phases, 0.1% formic acid in water (phase A) and acetonitrile (phase B) were used. The gradient elution was programmed as follows: 10% B for 0–5 min; 20% B for 40–45 min; 90% B for 45–55 min; and, finally, 10% B for 55.1–60 min. An electrospray ionization (ESI) source was used for mass spectrometry analysis in positive ionization mode. The ESI spray voltage, capillary voltage, capillary temperature, sheath gas (N_2) flow and auxiliary gas were 4.5 kV, −10 V, 275 °C, 30 arb, and 5 arb, respectively. The molecular weight range is 100–500 m/z in full scan mode.

2.3. Animal model and culture condition

The Oregon K wild-type flies and *esg*-Gal4 UAS-GFP flies ($n = 600$) were collected within 3d of eclosion, randomly divided into control and PSPE groups which contained 200 flies in each group. The all flies were reared at 25 °C and 50% humidity. Only males were tested and all flies were transferred to a fresh food source every three days.

2.4. Culture medium

The basal culture medium was boiled according to the previous method with slight modification (Wang et al., 2018). In short, 500 mL control medium contained 48 g corn flour, 48 g glucose, 6.7 g yeast, 4 g agar, and ethyl-p-hydroxybenzoate (0.4%). The experimental medium was added 0.5 and 2.0 mg/mL PSPE into control medium, 5 mL of the appropriate medium were added to each empty pipe.

2.5. Survival assays

To examine the effects of PSPE on the lifespan of flies, the wild-type flies were randomly allocated to the control or experimental groups. Living flies were transferred to a new pipe containing the same medium every three days. The maximum lifespan was regarded as the average lifespan of the 10% longest surviving flies in the study. The lifespan assays were repeated for three times.

2.6. Stress assays

To study the effect of PSPE on paraquat or H_2O_2 stress response, wild-type flies were reared on either control or PSPE diets for 25 days. After starving the specimens for 2 h, the flies were transferred to vials containing filter paper soaked with 1 mL 20 mM paraquat or 5% H_2O_2 in 6% glucose solution. The deaths were observed and recorded at every 2 h until no living flies remained.

2.7. Climbing assays

Climbing assays were used to evaluate locomotor function of flies, the assay was conducted as described by Peng (Peng et al., 2011). In short, wild-type flies were divided into control or PSPE groups. Ten flies were shaken into the bottom of the empty plastic vials, then given 20s to climb up. In each trial, we recorded the number of flies that climbed vertically to 5 cm and above. Each trial was performed three times and flies were tested at 0, 15, 30, and 40 days.

2.8. Food intake assays

The one hundred wild-type flies were treated in control or PSPE diets for ten days (20 flies per pipe). Then, the flies of each group were transferred to the same medium which contained 2.5% blue dye (wt/vol) for 1 h, respectively. The flies were collected and snap-frozen in liquid nitrogen, homogenized in distilled water, then filtered to remove residue and measured at 629 nm according to Su et al.' methods (Su et al., 2019).

2.9. Immunostaining and fluorescence microscopic imaging

Complete guts were removed and analyzed. The dissections were performed according to previous work (Wu et al., 2016). The intestines were removed from *esg*-Gal4 UAS-GFP flies treated with control or PSPE diets and then photographed using an inverted fluorescence microscope at 20 and 50 days.

In the LysoTracker-staining experiment, the midguts were dissected from wild-type flies that reared on control or PSPE diets for 20 days. Each gut was dissected in phosphate buffer and stained with 1 μM LysoTracker Red for 3 min. Images were captured using an inverted fluorescence microscope and the number of fluorescent spots were analyzed with Image J software.

2.10. Smurf assay

Wild-type flies were treated with control or PSPE diets for 10, 30, 40, and 50 days. The flies were then collected and maintained on media containing 2.5% (wt/vol) blue dye in the control medium for 9 h. Then, the flies were captured using microscope and the numbers of smurf flies were recorded. A fly was regarded as a "Smurf" when we observed blue coloration on the entire body (Rera et al., 2011).

2.11. Selective plates for bacterial cultures

The wild-type flies were reared on control or PSPE diets for 5 and 40 days, and then starved in empty pipes for 2 h. The midguts were removed, dissected, and homogenated. The homogenate coating was applied and prepared on selective plates according to a previous method (Guo et al., 2014).

2.12. SOD, CAT activity and MDA content

Wild-type flies were treated with control or PSPE diets for 45 days. Then, the flies were starved for 2 h, and stunned under N_2, so that the body weights of each group could be recorded. The flies were homogenized in ice-cold normal saline at a 1:49 dilution and then centrifuged at 2500*g* at 4 °C for 5 min. The supernatants were collected and stored at −80 °C and used to measure the SOD, and CAT activity and MDA content according to previous reports (Wang et al., 2018; Zou et al., 2015). The SOD activity was measured by xanthine oxidase methods (550 nm). Ammonium molybdate was used to measured CAT activity (405 nm). Thibabituric acid method was used to determine the MDA content (532 nm). The protein concentrations were measured by bicinchoninic acid method (562 nm).

2.13. Analysis of gene expression

Flies were reared for 45 days on control or PSPE diets and then frozen in liquid nitrogen for mRNA extraction. Total RNA was quantified using the commercial extraction reagent TRIzol, and High Capacity cDNA Reverse Transcription Kit to synthesize cDNA. In addition, real-time quantitative PCR (RT-PCR) was performed using SYBR Green with RT-PCR primer pairs (Supplementary Table S1). The gene expression of RP49 was used as internal control.

2.14. Statistical analyses

Statistical analysis of data was performed using Origin 2018 software. The data was shown as mean ± SD. Two-tailed Student's *t*-tests were used to compare sample means. Differences were regarded statistically significant when $p < 0.05$ and extremely significant when $p < 0.01$. The fluorescence intensity was analyzed by Image J software.

3. Results

3.1. Phytochemical profiles of extracts

The anthocyanins of PSPE were identified by LC-MS. The molecular ions and important fragment ions were compared with Zhao et al.'s report for identification (Zhao et al., 2014). In total, eight different compounds (including one unknown) were isolated and identified using UV and LC-ESI spectra. The basic structure of the anthocyanins isolated from PSPE was modified by structures that have been named in Fig. 1A. Eight peaks showed on the MS chromatogram of anthocyanins from PSPE (Fig. 1B). The mass spectrometry data of all the peaks were shown by LC-MS (Table 1).

3.2. PSPE extends the lifespan, improves physical activity and stress tolerance in flies

PSPE treated groups showed an increased mean lifespan compared to the control group, the mean lifespan was increased by 2.8% in the 0.5 mg/mL PSPE treated group compared to the control group, especially in the 2.0 mg/mL PSPE-treated group, where the mean lifespan was 14.5% longer than the corresponding control ($p < 0.05$) (Fig. 2A). To further study the effect of extending lifespan in PSPE diets was directly caused by dietary restriction (DR), we measured the food intake in flies. The results showed that there were no significant differences between PSPE-treated groups and control group, indicating that PSPE had no effect on food consumption (Supplementary Fig. S1).

The crawling experiment was used to evaluate whether PSPE enhanced physical activity of flies. In the study, climbing ability was enhanced by a PSPE-supplemented diet. In the control group, the mean climbing ability was 43.0%, whereas the climbing ability was improved by 6.9% in the 0.5 mg/mL PSPE treated group compared to the control group at 45 days, moreover, the climbing ability was increased by 23.3% in the 2.0 mg/mL PSPE-supplemented at 45 days ($p < 0.05$) (Fig. 2B).

As a strong oxidant, H_2O_2 can produce hydroxyl radicals in the body, and is often used in antioxidant experiments. After induction of H_2O_2, reactive oxygen species (ROS) content has been shown to increase and accelerate aging in *Drosophila melanogaster* (Guntur et al., 2016). Thus, this study researched whether PSPE improved stress tolerance to induce longevity. The results showed that the survival time was significantly increased by 6.2% and 18.2% against the H_2O_2 stress in the 0.5 mg/mL PSPE treated group and 2.0 mg/mL PSPE-treated group compared to the control group. Intensive H_2O_2 testing revealed that PSPE could effectively reduce oxidative stress induced by H_2O_2 (Fig. 2C).

Survival in the presence of a strong oxidant, paraquat, also declined as flies grow older (Piegholdt et al., 2016). In this study, the survival time was significantly increased by 6.3% in the 0.5 mg/mL PSPE treated group, furthermore, supplementation of 2.0 mg/mL PSPE partially reduced the paraquat-induced mortality rate and significantly increased the mean lifespan of treated flies by 21.0% compared to the control group (Fig. 2D). This demonstrates that dietary PSPE could delay aging.

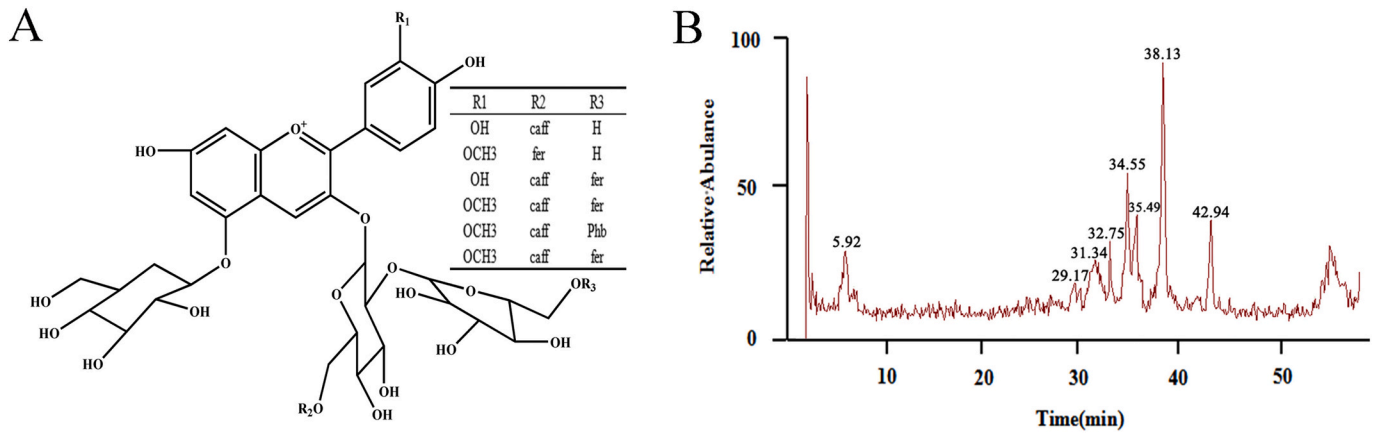

Fig. 1. (A) Structure of anthocyanins isolated from PSPE. Abbreviations: caff = caffeoyl, fer = feruloyl, phb = p-hydroxybenzoyl. (B) Major and unknown anthocyanin compounds isolated from PSPE.

Table 1
MS characteristics of eight compounds identified from PSPE.

Peak number	The name of compound	Retention time (min)	$[M - H]^-$ (*m/z*)	Main fragment ions	Contents of individual anthocyanins (%)
1	Unknown	5.92	156	79,463,949	11.56
2	Cyanidin 3-(6″-caffeoyl sophoroside)-5-glucoside	29.17	935	773,449,287	3.94
3	Peonidin 3-caffeoyl sophoroside-5-glucoside	31.34	949	787,463,301	17.68
4	Cyanidin 3-(6″-caffeoyl-6‴-feruloylsophoro-5-glucoside)	32.75	1111	949,449,287	5.32
5	Peonidin 3-(6″, 6‴-dicaffeoyl sophoroside)-5-glucoside	34.55	1111	949,463,385	9.39
6	Peonidin 3-(6″-caffeoyl-6‴-phydroxybenzoylsoph)-5-glucoside	35.49	1069	907,463,301	7.76
7	Peonidin 3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside	38.13	1125	963,463,301	31.88
8	Unknown	42.96	679	661,435,643	12.46

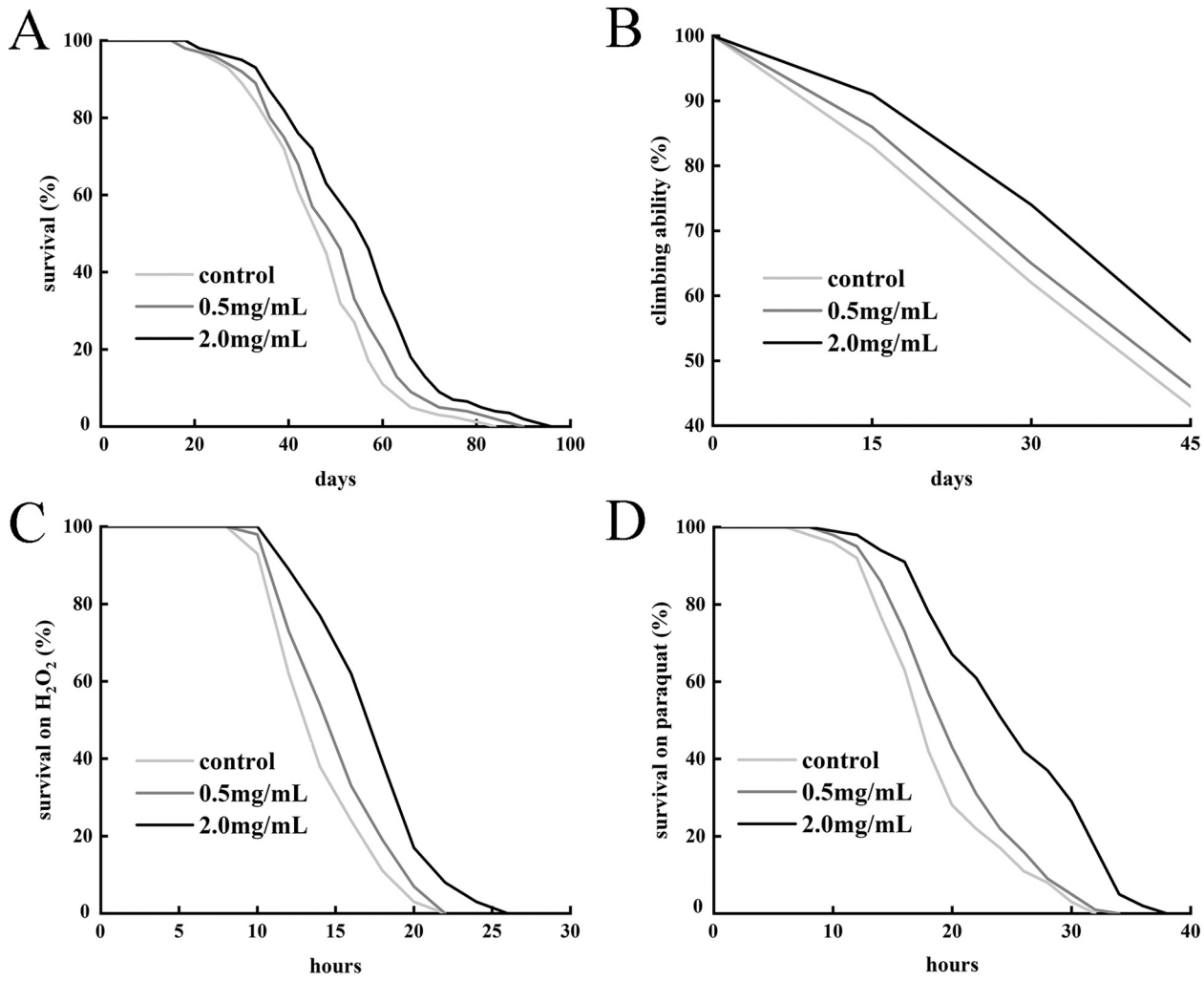

Fig. 2. Effect of PSPE on lifespan, physical activity and stress tolerance in flies. (A) Treatment with 0.5 and 2.0 mg/mL PSPE extended the lifespan of treated flies compared to the control group. The mean lifespan was extended by 2.8% and 14.5% in the 0.5 and 2.0 mg/mL PSPE treatments, respectively. (B) PSPE improved physical activity of flies, climbing ability was improved by 6.9% and 23.3% in the 0.5 mg/mL and 2.0 mg/mL PSPE-treated group at 45 days. (C) Supplementation of PSPE enhanced H_2O_2 stress tolerance in a dose-dependent manner. (D) PSPE diets increased tolerance to paraquat stress, the mean lifespan was significantly extended by 6.3% and 21.0% in the 0.5 and 2.0 mg/mL PSPE-treated group compared to the control group.

3.3. PSPE improves gut tissue homeostasis in flies

Intestinal integrity is an important marker of aged physiological health in numerous species (Tomoyo et al., 2013). We examined whether PSPE maintained intestinal homeostasis by analyzing proliferation rate of ISCs in flies, the results showed no significant differences between the control and PSPE-treated groups in the guts of 20-day-old guts of flies (Fig. 3A). However, in 50-day-old flies guts, the proliferation of ISCs was decreased by 9.7% compared to the control group in the supplementation of 0.5 mg/mL PSPE, moreover, the proliferation of ISCs significantly decreased by 32.3% in the 2.0 mg/mL PSPE treated group compared to the control group (Fig. 3B–C). The results showed that supplementation of PSPE preserved intestinal homeostasis in the aging flies' guts and further prolonged the lifespan.

Young flies have a well-developed homeostatic intestinal tract and are in homeostasis with low permeability. When flies enter the aging

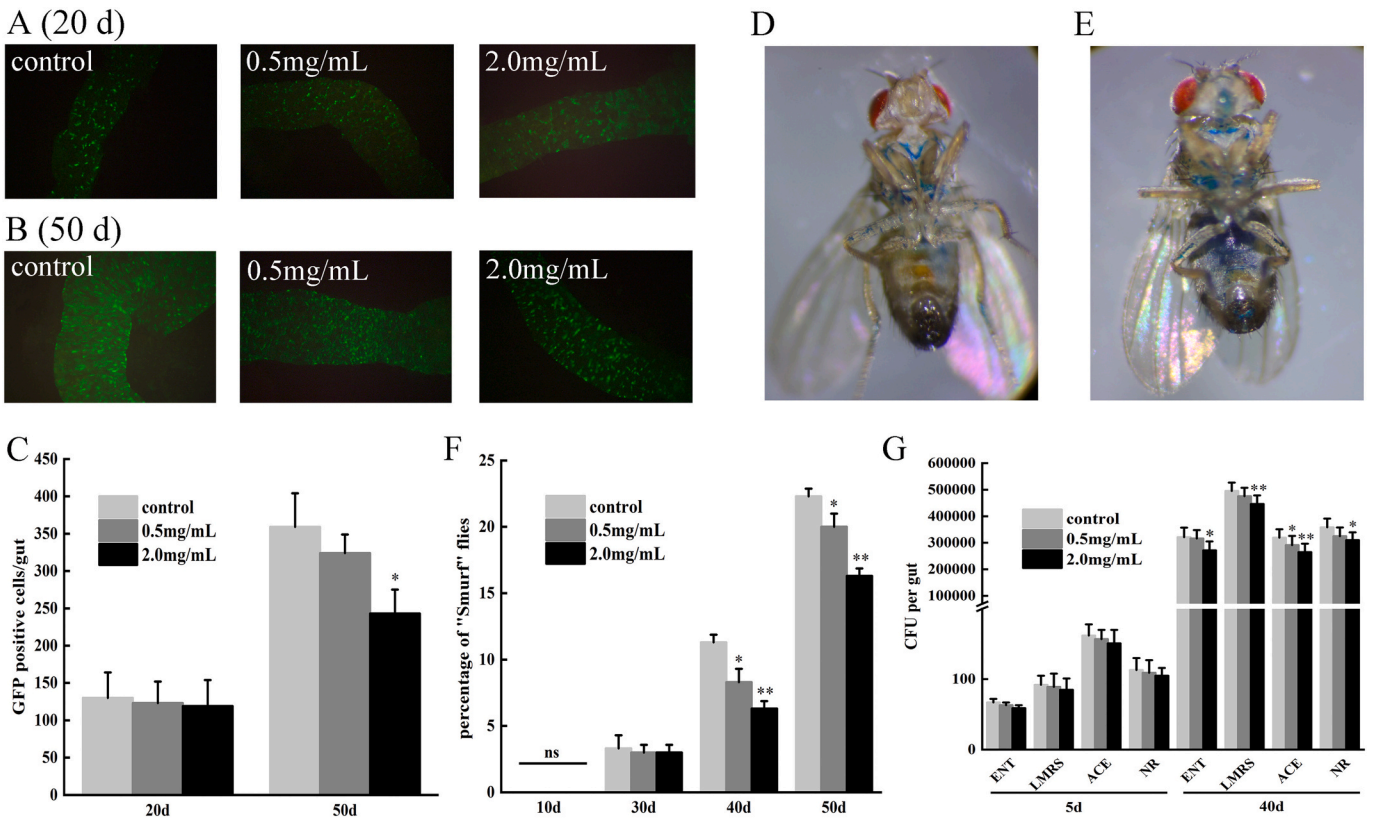

Fig. 3. Effect of PSPE on the proliferation of ISCs and intestinal integrity. (A) *Esg*$^+$ cells in control and PSPE treated groups in 20 - day - old flies' guts. (B) *Esg*$^+$ cells in control and PSPE treated groups in 50 - day - old flies' guts. (C) The quantification of *Esg*$^+$ cells in control and PSPE reared fly midguts. (D) In 10-day-old flies, the dye was only displayed in the proboscis and digestive tract. (E) In 30 - day - old aging flies, the dye was clearly visible throughout the body after feeding with FD&C Blue No.1. These were then regarded as "Smurfs". (F) The percentage of "Smurf" flies in control and PSPE treated at 10, 30, 40 and 50 days. (G) The midguts were homogenized from flies at 5 days and 40 days. The homogenates were coated on NR or selective plates, the number of CFUs was measured in control and PSPE treated flies (ns = nonsignificant, $*p < 0.05$, and $**p < 0.01$).

stage, the irregularity of their intestinal epithelial cells will break intestinal homeostasis (Wu et al., 2017) in this study, intestinal barrier dysfunction was tested by Smurf assay. As shown in Fig. 3D, the dye was only displayed in the proboscis and digestive tract of 10-day-old flies. However, in 30-day-old aging flies, the dye was clearly visible throughout the body after feeding with FD&C Blue No. 1, these flies were regarded as "Smurf" (Fig. 3E). The results showed that the percentage of "Smurf" flies significantly decreased in the 0.5 and 2.0 mg/mL PSPE treated groups compared to the control group at 40 and 50 days. In addition, "Smurf" flies significantly reduced by 10.0% and 26.9% in the 0.5 mg/mL and 2.0 mg/mL PSPE treated groups compared to the control group at 50 days, respectively (Fig. 3F).

Intestinal microorganisms have the potential to induce local and systemic inflammation. Under normal physiological conditions, the physical barrier of the intestinal perimetrium and the immune barrier of *Drosophila melanogaster* have a large number of signaling mechanisms to protect the host from direct microbial infection (Westfall et al., 2018). During the aging process, the intestinal flora increase abnormally, therefore, intestinal microorganisms were conducted by measuring CFUs from extracted flies' guts using selective plates to quantify how *Enterobacteriaceae* (*ENT*), *Lactobacilli* MRS (*LMRS*), *Acetobacteriaceae* (*ACE*) and other bacteria grew on NR. As shown in Fig. 3G, the number of CFUs was about 200 in flies' guts at 5 days and there were no significant differences between the control and PSPE treated groups. However, in aged flies (40 days old), the number of CFUs from PSPE-treated groups were significantly lower than the control group, indicating that PSPE improved the abnormal increasement of intestinal flora in flies' guts.

3.4. PSPE increases the antioxidant enzyme activities and antioxidant genes expression in flies

Cu-Zn-SOD enzyme and Mn-SOD enzyme are antioxidant enzymes distributed in lysosomes, cytoplasm, and nuclei and can catalyze superoxide anion radicals into harmless oxygen and water molecules, thus maintaining intracellular homeostasis (Tower, 2005). Cu-Zn-SOD enzyme activity was significantly higher than the corresponding control group in the PSPE-treated groups ($p < 0.05$). The Mn-SOD enzyme activity was increased by 4.7% in the 0.5 mg/mL PSPE treated group compared to the control group and there was no significance, however, the Mn-SOD enzyme activity was significantly increased by 32.7% following the supplementation of 2.0 mg/mL PSPE compared to the control group ($p < 0.01$) (Table 2).

CAT is an enzyme that can dismutate hydrogen peroxide into water and oxygen, and prevent hydrogen peroxide and oxygen from reacting

Table 2
Effect of PSPE on the antioxidant enzyme activity and in flies.

Group (mg/mL)	Cu-Zn-SOD (U/mg of pro)	Mn-SOD (U/mg of pro)	CAT (U/mg of pro)	MDA (nmol/mg of pro)
Control	179.67 ± 15.27	108.23 ± 8.30	51.49 ± 2.64	0.95 ± 0.08
0.5 mg/mL	208.72 ± 19.82*	115.88 ± 10.03	55.84 ± 2.65*	0.71 ± 0.07*
2.0 mg/mL	218.88 ± 18.78**	129.37 ± 11.74*	58.05 ± 1.37**	0.58 ± 0.09**

Data were represented as mean ± SD. $*p < 0.05$, $**p < 0.01$ versus the control group.

with iron chelate to form harmful hydroxyl groups. In this study, CAT activity was significantly increased by 8.7% and 15.03% in the 0.5 mg/mL and 2.0 mg/mL PSPE treatment compared to the control group ($p < 0.05$) (Table 2).

MDA content, reflects the lipid peroxidation rate and strength of the body, so the content of MDA is an important parameter that reflects the potential antioxidant capacity of the body. In the present study, MDA content significantly decreased by 22.6% and 45.2% in the 0.5 mg/mL and 2.0 mg/mL PSPE treatments, respectively ($p < 0.05$) (Table 2).

In the study, Cu-Zn-SOD and Mn-SOD mRNA expressions were significant increased by 49.2% and 87.0% in the 2.0 mg/mL PSPE-supplemented diets, respectively ($p < 0.01$) (Table 3). CAT gene expression was also increased by 9.9% in the 0.5 mg/mL PSPE diets, however, the CAT gene expression was significantly increased by 56.4% by the administration of 2.0 mg/mL PSPE (Table 3). MTH is a gene associated with longevity, down-regulating MTH could enhance the antioxidant capacity and reduce the production of free radicals in flies (Li et al., 2014). In this study, MTH gene expression was decreased by 9.7% in the 0.5 mg/mL PSPE diets and there was no significance, the MTH gene expression was significantly decreased by 31.0% after 2.0 mg/mL PSPE treatment (Table 3).

3.5. PSPE inhibits mTOR pathway and activates autophagy pathway in flies

The mTOR signaling pathway promotes substance metabolism, participates in apoptosis and autophagy, and plays a non-negligible role in various diseases. Inhibition of the mTOR pathway has been shown to extend the lifespan in a variety of organisms (Evans et al., 2011). Therefore, the study investigated whether the mTOR pathway in flies was inhibited by the supplementing of PSPE. The results showed that the upstream genes AKT-1, PI3K and mTOR were significantly inhibited, and the downstream gene 4E-BP was significantly increased in PSPE treated groups (Fig. 4A).

Autophagy is a process in which autophagosomal proteins or organelles are engulfed into vesicles and fused with lysosomes to form autophagososomes and degrade the contents (Bhukel et al., 2016). In the present study, levels of autophagy-related genes Atg1, Atg5, Atg8a, and Atg8b were significantly increased in PSPE groups (Fig. 4B). Furthermore, phagosome production was increased by lyso tracker staining in PSPE-treated flies in a dose-dependent manner (Fig. 4C-D).

4. Discussion

Purple sweet potatoes are rich in anthocyanin and other phenolic compounds with known antioxidant activities (Ju et al., 2011). It is previously reported that purple sweet potato have various functions, for example, anti-aging, anti-inflammation, anti-atherosclerosis (Sun et al., 2015). Therefore, PSPE was a potential functional food to delay senescence. It is confirmed that the efficacy of functional food tend to vary with sex in flies, in addition, the hormone of male flies was at a low level, therefore, the study used male flies as model mechanism (Chattopadhyay and Thirumurugan, 2020).

According to the previous study, the ratio of carbohydrate to protein was important in extending lifespan in flies (Wang et al., 2011). In the study, the basal medium mainly contained 0.72 g/mL carbohydrate, 0.075 g/mL protein, and the iron content was negligible.

Table 3
Effect of PSPE on the antioxidant gene expression in flies.

Group (mg/mL)	Cu-Zn-SOD /rp49	Mn-SOD /rp49	CAT /rp49	MTH /rp49
Control	1.00 ± 0.11	1.00 ± 0.23	1.0 ± 0.21	1.00 ± 0.22
0.5 mg/mL	1.18 ± 0.19	1.45 ± 0.36*	1.07 ± 0.22	0.86 ± 0.19
2.0 mg/mL	1.61 ± 0.31**	1.60 ± 0.31**	1.49 ± 0.23**	0.69 ± 0.14*

Data were represented as mean ± SD. *$p < 0.05$, **$p < 0.01$ versus the control group.

4.1. Improvement of physical activity and stress tolerance in flies after PSPE treatment

In the present study, the results showed that 0.5 and 2.0 mg/mL PSPE treatments increased lifespan by 2.8% and 14.5% in flies, respectively. The climbing ability and stress resistance to H_2O_2 and paraquat were also improved after PSPE treatment. These findings were similar to Wang and his colleagues who showed that purple sweet potato anthocyanin extended the lifespan, enhanced locomotor activity and improved resistance to stress in flies (Wang et al., 2016). Moreover, it is beneficial to slow aging in flies which were treated PSPE at early life, indicating that PSPE played an important role in the prevention of aging at initial life.

4.2. Improvement of gut tissue homeostasis in flies after PSPE treatment

Recent studies, have shown that maintaining intestinal homeostasis plays a primary role in flies. Homeostasis in the midgut is maintained by ISCs that produce two kinds of daughter cells, the one is used to maintain the stem cell population, and the other is to differentiate into enteroblast (EB), these cells are all expressed a transcription factor called *esg*, therefore, the expression of *esg* can be used to measure the rate of ISCs proliferation (Fan et al., 2015). According to the previous study, abnormal proliferation of ISCs accelerated aging progress and further triggered various diseases, thus, slowing down the rate of ISCs proliferation extended the lifespan in flies (Woodcock et al., 2015). In the present study, the proliferation of ISCs was significantly decreased in the 2.0 mg/mL PSPE-treated group compared to the control group in aging flies guts. This suggested that extension of lifespan was likely to due to decreased proliferation rate of ISCs in flies after PSPE treatment. Similar results were confirmed by Li et al. who studied that improvement of ISCs proliferation in the aging intestines extended the lifespan in flies (Li et al., 2013). The midgut tissue homeostasis was maintained by ISCs, decreased proliferation of ISCs promoted apoptosis of bad cells, thereby protecting intestinal homeostasis and extending the lifespan in flies.

Intestinal barrier dysfunction is a manifestation of intestinal homeostasis loss which is associated with shortened lifespan in *Drosophila* (Tomoyo et al., 2013). In the present study, the percentage of "Smurf" flies was significantly decreased in PSPE-treated flies, indicating that PSPE slowed down intestinal barrier dysfunction as the flies aged. In the aging flies, the intestinal flora increased sharply, and the imbalance led to a variety of diseases (Buchon et al., 2013). Our results showed that the intestinal flora was significantly reduced in guts of PSPE treated group. The results were in line with Fan and her colleagues who found that rapamycin slowed down the intestinal barrier dysfunction and reduced the number of CFUs, extending the lifespan in aged flies (Fan et al., 2015).

4.3. Enhancement of antioxidant activity in flies after PSPE treatment

SOD and CAT serving as primary endogenous antioxidants help to clear up free radicals and then slow down aging. The MDA content, is one of the most important products of membrane lipid peroxidation, which indirectly reflect the degree of tissue peroxidation damage (Gupta et al., 2005). In this study, the enzyme activity of Cu-Zn-SOD, Mn-SOD, and CAT were significantly enhanced after PSPE treatment. Similarly, SOD and CAT mRNA gene expressions were also significantly elevated. MTH mRNA expression was reduced after PSPE treatment. MDA content was decreased after PSPE treatment. The results were in agreement with Wang et al. who reported that the enzyme activity and gene expression of SOD and CAT were significantly increased by rosemary extract treatment, MDA content and MTH gene expressions were decreased in the supplementation of rosemary extract (Wang et al., 2017).

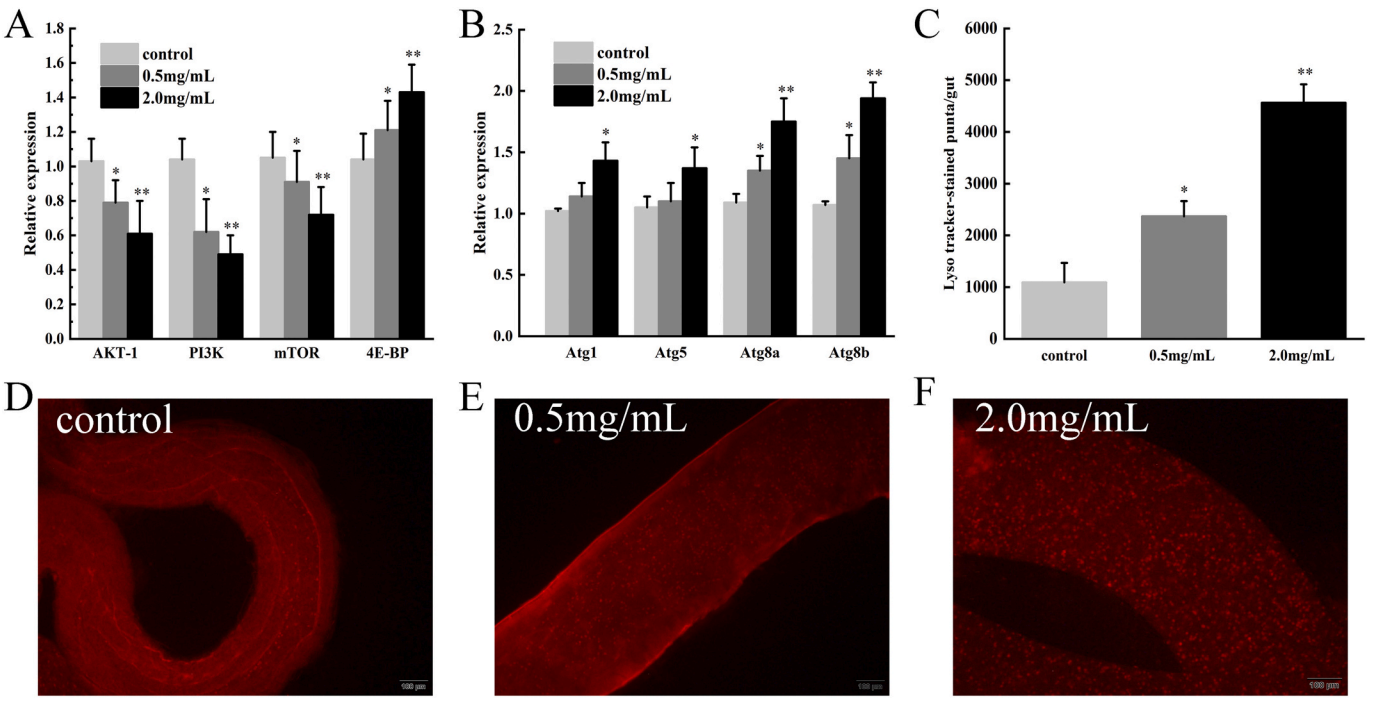

Fig. 4. Effect of PSPE on autophagy pathway in flies. (A) Relative mRNA expression levels of mTOR, AKT-1, PI3K were significantly inhibited, while 4E-BP was significantly increased. (B) Relative mRNA expression of autophagy - related genes, namely Atg1, Atg5, Atg8a, and Atg8b were up - regulated after PSPE treatment. (C) The formation of lysosomes increased in the supplementation of PSPE in a dose - dependent manner. (D) Relative fluorescence images of control group flies' midguts. (E) Relative fluorescence images of 0.5 mg/mL PSPE - treated flies' midguts. (F) Relative fluorescence of 2.0 mg/mL PSPE - treated flies' midguts. (*$p < 0.05$ and **$p < 0.01$).

4.4. The autophagy pathway participates in extension of lifespan in flies after PSPE treatment

The mTOR pathway is the most studied pathway associated with aging, inhibiting the mTOR pathway has been found to extend the lifespan of worms, fruit flies, and mammals (Cabo et al., 2014). In this study, the upstream genes of AKT-1, PI3K and mTOR levels were significantly decreased in PSPE-treated flies, whereas the downstream gene 4E-BP was markedly increased, suggesting that the mTOR pathway was inhibited after PSPE treatment. The results were consistent with Liu's reports which suggested that *Rhodiola rosea* extract decreased the growth of bladder cancer cells by inhibiting mTOR pathway and further extended the lifespan (Liu et al., 2012).

As a cellular process, autophagy plays an indispensable role in cell survival under some conditions of nutrition deficiency and normal or abnormal cell growth. Substantial reduction of phagosome in autophagy has been widely reported with the aging progress (Nazio et al., 2013). The previous study reported that Atg1, an evolutionarily preserved serine kinase, plays an important role in the prophase of autophagy as the last component of mTOR pathway and first component of the autophagy pathway (Kim et al., 2011). Lysosome is an organelle that decomposes proteins, nucleic acids, polysaccharides, and other biological macromolecules, which reflects the degree of autophagy (Bhukel et al., 2016). In the present study, the expression of autophagy-related genes were significantly increased in flies fed on PSPE and lysosome production was increased in the midgut epithelial cells after PSPE treatment, indicating that autophagy pathway was activated. It is similar to Su and his colleagues who showed that α-ketoglutarate extended the lifespan by significantly increasing the production of lysosomes in flies (Su et al., 2019).

5. Conclusions

PSPE extended the lifespan and increased climbing ability, decreased the mortality rates induced by paraquat and H_2O_2, improved intestinal homeostasis and intestinal barrier dysfunction, increased the activities of antioxidant enzymes and gene expression, inhibited mTOR and activated the autophagy pathway in flies. These results indicate PSPE is potential to be anti-aging products, and provide references for further anti-aging studies on human beings, and the improvement effect of PSPE treatment for lifespan and health span need further study at mid-life or later life in flies.

CRediT authorship contribution statement

In the manuscript, the red words which were signed have been modified.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2020.111190.

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