Biogerontology (2020) 21:669–682
https://doi.org/10.1007/s10522-020-09884-w

RESEARCH ARTICLE

Tectochrysin increases stress resistance and extends the lifespan of *Caenorhabditis elegans* via FOXO/DAF-16

Min Lu · Lin Tan · Xiao-Gang Zhou · Zhong-Lin Yang · Qing Zhu · Jian-Ning Chen · Huai-Rong Luo · Gui-Sheng Wu

Received: 12 February 2020 / Accepted: 23 May 2020 / Published online: 6 June 2020

Abstract Aging is related to the lowered overall functioning and increased risk for various age-related diseases in humans. Tectochrysin is a flavonoid compound and rich in a traditional Chinese Medicine *Alpinia oxyphylla* Miq., which has antioxidant, anti-inflammatory, anti-cancer, anti-bacterial, anti-diarrhea, hepatoprotective, and neuro-protective effects. Therefore, we tested if tectochrysin had an effect on aging in *Caenorhabditis elegans* (*C. elegans*). Our results showed that tectochrysin could extend the lifespan of *C. elegans* by up to 21.0%, delay the age-related decline of body movement, improve high temperature-stress resistance and anti-infection capacity, and protected worms against Aβ1-42-induced toxicity. Tectochrysin could not extend the lifespan of the mutants from genes *daf-2*, *daf-16*, *eat-2*, *aak-2*, *skn-1*, and *hsf-1*. Tectochrysin could increase the expression of DAF-16 regulated genes. The extension of lifespan by tectochrysin requires FOXO/DAF-16 and HSF-1. Overall, our findings suggest that tectochrysin may have a potential effect on extending lifespan and age-related diseases.

Keywords Tectochrysin · Aging · FOXO/DAF-16 · Age-related diseases · *Caenorhabditis elegans*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10522-020-09884-w) contains supplementary material, which is available to authorized users.

M. Lu · L. Tan · X.-G. Zhou · Z.-L. Yang · Q. Zhu · J.-N. Chen · H.-R. Luo (✉) · G.-S. Wu (✉)
Key Laboratory for Aging and Regenerative Medicine, Department of Pharmacology School of Pharmacy, Southwest Medical University, 319 Zhongshan Road, Luzhou 646000, Sichuan, China
e-mail: lhr@swmu.edu.cn

G.-S. Wu
e-mail: wgs@swmu.edu.cn

H.-R. Luo · G.-S. Wu
Key Laboratory of Medical Electrophysiology, Ministry of Education, Institute of Cardiovascular Research of Southwest Medical University, Luzhou 646000, Sichuan, China

Introduction

Aging is a natural phenomenon that occurs in -most living organisms. In humans, aging is associated with lowered overall functioning and increased risk for various age-related diseases (Castillo-Quan et al. 2015). Hence, researchers are keen on finding effective components that can promote healthy aging and extend lifespan. The natural decline of function occurring with aging could be prevented and / or reversed through various ways such as gene modifications, dietary interventions, and hormetic mechanism (Kunugi and Mohammed Ali 2019). Accumulating evidence indicated that traditional herbs could promote healthy aging and lengthen

healthy lifespans, such as *Ginseng Radix et Rhizoma* (Biswas et al. 2017), the extract of *Aralia chinensis* L (Zhang et al. 2015), *Notoginseng radix et rhizome* (Feng et al. 2015), *Dendrobium candidum* Wall. ex Lindl (Russo et al. 2019), *Calycophyllum spruceanum* (Benth.) (Peixoto et al. 2018), and *Curcuma longa* (Bielak-Zmijewska et al. 2019).

Alpinia oxyphylla Miq. is a traditional Chinese herbal medicinal against diarrhea, salivation, and diuresis (Zhang et al. 2018b). Accumulating evidence has demonstrated that the extracts from the *Alpinia oxyphylla* Miq. also exert significantly neuroprotective, antioxidant (Shi et al. 2006), anti-inflammatory (Shi et al. 2020), anti-tumor (He et al. 2010), and anti-diabetes effects (Xie et al. 2018). Flavonoids are the main active constituents of *Alpinia oxyphylla* Miq. (Chen et al. 2014). Tectochrysin (TEC) (Fig. 1a) is a flavonoid compound and rich in *Alpinia oxyphylla* Miq. Tectochrysin showed improved spatial memory performance in amnestic mice induced by Aβ1-42 (He et al. 2019). Tectochrysin could reduce pro-inflammatory mediator production by directly inactivating p-MEK1/2 (Hou et al. 2018). Tectochrysin could activate aryl hydrocarbon receptor (AhR, which mediates the toxicological effect) (Amakura et al. 2011). Tectochrysin exhibited its anti-diarrhea effect partially by affecting proteins NHE3 and AQP4 (Zhang et al. 2013). Given the multiple biological activities of tectochrysin, we were wondering if tectochrysin could affect the aging process. Because of its short lifespan, amenability to genetic manipulation, clear genetic background, and a proportion of homologous genes in humans, *C. elegans* is an excellent model for screening chemicals with longevity modulation effects (Ma et al. 2018). Here, we investigated if tectochrysin could extend the lifespan of *Caenorhabditis elegans*. We found that tectochrysin could improve anti-stress ability, extend lifespan, and improve the symptoms of geriatric diseases in *C. elegans*.

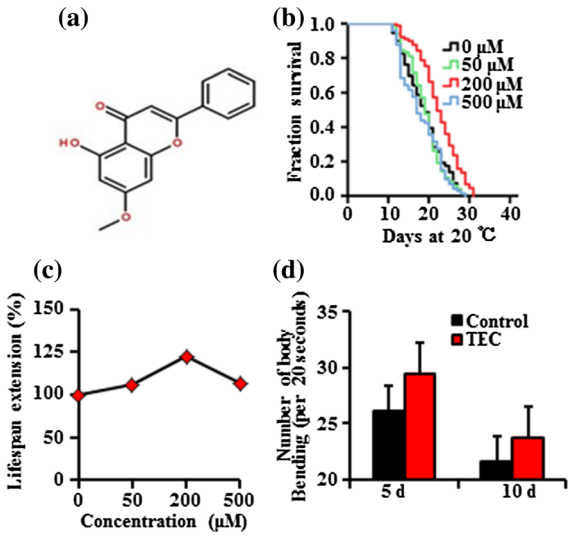


Fig. 1 Tectochrysin extends the lifespan of *C. elegans*. **a** The chemical structure of tectochrysin (TEC). **b** The survival of wild-type *C. elegans* (N2) treated with ddH_2O (control) and 50 μM, 200 μM, and 500 μM of tectochrysin. **c** The lifespan extension in fold changes under treatment of tectochrysin with different concentrations. **d** Body bending of wild-type *C. elegans* (N2) treated with or without 200 μM of tectochrysin. Each figure shows one experiment. The statistical significance represented by *p* value was calculated by *t*-test or log-rank test. The columns showed the mean value of one independent experiment with error bars representing SEM. The results of three repeated experiments and their statistical analysis were summarized in Supplementary Tables S1–3 (Supplementary information)

Methods

Strains and chemicals

All strains were obtained from *Caenorhabditis* Genetics Center (CGC) and maintained at appropriate temperature as described previously (Brenner 1974), unless otherwise stated. strains used in this study were as follows: N2 (Bristol, wild type), DA1116 *eat-2(ad1116) II.*, MQ887 *isp-1(qm150) IV.*, CB1370 *daf-2(e1370) III.*, TJ356 *daf-16(zls356) IV.*, CF1038 *daf-16(mu86) I.*, RB754 *aak-2(ok524) X.*, EU1 *skn-1(zu67) IV.*, PS3551 *hsf-1(sy441) I.*, CF1553 *muIs84 (sod-3::gfp)*, SJ4100 *zcIs13V (hsp-6::gfp)*, SJ4005 *zcIs4V (hsp-4::gfp)*, and CL4176 *dvls27 (myo-3/Aβ1-42 /let UTR, rol-6)*. All strains grew and were maintained on NGM plates seeded with *Escherichia coli* OP50. The CL4176 strain is temperature-sensitive maternal effect lethal, and maintains at 15 °C and shifts to 25 °C at L3 stage in the paralysis assay (Xu et al. 2019).

Tectochrysin was purchased from Shanghai Yuanye Bio-Technology Co., Ltd, and dissolved in ddH_2O. NGM plates containing tectochrysin were equilibrated overnight before use.

Lifespan assay

All strains were cultured for 2–3 generations without starvation, and lifespan analysis was conducted at 20 °C, unless otherwise stated. For each experiment, at least 60 worms were used. Late L4 larvae or young adults were transferred to NGM plates (9 cm, diameter) containing inactivated OP50 (60 °C for 35 min) and 50 μM of 5-fluoro-2′-deoxyuridine (FUDR, Sigma) to prevent self-fertilization. The day L4 larvae or young adults were transferred to experimental plates and were defined as experiment day 0. To ensure that tectochrysin retained its potency throughout the entire experiment, worms were transferred to fresh plates with or without tectochrysin every other day. Worms that did not respond to a mechanical stimulus were scored as dead, as described previously (Apfeld and Kenyon 1999). Worms were censored, if they crawled off the plate, displayed extruded internal organs, or died because of hatching progeny inside the uterus. The lifespan assay included at least three independent replicate experiments. Statistical analyses were carried out using SPSS packages. Kaplan–Meier lifespan analysis was carried out and *p* value was calculated using log-rank test.

Phenotypic assays

About 100 Late L4 larvae or young adult worms [wild-type *C. elegans* (N2)] were transferred to each plate with or without 200 μM of tectochrysin for 5 days or 10 days. Before the phenotype analysis, worms were transferred to fresh plates with inactivated OP50 every other day, and maintained at 20 °C.

In pathogen resistance assay, plates were seeded with live *Pseudomonas aeruginosa* (PA14) and cultured over-night before use. Worms were transferred to fresh NGM plate with live PA14, incubated at 20 °C, monitored every day, and scored as dead when they did not respond to the gentle touch with platinum wire pick (Pees et al. 2017).

In thermo-tolerance assay, worms were transferred to fresh plates with or without 200 μM of tectochrysin, and incubated at 35 °C, monitored every 2 h, and scored as dead when they did not respond to the gentle touch with platinum wire pick (Wilson et al. 2006).

In oxidative stress assay, worms were transferred to NGM plates containing paraquat (Sigma) and cultured at 20 °C (Zheng et al. 2017). Worms were monitored each day and scored as dead when they did not respond to the gentle touch with platinum wire pick. Kaplan–Meier lifespan analysis was carried out and *p* value was calculated using log-rank test.

Body bending assay was conducted as described previously (Huang et al. 2004). 3 worms were gently transferred to the plate with a drop of M9 buffer and left to acclimatize for 15 s at room temperature each time. Visualizing under a microscope, the complete body bending, defined as the maximum bend of the part of worm just behind the pharynx from one end to the opposite direction in a forward sinusoidal pattern, was manually counted for 20 s. The reverse bend in the same direction was not included in the count. In pharyngeal pumping assay, worms were transferred to fresh NGM plates. Visualizing under a microscope, the number of Pharynx pumping per 20 s was counted using a hand counter. The data was represented by histogram. The *p* value was obtained by *t*-test (Peixoto et al. 2019).

Each phenotype analysis included at least three independent replicate experiments. All the phenotype analyses except thermo-tolerance assay, were performed at 20 °C.

DAF-16::GFP localization assay

In each experiment, at least 30 worms of transgenic strain TJ356 *daf-16(zls356) IV.* were used to analyze the localization of DAF-16::GFP in the same condition as described for lifespan assay. L4 larvae worms were transferred to the plates with or without 200 μM of tectochrysin. Worms were cultured for 24 h at 20 °C. The location of DAF-16::GFP signal was monitored using a fluorescent microscope (Leica, DM6B). Live images were taken from at least 30 worms per group. The accumulation of fluorescent signal in nuclei was scored as described previously (Rezaizadehnajafi and Wink 2014). The assay included at least three independent replicate experiments. The *p* value was calculated using *t*-test.

Fluorescence intensity quantification assay

In fluorescence intensity quantification assays, we used SJ4005 *zcIs4 (hsp-4::gfp)*, SJ4100 *zcIs13(hsp-6::gfp)*, and CF1553 *muIs84 (sod-3::gfp)* to analyze whether tectochrysin affect the expression of genes: *hsp-4*, *hsp-6*, and *sod-3*, respectively. We observed the

distribution of green fluorescent protein and measured the fluorescence intensity in these transgenic strains SJ4005, SJ4100, and CF1553, respectively. Each plate contains 50 μM of FUDR to inhibit egg hatching, as in lifespan assays. About 100 Late L4 larvae or young adult worms (mutants) were transferred to the plates with or without 200 μM of tectochrysin, maintained at 20 °C with dead OP50 for 10 days. Then we took photos by fluorescence microscope (Leica DM6B). Live images were taken from at least 30 worms per group. At last, we used Image J to quantify the intensity of fluorescence. The assay included at least three independent replicate experiments (Peixoto et al. 2016).

Paralysis assay

Transgenic mutant strain CL4176 *dvls27 (myo-3/Aβ1-42 /let UTR, rol-6)* that carries human amyloid-β protein was used for paralysis assay (Xu et al. 2019). Worms were cultured on NGM plates seeded with dead OP50 until L3 larvae at 15 °C. Then worms were transferred to fresh NGM plates with or without 200 μM of tectochrysin, and were transferred to 25 °C incubator to induce Aβ1-42 expression. Worms were scored every 6 h for paralysis by tapping two times on their head using platinum wire pick till death of the last worm. Worms that did not show any movement were considered as paralyzed.

Chemotaxis assays

Many organisms use chemotaxis to seek out food sources, avoid noxious substances, and find mates. *C. elegans* has impressive chemotaxis behavior. To analyze whether *C. elegans* use chemotaxis to avoid tectochrysin, we performed chemotaxis assays (see Fig. 5c) using wild-type N2 worms as described previously (He et al. 2017; Margie et al. 2013). About 200 synchronized *C. elegans* were placed in the center of an assay NGM plate (diameter: 3.5 cm), which contained 200 μM of tectochrysin plus 2% tetramisole hydrochloride to paralyze the worms on one side of the plate (A side), and H_2O as solvent control plus 2% tetramisole hydrochloride on the opposite side of the plate (B side). After incubation at 20 °C for 1 h, Chemotaxis index (CI) was scored [CI = (number of worms on A side − number of worms on B side)/ number of worms on A side + number of worms on B side].

Quantitative RT-PCR assays

About 2,000 synchronized young adult worms were transferred to six NGM plates (9 cm, diameter) with or without 200 μM of tectochrysin and cultured at 20 °C for 24 h. Total RNA was extracted using RNAiso Plus (Takara) and converted to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The quantitative RT-PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) in Quantstudio 6 Flex system. The relative expression levels of genes were carried out using $2^{-\Delta\Delta CT}$ method and normalized to the expression of gene *cdc-42* (Lee et al. 2010). The *p* values were calculated using *t*-test. Partial quantitative RT-PCR primers were as follows:

cdc-42, 5′-CTGCTGGACAGGAAGATTACG-3′ (F)
5′-CTCGGACATTCTCGAATGAAG-3′ (R).
hsp-6, 5′-GGATGCTGGACAAATCTCTG-3′ (F)
5′-ACAGCGATGATCTTATCTCCA-3′ (R).
hsp-60, 5′-AAGGATATGGGAATTGCGACGGGA-3′ (F)
5′-TGTGCTCGATTCGCTTCTCGATCT-3′ (R).
hsp-16.2, 5′-CTGCAGAATCTCTCCATCTGAGTC-3′ (F)
5′-AGATTCGAAGCAACTGCACC-3′ (R).
sod-3, 5′-AGCATCATGCCACCTACGTGA-3′ (F)
5′-CACCACCATTGAATTTCAGCG-3′ (R).
irg-1, 5′-AAGCAGCATGCGTATTTTCA-3′ (F).
5′-GCAGCTTCTCCTTTTTCTCC-3′ (R)
F35E12.5, 5′-ACACAATCATTTGCGATGGA-3′ (F)
5′-GGTAGTCATTGGAGCCGAAA-3′ (R).

Statistical analysis

Data is presented as the means ± SEM unless specifically indicated. Statistical analyses included *t*-tests or log-rank test. All figures were generated using GraphPad Prism 6, SPSS 23 or MS Office 2010.

Results

Tectochrysin extends the lifespan of *C. elegans*

To determine the prolongevity property of tectochrysin, we treated wild-type *C. elegans* (N2) with a serial concentration of tectochrysin ranging from 0 to 500 μM. We found that animals raised at 20 °C on NGM plates containing 200 μM of tectochrysin displayed the largest lifespan extension by up to 21.0% ($p < 0.0001$). Tectochrysin exhibited a smaller but not significant lifespan extension effect with concentrations higher or lower than 200 μM (500 or 50 μM, Fig. 1b, c). The body movement of *C. elegans* decreases with aging (Newell Stamper et al. 2018). In this study, we selected the frequency of body bending as a measure that indicates nematode movement behavior as previous study (Zhang et al. 2018a). As shown in Fig. 1d, we found that upon treatment with tectochrysin, body movement was significantly increased at 5 days and 10 days of adults ($p < 0.0001$), suggesting tectochrysin could slow down the decline of body movement.

Tectochrysin improves stress resistance of *C. elegans*

C. elegans with extended lifespan resulted from genetic and non-genetic manipulations often present increased stress resistance (Denzel et al. 2019). Therefore, we examined the effect of tectochrysin on stress resistance to heat, oxidative, and pathogenic bacteria in *C. elegans*. As shown in Fig. 2a, tectochrysin treatment suppressed the lethality of heat stress in wild-type *C. elegans* (N2) and extended the lifespan of *C. elegans* to 35.8% ($p < 0.0001$). The molecular chaperone heat shock proteins can repair misfolded proteins, activate unfolded protein reaction and contribute to cellular homeostasis (Govindan et al. 2018; Tan et al. 2015). We found that the mRNA level of genes *hsp-6*, *hsp-60*, and *hsp-4* were higher in worms treated with tectochrysin ($p < 0.05$) (Fig. 2b). Furthermore, to test if tectochrysin regulate the expression of proteins HSP-4 and HSP-6, we fed tectochrysin to transgenic strains SJ4005 *zcIs4V (hsp-4::gfp)* and SJ4100 *zcIs13V (hsp-6::gfp)* expressing the fusion protein HSP-4::GFP and HSP-6::GFP, respectively. As shown in Fig. 2c, tectochrysin treatment showed increased expression of proteins HSP-4 and HSP-6 by SJ4005 *zcIs4V (hsp-4::gfp)* ($p < 0.0001$) and SJ4100 *zcIs13V (hsp-6::gfp)* ($p < 0.0001$), respectively.

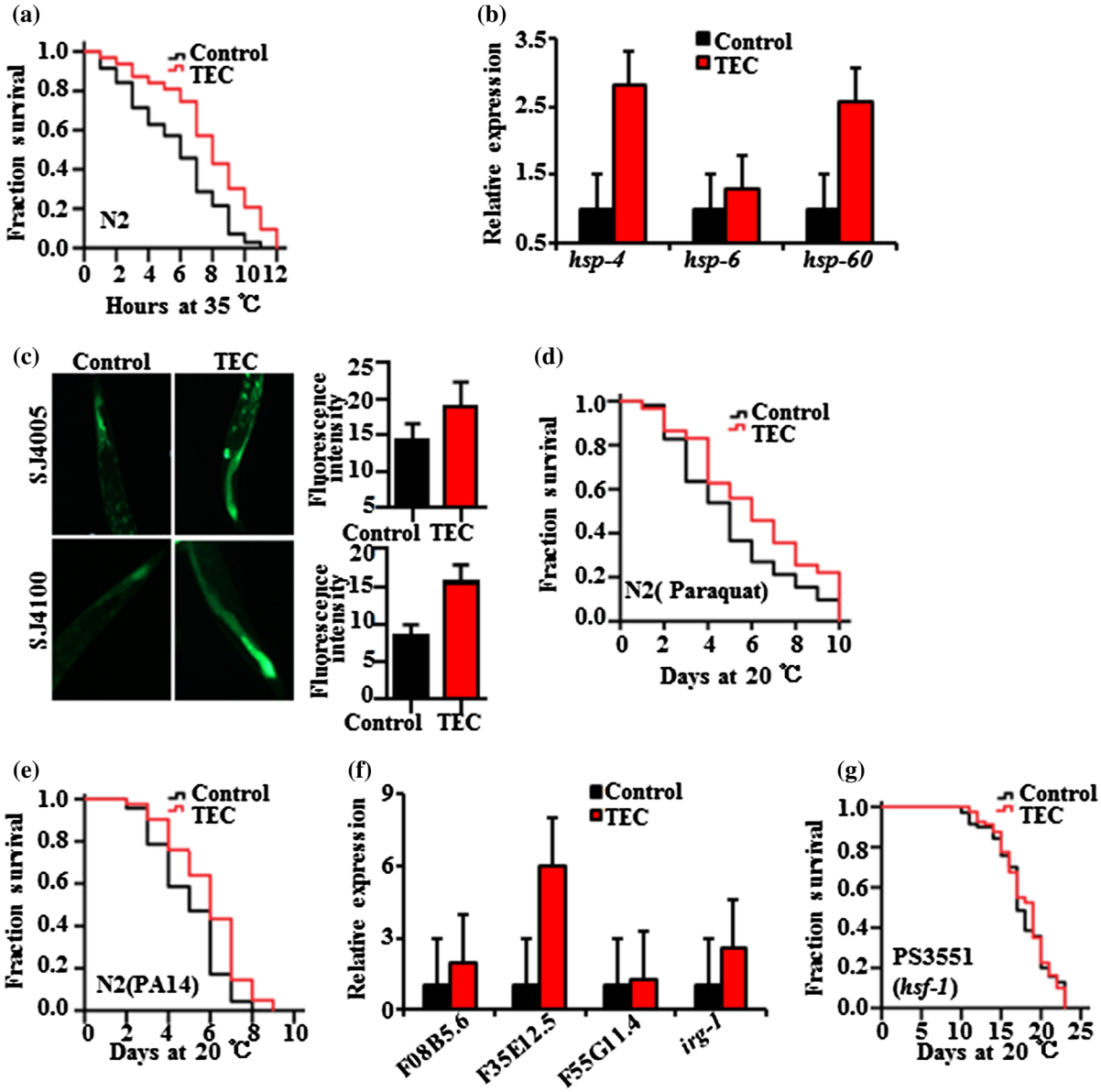
Paraquat is a free radical generating compound that can induce acute oxidative stress in cells. To analyze the anti-oxidative effect of tectochrysin, we measured the lifespan of wild-type *C. elegans* (N2) exposing to 20 mM of paraquat. As shown in Fig. 2d, tectochrysin treatment improved the survival rate of *C. elegans* exposed to paraquat and extended the lifespan to 20.9% ($p < 0.05$).

The pathogenic bacteria *pseudomonas aeruginosa* (PA14) was used to activate the immune response in *C. elegans* (Kurz and Tan 2004). To analyze the anti-pathogenic effect of tectochrysin, we measured the lifespan of wild-type *C. elegans* (N2) exposing to PA14. We found that tectochrysin suppressed the lethality of wild-type *C. elegans* feeded with PA14, and extended the lifespan of *C. elegans* to 17.8% ($p < 0.0001$) (Fig. 2e). Genes *irg-1*, *F55G11.4*, *F08G5.6*, and *F35E12.5* are immune responded (Kumar et al. 2019). To test if the anti-pathogenic effect of tectochrysin was associated with immune response, we measured the mRNA expression levels of these immune related genes in worms treated with tectochrysin. We found that the mRNA level of immune-related genes was significantly increased in *C. elegans* treated with tectochrysin ($p < 0.05$) (Fig. 2f). These results showed that tectochrysin could improve the health span of worms and increase their resistance to heat, oxidative, and pathogenic bacterial stress.

Heat shock factor 1 (HSF-1), an essential nuclear protein, is associated with heat-stress response, immunity and aging in *C. elegans* (Li et al. 2018; Seo et al. 2013). We found that tectochrysin could not extend the lifespan of *hsf-1* mutant PS3551 *hsf-1(sy441) I.* ($p > 0.05$) (Fig. 2g).

Tectochrysin decreases Aβ-induced toxicity in *C. elegans*

Alzheimer's disease (AD) is one of the major diseases in elderly population (Hou et al. 2018). The accumulation of peptide Aβ, which produced from amyloid precursor protein (APP) by proteolytic cleavages, is a key pathological event in the development of AD, causing synaptic dysfunction, neuronal death, and cognitive deficits (Jagust 2018). The CL4176 strain contains the transgene encoding human peptide


(a)
Control
TEC
Fraction survival
N2
Hours at 35 ℃
(b)
Control
TEC
Relative expression
hsp-4
hsp-6
hsp-60
(c)
Control
TEC
SJ4005
SJ4100
Fluorescence intensity
Control TEC
(d)
Control
TEC
Fraction survival
N2(Paraquat)
Days at 20 ℃
(e)
Control
TEC
Fraction survival
N2(PA14)
Days at 20 ℃
(f)
Control
TEC
Relative expression
F08B5.6
F35E12.5
F55G11.4
irg-1
(g)
Control
TEC
Fraction survival
PS3551
(hsf-1)
Days at 20 ℃

◀**Fig. 2** Tectochrysin improves stress resistance of *C. elegans*. **a** The survival analysis of wild-type *C. elegans* (N2) treated with or without 200 μM of tectochrysin at 35 °C. Tectochrysin can significantly extend the lifespan of *C. elegans* by up to 35.8% under heat stress, $p < 0.0001$. **b** The expression of *hsp-4*, *hsp-60*, and *hsp-6* at the mRNA level. The expression of heat-shock related genes showed significant difference. The columns showed the mean value of three independent experiments with error bars representing SEM ($p < 0.05$, two-tailed *t* test). **c** The picture of green fluorescent at tail in *C. elegans* SJ4005 *zcIs4V (hsp-4::gfp)* and SJ4100 *zcIs13V (hsp-6::gfp)* expressing HSP-4::GFP and HSP-6::GFP, respectively. The quantification of fluorescence intensity shows that the expression of HSP-4::GFP and HSP-6::GFP are significantly increased by the treatment of tectochrysin ($p < 0.0001$, two-tailed *t* test). The columns showed the mean value of one independent experiment with error bars representing SEM. **d** The survival curve of wild-type *C. elegans* (N2) treated with or without 200 μM of tectochrysin under oxidative stress induced by 20 mM of paraquat. Tectochrysin can significantly extend the lifespan of *C. elegans* by up to 20.9%, $p < 0.05$. **e** The survival curve of wild-type *C. elegans* (N2) treated with or without 200 μM of tectochrysin under pathogenic stress induced by *pseudomonas aeruginosa* (PA14). The figure shows tectochrysin can significantly extend the lifespan by up to 17.8%, $p < 0.0001$. **f** The mRNA expression of immune-related genes *irg-1*, *F55G11.4*, *F08G5.6*, and *F35E12.5*. The columns showed the mean value of three independent experiments with error bars representing SEM ($p < 0.05$, two-tailed *t* test). **g** The mean lifespan of *hsf-1* mutant PS3551 *hsf-1(sy441) I.* treated with or without 200 μM of tectochrysin at 20 °C. Each figure shows one representative experiment. The statistical significance represented by *p* value was calculated by *t*-test or log-rank test. The results from three repeated experiments and the statistical details were summarized in Supplementary Tables S4, 5, and S8 (Supplementary Information)

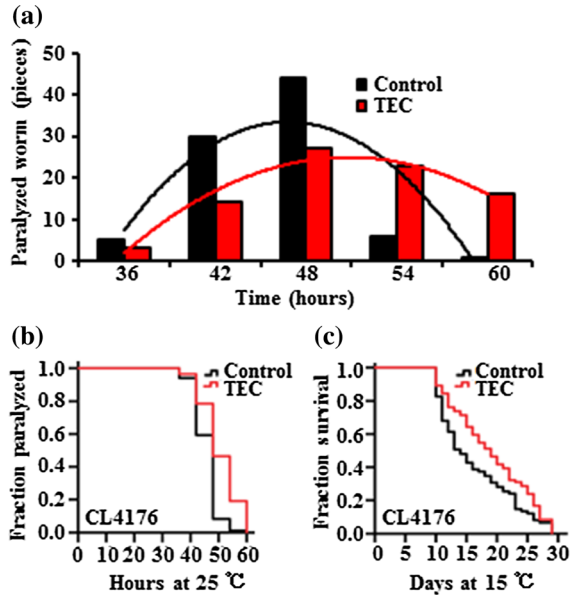


Fig. 3 Tectochrysin decreases Aβ-induced toxicity of *C. elegans*. **a**, **b** Tectochrysin significantly delayed the onset of paralysis of CL4176 *dvls27 (myo-3/Aβ1-42 /let UTR, rol-6)* strain caused by Aβ-induced toxicity at 25 °C ($p < 0.0001$, log-rank test). The number of worms paralyzed every six hours was fitted by normal distribution curves to indicate the peak period of paralysis. While Kaplan–Meier survival curves showed the onset of paralysis of CL4176 strain. **c** The mean lifespan of CL4176 strain treated with or without 200 μM of tectochrysin at 15 °C. Each figure shows one representative experiment. The statistical significance of the experiment *p* value was calculated by log-rank test. The results from three repeated experiments and the statistical details were summarized in Supplementary Tables S4 and S6 (Supplementary Information)

amyloid-β (Aβ1-42) is the genetic disease model of AD (Shanmuganathan et al. 2019). The expression of peptide Aβ could be induced at 25 °C and lead to paralysis of worms. We analyzed the body paralysis and lifespan of the CL4176 strain treated with tectochrysin. Before 48 h, the number of paralysis of worms treated without tectochrysin was higher than that of the treatment group. After 48 h, the number of paralysis of worms treated without tectochrysin was lower than that of the treatment group (Fig. 3a). We found that tectochrysin treatment significantly delayed the time of body paralysis in the CL4176 strain ($p < 0.0001$) (Fig. 3b) and extended the lifespan of the CL4176 strain by up to 14.8% ($p < 0.05$) (Fig. 3c), suggesting tectochrysin decreased the Aβ1-42-induced toxicity in *C. elegans*.

The effect of tectochrysin on the lifespan extension in *C. elegans* depends on FOXO transcription factor homologue DAF-16

Multiple signaling pathways, such as the insulin/IGF-1, TOR, AMPK, JNK and germline signaling, regulate aging and longevity (Yen et al. 2011). Mammalian FOXO transcription factor homologue DAF-16 could integrate different signals from these pathways to modulate aging and longevity. Via activated, DAF-16 shuttles from cytoplasm to nucleus and regulates the expression of downstream genes (Sun et al. 2017; Tissenbaum 2018). We tested if DAF-16 played a role in the effect of tectochrysin on lifespan extension. We observed the nucleus accumulation of DAF-16 in *C. elegans* under treatment of tectochrysin ($p < 0.0001$) (Fig. 4a). So we further investigated if tectochrysin regulated the expression of the downstream genes of

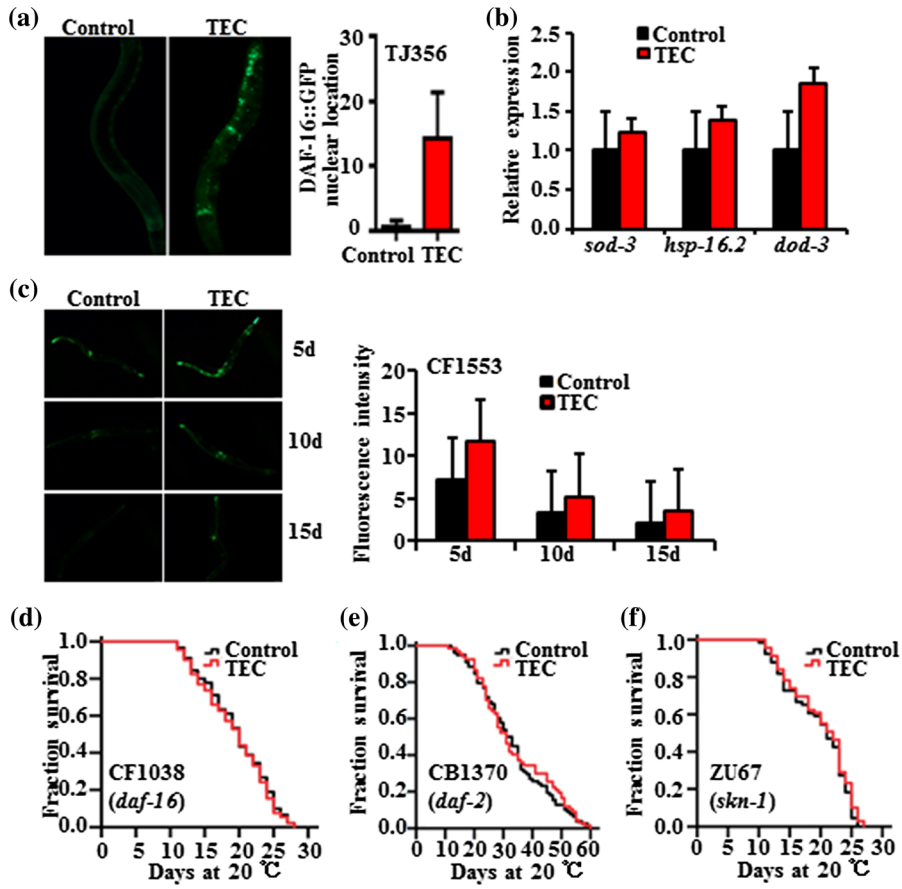


Fig. 4 The effect of tectochrysin on the lifespan extension in *C. elegans* depends on FOXO transcription factor homologue DAF-16. **a** Tectochrysion causes DAF-16 nuclear localization. Worms of transgenic strain TJ356 *daf-16(zls356) IV*. were treated with or without 200 μM of tectochrysin at 20 °C for 24 h. Tectochrysin significantly promoted the nuclear translocation of DAF-16, The columns showed the mean value of one independent experiment with error bars representing SEM ($p < 0.0001$, two-tailed t test). **b** The mRNA expression of genes regulated by *daf-16*. The columns showed the mean value of three independent experiments with error bars representing SEM ($p < 0.05$, two-tailed t test). **c** The quantification of fluorescence intensity of SOD-3::GFP in CF1553 *muIs84 (sod-3::gfp)*. Tectochrysin significantly increased the expression of SOD-3, The columns showed the mean value of one independent experiment with error bars representing SEM ($p < 0.0001$, two-tailed t test). **d** The mean lifespan of *daf-16* mutant CF1038 *daf-16(mu86) I.* treated with or without 200 μM of tectochrysin at 20 °C. **e** The mean lifespan of *daf-2* mutant CB1370 *daf-2(e1370) III.* treated with or without 200 μM of tectochrysin at 20 °C. **f** The mean lifespan of *skn-1* mutant EU1 *skn-1(zu67) IV* treated with or without 200 μM of tectochrysin at 20 °C. Each figure shows one representative experiment. The statistical significance of the experiment p value was calculated by t-test or log-rank test. The results from three repeated experiments and the statistical details were summarized in Supplementary Tables S4, 5, and S7, 8 (Supplementary Information)

daf-16. We found that the mRNA expression level of the downstream genes of *daf-16*, including *sod-3*, *hsp-16.2*, and *dod-3*, was significantly increased in *C. elegans* treated with tectochrysin (Fig. 4b). Among the downstream target genes of DAF-16, *sod-3* is involved in both stress resistance and longevity (Rangsinth et al. 2019). We examined expression level of *sod-3* at protein level in transgenic strain CF1553 expressing SOD-3::GFP fusion protein. We found that the expression of SOD-3 was significantly increased in *C. elegans* treated with tectochrysin ($p < 0.0001$) (Fig. 4c). Furthermore, to study whether tectochrysin extended lifespan depended on *daf-16*, we measured the lifespan of the null mutant of *daf-16* with tectochrysin treatment. Our result showed that tectochrysin could not extend the lifespan of *daf-16* mutant CF1038 *daf-16(mu86) I* (Fig. 4d).

Gene *daf-2* encoding the insulin/insulin-like growth factor (IGF-1) receptor, regulates PI3-kinase/AKT pathway and FOXO/DAF-16 (Kuningas et al. 2008). Gene *skn-1*, related to fat metabolism and oxidative stress, can be activated by DAF-16 (Park et al. 2009). We further measured the lifespan of the null mutant of *skn-1* and *daf-2* with tectochrysin treatment. Our results showed that tectochrysin could not extend the lifespan of mutants CB1370 *daf-2(e1370) III.* and EU1 *skn-1(zu67) IV.* (Fig. 4e, f).

Tectochrysin could not extend the long-lived mutants from genes involved in energy processing

Among many genetic and environmental interventions to delay aging so far, dietary restriction (DR) shows consistent lifespan extension with positive effects on health span ranging from *C. elegans* to monkeys (Colman et al. 2014; Lakowski and Hekimi 1998). Reducing pharyngeal pumping often leads to the decrease of food intake in worms (Chamoli et al. 2014). Firstly, to analyze whether worms avoid tectochrysin, we performed chemotaxis assay. We found worm did not avoid tectochrysin (Fig. 5a, b). Further, we also found that under the treatment of tectochrysin, the pharyngeal pumping in worms didn't reduce significantly until the 5th day of adult (Fig. 5c). Furthermore, we measured the lifespan of *eat-2* mutant treated with tectochrysin. The result showed that tectochrysin failed to extend the lifespan of long-lived *eat-2* mutant DA1116 *eat-2(ad1116) II.* (Fig. 5d). Further, the low-energy sensing AMP-activated protein kinase AMPK/aak-2 played a role in longevity induced by a DR regimen (Greer and Brunet 2009). While the gene *isp-1* encoding Rieske iron-sulfur polypeptide **1** is involved in mitochondrial electron transport and can activate the AMPK pathway by decreasing the level of ATP (Curtis et al. 2006). Our results showed that tectochrysin could not further extend the lifespan of the long-lived mutants RB754 *aak-2(ok524) X.* and MQ887 *isp-1(qm150) IV* (Fig. 5e, f).

Discussion

Tectochrysin is a main component of a traditional Chinese medicine *Alpinia oxyphylla* Miq., which has an effect for Center-Supplementing Qi-Boosting, is prescribed for diarrhea and loss of appetite (Zhang et al. 2018b). Tectochrysin also has antibacterial, antifungal, anti-cancer, anti-inflammatory, and anti-oxidation effects (Lee et al. 2003). But the anti-aging effect of tectochrysin is unknown. Here we found that tectochrysin could extend the lifespan of *C. elegans*, increase the stress resistance, and decrease Aβ-induced toxicity in *C. elegans.*

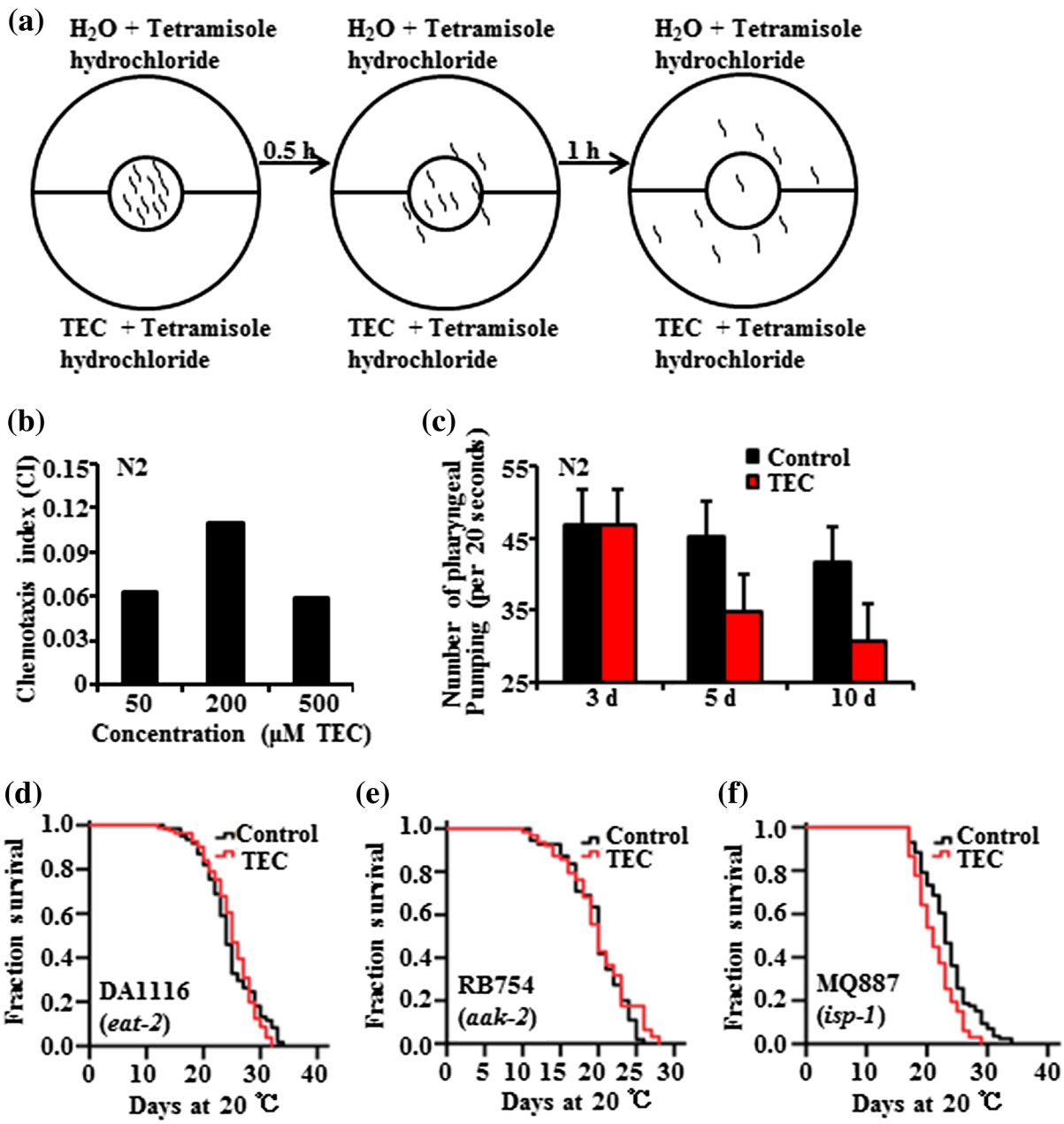
In *C. elegans*, increased lifespan is associated with increased expression of small heat shock proteins (HSPs) (Olsen et al. 2006). We found that tectochrysin could increase the expression of *hsp-4*, *hsp-6*, and *hsp-60* at mRNA level, while increase the expression of *hsp-4* and *hsp-6* at protein level. The expression of *hsp-4* and *hsp-6* are the positive markers of unfold protein reaction (UPR), which can respond to the stress conditions (Jovaisaite et al. 2014). Heat shock factor 1 (HSF-1), an essential nuclear protein, is associated with heat-stress response, immunity and aging in *C. elegans* (Li et al. 2018; Seo et al. 2013). We found that tectochrysin could not extend the lifespan of *hsf-1* mutant, suggesting tectochrysin required *hsf-1* to extend the lifespan of *C. elegans*.

FOXO/DAF-16 contributes to the regulation of development, lifespan, and stress resistance in worms. Thus we investigated if tectochrysion extended the lifespan of worms depending on DAF-16. Our result showed that tectochrysin didn't extend the lifespan of the *daf-16* mutant CF1038 *daf-16(mu86) I.* Meanwhile, the increased nuclear translocation of DAF-16::GFP was further identified in transgenic strain TJ356 *daf-16(zls356) IV* exposed to tectochrysin. Furthermore, *daf-16* related genes: *sod-3*, *dod-3*, and *hsp-16.2* were also up-regulated following the tectochrysin treatments at the mRNA level. SOD-3 encodes a Fe/Mn superoxide dismutase, which contributed to the oxidative stress resistance, and reduced with aging (Rangsinth et al. 2019). We found that tectochrysin could slow down the decline of the expression of SOD-3 with aging in worms. Above results suggest that tectochrysin extends the lifespan of *C. elegans* via DAF-16. The insulin-like receptor DAF-2 regulates the activity of DAF-16 in worms. Furthermore, tectochrysin could not significantly extend the lifespan of *daf-2* mutant CB1370 *daf-2(e1370) III*. This result suggests that either tectochrysin regulates IIS pathway to extend the lifespan of *C. elegans*, or the effect of tectochrysin on the lifespan extension is not strong enough to distinguish

from the extremely long lifespan of CB1370 *daf-2(e1370) III*.

We found worm did not avoid tectochrysin. Under the treatment of tectochrysin, the pharyngeal pumping in worms didn't reduce significantly until the 5th day of adult. Tectochrysin couldn't extend the lifespan of mutants from genes *aak-2*, *eat-2*, and *isp-1*. These results suggested that the effect of tectochrysin on the lifespan extension might be not strong enough to distinguish from the lifespan of these long-lived mutants.

Hormesis is biological phenomena characterized by dose–response relationships displaying low-dose stimulation and high-dose inhibition. It is classically described by an inverted U-shaped or J-shaped dose response curve (Calabrese and Baldwin 2002).



◀**Fig. 5** Tectochrysin could not extend the long-lived mutants from genes involved in energy processing. **a**, **b** The process of chemotaxis assay. Synchronized wild-type worms treated were placed in the center of assay plates containing 200 μM tectochrysin plus 2% tetramisole hydrochloride to paralyze the worms on one side of the plate (A side), and H_2O as solvent control plus 2% tetramisole hydrochloride on the opposite side of the plate (B side). After incubation at 20 °C for1 h, CI was scored, CI > 0. It means that *C. elegans* do not use chemotaxis to avoid tectochrysin. **c** The pharyngeal pumping of worms treated with or without 200 μM of tectochrysin. There is no significant difference between the pharyngeal pumping of worms treated and non-treated controls at 3 days of adult ($p > 0.05$). The pharyngeal pumping was significantly reduced by tectochrysin at 5 or 10 days of adult, $p < 0.0001$. **d** The mean lifespan of *eat-2* mutant DA1116 *eat-2(ad1116) II.* treated with or without 200 μM of tectochrysin at 20 °C. **e** The mean lifespan of *aak-2* mutant RB754 *aak-2(ok524) X.* treated with or without 200 μM of tectochrysin at 20 °C. **f** The mean lifespan of *isp-1* mutant treated with or without 200 μM of tectochrysin at 20 °C. Tectochrysin could not further extend the mean lifespan of *isp-1* mutant MQ887 *isp-1(qm150) IV.* Each figure shows one representative experiment. The statistical significance represented by *p* value was calculated by *t*-test or log-rank test. The results from three repeated experiments and the statistical details were summarized in Supplementary Tables S4, S9 and S10 and Supplementary Figure S1 (Supplementary Information)

Meanwhile, lifespan extension from stress hormesis by thermal stress and oxidative stress also occurred in *C. elegans.* (Cypser and Johnson 2003; Heidler et al. 2010). Previous studies found that many compounds, such as metformin, curcumin, and tubers extract, regulated the aging process through hormesis (Govindan et al. 2018; Piskovatska et al. 2020; Stępień et al. 2020). The dose–response curve of tectochrysin is an inverted U-shaped. In *C. elegans*, we also observed increased resistance to stress and lifespan extension by supplementing with tectochrysin. Meanwhile, tectochrysin increased the mRNA level of heat-shock response, immune, and DAF-16 related genes. Given these results, we speculated tectochrysin might partly extend nematode life through hormesis. However, the cellular mechanisms involved in hormesis are still unclear and need further investigation.

In summary, we found that tectochrysin could delay the progress of age-related disease by reducing Aβ1-42-induced toxicity and increase stress resistance in *C. elegans*, and extend the lifespan of *C. elegans* via FOXO/DAF-16. It is worth to investigate the beneficial effect of tectchrysin, especially the lifespan extension and treatment for age-related disease of tectochrysin in mammals.

Acknowledgements We thank the *Caenorhabditis* Genetic Center (CGC) for providing the worm strains, which is funded by NIH Office of Research Infrastructure Program (P40OD010440). Financial supports were received from the Natural Science Foundation of China (Grant Nos. 81771516 and 81671405) and the Science and Technology Cooperation Project of Luzhou and Southwest Medical University (2016LZXNYD-Z05).

Author contributions The study was conceived and designed by H.-R.L and G.-S.W. Material preparation, data collection and analysis were performed by M.L., L.T. Q.Z., and Z.-L.Y. The first draft of the manuscript was written by M.L, and then the manuscript was revised by J.-N.C., X.-G.Z., H.-R.L and G.-S.W. All authors commented on previous versions of the manuscript. All the authors have read and approved the final version.

Compliance with ethical standards

Conflict of interest All authors declare that they do not have competing interests.

References

Amakura Y, Tsutsumi T, Nakamura M, Handa H, Yoshimura M, Matsuda R, Yoshida T (2011) Aryl hydrocarbon receptor ligand activity of commercial health foods. Food Chem 126:1515–1520. https://doi.org/10.1016/j.foodchem.2010.12.034

Apfeld J, Kenyon C (1999) Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. Nature 402:804–809. https://doi.org/10.1038/45544

Bielak-Zmijewska A, Grabowska W, Ciolko A, Bojko A, Mosieniak G, Bijoch Ł, Sikora E (2019) The role of curcumin in the modulation of ageing. Int J Mol Sci 20:1239. https://doi.org/10.3390/ijms20051239

Biswas T, Mathur AK, Mathur A (2017) A literature update elucidating production of *Panax* ginsenosides with a special focus on strategies enriching the anti-neoplastic minor ginsenosides in ginseng preparations. Appl Microbiol Biotechnol 101:4009–4032. https://doi.org/10.1007/s00253-017-8279-4

Brenner S (1974) The genetics of *Caenorhabditis elegans*. Genetics 77:71–94

Calabrese EJ, Baldwin LA (2002) Hormesis and high-risk groups. Regul Toxicol Pharmacol 35:414–428. https://doi.org/10.1006/rtph.2001.1529

Castillo-Quan JI, Kinghorn KJ, Bjedov I (2015) Genetics and pharmacology of longevity: the road to therapeutics for healthy aging. Adv Genet 90:1–101. https://doi.org/10.1016/bs.adgen.2015.06.002

Chamoli M, Singh A, Malik Y, Mukhopadhyay A (2014) A novel kinase regulates dietary restriction-mediated

longevity in *Caenorhabditis elegans*. Aging Cell 13:641–655. https://doi.org/10.1111/acel.12218

Chen F, Li HL, Tan YF, Guan WW, Zhang JQ, Li YH, Zhao YS, Qin ZM (2014) Different accumulation profiles of multiple components between pericarp and seed of *Alpinia oxyphylla* capsular fruit as determined by UFLC-MS/MS. Molecules 19:4510–4523. https://doi.org/10.3390/molecules19044510

Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM (2014) Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat Commun 5:3557. https://doi.org/10.1038/ncomms4557

Curtis R, O'Connor G, DiStefano PS (2006) Aging networks in *Caenorhabditis elegans*: AMP-activated protein kinase (*aak-2*) links multiple aging and metabolism pathways. Aging Cell 5:119–126. https://doi.org/10.1111/j.1474-9726.2006.00205.x

Cypser JR, Johnson TE (2003) Hormesis in *Caenorhabditis elegans* dauer-defective mutants. Biogerontology 4:203–214. https://doi.org/10.1023/a:1025138800672

Denzel MS, Lapierre LR, Mack HID (2019) Emerging topics in *C. elegans* aging research: Transcriptional regulation, stress response and epigenetics. Mech Ageing Dev 177:4–21. https://doi.org/10.1016/j.mad.2018.08.001

Feng S, Cheng H, Xu Z, Shen S, Yuan M, Liu J, Ding C (2015) Thermal stress resistance and aging effects of *Panax notoginseng* polysaccharides on *Caenorhabditis elegans*. Int J Biol Macromol 81:188–194. https://doi.org/10.1016/j.ijbiomac.2015.07.057

Govindan S, Amirthalingam M, Duraisamy K, Govindhan T, Sundararaj N, Palanisamy S (2018) Phytochemicals-induced hormesis protects *Caenorhabditis elegans* against alpha-synuclein protein aggregation and stress through modulating HSF-1 and SKN-1/Nrf2 signaling pathways. Biomed Pharmacother 102:812–822. https://doi.org/10.1016/j.biopha.2018.03.128

Greer EL, Brunet A (2009) Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. Aging Cell 8:113–127. https://doi.org/10.1111/j.1474-9726.2009.00459.x

He Z-H, Ge W, Yue GG-L, Lau CB-S, He M-F, But PP-H (2010) Anti-angiogenic effects of the fruit of *Alpinia oxyphylla*. J Ethnopharmacol 132:443–449. https://doi.org/10.1016/j.jep.2010.08.024

He Q, Huang G, Chen Y, Wang X, Huang Z, Chen Z (2017) The protection of novel 2-arylethenylquinoline derivatives against impairment of associative learning memory induced by neural Aβ in *C. elegans* Alzheimer's disease model. Neurochem Res 42:3061–3072. https://doi.org/10.1007/s11064-017-2339-0

He B, Xu F, Yan T, Xiao F, Wu B, Wang Y, Bi K, Jia Y (2019) Tectochrysin from *Alpinia Oxyphylla* Miq. alleviates Aβ(1–42) induced learning and memory impairments in mice. Eur J Pharmacol 842:365–372. https://doi.org/10.1016/j.ejphar.2018.11.002

Heidler T, Hartwig K, Daniel H, Wenzel U (2010) *Caenorhabditis elegans* lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. Biogerontology 11:183–195. https://doi.org/10.1007/s10522-009-9239-x

Hou R, Han Y, Fei Q, Gao Y, Qi R, Cai R, Qi Y (2018) Dietary flavone tectochrysin exerts anti-inflammatory action by directly inhibiting MEK1/2 in LPS-primed macrophages. Mol Nutr Food Res 62:1700288. https://doi.org/10.1002/mnfr.201700288

Huang C, Xiong C, Kornfeld K (2004) Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 101:8084–8089. https://doi.org/10.1073/pnas.0400848101

Jagust W (2018) Imaging the evolution and pathophysiology of Alzheimer disease. Nat Rev Neurosci 19:687–700. https://doi.org/10.1038/s41583-018-0067-3

Jovaisaite V, Mouchiroud L, Auwerx J (2014) The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. J Exp Biol 217:137–143. https://doi.org/10.1242/jeb.090738

Kumar S, Egan BM, Kocsisova Z, Schneider DL, Murphy JT, Diwan A, Kornfeld K (2019) Lifespan extension in C. *elegans* caused by bacterial colonization of the intestine and subsequent activation of an innate immune response. Dev Cell 49(100–117):e6. https://doi.org/10.1016/j.devcel.2019.03.010

Kuningas M, Mooijaart SP, van Heemst D, Zwaan BJ, Slagboom PE, Westendorp RGJ (2008) Genes encoding longevity: from model organisms to humans. Aging Cell 7:270–280. https://doi.org/10.1111/j.1474-9726.2008.00366.x

Kunugi H, Mohammed Ali A (2019) Royal jelly and its components promote healthy aging and longevity: from animal models to humans. Int J Mol Sci 20:4662. https://doi.org/10.3390/ijms20194662

Kurz CL, Tan MW (2004) Regulation of aging and innate immunity in *C. elegans*. Aging Cell 3:185–193. https://doi.org/10.1111/j.1474-9728.2004.00108.x

Lakowski B, Hekimi S (1998) The genetics of caloric restriction in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 95:13091–13096. https://doi.org/10.1073/pnas.95.22.13091

Lee S, Kim KS, Park Y, Shin KH, Kim BK (2003) In vivo antioxidant activities of tectochrysin. Arch Pharm Res 26:43–46. https://doi.org/10.1007/bf03179930

Lee SJ, Hwang AB, Kenyon C (2010) Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. Curr Biol 20:2131–2136. https://doi.org/10.1016/j.cub.2010.10.057

Li F, Ma X, Cui X, Li J, Wang Z (2018) Recombinant buckwheat glutaredoxin intake increases lifespan and stress resistance via *hsf-1* upregulation in *Caenorhabditis elegans*. Exp Gerontol 104:86–97. https://doi.org/10.1016/j.exger.2018.01.028

Ma L, Zhao Y, Chen Y, Cheng B, Peng A, Huang K (2018) *Caenorhabditis elegans* as a model system for target identification and drug screening against neurodegenerative diseases. Eur J Pharmacol 819:169–180. https://doi.org/10.1016/j.ejphar.2017.11.051

Margie O, Palmer C, Chin-Sang I (2013) *C. elegans* chemotaxis assay. J Vis Exp 74:50069

Newell Stamper BL, Cypser JR, Kechris K, Kitzenberg DA, Tedesco PM, Johnson TE (2018) Movement decline across lifespan of *Caenorhabditis elegans* mutants in the insulin/insulin-like signaling pathway. Aging Cell 17:e12704. https://doi.org/10.1111/acel.12704

Olsen A, Vantipalli MC, Lithgow GJ (2006) Lifespan extension of *Caenorhabditis elegans* following repeated mild hormetic heat treatments. Biogerontology 7:221–230. https://doi.org/10.1007/s10522-006-9018-x

Park SK, Tedesco PM, Johnson TE (2009) Oxidative stress and longevity in *Caenorhabditis elegans* as mediated by SKN-1. Aging Cell 8:258–269. https://doi.org/10.1111/j.1474-9726.2009.00473.x

Pees B, Kloock A, Nakad R, Barbosa C, Dierking K (2017) Enhanced behavioral immune defenses in a *C. elegans* C-type lectin-like domain gene mutant. Dev Comp Immunol 74:237–242. https://doi.org/10.1016/j.dci.2017.04.021

Peixoto H, Roxo M, Krstin S, Röhrig T, Richling E, Wink M (2016) An anthocyanin-rich extract of acai (*Euterpe precatoria* Mart.) s in *Caenorhabditis elegans*. J Agric Food Chem 64:1283–1290. https://doi.org/10.1021/acs.jafc.5b05812

Peixoto H, Roxo M, Koolen H, da Silva F, Silva E, Braun MS, Wang X, Wink M (2018) *Calycophyllum spruceanum* (Benth), the amazonian "tree of youth" prolongs longevity and enhances stress resistance in *Caenorhabditis elegans*. Molecules 23:534. https://doi.org/10.3390/molecules23030534

Peixoto H, Roxo M, Silva E, Valente K, Braun M, Wang X, Wink M (2019) Bark extract of the amazonian tree *Endopleura uchi* (Humiriaceae) extends lifespan and enhances stress resistance in *Caenorhabditis elegans*. Molecules 24:915. https://doi.org/10.3390/molecules24050915

Piskovatska V, Storey KB, Vaiserman AM, Lushchak O (2020) The use of metformin to increase the human healthspan. Adv Exp Med Biol 1260:319–332. https://doi.org/10.1007/978-3-030-42667-5_13

Rangsinth P, Prasansuklab A, Duangjan C, Gu X, Meemon K, Wink M, Tencomnao T (2019) Leaf extract of *Caesalpinia mimosoides* enhances oxidative stress resistance and prolongs lifespan in *Caenorhabditis elegans*. BMC Complement Altern Med 19:164–164. https://doi.org/10.1186/s12906-019-2578-5

Rezaizadehnajafi L, Wink M (2014) EPs7630(®) from *Pelargonium sidoides* increases stress resistance in *Caenorhabditis elegans* probably via the DAF-16/FOXO pathway. Phytomedicine 21:547–550. https://doi.org/10.1016/j.phymed.2013.10.027

Russo GL, Spagnuolo C, Russo M, Tedesco I, Moccia S, Cervellera C (2019) Mechanisms of aging and potential role of selected polyphenols in extending healthspan. Biochem Pharmacol. https://doi.org/10.1016/j.bcp.2019.113719

Seo K, Choi E, Lee D, Jeong DE, Jang SK, Lee SJ (2013) Heat shock factor 1 mediates the longevity conferred by inhibition of TOR and insulin/IGF-1 signaling pathways in *C. elegans*. Aging Cell 12:1073–1081. https://doi.org/10.1111/acel.12140

Shanmuganathan B, Sathya S, Balasubramaniam B, Balamurugan K, Devi KP (2019) Amyloid-β induced neuropathological actions are suppressed by *Padina gymnospora* (Phaeophyceae) and its active constituent α-bisabolol in Neuro2a cells and transgenic *Caenorhabditis elegans* Alzheimer's model. Nitric Oxide 91:52–66. https://doi.org/10.1016/j.niox.2019.07.009

Shi G-F, An L-J, Jiang B, Guan S, Bao Y-M (2006) *Alpinia* protocatechuic acid protects against oxidative damage in vitro and reduces oxidative stress in vivo. Neurosci Lett 403:206–210. https://doi.org/10.1016/j.neulet.2006.02.057

Shi W, Zhong J, Zhang Q, Yan C (2020) Structural characterization and antineuroinflammatory activity of a novel heteropolysaccharide obtained from the fruits of *Alpinia oxyphylla*. Carbohydr Polym 229:115405. https://doi.org/10.1016/j.carbpol.2019.115405

Stępień K, Wojdyła D, Nowak K, Mołoń M (2020) Impact of curcumin on replicative and chronological aging in the *Saccharomyces cerevisiae* yeast. Biogerontology 21:109–123. https://doi.org/10.1007/s10522-019-09846-x

Sun X, Chen WD, Wang YD (2017) DAF-16/FOXO transcription factor in aging and longevity. Front Pharmacol 8:548. https://doi.org/10.3389/fphar.2017.00548

Tan L, Wang S, Wang Y, He M, Liu D (2015) Bisphenol A exposure accelerated the aging process in the nematode *Caenorhabditis elegans*. Toxicol Lett 235:75–83. https://doi.org/10.1016/j.toxlet.2015.03.010

Tissenbaum HA (2018) DAF-16: FOXO in the context of *C. elegans*. Curr Top Dev Biol 127:1–21. https://doi.org/10.1016/bs.ctdb.2017.11.007

Wilson MA, Shukitt-Hale B, Kalt W, Ingram DK, Joseph JA, Wolkow CA (2006) Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. Aging Cell 5:59–68. https://doi.org/10.1111/j.1474-9726.2006.00192.x

Xie Y, Xiao M, Ni Y, Jiang S, Feng G, Sang S, Du G (2018) *Alpinia oxyphylla* Miq. extract prevents diabetes in mice by modulating gut microbiota. J Diabetes Res 2018:4230590. https://doi.org/10.1155/2018/4230590

Xu J, Yuan Y, Zhang R, Song Y, Sui T, Wang J, Wang C, Chen Y, Guan S, Wang L (2019) A deuterohemin peptide protects a transgenic *Caenorhabditis elegans* model of Alzheimer's disease by inhibiting Aβ(1–42) aggregation. Bioorg Chem 82:332–339. https://doi.org/10.1016/j.bioorg.2018.10.072

Yen K, Narasimhan SD, Tissenbaum HA (2011) DAF-16/Forkhead box O transcription factor: many paths to a single Fork(head) in the road. Antioxid Redox Signal 14:623–634. https://doi.org/10.1089/ars.2010.3490

Zhang J, Wang S, Li Y, Xu P, Chen F, Tan Y, Duan J (2013) Anti-diarrheal constituents of *Alpinia oxyphylla*. Fitoterapia 89:149–156. https://doi.org/10.1016/j.fitote.2013.04.001

Zhang J, Lu L, Zhou L (2015) Oleanolic acid activates *daf-16* to increase lifespan in *Caenorhabditis elegans*. Biochem Biophys Res Commun 468:843–849. https://doi.org/10.1016/j.bbrc.2015.11.042

Zhang J, Xue X, Yang Y, Ma W, Han Y, Qin X (2018a) Multiple biological defects caused by calycosin-7-O-β-D-glucoside in the nematode *Caenorhabditis elegans* are associated with the activation of oxidative damage. J Appl Toxicol 38:801–809. https://doi.org/10.1002/jat.3588

Zhang Q, Zheng Y, Hu X, Hu X, Lv W, Lv D, Chen J, Wu M, Song Q, Shentu J (2018b) Ethnopharmacological uses, phytochemistry, biological activities, and therapeutic applications of *Alpinia oxyphylla* Miquel: a review. J Ethnopharmacol 224:149–168. https://doi.org/10.1016/j.jep.2018.05.002

Zheng SQ, Huang XB, Xing TK, Ding AJ, Wu GS, Luo HR (2017) Chlorogenic acid extends the lifespan of

Caenorhabditis elegans via insulin/IGF-1 signaling pathway. J Gerontol A Biol Sci Med Sci 72:464–472. https://doi.org/10.1093/gerona/glw105