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Early Exposure to Polyphenol-Rich Sugarcane Extract (PRSE) Mitigates Aging While Enhancing Thermotolerance in *C. elegans*

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Abstract: Previous research has established that polyphenols increase lifespan and stress resistance in *C. elegans*. However, the effects of timing of exposure on the extension of lifespan and the mechanisms involved are not completely understood. This study aims to clarify the influence of the timing of polyphenol-rich sugarcane extract (PRSE) administration on the lifespan of *Caenorhabditis elegans*. *C. elegans* worms were subjected to a diet containing PRSE powder at various larval stages until their death. The optimal concentration for lifespan extension was found to be 5 mg/mL PRSE, which increased the lifespan of N2 worms by 18.12% compared to the control group. Mutant strains were also tested, and worm thermotolerance assays were used to assess age-related health at different life stages. Lifespan was notably extended when PRSE was introduced at the first larval stage and 15 h thereafter. No significant extension of lifespan was observed when introduced later in life. The lifespan of DAF-16 and DAF-2 mutants remained unaffected by PRSE. Improved thermotolerance was observed, especially in young and middle-aged worms. Early initiation and sustained intake of PRSE might improve the heat stress and lifespan, implicating the insulin/IGF-1 signaling pathway as a likely mediator in *C. elegans*.

Keywords: PRSE; lifespan; DAF-2; DAF-16; healthspan; mechanisms; *Caenorhabditis elegans*; heat stress

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1. Introduction

The primary risk factor for non-communicable diseases (NCDs) such as cardiovascular disease, cancer, diabetes, and neurodegenerative disorders is aging [1]. The increasing prevalence of these diseases worldwide can be partially attributed to demographic shifts towards an aging population, epidemiological transitions, and modern lifestyles that include higher caloric intake and reduced physical activity [2]. In light of this, recent healthcare efforts have extended beyond merely prolonging lifespan to improving "healthspan", defined as the period of life spent in good health. Novel interventions ranging from genetic manipulations and behavioural modifications to pharmacological treatments have shown promise in delaying the onset of age-associated diseases [3,4].

The molecular pathways underlying the aging process have become the focus of investigation. Signaling pathways such as insulin/IGF-1 and mechanisms involving the mitochondrial function have been particularly emphasized [5]. These pathways, along with the beneficial effects of caloric restriction, are thought to play significant roles in aging and the pathogenesis of NCDs [6–8].

Among the various strategies for mitigating the effects of aging and associated diseases, dietary interventions have garnered substantial attention. Specifically, diets rich in polyphenols have shown potential in reducing oxidative stress, inflammation, and the detoxification of harmful agents [9]. A broad array of plant foods, including fruits, vegetables, teas, and whole grains, have been identified as rich sources of polyphenols [10]. Polyphenol-rich sugarcane, for example, has been noted for its flavonoid and phenolic acid content, showing promising results in anti-aging and antioxidant activity [11].

Model organisms like the nematode *Caenorhabditis elegans* (*C. elegans*) have emerged as invaluable tools for investigating the long-term effects of polyphenols on aging and healthspan. The short lifespan, availability of mutant strains, and easily quantifiable markers of aging in *C. elegans* make it an ideal model for such studies [12,13]. They have shown great promise in trials to assess aging. For example, when a cocoa supplement, rich in polyphenols, was administered to *C. elegans* from their L1 stage until their death, there was a notable extension of the worms' lifespans [14]. Given this context, it was aimed to address a critical gap in the literature by focusing on the effects of early exposure to polyphenol rich sugarcane extract (PRSE), a food uniquely rich in a specific blend of polyphenols, on the lifespan of *C. elegans*. The study aimed to establish a foundational understanding that could inform future translational research. This research has the potential to shape evidence-based dietary guidelines aimed at enhancing healthy aging and reducing age-related diseases in humans.

2. Materials and Methods

2.1. Polyphenol-Rich Sugarcane Extract and Treatment

Polyphenol-rich sugarcane extract (PRSE) is a patented product provided by The Product Makers (Keysborough, Victoria, Australia). PRSE used in this study was made using a patented proprietary process that starts with the juice of the sugarcane plant *Saccharum officinarum*, which is initially converted into molasses by vacuum evaporation [15]. The molasses (80 Brix) is then reduced to 50 Brix with deionized water. This feedstock is treated with 95% food grade ethanol in another proprietary process which precipitates unwanted material and recovers the supernatant. This supernatant, after vacuum evaporation to remove ethanol and water, results in a 70 Brix syrup-like product, enriched in polyphenols, flavonoids, sugars, organic and amino acids, minerals, and salts. This product, after diluting to 20 Brix with deionized water, is mixed with FPX-66 resin (Dowex^{TM} ion exchange resins) manufactured by DuPont ^{TM} in Wilmington, DE, USA which preferentially binds to hydrophobic compounds (mainly the polyphenols and flavonoids), allowing the hydrophilic compounds (sugars, acids, minerals, and salts) to be washed free. This resin water washing process is repeated three times and the material bound to the resin is removed with 80% ethanol and vacuum evaporated to a 40 Brix product which is spray-dried to a free-flowing powder to produce PRSE [16].

This powder contains a unique mixture of phenolic acids, glycone and aglycone flavonoids, including chlorogenic acid, syringic acid, diosmin, diosmetin and some chelated with divalent anions (Mg, Ca, Zn) that deliver the antioxidant and antidiabetic functions of PRSE followed by its polyphenolic composition through liquid chromatography-mass spectrometry (LCMS); analysis has been discussed previously and quercetin was not identified in PRSE [15,16].

One single sample of PRSE was used in this study. The PRSE was prepared with M9 buffer in different concentrations, ranging from 1 mg/mL to 5 mg/mL. This PRSE suspension was then applied to a layer of *E. coli OP50* bacteria that had been cultured overnight on a 60 × 10 mm NGM (nematode growth medium) agar plate containing 5 mL of NGM agar. The application was performed at a 2:1 volume ratio, with 400 µL of the PRSE suspension combined with 200 µL of *E. coli OP50*. Consequently, the diluted PRSE's final concentration ranged between 0.02 mg/mL and 0.1 mg/mL. Introduction of the PRSE suspension was from the first larval stage (L1) onwards, in which plates of each treatment in triplicate were maintained at 20 °C.

2.2. Escherichia coli OP50 Culture Conditions and Maintenance of C. elegans

Escherichia coli OP50 (*E. coli OP50*), wild-type N2 (Bristol), CB1370 *daf-2* (*e1370*) *III*., and GR1307 *daf-16* (*mgDf50*) *I*. *C. elegans* strains were purchased from the Caenorhabditis Genetics Center (Minneapolis, MN, USA). Concentrated *E. coli OP50* was prepared by diluting 1 g of cultured *E. coli OP50* pellet in 12 mL of M9 buffer. In order to arrest age-synchronized L1 wild-type N2 (Bristol) nematodes in M9 buffer, gravid adult nematodes were bleached in the mixture of 1 mL bleach and 0.5 mL of 5N NaOH. Eggs were kept in 3 mL of M9 buffer for 48 h [17]. All cultured nematodes on NGM plates were maintained at 20 °C during their entire lifetime.

2.3. Lifespan Assay

Wild-type N2, CB1370 *daf-2* (*e1370*) *III*. and GR1307 *daf-16* (*mgDf50*) *I*. arrested L1 worms were cultured on 60 × 10 mm NGM plates in triplicate and spotted with *E. coli OP50*.

The lifespan assay was performed without using 5′-fluorodeoxyuridine (FUdR), as FUdR may increase longevity and worms were picked and transferred onto fresh plates daily until they stopped laying eggs to maintain age-synchronized nematodes on NGM plates. After day 10, the worms were transferred to new plates every three days. The number of dead and live worms was checked by touch provocation with the platinum loop and scored daily [18,19]. Lifespan was counted from L1 at day 1 until all the worms had died. Worms that crawled off the plates were excluded from the analysis. The mean, median, and maximum lifespans of wild-type N2 worms were reported, and their respective survival curves plotted.

The most effective concentration was identified as 5 mg/mL, which has been chosen for subsequent PRSE exposure timing experiments.

2.4. Timing of Exposure to PRSE

To explore the effect of timing of exposure to PRSE treatment, N2 worms were exposed to 5 mg/mL of a suspension of PRSE at varying timepoints of their life cycle (i.e., L1 stage, 15 h after L1 stage, 24 h after L1 stage, 34 h after L1 stage and 48 h after L1 stage) until the death of all worms as illustrated in Figure 1.

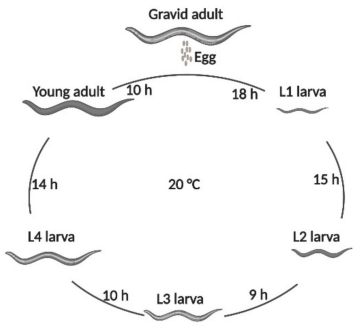

Figure 1. Development of *C. elegans* at 20 °C based on hours, modified from the reference [20]. Four larval stages followed by adulthood and normal standard temperature condition were shown. To determine the effect of timing of exposure to PRSE, intervention of PRSE supplement (5 mg/mL) categorized based on hours from L1 stage (L1, L1 + 15 h, L1 + 24 h, L1 + 34 h, and L1 + 48 h).

2.5. Thermotolerance Assay

The thermotolerance assay was performed by shifting 5 mg/mL of PRSE treated and untreated gravid adult wild-type N2 (Bristol), *daf-2* (*e1370*) *III* and *daf-16* (*mgDf50*) *I* hermaphrodites reached to day 4, day 8, and day 12 grown onto NGM plates at 20 °C 60 × 10 mm NGM plates containing *E. coli OP50* lawn maintained at 35 °C. Each hour, worms were scored by touch provocation for dead and alive worms until all worms died due to heat stress. The mean, median, and maximum lifespans of worms were calculated, and their survival curves were plotted.

2.6. Statistical Analysis

All analyses were carried out using IBM SPSS® statistics software (version 26.0), while figures were created using GraphPad Prism 9. All data were represented as their mean ± standard error mean (mean±SEM) unless specifically stated. Survival curves were analysed using Kaplan–Meier curves. *P* values for the survival function were generated using the log rank (Mantel–Cox) test to compare between treated and untreated groups. A p value of < 0.05 was considered statistically significant. A one-way ANOVA followed by a Tukey's test was used to estimate differences between groups for maximum lifespan in which the maximum lifespan was defined as the average f the 10 longest-lived worms.

3. Results

3.1. Supplementation of PRSE Extends C. elegans Lifespan

Under standard laboratory conditions, worms were given a dosage of 15 mg/mL of the PRSE starting from the L1 stage and continuing until their death. The worms had a mean lifespan of 16.49 ± 0.36 days, a median lifespan of 17.00 ± 0.35 days, and a maximum lifespan of 19.70 ± 0.23 days (Figure 2A,B, Table 1). After supplementing the worms with PRSE, the mean and median lifespan of worms significantly increased to 19.48 ± 0.37 days and 19.00 ± 0.69 days, respectively (maximum lifespan, 25 days). At a dose of 5 mg/mL PRSE supplementation, the mean and median lifespan increased by 18.12% and 11.76%, respectively ($p < 0.05$); (Table 1). The maximum lifespan at a dose of 1 mg/mL PRSE significantly decreased to 19.07 ± 0.20 days, while at a dose of 2 mg/mL, 3 mg/mL, 4 g/mL, and 5 mg/mL PRSE supplementation, the maximum lifespan significantly increased to 20.77 ± 0.33, 21.70 ± 0.46, 23.63 ± 0.27 and 25.17 ±0.32 days, respectively, compared to the control group ($p < 0.05$); (Figure 2B, Table 1).

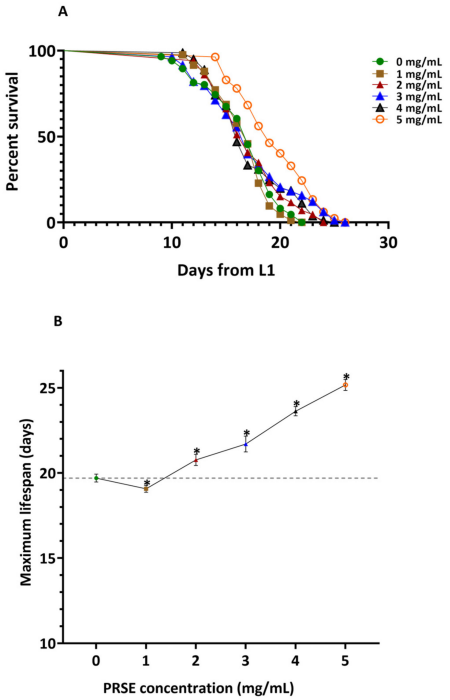

Figure 2. PRSE supplementation extended the lifespan of *c. elegans* worms. (**A**) Survival curves of wild-type N2 worms in response to different concentrations of PRSE. Mean lifespan was extended by 5 mg/mL PRSE (* $p < 0.05$). (**B**) Dose–response curve of wild-type N2 maximum lifespan at stated concentrations.

Table 1. The effect of different concentrations of PRSE on the lifespan of wild-type N2 *C. elegans*.

PRSE Concentration (mg/mL)	Sample Size (n)	Mean Lifespan (Days) ± SEM	% Extension Compared to Control	Median Lifespan (Days) ± SE	% Extension Compared to Control	Maximum Lifespan(Days) ± SEM
0	86	16.49 ± 0.36		17 ± 0.35		19.70 ± 0.23
1	83	16.65 ± 0.28	0.98	17 ± 0.31	0.00	19.07 ± 0.20 *
2	87	17.12 ± 0.35	3.81	17 ± 0.41	0.00	20.77 ± 0.33 *
3	83	17.12 ± 0.46	3.83	17 ± 0.34	0.00	21.70 ± 0.46 *
4	81	17.15 ± 0.38	4.00	17 ± 0.32	0.00	23.63 ± 0.27 *
5	82	19.48 ± 0.37 *	18.12 *	19 ± 0.69 *	11.76 *	25.17 ± 0.32 *

* Denoted that the comparison between treatments and control group ($p < 0.05$) was statistically significant.

3.2. PRSE Supplement Intervention Beginning at Early Stages Extends the Lifespan of C. elegans

A quantity of 5 mg/mL of PRSE intervention at the L1 stage significantly extended the mean lifespan of worms to 18.14 ± 0.45 days (10.67%) in which the maximum lifespan significantly increased to 22.97 ± 0.37 days ($p < 0.05$); (Figure 3A,B, Table 2). Intervention of the PRSE at 15 h after L1 stage significantly extended the mean, median, and maximum lifespan to 20.37 ± 0.33 (24.26%), 20 ± 0.35 (11.1%), and 24.40 ± 0.18 days, respectively, compared to the control group ($p < 0.05$). PRSE intervention at 34 h after the L1 stage and 48 h after the L1 stage also significantly increased the maximum lifespan to 20.70 ± 0.25 and 20.40 ± 0.20 days compared to the control group, while the mean and median lifespans were unaffected by PRSE intervention ($p < 0.05$); (Figure 3A,B, Table 2).

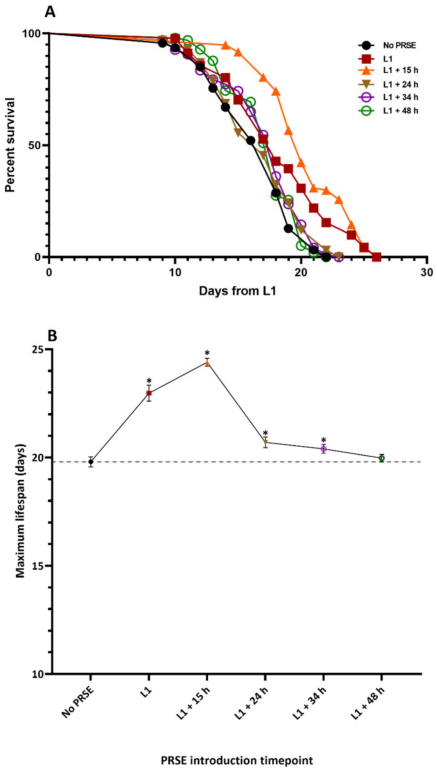

Figure 3. Timing of exposure to PRSE (5 mg/mL) survival curves for treated and untreated wild-type N2 worms. (**A**) Survival curves of wild-type N2 worms in response to different time points of PRSE intervention. (**B**) Maximum lifespan curve beginning from a baseline of 19.80 ± 0.23 days of wild-type N2 worms in response to different time points of PRSE introduction (* $p < 0.05$).

Table 2. The effect of exposure to PRSE supplement at different time points of life cycle on wild-type N2 *C. elegans* lifespan.

Timing of Exposure to PRSE	Sample Size (n)	Mean Lifespan (Days) ± SEM	% Extension Compared to Control	Median Lifespan (Days) ± SE	% Extension Compared to Control	Maximum Lifespan (Days) ± SEM
No PRSE	94	16.39 ± 0.35		18 ± 0.49		19.80 ± 0.23
L1	91	18.14 ± 0.45 *	10.67 *	18 ± 0.57	0.00	22.97 ± 0.37 *
L1 + 15 h	97	20.37 ± 0.33 *	24.26 *	20 ± 0.35 *	11.1 *	24.40 ± 0.18 *
L1 + 24 h	99	16.61 ± 0.36	1.36	18 ± 0.65	0.00	20.70 ± 0.25 *
L1 + 34 h	97	16.94 ± 0.35	3.32	18 ± 0.34	0.00	20.40 ± 0.20 *
L1 + 48 h	98	17.05 ± 0.28	4.01	18 ± 0.22	0.00	19.70 ± 0.17

* Denoted that the comparison between treatments and control group was statistically significant ($p < 0.05$).

3.3. Supplementation of PRSE at Early Stage Extends Lifespan through the Insulin/IGF-1 Signaling (IIS) Pathway

PRSE intervention at the L1 stage failed to prolong the lifespan of *daf-16* (*mgDf50*) *I* mutants. On the other hand, *daf-2* (*e1370*) *III* could not extend the lifespan ($p > 0.05$); (Figure 4, Table 3). The average lifespan is subject to considerable variation due to factors such as experimental conditions, genetic makeup, and environmental influences. Despite efforts to maintain uniform experimental conditions, differences arising from conducting experiments at various times are unavoidable. This is a recognized obstacle in lifespan research using model organisms. Such variability might account for the observed shorter mean lifespan of N2 untreated worms compared to DAF-16 [21].

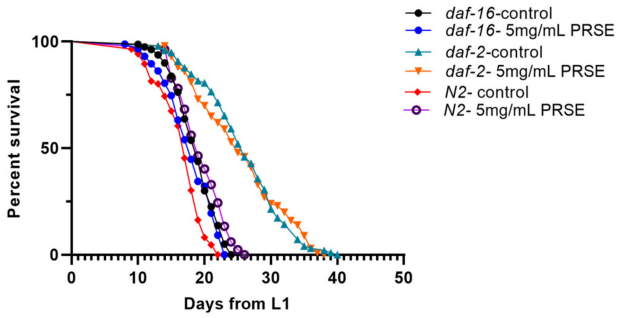

Figure 4. Survival curves of *daf-2* (*e1370*) *III*, *daf-16* (*mgDf50*) *I* and wild-type N2 nematodes in response to 5mg/mL of PRSE supplementation.

Table 3. The effect of exposure to PRSE supplement on lifespan of *daf-16 (mgDf50) I*, *daf-2 (e1370)* III mutants.

Strain/Treatment	Sample Size (n)	Mean Lifespan (Days) ± SEM	% Reduction Compared to Control	Median Lifespan (Days) ± SE	% Reduction Compared to Control
daf-16 (*mgDf50*) *I* 0 mg/mL-control	80	18.55 ± 0.35		19 ± 0.55	
daf-16 (*mgDf50*) *I* 5 mg/mL	87	17.76 ± 0.40	−4.27	18 ± 0.58	−5.26
daf-2 (*e1370*) *III* 0 mg/mL-control	98	25.82 ± 0.63		26 ± 0.93	
daf-2 (*e1370*) *III* 5 mg/mL	100	25.33 ± 0.68	−1.88	25 ± 1.11	−3.85

3.4. PRSE Supplementation Moderates Thermotolerance in C. elegans Nematodes

PRSE significantly extended the mean survival times of N2 *C. elegans* to 9.06 ± 0.12 h (15.1%), 7.79 ± 0.12 h (10.3%) and 6.61 ± 0.10 h (7.4%) at day 4, day 8, and day 12 compared

to each day of the control group, respectively ($p < 0.05$); (Figure 5A–C, Table 4). The median survival times of N2 PRSE-treated nematodes were found to extend significantly with PRSE to 9.00 ± 0.12 h (12.5%), 8.00 ± 0.21 h (14.3%) and 7.00 ± 0.08 h (16.7%) at day 4, day 8, and day 12 compared to each day's control group, respectively ($p < 0.05$); (Figure 5A–C, Table 4). PRSE supplementation on *daf-2* (*e1370*) *III* mutant significantly increased the mean survival time to 8.84 ± 0.20 h (8.82%) and 9.87 ± 0.21 h (8.38%) at day 8 and day 12 of the lifespan compared to each day's control group ($p < 0.05$); (Figure 5B,C, Table 4). The median survival time of *daf-2* (*e1370*) mutant treated with PRSE was significantly extended by 10 ± 0.15 h (11.11%) at day 8 compared to the control group ($p < 0.05$, Figure 5B, Table 4). PRSE supplementation significantly increased the mean survival time of *daf-16* (*mgDf50*) mutants at day 4 and day 8 by 6.94 ± 0.21 h (8.92%) and 5.06 ± 0.15 h (11.54%) compared to each day's control group ($p < 0.05$); (Figure 5A,B, Table 4).

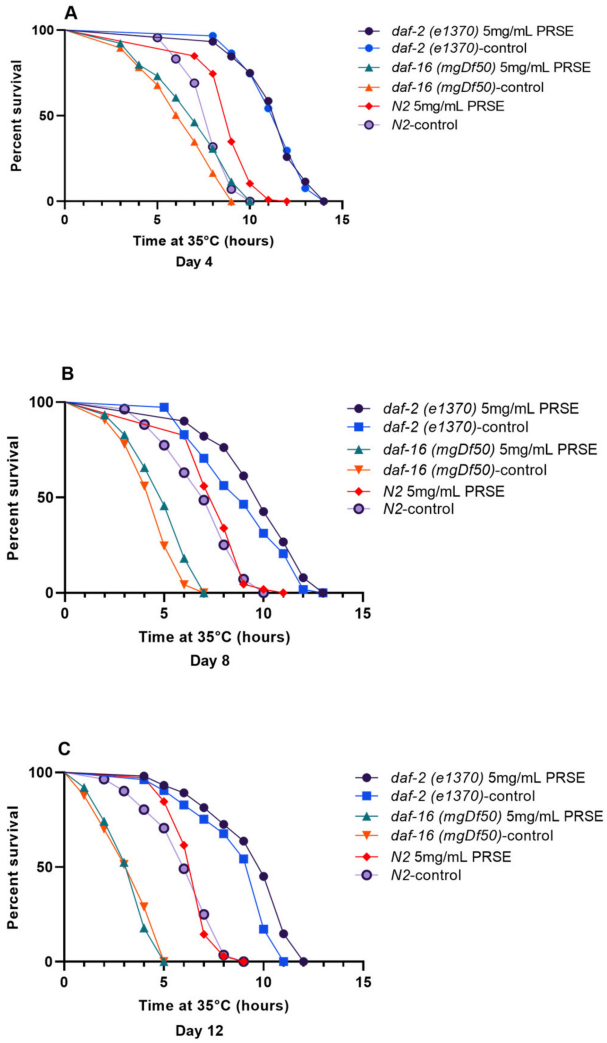

Figure 5. Survival curves at 35 °C for wild-type N2 (Bristol) (**A**), *daf-2* (*e1370*) *III* (**B**) and *daf-16* (*mgDf50*) *I*. (**C**) nematodes treated with and without 5 mg/mL of PRSE at day 4, day 8, and day 12.

Table 4. Mean and median survival times based on hours for 5mg/mL PRSE treated and untreated wild-type N2, *daf-2* (*e1370*) *III* and *daf-16* (*mgDf50*) *I C. elegans* strains.

Strain	Treatment	Day	Sample Size (n)	Mean Survival ± SEM	% Extension/Reduction Compared to Control	Median Survival ± SE	% Extension/Reduction Compared to Control
N2	Control PRSE	4	113 106	7.87 ± 0.12 9.06 ± 0.12 *	15.1 *	8.00 ± 0.12 9.00 ± 0.12 *	12.5 *
N2	Control PRSE	8	111 109	7.06 ± 0.18 7.79 ± 0.12 *	10.3 *	7.00 ± 0.25 8.00 ± 0.21 *	14.3 *
N2	Control PRSE	12	112 104	6.15 ± 0.16 6.61 ± 0.10 *	7.4 *	6.00 ± 0.21 7.00 ± 0.08 *	16.7 *
daf-2 (*e1370*)	Control PRSE	4	118 104	11.49 ± 0.14 11.49 ± 0.16	−0.01	12 ± 0.19 12 ± 0.13	0
daf-2 (*e1370*)	Control PRSE	8	112 101	9.07 ± 0.21 9.87 ± 0.20 *	8.82 *	9 ± 0.38 10 ± 0.15 *	11.11 *
daf-2 (*e1370*)	Control PRSE	12	105 102	8.84 ± 0.21 9.58 ± 0.21 *	8.38 *	10 ± 0.20 10 ± 0.26	0
daf-16 (*MgDf50*)	Control PRSE	4	115 104	6.37 ± 0.18 6.94 ± 0.21 *	8.92 *	7 ± 0.27 7 ± 0.33	0
daf-16 (*MgDf50*)	Control PRSE	8	118 105	4.53 ± 0.12 5.06 ± 0.15 *	11.54 *	5 ± 0.13 5 ± 0.20	0
daf-16 (*MgDf50*)	Control PRSE	12	107 101	3.39 ± 0.13 3.37 ± 0.12	−0.5	4 ± 0.21 4 ± 0.14	0

* Denoted that the comparison between treatments and each control group was statistically significant ($p < 0.05$).

4. Discussion

The influence of long-term PRSE supplementation on the lifespan of *C. elegans*, starting from the first larval stage (L1), has been shown to be concentration-dependent. Specifically, the mean and median lifespans were extended by 18.12% and 11.76%, respectively, at a concentration of 5 mg/mL. The maximum lifespan also showed significant increases across various concentrations of PRSE supplementation. Polyphenols are commonly found in various foods and beverages, including fruits, vegetables, olive oil, chocolate, legumes, tea, wine, and coffee [9,22]. Consumption of these polyphenol-rich foods is considered a promising approach for preventing chronic diseases and improving overall health [9,22].

Using live *E. coli* as a food source in *C. elegans* lifespan studies is a widespread method. This technique is preferred because live *E. coli* offers a natural and comprehensive nutritional environment for worms. The belief is that the active metabolic processes of live *E. coli* play a crucial role in the proper growth and wellbeing of *C. elegans* [23], However, it is crucial to consider that live bacteria can also produce metabolites that might independently affect the worms, potentially confounding the results of experiments designed to test specific interventions like PRSE. Therefore, to ensure that the observed effects are due to the PRSE and not bacterial metabolites, it can be beneficial to conduct control experiments using metabolically inactive *E. coli* (UV inactive bacteria). Polyphenols found in sugarcane may provide health advantages [15]. The rinds of sugarcane can serve as an abundant resource of phenolic compounds, which are known for their antioxidant capabilities [24]. Sugarcane-derived polyphenols have the potential to positively impact cell functions, which can be conducive to overall health [16]. The reported health benefits of sugarcane juice may be attributed to its capacity to neutralize free radicals, reduce iron complexes, and curb lipid peroxidation [25].

Supplementing with PRSE at a concentration of 5 mg/mL during early stages (L1 and 15 h after L1) significantly extended the mean and median lifespans of *C. elegans*. However, future studies could explore whether concentrations above 5 mg/mL offer enhanced effects or exhibit different outcomes, providing further insights into PRSE's impact on the worm lifespan. The debate on early nutritional programming and its impact on longevity and late-life health through gene-nutrient interactions is ongoing [26,27]. Recent findings suggest that nutrition plays a crucial epigenetic role in modulating aging-associated diseases such as diabetes, cancer, and obesity, which in turn affects longevity [28]. In humans, the first 1000 days of life, from conception to 24 months after birth, are critical for early-life programming [29]. In accordance with our observations involving PRSE, where a significant mean lifespan extension was documented in *C. elegans* following exposure from the L1

larval stage (an increment of 10.67% compared to the control group) and 15 h post-L1 stage (an increment of 24.26% relative to controls), Munasighe et al., [14] similarly reported a statistically significant extension in mean lifespan. In their investigation, *C. elegans* subjected to cocoa exposure from the L1 stage until death or adult day 1 experienced an 8.9% increase in lifespan compared to a control cohort. Notably, the PRSE treatment administered 15 h post-L1 stage yielded a more substantial impact on mean lifespan compared to initiation at the L1 stage alone, with the median lifespan being extended by 11.1% relative to the control group. In order to understand the mechanisms underlying the lifespan extension caused by PRSE in *C. elegans*, established genetic pathways were investigated, with known longevity extension properties. The regulation of lifespan significantly hinges on the roles of DAF-16 knockout and DAF-2 knockout [30,31]. It was found that the lifespans of DAF-2 and DAF-16 mutants were not extended by the 5 mg/mL PRSE intervention, suggesting that PRSE supplementation at an early stage extends lifespan through the insulin/IGF-1 signaling (IIS) pathway. The lifespan of *Caenorhabditis elegans* is controlled by the insulin/insulin-like growth factor-1 (IGF-1) signaling pathway mediated by the DAF-2 receptor. This pathway activates a PI 3-kinase/Akt pathway, leading to phosphorylation of the DAF-16 transcription factor and ultimately shortening the lifespan. However, disrupting Akt-consensus phosphorylation sites in DAF-16 does not significantly affect the lifespan. Additionally, the DAF-2 pathway regulates DAF-16 localization in the nucleus and perturbing sensory neurons or germ cells can extend the lifespan through DAF-16. These findings highlight the intricate and multifaceted nature of the DAF-16-dependent pathways involved in regulating aging in *C. elegans* [32]. The focus on DAF-16 localization using a DAF-16::GFP strain is a pivotal aspect of our future research direction. This approach will provide valuable insights into how PRSE influences the nuclear localization and activity of DAF-16, a key factor in lifespan regulation. Additionally, assessing the role of HSF-1, known for its interaction with DAF-16 and significant impact on thermotolerance and longevity, will further enhance our understanding of the molecular mechanisms through which PRSE affects aging in *C. elegans*. This comprehensive analysis will deepen our knowledge of the genetic pathways involved in aging.

The ability to survive heat stress relies on the mechanisms involved in maintaining proteostasis [33]. Aging leads to changes in proteostasis, resulting in a decreased ability to withstand heat stress. Our findings demonstrated that young and middle-aged wild-type *C. elegans* worms were more resilient to heat stress compared to older worms. Interestingly, treatment with 5 mg/mL of PRSE at the L1 stage significantly enhanced survival across all age groups. In alignment with our observations, Shakeri et al. [34] demonstrated that 4 g/kg sugarcane polyphenol compound could mitigate the detrimental impacts of heat stress on the growth and meat quality of broiler chickens. Previous research evaluating the effects of epicatechin and catechin on thermal stress in *C. elegans* revealed that both compounds improved survival, particularly in the later stages of adulthood, with greater protective effects compared to young worms [35]. In the nematode *Caenorhabditis elegans*, reduced insulin-like signaling hyperactivates DAF-16 transcription factors, resulting in longer-lived, stress-resistant worms [36].

Quercetin has been observed to prolong the lifespan, mitigate age-related mobility reduction, and expedite recovery from heat stress, as well as diminish reactive oxygen species. These outcomes are likely achieved via the manipulation of the insulin-like signaling (ILS) pathway. It was discovered that the transcription factors DAF-16 and SKN-1 are central to these changes. Interestingly, polyphenol compounds were also found to boost *C. elegans* resistance to heat stress; this is another piece of evidence that supports our finding [37]. SKN-1 activation in *C. elegans* delays degenerative tissue changes during aging and preserves glutathione levels, potentially ensuring longevity through different mechanisms under different conditions [38]. The primary polyphenolic compounds identified in PRSE are apigenin, luteolin, and tricin [16]. Luteolin and apigenin are dietary flavones known for their antioxidant, anti-inflammatory, anti-cancer, and neuroprotective properties [39]. Luteolin is particularly effective, reducing oxidative stress and regulating

stress-response pathways more significantly than apigenin. Both flavones activate key signaling pathways, aiding in cellular protection and stress response [40]. It was demonstrated that acid hydrolysates from A. auricula polysaccharides significantly increased the activities of antioxidant enzymes (SOD, CAT, GR) and levels of antioxidants (GSH) in *C. elegans*. They also observed the upregulation of antioxidant-related genes, suggesting that these compounds could improve the antioxidant defense system, potentially linked to aging prevention [40]. Polyphenols positively influence *Caenorhabditis elegans*' redox balance by enhancing its antioxidant mechanisms, with effects varying by polyphenol type. The integration of biochemical and molecular techniques to quantify the tissue antioxidant levels of *C. elegans* can enhance our understanding of these impacts. Future research could focus on examining the effects of luteolin, tricin, and apigenin, which are key polyphenols in PRSE, specifically looking into their antioxidant properties in *C. elegans*. This could provide valuable insights into their potential benefits.

As shown in Figure 6, the *C. elegans* IIS pathway involves key genes: daf-2, encoding an insulin/IGF-1 receptor, and daf-16, a FOXO transcription factor. Under conditions of abundant nutrition, insulin-like peptides bind to DAF-2, initiating a signaling cascade through AGE-1, PDK-1, and AKT-1/2 kinases. This cascade leads to the phosphorylation of DAF-16, preventing its nuclear localization. Activated DAF-16 promotes longevity, stress resistance, and the expression of stress-response genes [41]. Consequently, the activation of the IIS pathway, inhibiting DAF-16, hinders the expression of genes related to longevity, metabolism, autophagy, and stress resistance, explaining why mutations in daf-2 extend the lifespan while daf-16 mutations accelerate aging [42,43]. In addition to DAF-16, the transcription factors HSF-1 and SKN-1/Nrf also play vital roles downstream of the IIS pathway, contributing to lifespan extension. Loss of function mutations in HSF-1 have been shown to shorten the lifespan in daf-2 mutant organisms [44].

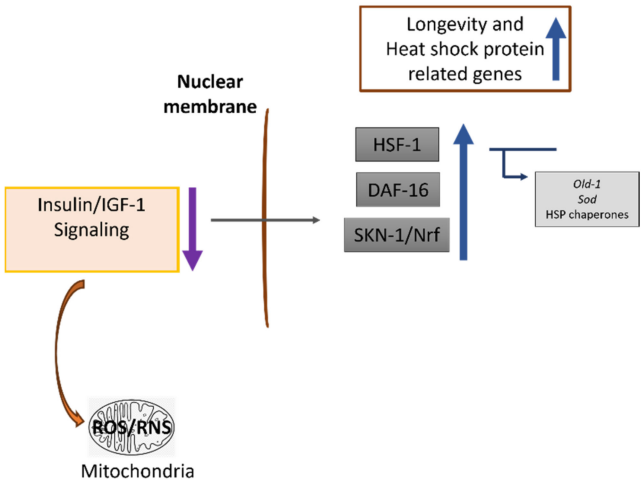

Figure 6. Insulin/IGF-1 signaling (IIS) in *C. elegans*. The inhibition of transcription factors like DAF-16/FOXO, SKN-1/Nrf, and HSF-1 ultimately leads to the suppression of downstream genes associated with stress resistance and longevity in *C. elegans*.

Wilson et al. [45] suggested that blueberry polyphenols have a protective effect against low levels of free radicals in cells. It is also possible that blueberry polyphenols can modulate the activity of signaling pathways involved in the response to thermal stress. This aligns with our findings, as the survival rate of *C. elegans* mutants treated with 5 mg/mL of PRSE (DAF-2 and DAF-16 mutants) was not improved compared to untreated worms. These results support the notion that the insulin-like signaling (ILS) pathway regulates the *C. elegans* lifespan by modifying stress reactions and DAF-16, a key transcription factor, is crucial in this process [46]. Reduced ILS prompts heat tolerance through protein translation, while certain genes adjust post-transcriptionally during heat stress. This alteration in ILS

conditions DAF-16-induced metabolic changes, preparing for acute stress responses and influencing survival [46].

5. Conclusions

In summary, this study found that supplementing with PRSE at an early stage of the *C. elegans* life cycle significantly extended the lifespan and improved heat stress resistance. These effects might be attributed to the activation of the insulin/IGF-1 signaling (IIS) pathway. The IIS pathway, when activated, enhances stress resistance and the expression of stress-related genes, ultimately leading to increased longevity. These findings shed light on the role of PRSE in influencing aging processes and underscore the importance of early-life nutritional interventions in promoting longevity and stress resilience. Further research is beneficial for underlying the molecular mechanisms involved in aging.

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