Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 5762922, 12 pages
https://doi.org/10.1155/2023/5762922

Research Article

Protective Effects of *Artemisia selengensis* Turcz. Leaf Extract on *Caenorhabditis elegans* under High Glucose Diet

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Received 9 February 2023; Revised 18 April 2023; Accepted 21 April 2023; Published 9 May 2023

Academic Editor: Miguel Rebollo-Hernanz

Artemisia selengensis Turcz. (AST), as a common edible and medicinal herb, exerts multiple biological activities including antioxidant, antihyperuricemia, and inhibition of α-glucosidase activities. However, limited information is available about its beneficial effects on high glucose toxicity in *Caenorhabditis elegans* (*C. elegans*). In the current study, *Artemisia selengensis* Turcz. leaf extract (ASTE), rich in caffeoylquinic acids, was found to alleviate high glucose-induced damages in *C. elegans* by extending the lifespan, increasing the body bending and pharyngeal pumping rates, and by reducing glucose and lipid accumulation and oxidative stress. Further results demonstrated that ASTE extended the lifespan of *C. elegans* on high glucose diet by inhibiting the insulin/IGF-1 signaling (IIS), and activating SKN-1 and AAK-2 signaling pathways. In conclusion, our findings reveal the direct evidence for the protective effects of ASTE against high glucose toxicity.

1. Introduction

During the last decades, the dietary habit of people has gradually shifted to high sugar and high lipid. Diets enriched in glucose, one of the most extensively studied sugar, have been reported to cause several metabolic-related diseases such as diabetes mellitus and obesity [1]. Recently, growing evidence has emerged that high glucose diet could also lead to accelerated aging in different organisms, thus resulting in reduced longevity and locomotion, and increased neuronal defects [2–4]. Nutritional intervention has been considered to be an effective approach to attenuate the detrimental effects induced by high glucose diet and promote healthy aging [5, 6].

Artemisia selengensis Turcz. (AST), as a widely edible vegetable in the central region of China, has been reported to possess many bioactivities such as antioxidation, antidiabetic, and anticancer activities [7–9]. Recent study also reveals that ASTE is a potential α-glucosidase inhibitor, which may be active in regulating glucose metabolism [10]. Moreover, caffeoylquinic acids, the major components of ASTE, have been suggested to mediate glucose metabolism and alleviate the impact of diabetes complications [11]. Emerging evidence demonstrates that sustained elevated plasma glucose level affects organismal aging process [3]. Several natural ingredients that regulate glucose metabolism have been found to prevent the negative impact of glucose on longevity [12, 13]. However, little is known about the effects of ASTE in alleviating the detrimental impacts and delaying the accelerated aging induced by high glucose diet.

Due to the short lifespan, easy cultivation, and facile gene manipulation, *C. elegans* has been utilized as a powerful tool to explore the metabolic and phenotypic effects of high glucose diet [14]. In addition, nutrient-sensing pathways involving IIS, AAK-2/AMP-activated protein kinase (AMPK), and SKN-1/nuclear factor erythroid 2-related

factor 2 (Nrf2) can regulate glucose level and aging process by sensing nutrient or metabolites, and these pathways are highly conserved between nematodes and mammals [15]. Thus, natural ingredients identified, with protective effects against glucotoxicity on *C. elegans*, may have the potential to promote human health in high sugar environments.

In the present study, the effects and underlying mechanisms of ASTE on alleviating high glucose-induced damages were investigated by employing the *C. elegans* model. This research reveals the potential of ASTE in preventing glucose toxicity and promoting healthy aging.

2. Materials and Methods

2.1. Materials and Chemicals. AST was from Wuhan Hexiangyuan Agricultural Development Co., Ltd (Hubei, China). 2′,7′-dichlorodihydro fluorescein diacetate (H_2DCF-DA), 5-fluoro-2-deoxyuridine (FUDR), and oil-red-O stock were from Sigma-Aldrich (St. Louis, MO, U.S.A.). The Superoxide Dismutase (SOD) assay kit, reduced glutathione (GSH) assay kit, glucose assay kit, and triglyceride assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China).

2.2. Preparation of ASTE. ASTE was obtained from our laboratory according to our previous study [16]. In brief, *Artemisia selengensis* Turcz. leaves were ground to powder and extracted with 50% ethanol three times, then partitioned with ethyl acetate to obtain the crude extracts. Once removing the solvents, the extract was loaded onto a resin HP-20 column to purify. Afterwards, the eluted fractions with 50% ethanol were concentrated and freeze-dried to yield ASTE. The ASTE was diluted in dimethyl sulfoxide (DMSO) to prepare 60 mg/mL stock solution.

2.3. Strains and Cultivation of Nematodes. The strains including N2 (wild type), CB1370 [daf-2(e1370)III], CF1038 [daf-16 (mu86)I], RB754[aak-2(ok524)X], and EU1 [skn-1 (zu67)IV] were obtained from Caenorhabditis Genetics Center (CGC). Nematodes were grown on nematode growth medium (NGM) plate with live *Escherichia coli* (*E.coli*) OP50 at 20°C. The age-synchronized larvae were prepared according to the standard protocols previously described [17].

2.4. Lifespan Assay. The late L4-stage synchronized worms were transferred into a Transwell-96 well plate containing liquid S-complete medium with/without glucose [18], and treated with 0.6% DMSO (control) or various concentrations of ASTE (60, 240, and 360 μg/mL). The FUDR and heat-killed *E. coli* OP50 were also added. For glucose addition, sterilized stock solution of glucose was added to the liquid S-complete medium to the desired concentration (1%, 2%, and 4% glucose, respectively). The living number of worms was recorded until 100% mortality.

2.5. Motility Assays. Worms were cultured as outlined in the lifespan assay. On the 3rd, 6th, and 9th day of cultivation, worms were moved onto new NGM plates, and the number of body bending and pharyngeal pumping rates in 20 s was counted using an anatomic microscope according to a previous report [19]. Each trial was repeated 3 times with at least 30 worms.

2.6. Intracellular ROS Assay. The nematodes were removed into a black 96-well plate (30 worms per well) after culturing for 7 days as described in Section 2.4 and then treated with 50 μM fresh H_2DCF-DA for 2 h at 37°C [20]. The fluorescence intensity was then recorded every 20 min at an excitation/emission wavelength of 485/530 nm.

2.7. GSH Contents and SOD Activities Determination. Worms were prepared as outlined in Section 2.4. After treated for 3 days, the nematodes (approximately 2000 worms per group) were collected with M9 buffer, ground in a tissue grinder, and successively centrifuged to obtain the supernatant [21]. The GSH contents and SOD activities were analyzed by commercial kits (Nanjing Jiancheng Bioengineering Inst., Jiangsu, China) based on the manufacturers' instructions. The results were normalized to the protein content, which was analyzed by a BCA kit (Nanjing Jiancheng Bioengineering Inst., Jiangsu, China).

2.8. Glucose and Triglyceride Quantification. On the 3rd day of adulthood, the nematodes (approximately 2000 worms per group) were collected and washed with M9 buffer for 3 times, then homogenized and centrifuged to obtain the samples for subsequent assays. The glucose and triglyceride contents were then quantified by the commercial kits (Nanjing Jiancheng Bioengineering Inst., Jiangsu, China).

2.9. Oil-Red O Staining. Based on the previous report, the lipid droplets of nematodes were detected by Oil-Red O staining [22]. In brief, after treatment for 3 days, the worms were collected and fixed with 2% paraformaldehyde for 30 min. Afterwards, the working solution of Oil-Red O was added and incubated overnight for staining. The photographs of stained worms were then recorded under an inverted microscope. The intensity of the stained fat of each worm was quantified using ImageJ software.

2.10. Quantitative RT-PCR Analysis. Worms were collected on the 3rd day of adulthood treated as for lifespan assay. RNA in each group was extracted and converted to cDNA (Takara), as described previously [16]. By using SYBR Green PCR Master Mix (Takara), the qRT-PCR reaction was carried out on a qTOWER 2.0 PCR system (Analytik JenaAG, Germany). The mRNA expression level of gene was quantified by $2^{-\Delta\Delta CT}$ method, and act-1 was chosen as

endogenous control. The primer sequences of above-mentioned genes are presented in Supporting Information Table S1.

2.11. Statistical Analysis. Data were expressed as means ± SD or SEM. Survival analysis was performed by GraphPad Prism version 7.0 (San Diego, CA, USA) with the log-rank test. Student's *t*-test or one-way analysis of variance with Duncan's test was applied for comparison among different groups, and statistical significance was defined as $p < 0.05$.

3. Results

3.1. High Glucose Diet Decreased the Lifespan of C. elegans. To evaluate the effect of glucose on the lifespan of *C. elegans*, we exposed wildtype worms to glucose at a range of concentrations. The lifespan assay was then carried out. In comparison with the control, worms fed with 1%, 2%, and 4% glucose markedly decreased the mean lifespan by 14.58%, 22.22%, and 31.50%, respectively (Figure 1(a), Table 1, $p < 0.01$). Since the epidermis of *C. elegans* treated with 4% glucose was slightly wrinkled during the experiment, we used 2% glucose for the subsequent experiments.

3.2. ASTE Extended the Lifespan of C. elegans on High Glucose Diet. The major phytochemical compounds in ASTE were chlorogenic acid, 1,4-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid, as we have previously reported [16]. The HPLC profile of ASTE is shown in supplementary information Figure S1. Based on our previous study, these plant-derived polyphenols have great benefits on antiaging activity, therefore, we further examined whether ASTE could protect worms from the negative impact of a high-glucose diet on lifespan. The results showed that high glucose diet (2%) feeding decreased the lifespan by 28.42%, while ASTE at 240 μg/mL and 360 μg/mL notably prolonged the mean lifespan of worms by 28.70% and 27.93%, respectively, ($p < 0.001$ and $p < 0.01$, Figure 1(b), Table 2). These findings confirmed that ASTE can alleviate the adverse impacts of high glucose on longevity. In addition, the dose of 240 μg/mL ASTE was applied for the subsequent experiments due to its remarkable effect.

3.3. ASTE Attenuated the Negative Effects of High Glucose Diet on Healthspan. In addition to a reduction in lifespan, a high glucose diet also had adverse effects on healthspan [23, 24]. With increasing age, the locomotion ability of nematodes decreased in both the normal diet and high glucose diet group; however, the pharyngeal pumping rates and body-bending rates of worms grown on high glucose diet were further decreased compared with the normal diet group (Figures 2(a) and 2(b), $p < 0.001$). In addition, the results also revealed that the pharyngeal pumping and body bending rates obviously recovered to a certain extent in worms fed with 2% glucose after the addition of ASTE, but did not return to the level of the normal diet group. Taken together, ASTE, as a natural plant-derived ingredient, partially mitigated the damage to the healthspan of *C. elegans* caused by high glucose diet.

3.4. ASTE Enhanced the Antioxidant Activity in C. elegans. On the basis of previous reports, excessive glucose intake may lead to an enhanced oxidative stress by increasing the level of cellular reactive oxygen species (ROS), thereby accelerating the aging process [14, 25]. To verify whether ASTE alleviated the detrimental effects of high glucose diet via decreasing the oxidative stress, we examined the accumulation levels of ROS and the activity of some antioxidant enzymes. As displayed in Figure 3(a), the intracellular ROS level of worms on high glucose diet was significantly higher than that of worms on normal diet ($p < 0.05$). However, supplementation with ASTE considerably reduced the ROS accumulation in the worms on high glucose diet. Besides, the SOD activity and GSH content of worms on high glucose diet significantly decreased by 39.30% and 40.93%, respectively, compared with that in worms on normal diet (Figures 3(b) and 3(c), $p < 0.001$ and $p < 0.01$). With the addition of ASTE, the SOD activity and GSH content of worms on high glucose diet considerably increased by 39.83% and 74.39% ($p < 0.01$). Abovementioned data demonstrated that ASTE enhanced the antioxidant activity of *C. elegans* by activating the antioxidant enzymes and lowering ROS accumulation.

3.5. ASTE Regulated the Glucose and Fat Metabolism of C. elegans. It has been reported that six caffeoylquinic acid compounds identified from *Artemisia selengensis* Turcz. (AST) could inhibit α-glucosidase activity [10]. To assess whether ASTE could actually mediate the glucose metabolism *in vivo*, we checked the glucose content in worms on high glucose diet. As expected, the glucose content of nematodes incubated with 2% glucose was obviously increased by 5.6-fold ($p < 0.001$), compared with that in the normal diet group, whereas ASTE remarkably declined the glucose content in worms on high glucose diet (Figure 4(a), $p < 0.01$). Meanwhile, the triglyceride contents of worms treated with high glucose significantly increased by 2.26-fold, suggesting that the dietary glucose was indeed absorbed by *C. elegans* and stored as fat (Figure 4(b), $p < 0.01$). Furthermore, supplementation of ASTE considerably reduced the triglyceride contents of worms on high glucose diet by 19.64% ($p < 0.01$). Consistently, the Oil-Red O staining results also confirm that ASTE could reduce the fat contents in *C. elegans* (Figures 4(c) and 4(d)). To further determine the mechanisms of ASTE in regulating the glucose and fat metabolism, we examined the expression of related genes in Figure 4(e). In *C. elegans*, oga-1 has been reported to encode an active O-GlcNAcase, which was related to glucose uptake [26]. The results showed that the expression of oga-1 was obviously increased by 2.82-fold in worms on high glucose diet, whereas ASTE decreased the expression of oga-1 by 0.73-fold (Figure 4(e), $p < 0.001$). Meanwhile, the expressions of lbp-5 (intracellular lipid-binding protein) and acs-2 (acyl-CoA synthetase) were

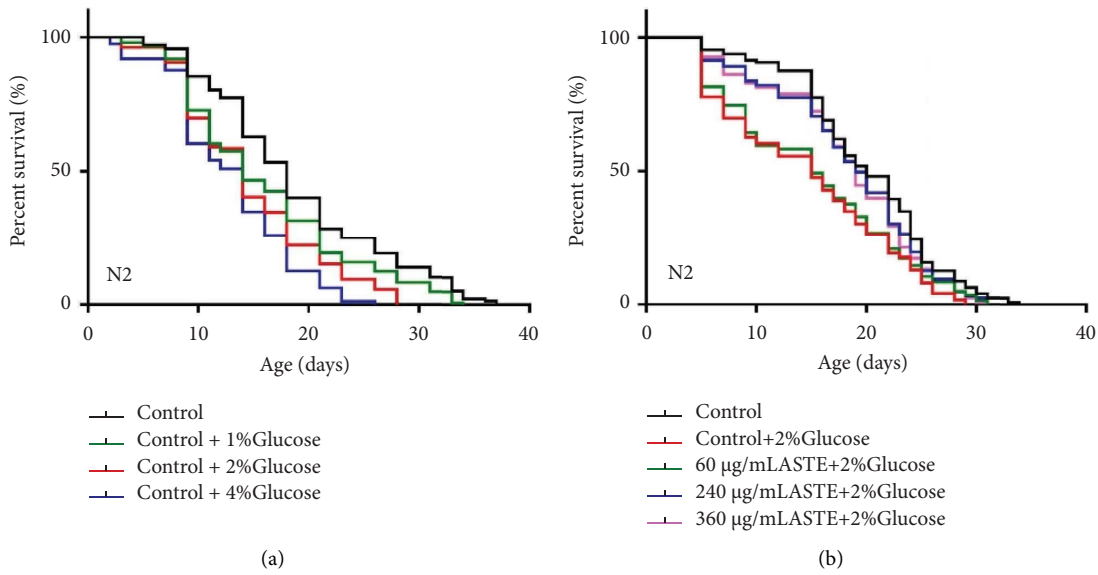

Figure 1: ASTE extended the lifespan of *C. elegans* on high glucose diet: (a) lifespan curves of N2 treated with DMSO (control) or 1%, 2%, and 4% glucose and (b) lifespan curves of N2 treated with 0.6% DMSO (control) or different doses of ASTE on a high glucose diet (2%).

Table 1: Effects of different doses of glucose on the lifespan of *C. elegans*.

Treatment	Mean lifespan ± SEM (days)	Percentage change	Number of worms	*p* value
Control	18.86 ± 0.69^{a}	—	137	—
1% glucose	16.11 ± 0.64^{b}	14.58%	145	0.0041
2% glucose	14.67 ± 0.50^{c}	22.22%	161	<0.0001
4% glucose	12.92 ± 0.44^{d}	31.50%	167	<0.0001

Different letters (a, b, c, and d) indicated a significant difference among different groups. *p* values were analyzed by the log-rank test.

Table 2: Effects of ASTE on the lifespan of *C. elegans* on 2% glucose diet.

Treatment	Mean lifespan ± SEM (days)	Percentage change	Worms	*p* value
Control	19.81 ± 0.59	—	129	—
2% glucose	14.18 ± 0.73	28.42%	126	$<0.0001^{a}$
60 μg/mL ASTE + 2% glucose	14.88 ± 0.68	4.94%	146	0.3154^{b}
240 μg/m ASTE + 2% glucose	18.25 ± 0.64	28.70%	131	0.0009^{c}
360 μg/mL ASTE + 2% glucose	18.14 ± 0.63	27.93%	124	0.0024^{d}

Letter a meant the comparison between the 2% glucose group and the control group. Letters (b, c, and d) meant the comparison between the 60 μg/mL, 240 μg/mL, and 360 μg/mL ASTE group and the 2% glucose group, respectively. *p* values were analyzed by the log-rank test.

significantly reduced by 0.53-fold and 0.57-fold, respectively, (Figure 4(e), $p < 0.001$ and $p < 0.01$), while the expression of fat-6 (stearoyl-CoA desaturase) was increased by 2.77-fold in worms on high glucose diet. On the contrary, ASTE notably upregulated the expressions of lbp-5 (1.99-fold) and acs-2 (3.75-fold), and inhibited the expression of fat-6 (0.11-fold) in worms on high glucose diet. Above all, these results indicated that ASTE played an important role in reducing glucose and fat contents in *C. elegans*.

3.6. ASTE Extended the Lifespan of C. elegans on High Glucose Diet by Regulating the IIS Pathway. The IIS pathway was reported to regulate aging, stress resistance, metabolism, and development, while inhibiting IIS pathway was an effective approach to promote the longevity [27, 28]. Therefore, we examined whether ASTE-induced lifespan extension on high glucose diet relied on the IIS pathway. The results showed that daf-2 and daf-16, which were two critical regulatory factors of the IIS pathway, abolished ASTE-induced lifespan extension (Figures 5(a) and 5(b), Table S2), demonstrating that daf-2 and daf-16 were indispensable for the ASTE-mediated longevity promotion effect on high glucose diet. Moreover, compared with the normal diet group, the expressions of daf-2, age-1, and akt-2 were significantly increased in the 2% glucose diet group (Figure 5(c), $p < 0.05$). Meanwhile, the expressions of critical transcription factor daf-16 and its downstream

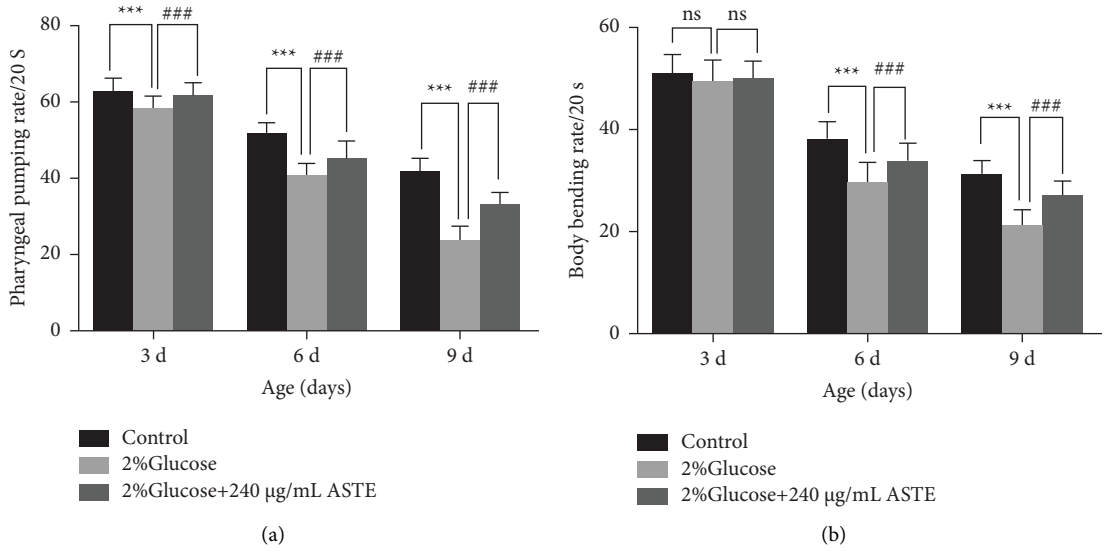

Figure 2: ASTE promoted the healthspan of *C. elegans* on high glucose diet. The effect of ASTE on the rates of (a) pharyngeal pumping and (b) body bending of worms on the days 3, 6, and 9 of adulthood. Statistical significance was performed by two-tailed Student's t test. $^{***}p < 0.001$ represented the comparison between the 2% glucose group and the normal diet group, and $^{\#\#\#}p < 0.001$ represented the comparison between the ASTE group and the 2% glucose group.

stress-response genes sod-3 (superoxide dismutase), mtl-1 (metallothionein), and hsp-12.3 (heat shock proteins) were markedly inhibited in the 2% glucose diet group ($p < 0.05$). However, the supplementation of ASTE on 2% glucose diet obviously decreased the expressions of daf-2, age-1, and akt-2, and elevated the expressions of daf-16, sod-3, mtl-1, and hsp-12.3 (Figure 5(c), $p < 0.01$). Taken together, these data indicated that ASTE prolonged the lifespan of *C. elegans* on high glucose diet by inhibiting the IIS pathway.

3.7. ASTE-Induced Lifespan Extension on High Glucose Diet Was Dependent on skn-1 and aak-2. SKN-1, a homologue of mammalian Nrf2, was found to activate phase II detoxification and antioxidative stress response pathway in *C. elegans* [29]. In addition, aak-2, a homologue of α-catalytic subunits of mammalian AMPK, was reported to regulate oxidative stress resistance and energy metabolism in nematodes [30]. Considering that ASTE could decrease the ROS level in worms on high glucose diet, we further examined whether the lifespan extension effect of ASTE was relied on skn-1 and aak-2. The results showed that ASTE failed to prolong the lifespan of skn-1 and aak-2 null mutants (Figures 6(a) and 6(c), Table S2), indicating that skn-1 and aak-2 were required for ASTE-induced lifespan extension on high glucose diet. In addition, the expressions of skn-1 and aak-2 were significantly inhibited in the high glucose diet group in comparison to the normal one, whereas ASTE intervention obviously upregulated the expressions of skn-1 and aak-2 (Figures 6(b) and 6(d), $p < 0.01$). These findings suggest that ASTE might prolong the lifespan of worms grown on high glucose diet by activating skn-1 and aak-2.

4. Discussion

There is growing evidence that excess glucose intake affects the lifespan of *C. elegans* [14, 31]. One previous study has demonstrated that ASTE is a potential resource of α-glucosidase inhibitor, which might help to reduce the postprandial blood glucose level [10]. However, little is known about the effects of ASTE on the lifespan of *C. elegans* on high glucose diet. Therefore, by employing the *C. elegans* model, we investigated the benefits of ASTE in face of high glucose diet. Herein, we found that ASTE, rich in caffeoylquinic acids, was able to partially rescue the shortened lifespan and mitigate the damage to the healthspan caused by high glucose diet. Moreover, ASTE decreased the oxidative stress and the glucose and fat contents in worms grown on high glucose diet. The protective effects of ASTE were mediated by inhibiting the IIS pathway and activating skn-1 and aak-2.

It is well known that a consistently large amount of dietary sugar can lead to the increase in obesity, diabetes, and cardiovascular diseases [32, 33]. However, there is limited information about the direct effects of a high sugar diet on aging, especially on longevity. Our results indicated that addition of glucose shortened the lifespan and induced damage to the healthspan of *C. elegans*. ASTE, as a natural plant-derived ingredient, was found to extend the lifespan and improve the locomotion ability of *C. elegans*, thus delaying aging induced by a high glucose diet. Besides, ASTE has been reported to inhibit α-glucosidase [10], suggesting that ASTE has potential in regulating glucose metabolism. This was supported by the current observation that ASTE obviously decreased the glucose content in *C. elegans*.

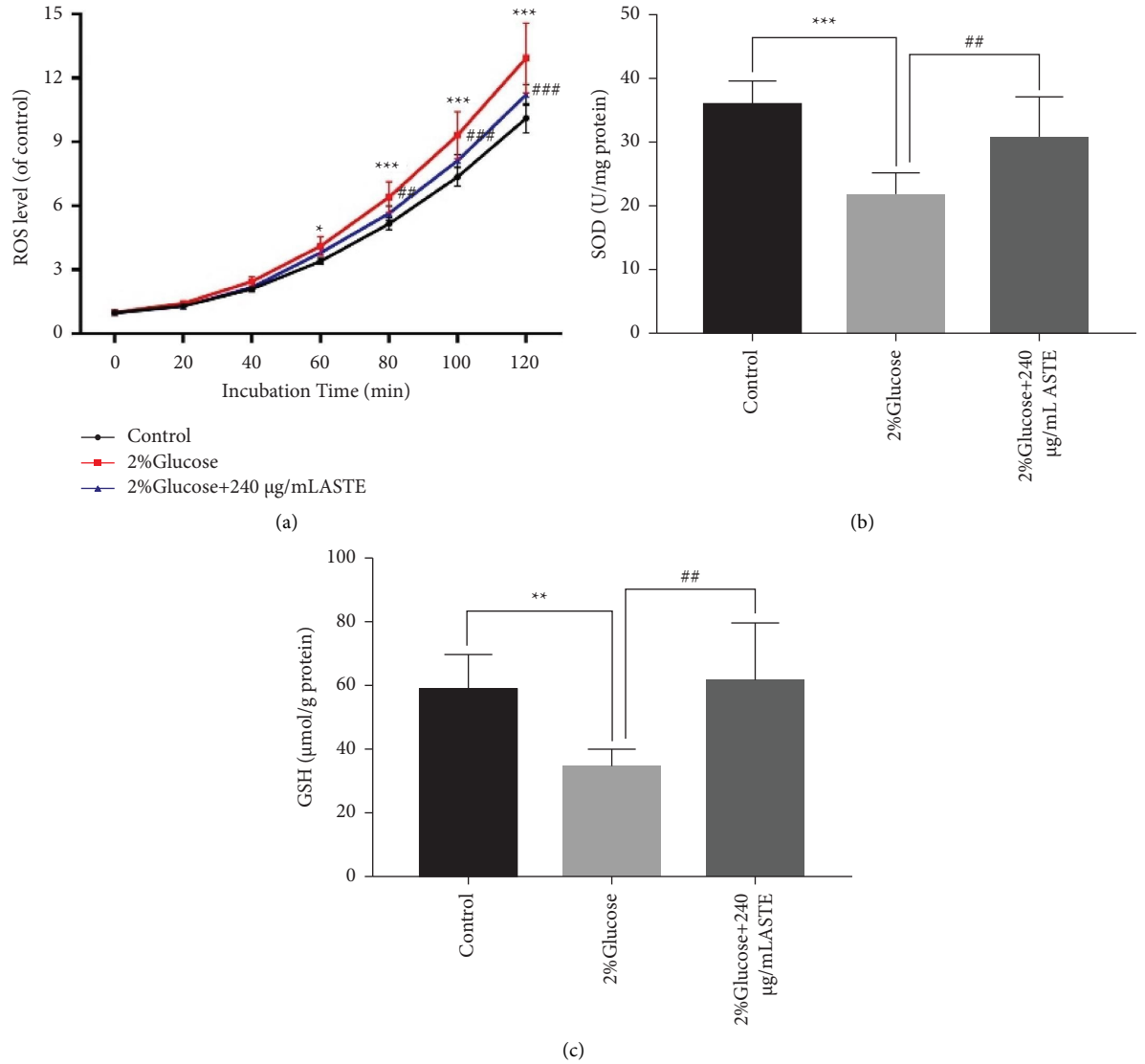

Figure 3: Effects of ASTE on ROS levels and antioxidant enzyme activities of nematodes on high glucose diet: (a) intracellular ROS levels, (b) SOD activities, and (c) GSH contents in worms on normal diet or high glucose diet. Statistical analysis was performed by two-tailed Student's t test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ represented the comparison between the 2% glucose group and the normal diet group, while $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ represented the comparison between the ASTE group and the 2% glucose group.

Moreover, ASTE was found to remarkably reduce the fat level in *C. elegans*, which was consistent with a previous report that *Lonicera japonica* extracts rich in caffeoylquinic acids could decrease fat accumulation in worms [34]. Taken together, our findings demonstrated that ASTE not only promoted longevity and fitness, but also regulated the glucose and fat metabolism of *C. elegans* on high glucose diet.

Excessive glucose intake may lead to an enhanced oxidative stress by increasing the level of cellular reactive oxygen species (ROS), thereby accelerating the aging process. Specifically, sustained hyperglycemia induces the formation of ROS via increasing the formation of advanced glycation end products, activating protein kinase C and mitochondrial electron transport chain, thus causing damages to various macromolecules including protein, DNA, and lipids in the long run [35, 36]. Therefore, removing the excessive ROS induced by hyperglycemia was considered to be an effective strategy to alleviate diabetes complications [37]. In our study, high glucose feeding increased the oxidative stress in *C. elegans* through accumulating ROS and reducing the SOD activity and GSH content, while ASTE treatment significantly decreased the oxidative stress induced by high glucose. Combined with the fact that ASTE could inhibit the activity of α-glucosidase [10], our results were in line with a previous study that inhibition of α-glucosidase was responsible for decreased oxidative stress [36]. Above all, it is conceivable that the reduction of oxidative stress may contribute to the longevity extension effect of ASTE in *C. elegans* on high glucose diet.

A few previous studies have demonstrated that an increased dietary glucose and lipid intake are closely associated

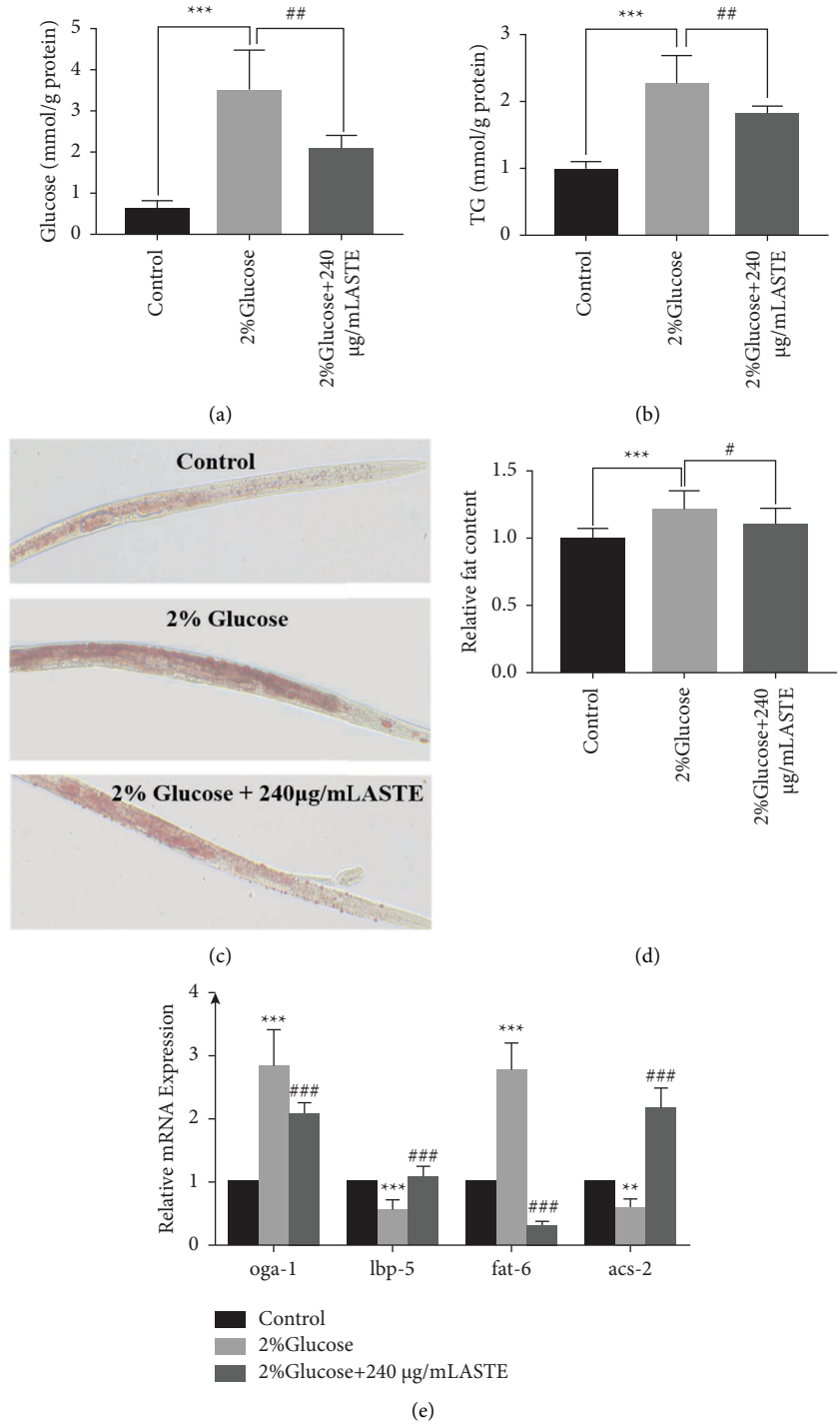

Figure 4: Effects of ASTE on glucose and fat metabolism of *C. elegans* on high glucose diet. The glucose content (a) and triglyceride contents (b) Oil-Red-O staining pictures of worms (c) and relative quantification of fat content (d) by Oil-Red-O staining. (e) The mRNA expression levels of oga-1, lbp-5, fat-6, and acs-2. Statistical significance was performed by two-tailed Student's *t* test. $^{**}p < 0.01$, and $^{***}p < 0.001$ meant the comparison between the 2% glucose group and the control group, while $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ meant the comparison between the ASTE group and the 2% glucose group.

with metabolic dysregulation, which ultimately decreases the lifespan of *C. elegans* [3, 14]. Based on the fact that some genes involved in regulating glycolipid metabolism also mediated longevity, we further investigated whether ASTE extended the lifespan of *C. elegans* on high glucose diet through modulating the metabolic-related genes. The glycosylation gene oga-1 has been reported to affect glucose uptake in *C. elegans*, and inactivation of oga-1 leads to reduced glucose uptake and prolonged lifespan [38, 39]. In the current study, ASTE could significantly inhibit the

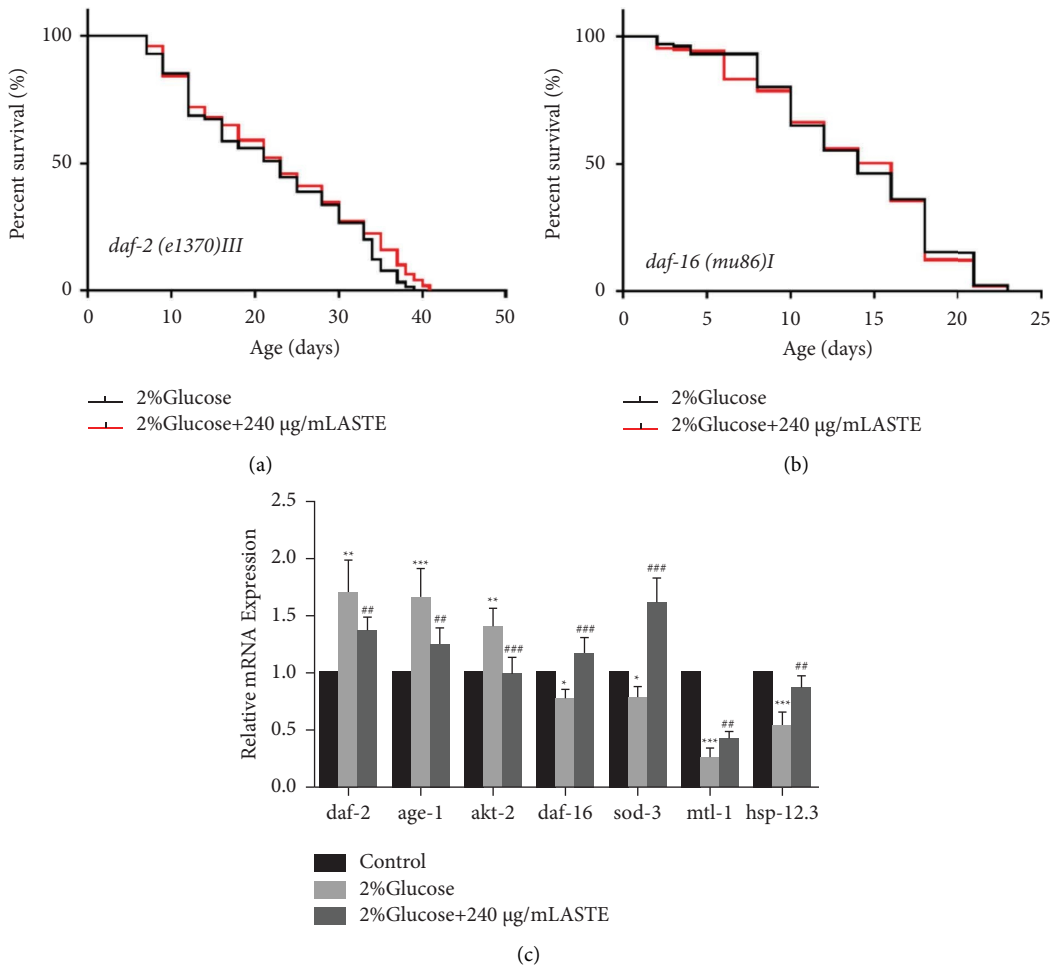

FIGURE 5: ASTE extended the lifespan of *C. elegans* on high glucose diet by regulating the IIS pathway. Lifespan curves of (a) daf-2 (e1370), (b) daf-16 (mu86) mutant strains treated with DMSO or 240 μg/mL ASTE on 2% glucose diet. (c) The mRNA expressions of daf-2, age-1, akt-2, daf-16, sod-3, mtl-1, and hsp-12.3. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ represented the comparison between the 2% glucose group and the normal diet group, while $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ represented the comparison between the ASTE group and the 2% glucose group.

expression of oga-1 in worms grown on high glucose diet, which contributed to the decrease of glucose content as well as the lifespan extension in *C. elegans*. Furthermore, deficiency of lbp-5, an intracellular lipid chaperone gene, leads to increased ROS production and fat accumulation [40]. Our results demonstrated that the expression of lbp-5 in worms fed with high glucose was remarkably elevated by ASTE, thus leading to reduced fat accumulation and enhanced antioxidant capacity. Collectively, these findings confirmed that the lifespan promotion effect of ASTE was tightly correlated to its regulation of glycolipid metabolism.

In *C. elegans*, the IIS pathway has been well known to regulate growth, reproduction, metabolism, and aging, while inhibiting the IIS pathway is an effective strategy to prolong lifespan [41, 42]. Thus, we examined whether the longevity promotion effect of ASTE on high glucose diet was dependent on the IIS pathway. In the current study, the lifespan extension was completely abolished in the daf-2 and daf-16 null mutants, which were two critical regulatory factors of the IIS pathway, indicating that daf-2 and daf-16 were required for ASTE to prolong the lifespan on high glucose diet. Besides, a previous study has reported that glucose-enriched diets shorten the lifespan of *C. elegans* by downregulating the DAF-16/FOXO, but the mechanism by which high glucose inhibiting DAF-16/FOXO remains unclear [43]. Our results revealed that high glucose feeding significantly upregulated the expressions of daf-2, age-1, and akt-2, and downregulated the expressions of daf-16, sod-3, mtl-1, and hsp-12.3, indicating that high glucose inhibited the activity of DAF-16/FOXO by activating the IIS pathway, and ultimately reduced the lifespan of worms. However, ASTE addition was proved to upregulate DAF-16/FOXO and its downstream target genes by downregulating the IIS pathway. This was in line with a previous report that chlorogenic acid, one of the main bioactive ingredients of ASTE, extended the lifespan of *C. elegans* by modulating the IIS pathway [44].

It has been well documented that overconsumption of glucose may result in increased cellular oxidative stress, which negatively impacts glucose metabolism and apoptosis

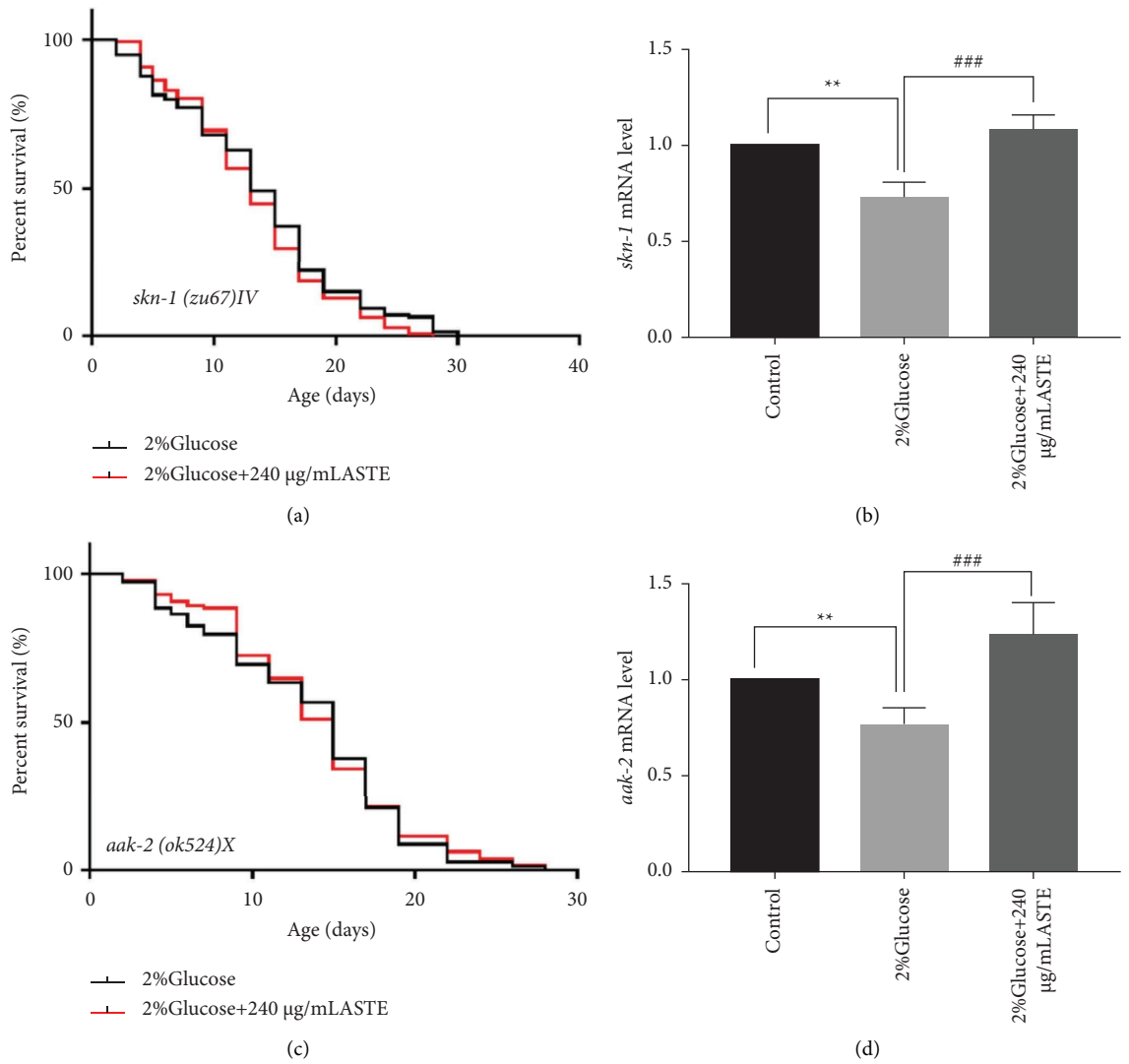

FIGURE 6: ASTE extended the lifespan of *C. elegans* on high glucose diet by activating skn-1 and aak-2. Lifespan curves of (a) skn-1 (zu67), (c) aak-2 (ok524) mutant strains treated with DMSO or 240 μg/mL ASTE on 2% glucose diet. The mRNA expressions of (b) skn-1 and (d) aak-2. $^{**}p < 0.01$ represented the comparison between the 2% glucose group and the normal diet group, while $^{###}p < 0.001$ represented the comparison between the ASTE group and the 2% glucose group.

[25]. The transcription factor SKN-1, ortholog of the mammalian Nrf2 in *C. elegans*, plays a key role in regulating oxidative stress response pathway and longevity [45]. Considering that ASTE could reduce the oxidative stress in worms on high glucose diet, we investigated the role of SKN-1 in ASTE-mediated lifespan extension. Our results indicated that high glucose treatment obviously inhibited the expression of skn-1, which was consistent with a previous report that high glucose intervention shortened the lifespan of worms by inactivating SKN-1 [46]. Nevertheless, ASTE treatment considerably upregulated the skn-1 and failed to extend the lifespan of skn-1 deletion mutants, indicating that skn-1 was required for the lifespan extension effect of ASTE on high glucose diet. Besides, another study suggested that constitutive activation of SKN-1 could protect the worms from fat accumulation when grown on a high glucose diet [47]. Thus, future experiments are needed to investigate the role of SKN-1 in ASTE-regulated fat metabolism.

AMPK, as a crucial cellular energy sensor, has been reported to regulate energy homeostasis and metabolism, and high cellular energy levels caused by a glucose-rich diet will lower the AMP/ATP ratio and inactive AMPK [3, 48].This was supported by the fact that high glucose uptake disrupted aak-2, a homologue of AMPK, and led to survival reduction in *C. elegans* [49]. Consistently, our results showed that high glucose treatment downregulated the aak-2, while ASTE addition enhanced the expression of aak-2. Combining with the fact that ASTE failed to prolong the lifespan of aak-2 null mutants on high glucose diet, we speculated that ASTE extended the lifespan of *C. elegans* by activating akk-2. These findings were consistent with the previous studies showing that the main compounds of

ASTE, such as chlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid were reported to activate AMPK signaling pathway and regulate lipid metabolism [50, 51]. Taken together, it is conceivable that in addition to extending the lifespan, the activation of aak-2 might contribute to the lipid-lowering effect of ASTE.

5. Conclusion

In summary, this study demonstrates that ASTE protects *C. elegans* from glucotoxicity by extending the lifespan, strengthening the locomotive abilities, decreasing the oxidative stress, and lowering the accumulation of glucose and lipid. Meanwhile, the mechanistic study reveals that ASTE can reverse survival reduction by regulating skn-1, aak-2, and IIS pathway. Our findings reveal the beneficial effects of ASTE in the face of unhealthy high-sugar diet, thus broadening the application of ASTE for health promotion.

Data Availability

The data used to support the findings of this study are available from the corresponding author.

Additional Points

Practical Applications. In the present study, the protective effects, and mechanisms of ASTE against high glucose toxicity were systemically evaluated in the *C. elegans* model. Our findings suggest that ASTE may serve as novel nutraceutical for enhancing the health of people in the face of high sugar diet. In addition, our results also reveal its potential as the functional foods for antiobesity and antidiabetes application.

Disclosure

Rong Li and Mingfang Tao are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Rong Li performed data curation, project administration, methodology, writing–review and editing, and writing–original draft. Mingfang Tao performed methodology, investigation and data curation, and formal analysis. Tingting Xu performed methodology and data curation. Jinsong Wang performed methodology and data curation. Rui Duan performed formal analysis. Ting Wu performed resources, formal analysis, and writing–review and editing. Siyi Pan collected resources and performed writing–review and editing. Xiaoyun Xu conceptualized the study, collected resources, supervised the study, and performed writing–review and editing. The authors Rong Li and Mingfang Tao contributed equally to this work.

Acknowledgments

This work was supported by Wuhan Applied Foundational Frontier Project (2020020601012268), the Key Research and Development Project in Hubei Province (grant no. 2022BCE057), the Technology Innovation Project in Hubei Province (grant no. 2022BEC031), and the Major Science and Technology Projects in Hubei Province (grant no. 2022BCE060).

Supplementary Materials

Figure S1: the HPLC fingerprint of ASTE and mixed standard. Chlorogenic acid for peak 1, 1,4-dicaffeoylquinic acid for peak 2, 3,4-dicaffeoylquinic acid for peak 3, 1,5-dicaffeoylquinic acid for peak 4, 3,5-dicaffeoylquinic acid for peak 5, and 4,5-dicaffeoylquinic acid for peak 6. Supplementary Table S1: primers used in this research. Supplementary Table S2: effects of ASTE on lifespan of mutants on 2% glucose diet[a]. (*Supplementary Materials*)

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