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Aloe vera Gel Extract Prolongs Lifespan in *Caenorhabditis elegans*

Şeyda Berk [1,2,a,*]

[1] *Department of Molecular Biology and Genetics, Faculty of Science, Sivas Cumhuriyet University, Sivas, Türkiye.*
[2] *Advanced Technology Research Center (CUTAM), Sivas Cumhuriyet University, Sivas, Türkiye.*
**Corresponding author*

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ABSTRACT

Caenorhabditis elegans (*C. elegans*), with its superiority and physiological aging properties, has become a widely recognized model system in research on aging, longevity mechanisms, age-related diseases, and drug screening. Lifespan-extending mutations in *C. elegans* are known to slow the aging process by interfering with a number of signaling pathways such as the AMP-activated protein kinase (AMPK), mechanistic target of rapamycin (mTOR) and insulin/IGF-1 signaling (IIS) pathways. We aimed to see how *Aloe vera* (*A. vera*) gel affects the fertilization and lifespan of *C. elegans*. In the presence of all *A. vera* gel concentrations (0.312 - 5 mg/mL), the fertilization capacity of N2 worms increased and extended their lifespan as well as increased their body size. We found that in N2 worms cultured with 2.5 mg/ml *A. vera* gel, *sgk-1*, *age-1*, and *let-363* mRNA expression was significantly increased, while *rsks-1* mRNA expression was significantly decreased. We therefore conclude that it may extend lifespan through a mechanism specifically dependent on mTOR signaling. All these observations will provide a new perspective on mammalian life extension through the model organism *C. elegans*.

Keywords: *Aloe vera*, *C. elegans*, Lifespan, Ageing, Fertilization.

[a] *sberk@cumhuriyet.edu.tr* *https://orcid.org/0000-0003-4687-0223*

Introduction

Anti-aging and living a long, healthy life free of illness have been goals since ancient times, despite the fact that aging is a complicated phenomenon that is still not fully understood. *C. elegans,* a small nematode, is now a popular model system for studies on aging. Research using *C. elegans* in multiple labs has greatly advanced our basic understanding of aging as a biological process regulated by cellular signaling pathways and gene expression programs [1]. The isolation of long-lived *C. elegans* mutants sparked a scientific journey that has yielded numerous insights into the mechanisms that regulate lifespan, with implications for human longevity [2]. The requirement for extended periods of time (months to years) in mammalian systems presents the largest obstacle to lifespan extension research. The adaptable, seemingly straightforward model organism *C. elegans*, which has a brief lifespan (measured in days), has been used to overcome this. Its fundamental systems and mechanisms are strikingly similar to those of mammals [3]. Furthermore, a number of investigations have demonstrated that the mechanisms for longevity found in *C. elegans* are also found in flies, mice, and humans in comparable patterns [4]. First observed in *C. elegans*, the positive correlation between extended healthy longevity and modulation of insulin/IGF-1 signaling was later confirmed in flies and mice [1]. This gives studies on the longevity of *C. elegans* greater context. It also requires less time and is simple to use [3]. The mTOR pathway is another important pathway that connects metabolism and nutrient availability to longevity. This pathway regulates numerous downstream signaling pathways that control cell proliferation, growth, survival, motility, and protein synthesis [5]. Research on *C. elegans* has demonstrated that mTOR activity inhibition increases worm lifespan. The IIS pathway and the mTOR pathway are most likely separate pathways that contribute to longevity, though there may be some overlap [6].

The effects of bioactive secondary metabolites of plant foods on the prevention of oxidative stress-related diseases have received excessive attention. Many secondary metabolites, including polyphenols, ascorbic acid, and carotenoids, have been shown to reduce oxidative stress caused by reactive oxygen species (ROS) and this situation is described as oxidative damage (or oxidative stress) theory [7]. Free radicals, or ROS, are involved in oxidative stress-related illnesses and aging. In order to combat ROS damage, the antioxidant defense system and aerobic metabolism coevolved [8]. When ROS production is out of control relative to antioxidant activity, biomolecules such as DNA, lipids, and proteins can be damaged. Aging and other chronic illnesses like cancer, heart disease, atherosclerosis, myocardial infarction, diabetes, and other degenerative diseases in human can be mediated by these activities [9]. The relationship between aging and oxidative damage accumulation, as well as antioxidant defense levels, has been supported by oxidative damage theory [7]. *C. elegans* mutants with reduced insulin/IGF-1 signaling (IIS) are resistant to

oxidative stress, and *C. elegans* mutants with increased resistance to a ROS-producing compound (juglone) have been identified [10].

A. vera is a genus of perennial succulent or xerophyte that belongs to the Liliaceae family. It has elongated, peaked leaves that store a large amount of water in their tissue, and it can be stemless or have very short stems. Additionally, the inner leaf pulp, which makes up the majority of the plant volume, is composed of large thin-walled parenchymal cells containing *A. vera* gel and is also referred to as inner leaf, inner leaf fillet, or fillet [11]. Pharmacologically active components found in aloe have a variety of biological effects, such as fungicidal, immunomodulating, antiviral, antibacterial, anti-inflammatory, and anticancer properties [12].

In present study, we investigated the effects of *A. vera* gel extracts on the fertility and survival of *C. elegans* in N2 worms. Furthermore, we assessed variations in the mRNA expression of the insulin/IGF signaling (IIS) and rapamycin (TOR) signaling components, *sgk-1 (serine/threonine-protein kinase sgk-1), age-1/PI3K (phosphatidylinositol 3-kinase age-1), let-363/TOR (target of rapamycin homolog)*, and *rsks-1/S6K (ribosomal protein S6 kinase beta)*, which are two important nutrient-sensing pathways in the most well-validated anti-aging research.

Materials and Methods

A. vera Gel Extract Preparation

Fresh, mature, healthy leaves of commercially purchased *A. vera* were thoroughly cleaned with water, then the spikes on the edges were removed and cut into transverse pieces. The dense epidermis was carefully separated from the parenchyma with a sharp knife. Before homogenization in a blender, the parenchyma was again gently washed with distilled water at ambient conditions to extract surface exudates. To remove fibers, the homogenized gel was centrifuged at 3000 rpm for 30 min. The supernatant was obtained by vacuum filtering through a filter paper (Whatman, Turkey) to obtain fresh *Al. vera* gel. The resulting aqueous *A. vera* gel was used directly and a new batch of gel was prepared each time [13].

C. elegans and Escherichia coli Strains

For this investigation, the OP50 strain of *E. coli* and the wild-type *C. elegans* (N2 Bristol) were procured from the Caenorhabditis Genetics Centre (CGC) at the University of Minnesota (Minneapolis–St. Paul, MN 55455, USA).

Maintenance of C. elegans

Worms were maintained in accordance with Brenner's previously instruction [14]. Briefly, worms were grown at 20 °C on nematode growth medium (NGM) agar [Preparation of NGM agar plates: 3 g NaCl, 17 g agar and 2.5 g peptone 975 mL H_2O were added, mixed and autoclaved. After the autoclave mixture was cooled to 55 °C, 1 mL of 1 M $CaCl_2$, 1 mL of 5 mg/ml cholesterol in ethanol, 1 ml of 1 M $MgSO_4$ and 25 mL of 1 M KPO_4 (pH:6) buffer were added and the prepared NGM solution was dispensed into petri dishes (60x15mm)] seeded with *E. coli* OP50 (food).

Worms cultivated on NGM plates were transferred using the "chunking" technique. This method involved taking a sterile scalpel or spatula and moving a piece of agar from one old plate to a new one. There were worm stock transfers every one to three generations. The method of controlling worms involved observing them through a dissecting microscope. Obtaining axenized *C. elegans* eggs and synchronous cultures of *C. elegans* were carried out according to the method previously described by Özdemir et al. [15]

Fertilization Assay

Age-matched adult worms (one worm per well) were cultured at 20°C in 24-well plates containing 1 mL/well S Medium [1L S Basal, 10 mL 1 M potassium citrate pH: 6, 10 mL trace metals solution (1.86 g disodium EDTA, 0.69 g $FeSO_4$ •7 H_2O, 0.2 g $MnCl_2$•4 H_2O, 0.29 g $ZnSO_4$•7H_2O, 0.025 g $CuSO_4$•5 H_2O, dissolved in 1L H_2O), 3 mL 1 M $CaCl_2$, 3 mL 1 M $MgSO_4$] with 3L of *E. coli* OP50 and increasing concentrations of *A. vera* gel extract (0.312 mg/mL, 0.625 mg/mL, 1.250 mg/mL, 2.5 mg/mL, and 5 mg/mL). The number of eggs laid in each well over a seven-day period was counted under a microscope. The experiments were repeated twice, and each experiment was carried out in quadruplicate.

Lifespan Assay

The L4 stage worms were placed in 24-well plates with 1 mL/well S (about 12 worms per well). In medium containing 3µL of *E. coli* OP50, worms were cultured at 20°C for the lifespan assay while increasing concentrations of *A. vera* gel extract (0.312 - 5 mg/mL) were added. To prevent reproduction, 5-Fluoro-2'-deoxyuridine (FUDR), was utilized. Worms were counted under a microscope every day, and the medium was changed every seven days. After being gently prodded to move the plate, animals were considered dead if they did not move. In order to analyze the body size of worms, photomicrographs taken at 0, 7, and 14 days were obtained using the Zeiss Axiovert A1 in conjunction with the Zeiss Digital Microscope Camera (AxioCam ICc 5). Body size was determined using ImageJ software. GraphPad Prism was used to analyze the data. The experiments were repeated twice, with each experiment being carried out in duplicate.

RNA Isolation and cDNA Synthesis

Preparation of worm samples for RNA isolation was carried out according to the previously described method [15]. Total RNA was isolated using a commercially available kit according to the manufacturer's instructions (HibriGen Biotechnology, Istanbul, Turkey, Total RNA Isolation kit; Cat. No: MG-RNA-01-100). The cDNA synthesis has been performed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA; Cat. No: 1708891) for obtaining cDNA product with 500 ng/µL of RNA according to manufacturer's recommendations. The cDNA that was produced was either immediately examined by qPCR or was kept at +4°C for further use.

Quantitative Real-time PCR (RT-qPCR)

RT-qPCR was used to assess the expression *sgk-1, age-1, let-363* and *rsks-1* mRNA in N2 L4 stage worms. The SYBR green assay and the StepOnePlusTM Real Time PCR system (Applied Biosystems, USA) were used for quantitative PCR, which was done according to the manufacturer's instructions for real-time amplifications.

The assay was carried out in a total reaction volume of 20 µL with 10 µL of BlasTaqTM 2X qPCR Master Mix (Abm, Canada, USA), 0.5 µL of each primer, and 2 µL of cDNA template. Table 1 contains primer information. All reactions were carried out in 96-well plates. The PCR was carried out using the real-time instrument in accordance with the manufacturer's instructions. Typically, 3 minutes of initial denaturation at 95°C was followed by 40 cycles of 10 seconds denaturation at 95°C, 30 seconds annealing at 60°C, and 15 seconds extension at 72°C. For the purpose of melting curve analysis, a cycle of dissociation steps (58°C for 1 minute followed by 0.5°C for 10 seconds up to 95°C) was added. At the end of each PCR experiment, a melting curve was generated to confirm the presence of a single peak and rule out the synthesis of primer dimers and non-specific products. For each gene, two biological samples were collected and measured twice. *act-1* and *cdc-42* reference genes were determined as the most stable genes for N2 strain in the study conducted by our research group [15]. Therefore, *act-1* and *cdc-42* were used to normalize mRNA levels using the method as previously described [16].

Table 1. Primer sequences and gene information for RT-qPCR

Gene symbol	Gene description	Sequence name	Function	Primer sequence	Reference
act-1	Actin-1	T04C12.6	Cytoskeletal structural protein	F: 5'-CTCTTGCCCCATCAACCATG-3' R: 5'-CTTGCTTGGAGATCCACATC-3'	[17]
cdc-42	Cell division control protein 42 homolog	R07G3.1	RHO GTPase	F: 5'-CTGCTGGACAGGAAGATTACG-3' R: 5'-CTCGGACATTCTCGAATGAAG-3'	[17]
rsks-1	Ribosomal protein S6 kinase beta	Y47D3A.16	ribosomal protein S6 kinase activity	F: 5'-CCGTTTGTGGGATTCACC-3' R: 5'-TGGCTTTCTCGGGCTCTT-3'	[18]
let-363	Target of rapamycin homolog	B0261.2	protein serine/threonine kinase activity	F: 5'-GCCACTCTCTGATTACCCTGT-3' R: 5'-GTGAGCCGCGTGTTTCAAAT-3'	[19]
sgk-1	Serine/threonine-protein kinase sgk-1	W10G6.2	Phosphatidylinositol-3,4,5-trisphosphate binding activity and protein serine/threonine kinase activity	F: 5'-AAGACTGTTGACTGGTGGTG-3' R: 5'-AGACGAAGTGGCTGGTTG-3'	[20]
age-1	Phosphatidylinositol 3-kinase age-1	B0334.8	1-phosphatidylinositol-3-kinase activity and 1-phosphatidylinositol-4-phosphate 3-kinase activity	F: 5'-GCTGCTCCGTGCAGAGATTG-3' R: 5'-CACGGAGGTAAGCTTCCATC-3'	[21]

The relative expression of genes was calculated using the comparative threshold method, $2^{\Delta Ct}$. Primers were purchased from BMLabosis (Ankara, Turkey). Ct values >35 did not yield consistent results and were considered below the detection limit of the assay.

Statistical Analysis

All experiments were repeated at least twice. The outcomes of the repeated experiments were comparable. The statistical software GraphPad Prisim 6.0 (GraphPad software, San Diego, CA) was utilized for the analysis. For survival, log-rank tests were used. A one-way ANOVA test was used to compare statistical results between groups. When significant differences were discovered, the Newman-Keuls test was used to compare groups. $p<0.05$ was considered statistically significant in all analyses. The data is shown as mean SEM.

Results

Effects of A. vera Gel Extract on C. elegans Fertilization

Our results showed that *A. vera* gel extract at concentrations of 0.312 - 5 mg/mL had a significant stimulatory effect on the number of eggs laid by *C elegans* N2 worms (Figure 1). While it was observed that the number of eggs laid increased significantly compared to the control group at all *A. vera* concentrations applied to wild-type N2 worms, we observed the highest increase at 0.625 and 2.5 mg/ml.

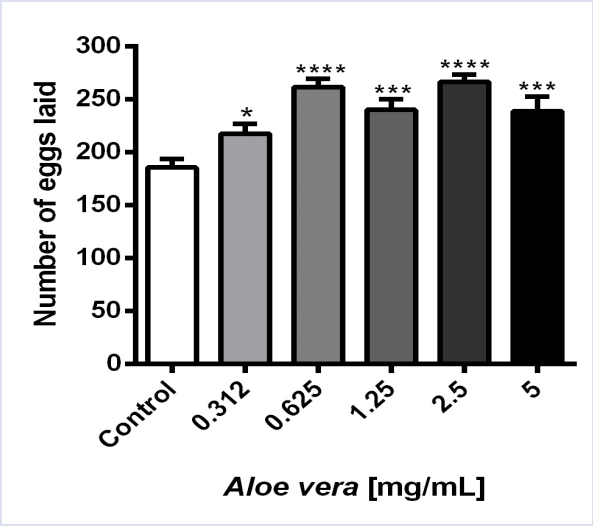
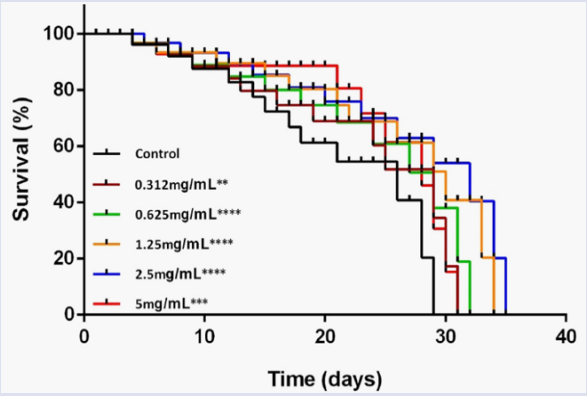


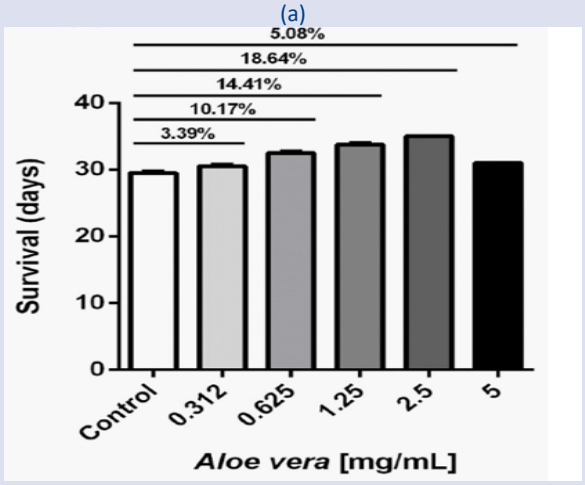
Figure 1. The effect of *A. vera* gel extract on egg-laying in N2 worms. The experiments were carried out on synchronized populations. Worms were assessed to be exposed to *A. vera* gel extract throughout their development from egg to young adulthood for the experiment, in which exposure to *A. vera* gel extract was shown at 7 days (the method by which L4 individuals attain fertile adulthood) according to the worm's developmental stage. During the egg stage, synchronized populations of wild-type and mutant *C. elegans* were transplanted to 24-well plates containing liquid media with *A. vera* gel extract (0,312 - 5 mg/mL). Significance relates to a comparison of egg-laying behaviour in *C. elegans* exposed to *A. vera* and control results. The number of eggs laid by each worm was counted every day for 7 days. Values represent the mean ±SEM of two independent experiments in quadruplicate. *p < 0.05, ***p<0.001, ****p<0.0001; n=3.

Effects of A. vera Gel Extract on Lifespan of C. elegans

We confirmed the effects of *A. vera* on *C. elegans* lifespan by survival analysis. Figure 2A shows the survival curve analysis results of N2 worms (Figure 2A). *A. vera* gel extract at concentrations of 0.312 mg/mL, 0.625 mg/mL, 1.250 mg/mL, 2.5 mg/mL and 5 mg/mL increased the average lifespan of N2 worms by 3.39%, 10.17%, 14.41%, 18.64% and 5.08%, respectively (Figure 2B). We determined 2.5 mg/mL *A. vera* gel extract as the concentration that most prolongs the lifespan of N2 worms.



(a)

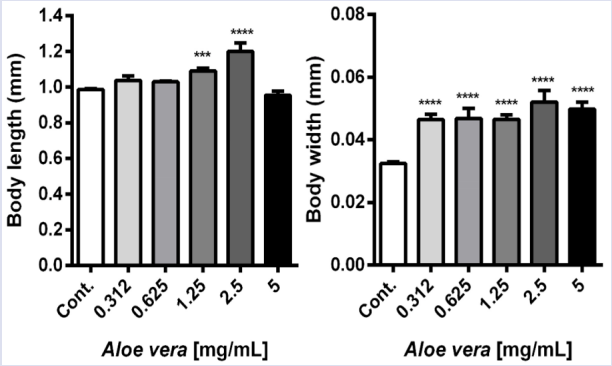


(b)

Figure 2. (A) Lifespan survival curves of N2 worms. (B) Graph showing the % increase in lifespan in the presence of *A. vera* gel extract compared to control. The experiments were carried out on synchronized populations. The synchronized populations of wild-type *C. elegans* were transplanted to 24-well plates containing liquid media with *A. vera* gel extract (0,312 - 5 mg/mL). Significance relates to a comparison of lifespan in *C. elegans* exposed to *A. vera* and control results. Values represent the mean ±SEM of two measurements from two independent experiments. *$p < 0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.000$.

Body Size Measurement

In order to reveal the changes in the body size of *C. elegans* after 14 days of *A. vera* gel extract at concentrations of 0.312 - 5 mg/mL, we measured body size of N2 worms (Figure 3). We observed that the body length of wild-type N2 animals increased significantly compared to the control as a result of *A. vera* treatment at concentrations of 1.250 mg/mL, 2.5 mg/mL (Figure 3A), while body width increased significantly at all applied concentrations (Figure 3B).



(a) (b)

Figure 3. Body size of wild-type N2 strains grown in S-medium including different concentrations of *A. vera* gel extract (0.312 - 5mg/mL). Body length (A) and body width (B) of N2 grown in S-medium including *A. vera* gel extract compared with control. Body length and width size derived N2 worms after 14 days is in mm using ImageJ software. Values represent the mean ±SEM of two independent experiments in duplicate. ****$p<0.0001$, ***$p<0.001$.

mRNA Expression Analysis in C. elegans

The mRNA expression of *sgk-1, age-1/PI3K, let-363/TOR*, and *rsks-1/S6K* mRNA expression of N2 worms cultured in S medium containing 2.5 mg/mL *A. vera* gel extract were determined by quantitative RT-PCR (Figure 4).

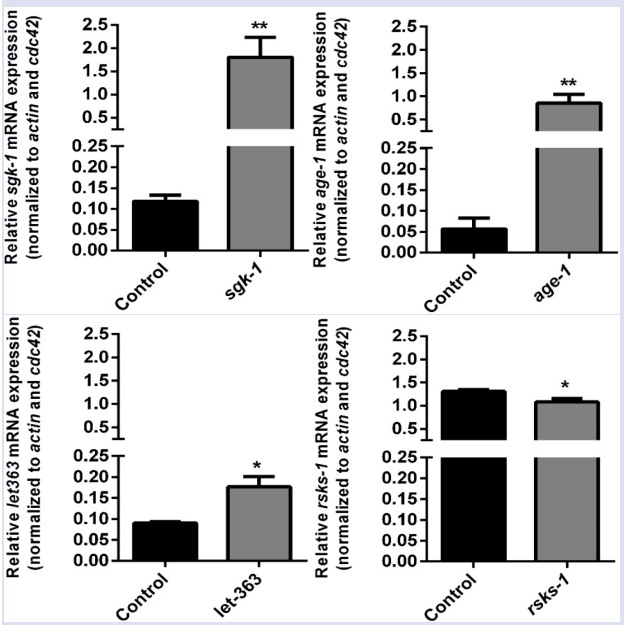


Figure 4. *Sgk-1, age-1, let-363* and *rsks-1* mRNA expression in N2 worms. *sgk-1, age-1, let-363* and *rsks-1* mRNA expression was measured relative to the levels of the house-keeping genes *cdc-42* and *act-1*. Values represent the mean ±SEM of two measurements from two independent experiments. *$p < 0.05$, **$p<0.01$.

Since we obtained the highest egg production and longevity at 2.5 mg/mL *A. vera* concentration, we performed mRNA analyses in the presence of this concentration. Compared with the control, *sgk-1, age-1* and *let-363* mRNA expression increased significantly in N2 worms cultured with *A. vera* gel extract, while *rsks-1* mRNA expression significantly decreased (Figure 4).

Discussion

To our knowledge, there has been no previous study how adding *A. vera* gel extract to *C. elegans* growth medium affects fertilization and longevity. In this study we investigated the effects of *A. vera* gel extract on *in vivo* fertilization, lifespan and body size in N2 worms. We also evaluated differential mRNA expression of components of the insulin/IGF signalling (IIS) and target of rapamycin (TOR) pathways, which have been associated with two major nutrient sensing pathways of the best validated antiaging interventions.

In the literature, *C. elegans* drug applications have largely been carried out on NGM agar growth medium. Therefore, the high bioavailability of the drug applied to the worms in the agar medium has always been questionable. Therefore, when drug applications are carried out on NGM agar medium, it is recommended to apply the drug at high concentrations for bioavailability in *C. elegans*. Liquid culture conditions are preferred because the bioavailability efficiency of the drug is high [22]. Our study team cultured worms in liquid culture medium to increase the bioavailability of *A. vera* gel extract by worms. Therefore, while it increased the bioavailability of drugs/extracts applied to worms in liquid culture medium, it also allowed the culture medium and drugs/extracts to be refreshed more easily at regular intervals.

We found that the egg-laying capacity of all concentrations of *A. vera* gel extract applied to wild-type N2 worms was significantly increased compared to the control group. We observed the highest egg yield at 2.5 mg/ml *A. vera* concentration. According to the longevity analysis results, wild-type N2 worms extended their lifespan in the presence of all concentrations of *A. vera* gel extract. We determined that 2.5 mg/ml *A. vera* was the concentration that most increased the longevity of N2 worms. As expected, it was found that the lifespan of the worms was extended independently of concentration. Stated differently, survival did not increase further with increasing inhibitor concentration. There are no studies in the literature investigating the effects of *A. vera* extract on *C. elegans* fertility and longevity, but the effects of *A. vera* have been investigated in *Drosophila melanogaster*, another important model organism [23]. According to the findings of the study, adding *A. vera* extract (5ml/L) to the diet prolonged adult lifespan in both male and female flies without reducing fertility. The findings of this study are consistent with our findings, and *C. elegans* dietary supplementation containing *A. vera* extract prolonged both fertility and adult lifespan.

Another study examining the effects of long-term *A. vera* intake on the growth of Fischer 344 male rats showed that ingestion of *A. vera* by rats led to an increase in body weight [24]. They also suggested that *A. vera* intake did not cause any significant adverse effects in rats. According to the body size analysis results of our current study, when N2 worms were exposed to *A. vera* gel extract, significant increases in body length and width occurred in wild-type N2 worms, according to the results of its effect on body size. The fact that we observed a significant increase, especially in the body width of the worms, at all *A. vera* concentrations applied to the worms confirms the results of the study conducted with rats.

The insulin/IGF signaling (IIS) and rapamycin target (TOR) pathways are the two main nutrient sensing pathways associated with the most effective antiaging therapies that have been validated. When these pathways are activated, autophagy, mitochondrial biogenesis, and the expression of detoxifying and antioxidant enzymes are all induced. These processes taken together can enhance cellular function [25]. Diet composition influences the aging process in terms of lifestyle, a longer lifespan is linked to a high intake of phytochemicals and a low protein diet [25]. Notably, a recent analysis indicates that human longevity has a heritability of less than 10%, suggesting that lifestyle decisions have a significant impact on both longevity and aging [26].

Many natural substances can act by prolonging the lifespan of nematodes by regulating some important cellular signaling pathways [25]. The insulin pathway, which is triggered by food intake, is one of these cellular pathways that regulates the lifespan of *C. elegans* [5]. This pathway includes *daf-16*, the sole homolog of the FOXO family of transcription factors, multiple conserved protein kinases, and *daf-2*, the homolog of the human insulin receptor. In nematodes, insulin-like peptides bind to *daf-2* and activate intracellular signaling. *Daf-2* signaling results in phosphorylation of *age-1*, *pdk-1*, and *akt-1/2* inactivates DAF-16/FOXOs by retaining the protein in the cytosol. In the absence of *daf-2*, *age-1*, and *akt-1/2*, as when there is a lack of food, *daf-16* migrates to the nucleus and induces the expression of genes associated with longevity [5].

Nutrients and amino acids trigger the target of rapamycin (TOR), which is another pathway triggered by food consumption. In response to the presence of amino acids and growth factors, the TOR (target of rapamycin) pathway regulates growth and reproduction. TOR, as a nutrient sensor, mediates the metabolic response to dietary restriction; it is a conserved lifespan-extension paradigm with numerous connections to many other signaling pathways. In line with this, inhibiting TOR activity increased lifespan in several species [27]. The TOR ortholog in *C. elegans*, *let-363*, is a serine/threonine kinase, but no protein has been identified as a direct phosphorylation target of TORC1 or TORC2 for nearly all of the TOR-regulated processes in *C. elegans* [28]. p70 ribosomal S6 kinase (S6K), is a downstream target of mTOR and rapamycin, and *in vitro* studies in mammalian

cell culture and other model organisms have revealed that S6K is a direct phosphorylation target of mTORC1 [29]. Reduced translation of the S6K protein is the outcome of mTOR signaling inhibition [30]. Since reducing the expression of the S6K homolog in *C. elegans* (*rsks-1*) alone is sufficient to extend lifespan, this mechanism most likely contributes to lifespan extension via mTOR inhibition. Reduced *S6K/rsks-1* transcription and deletion mutations have been shown to increase *C. elegans* lifespan [31].

In mammalian cell culture, *sgk* inhibits FoxO3 activity [32], and *sgk-1* is thought to reduce lifespan in *C. elegans* through inhibiting DAF-16/FoxO activity [33]. However, some studies have shown that sgk-1 mutations reduce the longevity of *C. elegans* [34-36], controversy the lifespan extension phenotype of *sgk-1* following RNAi knockdown. [33]. Additionally, they proposed that the mechanisms by which *Akt/PKB* and *sgk-1* influence lifespan, stress resistance, and FoxO transcription factor activity in *C. elegans* are distinct. In summary, while *sgk-1* and *age-1* limit lifespan by inhibiting DAF-16/FoxO activity, reduced transcription and deletion mutation of *let-363/TOR* and *S6K/rsks-1* are known to contribute to lifespan extension through mTOR inhibition. However, as mentioned above, processes that occur contrary to this observed lifespan extension phenotype are also included in the literature. Based on all these findings, studies on *C. elegans* aging and longevity remain complex, especially in associating these two processes with signaling pathways. In our current study, after adding *A. vera* extract to the diet of N2 worms, an increase in *sgk-1, age-1* and *let-363* mRNA expression was observed, while a decrease in *rsks-1* mRNA expression was observed. This suggested that the decrease in *rsks-1* expression in the presence of *A. vera* may extend the lifespan of N2 worms, especially through the inhibition of the TORC1 pathway.

According to some research, ROS may cause oxidative stress, which would shorten *C. elegans* life span above a certain concentration range. Furthermore, antioxidants with the capacity to scavenge excess radicals may increase longevity and postpone the negative effects of aging [37]. Many studies have shown that some plant extracts or phytochemicals extracted from plants can support stress tolerance and longevity in *C. elegans* through various signaling pathways [38]. The Aloe contains a large amount of bioactive compounds such as fatty acids, anthraquinones , flavonoids, lectins, terpenoids, mono- and polysaccharides, sterols (campesterol, β-sitosterol), enzymes, tannins, salicylic acid, vitamins and minerals [39]. This highly bioactive component of *A. vera* also causes it to have high antioxidant activity. A study by Ray et al. showed that *A. vera* gel extracts have high antioxidant capacity [40]. In our study, the phytochemicals contained in *A. vera* may have had a stimulating effect on longevity in N2 worms by acting as antioxidants that mainly scavenge ROS.

In conclusion, we showed that *A. vera* gel extracts applied to N2 worms at all concentrations increased fertilization capacity and longevity. Additionally, increases in body length and width of N2 worms were observed in presence of *A. vera*. We observed that *sgk-1, age-1* and *let-363* mRNA expression significantly increased, while *rsks-1* mRNA expression significantly decreased in N2 worms cultured with 2.5mg/ml *A. vera* gel extract. We therefore conclude that it may extend lifespan through a mechanism specifically dependent on mTOR signaling. However, we measured only mRNA and not actual protein levels of IIS and mTOR signaling pathway components. More research is needed on this topic as mRNA levels do not necessarily reflect protein levels. It would also be useful to examine the protein expression levels of IIS and mTOR components, which play an active role in longevity and fertility processes. Our other conclusion is that the phytochemicals found in *A. vera* may extend the lifespan of N2 worms, primarily by acting as antioxidants. Therefore, it would be useful to elucidate these mechanisms with molecular and biochemical experiments in the future. All these observations will provide a new perspective on mammalian life extension through the model organism *C. elegans*.

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Conflict of Interest

The author state that did not have conflict of interests

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