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Ultraviolet-A triggers photoaging in model nematode *Caenorhabditis elegans* in a DAF-16 dependent pathway

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Abstract Ultraviolet radiations (UV) are the primary causative agent for skin aging (photoaging) and cancer, especially UV-A. The mode of action and the molecular mechanism behind the damages caused by UV-A is not well studied, in vivo. The current study was employed to investigate the impact of UV-A exposure using the model organism, *Caenorhabditis elegans*. Analysis of lifespan, healthspan, and other cognitive behaviors were done which was supported by the molecular mechanism. UV-A exposure on collagen damages the synthesis and functioning which has been monitored kinetically using engineered strain, col-19:: GFP. The study results suggested that UV-A accelerated the aging process in an insulin-like signaling pathway dependent manner. Mutant (*daf-2*)-based analysis concrete the observations of the current study. The UV-A exposure affected the usual behavior of the worms like pharyngeal movements and brood size. Quantitative PCR profile of the candidate genes during UV-A exposure suggested that continuous exposure has damaged the neural network of the worms, but the mitochondrial signaling and dietary restriction pathway remain unaffected. Western blot analysis of HSF-1 evidenced the alteration in protein homeostasis in UV-A exposed worms. Outcome of the current study supports our view that *C. elegans* can be used as a model to study photoaging, and the mode of action of UV-A-mediated damages can be elucidated which will pave the way for drug developments against photoaging.

Keywords UV-A · *C. elegans* · Photoaging · Lifespan · IIS pathway

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Introduction

Ultraviolet radiations (UV) are one of the electromagnetic radiations emitted by the Sun. The ozone layer in the atmosphere blocks most of the UV radiations. The major forms of UV include UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). Among these, the ozone layer completely and partially blocks UV-C and UV-B, respectively. But UV-A can penetrate the ozone layer and can reach Earth's crust. According to the World Health Organization (WHO), 90–95 % of UV radiations that reach Earth are UV-A. Mild exposure to UV-A will help in increasing the melanin production in the skin (Watanuki et al. 2014) which enhances the innate immunity. In addition, UV-A is the major factor in producing vitamin D than UV-B (McKenzie et al. 2012).

However, continuous exposure may lead to chronic irreversible damages ranging from sun burns to skin cancer. McKenzie et al. (2009) mentioned that during summer season, there will be enough UV to photosynthesize vitamin D in 1 min in the mid-latitude regions. However,

if the exposure time is further extended to 15 min, it will lead to skin damage (McKenzie et al. 2009). In addition, long-term exposure of skin against UV radiations leads to premature aging or photoaging, which is characterized by wrinkle formation, hyperpigmented lesions or age spots, and decrease in the integrity of the skin (Puglia et al. 2014). Photoaging can be characterized by fragmentation and reduced production of collagen in the skin. Lower doses of UV radiation in the skin affect the activity of collagen (Wang et al. 2014). As the level of UV exposure increases, the collagen that is already present in the extracellular matrix will breakdown, and also, the synthesis of new collagen will be hindered (Pandel et al. 2013).

Exposure to UV-B leads to the degradation of collagen followed by the upregulation of matrix metalloproteinase (MMP) and reactive oxygen species (ROS) production which will result in photoaging (Wang et al. 2014). However, the role of UV-A, in eliciting photoaging, is less studied. As UV-A is 10 to 100 times abundant in natural sunlight and because of its capacity to penetrate the skin more deeply than UV-B (Hung et al. 2015), it is necessary to focus more on UV-A-mediated photoaging process.

All age-related diseases are induced by the universal phenomenon, aging, which can be analyzed by the random but progressive accumulation of damaged cells, tissues, and organs. These are irreversible damages that depend on the genetic factors of an individual (Juckett 2010). In the last two decades, several reports suggest that there is an unexpected periodic pattern in the prevalence and mortality of age-related diseases (Juckett and Rosenberg, 1991). In the current scenario, age-related diseases are predominant in causing death. Death caused by cancers, especially skin cancer, is a major threat to mankind (Juckett 2010).

Model organisms such as mice (Kong et al. 2014) and rats (Barcelos et al. 2014) have been routinely used to study photoaging. UV-A exposure to the skin of nude mice causes pale coloring of the skin that lead to decreased blood supply and eventually to apoptosis and necrosis (Hung et al. 2015). A recent study used hen as a model to check the level of vitamin D in eggs upon exposure to UV radiation (Kuhn et al. 2015). Researchers favor these models because of the ease of identifying the physiological changes in the animals during the course of exposure. However, these studies may take an extended duration. Even though the UV-mediated response was studied in higher model organisms, the molecular mechanism behind the UV-A-mediated photoaging was not yet established.

The model nematode *Caenorhabditis elegans* which are widely used as a model in aging studies (Fawcett et al. 2015; Chondrogianni et al. 2015) were used to study the physiological and molecular changes in a biological system during UV-A exposure. Many natural compounds with antioxidants that have anti-aging activity have been successfully tested in this model (Zheng et al. 2014; Sonani et al. 2014). This microscopic model system is preferred due to its short life cycle and transparent cuticle, which enables to monitor the physiological changes through a microscope. Moreover, it facilitates to study the regulation of single gene, which can make a significant change in lifespan or any other physiological activity, through RNAi-mediated approach (Qian et al. 2015).

In the current study, we tried to understand the mode of action of UV-A exposure upon *C. elegans* by analyzing the changes in physiological and cognitive behaviors in the host, along with the molecular changes. It was observed that the lifespan and healthspan was decreased drastically upon exposure, as UV-A has triggered photoaging in *C. elegans.*

Materials and methods

Nematodes, reagents, and equipments

The wild type *C. elegans* strain (Bristol N2), *col-19*::GFP strain (TP12), and *daf-2* mutant (CB1370) were obtained from *Caenorhabditis* Genetics Center, University of Minnesota, USA. All strains were grown in nematode growth medium (NGM) at 20 °C as described (Brenner 1974). The uracil auxotroph, *E. coli* OP50, was used as food source for *C. elegans*. All experiments were done in triplicates with age-synchronized young adult worms. Synchronization was done by bleaching of gravid adults (Sivamaruthi et al. 2011).

C. elegans were exposed to UV-A at a constant wavelength of 365 nm for 2, 4, and 6 h using a UV transilluminator. All other chemicals used were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Confocal microscopy

Transgenic strain of *C. elegans* after exposure along with control worms were washed separately using M9 buffer and placed in a drop of sodium azide solution on

2 % agarose pad and viewed through Confocal laser scanning microscope (Carl Zeiss, Germany). The intensity profile of GFP was read using the Zen software provided with the microscope (Durai et al. 2011).

Collagen quantification assay

Quantification of collagen inside the worms was done using a Sircol Collagen assay kit method. Briefly, 50 μg of total protein sample was taken and incubated with sircol dye for 30 min. Further, the proteins were collected as pellet through centrifugation, and excess dye was washed off through specific acid salt reagent. The pellet was further dissolved in alkali reagent and checked for absorbance at 555 nm, which was plotted against a standard graph.

Lifespan assay

Lifespan assay was carried out in both solid and liquid conditions as described (Sivamaruthi et al. 2011) with some modifications. In solid assay, a known number of ~20 UV-A-exposed young adults were positioned on solid NGM plates seeded with *Escherichia coli* OP50. Similarly, a known number of UV-A-exposed worms were maintained with M9 buffer and *E. coli* OP50 in a 24-well microtiter plate for liquid assay. The worms were monitored during every 24 h. The worms were transferred to fresh media on every alternate day to replenish food and also to avoid false positive data due to the presence of young ones. Worms were considered as dead when they did not respond to a gentle tap or touch with a platinum wire pick. Nematodes unexposed to UV-A were considered as control.

Pharyngeal pumping assay

To determine the pumping rate, worms (~10 young adults) after exposure to UV-A for 2, 4, or 6 h were placed on NGM plates seeded with *E. coli* OP50. Pharyngeal pumping was observed once at every 24-h interval using a stereomicroscope (Nikon SMZ1000, Japan) for ten consecutive seconds. Worms unexposed to UV-A were considered as control.

Egg laying assay

Similar to the pharyngeal pumping assay, worms (~10 young adults) after exposure to UV-A for 2, 4, or 6 h were placed individually on NGM plates seeded with *E. coli* OP50. The number of eggs laid was counted once at every 24-h interval as described (Kesika et al. 2011). Worms were transferred to new plates after every 24 h. Worms unexposed to UV-A were considered as control. The experiment was carried out in three independent trials.

Drop assay

Drop assay was performed with a method developed by Hilliard et al. (2002) with some modifications. Briefly, worms after exposure to UV-A for 2, 4, or 6 h were allowed to crawl on unseeded NGM plates. Worms unexposed to UV-A was considered as control. A drop of repellent (Glycerol) with ~5–10 nl was kept on the tail of the worm without touching it or disturbing its forward movement. The drop will reach the anterior amphid sensory organ through capillary action. Within 1–3 s, worms will move backward by sensing the repellent. If the worm continues to move forward, then the neural network is probably damaged.

Osmosensation assay

Briefly, a pinch of Bromophenol blue, which acts as an indicator, was mixed with 4 M NaCl solution. A circular ring with ~1 cm diameter was dipped in the solution and placed in the center of an unseeded NGM plate and marked, without tampering the media. A ring was formed, which was visible with the dye, and then, it was allowed to absorb by the plate for 5–10 min. UV-A-exposed worms were placed inside the ring and observed for 10 min along with control. The trial was done independently with all different exposures.

Total RNA isolation and qPCR analysis

Synchronized populations of wild type young adult worms were generated from eggs at 20 °C on the standard food source. Worms were collected from *E. coli* OP50 lawns in M9 buffer at room temperature and washed several times with M9 buffer. Worms were then exposed to UV-A for 2, 4, and 6 h. Worms unexposed to UV-A were kept as control. The experimental worms were washed and treated with TRIzol reagent (RNA X Press reagent, Sigma) for isolating total RNA and were reverse-transcribed using oligodT primer and MultiScribe™ Reverse Transcriptase (Applied Biosystems) enzyme. After first-strand synthesis,

quantitative PCR (qPCR) was carried out to analyze the expression pattern of candidate genes that regulate lifespan and healthspan using gene-specific primers. The expression data was represented as upregulated or downregulated by normalizing the 0-h control values and internal control actin. The sequences of the primers are given in Table 1.

Western blot analysis

To monitor the changes at protein level, total proteins were isolated from the UV-A-exposed samples by following standard protocols. Sixty micrograms of each protein sample (exposed and control) was boiled to break the complex proteins and separated in 12 % SDS-PAGE. A PVDF membrane was used to transfer the proteins from the gel at a constant voltage (15 V) for 3 h. Immunodetection was performed by using specific antibodies against candidate protein. The antibodies used in the present study include rabbit polyclonal antibody raised against heat shock factor of human origin, HSF-1 (Santa Cruz Biotechnology, Inc.) at 1:1000 dilution and monoclonal anti-actin purified mouse immunoglobulin (Sigma-Aldrich) at 1:1000 dilution, followed by exposure to corresponding secondary antibody for 4 h. The membrane was developed by transferring to 1X Alkaline Phosphatase (AP) buffer containing substrate NBT and 5-bromo-4-chloro-3-indolyphosphate until intense bands were observed in the membrane (Durai et al. 2014). Further, quantification of the bands developed was done using ImageLab software.

Statistical analysis

All the experiments were done in triplicates, and one-way ANOVA (SPSS 17) was used to compare the mean values of each treatment. The data were represented as average of three independent experiments. Significant differences between the means of parameters were determined by using the Duncan's test ($p < 0.05$) comparing between the groups control vs treated.

Results

UV-A induces damage in the nematode

UV-A is known to cause direct impact on the outer epidermal layer of humans. Since the model host lacks a proper epidermal layer as that of humans, the preliminary aim was to confirm whether UV-A can cause any damage in the nematode. For this, transgenic strain of collagen tagged with GFP, *col-19*::GFP strain (TP12), was exposed to UV-A for 2, 4, and 6 h. The *col-19* gene is an adult specific marker and is also essential for the normal structure of the alae in adult *C. elegans* (Thein et al. 2003). In this strain, the *col-19* gene is fused with GFP and localized in the cuticle. After exposure, the worms were viewed and imaged using a confocal laser scanning microscope. It was observed that the level of fluorescence was altered significantly upon exposure to UV-A (Fig. 1a). This suggested that UV-A can cause damage to the cuticular layer of the worms. To further confirm this, the level of expression of *col-19* in wild type nematodes during exposure was analyzed. A marked change in the level of expression of *col-19* was observed (Fig. 1b). Additionally, the rate of collagen synthesis after exposure was monitored quantitatively. The reduced levels of collagen indicate that the host lost its capacity to synthesize collagen (Fig. 1c).

Table 1 List of primers

Gene name	Forward primer	Reverse primer
col-19	CACACAAATGCTCCACCAAC	CTGGATTTCCCTTCTGTCCA
daf-2	TCGAGCTCTTCCTACGGTGT	CATCTTGTCCACCACGTGTC
daf-16	TGGTGGAATTCAATCGTGAA	ATGAATATGCTGCCCTCCAG
age-1	ATAGAGCTCCACGGCACTTT	TGTCAAGCACGTTTTCTTCG
utx-1	GCAGAACACCAGCTCATCAG	ATCAACGCCATTCTTCTCGC
egl-8	CGTATCGTTGCGCTTCTCA	AGTAGTGACACAGCGGTTG
egl-30	TCAGAAAGGCGGAAGTGGAT	GGTTCTCGTTGTCACACTCG
dgk-1	GTTGGGGAAGTGGTGCAAAT	GCGAGCTTGGATTGGATGAG
goa-1	TGTTCGATGTGGGAGGTCAA	TCGTGCATTCGGTTTGTTGT

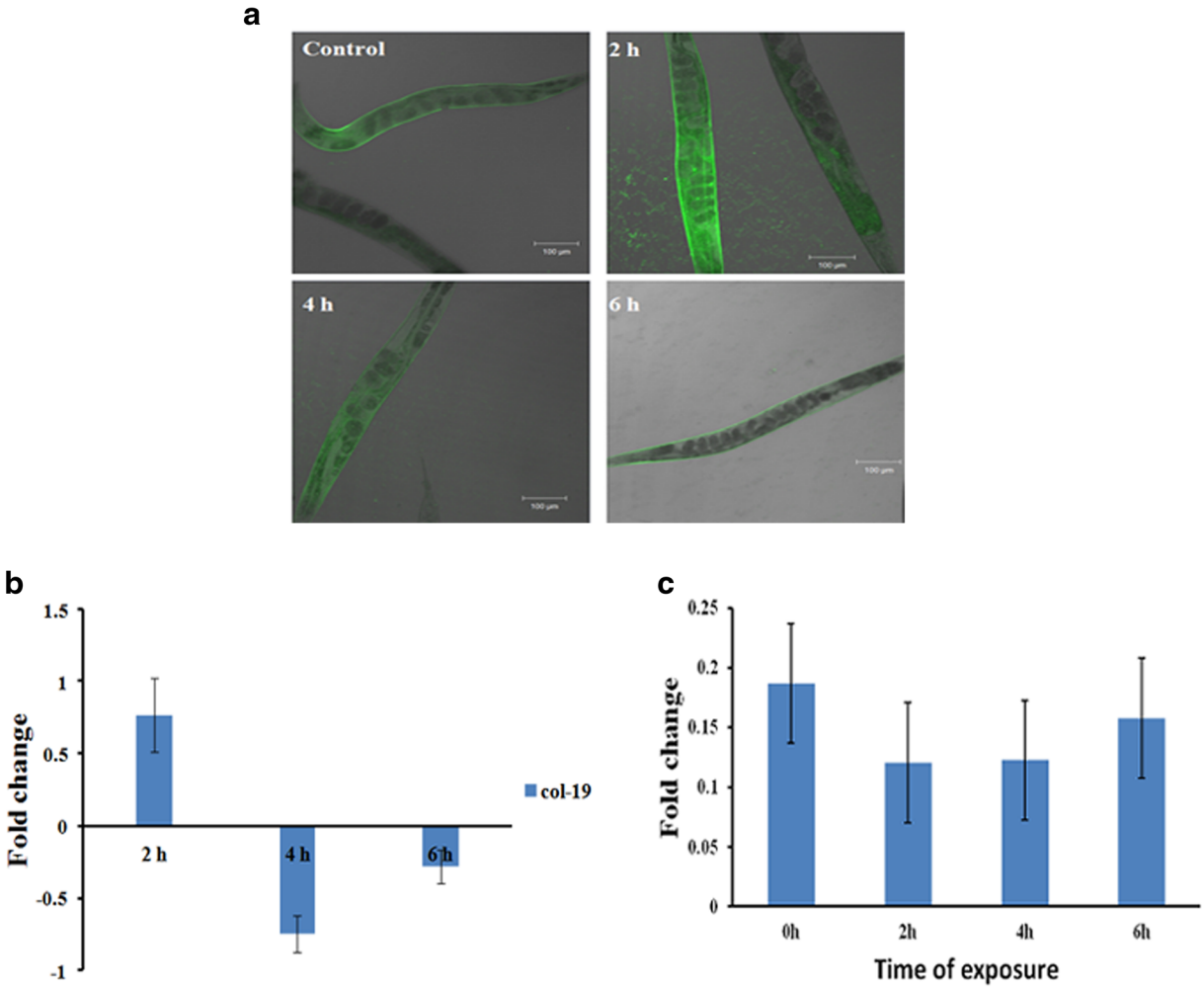

Fig. 1 Effect of UV-A in nematode. **a** Confocal images showing differential regulation of *col-19* in the transgenic strain TP12. **b** qPCR analysis showing altered expression of *col-19* in wild type *C. elegans*. **c** Quantification of collagen inside the worms after exposure showing decrease in the level of collagen

UV-A alters lifespan of *C. elegans*

To understand the physiological damage caused by UV-A to the nematode, the total lifespan of wild type as well as *daf-2* mutants in both solid and liquid media after UV-A exposure was analyzed. DAF-16 is the effector of DAF-2/DAF-16 pathway which regulates the expression of many aging regulating factors in *C. elegans*. DAF-2 phosphorylates DAF-16 and prevents it from integrating into nucleus and activating the regulatory genes. Knockdown of DAF-2 allows the activation of DAF-16, and these mutants were known for their extended lifespan (Murphy and Hu 2013; Sonani et al. 2014).

After exposure, wild type worms survived up to 15, 13, and 14 days in liquid and 15, 15, and 10 days in solid media whereas *daf-2* mutants survived up to 40, 35, and 30 days in liquid and 42, 38, and 33 days in solid media, after 2, 4, and 6 h of UV exposure, respectively (Fig. 2a–d). These observations clearly indicate that UV-A significantly reduced the lifespan of the nematode. Further, to confirm the role of DAF-2/DAF-16 pathway, which is widely known as insulin-like signaling (IIS) pathway, during UV exposure, qPCR analysis of selected candidate genes (*daf-2*, *daf-16*, *age-1*, *utx-1*) were performed. It was observed that *daf-2* and *utx-1* were upregulated during the course of exposure whereas *daf-16* was downregulated. In the case of *age-1*, it was found to be downregulated during the initial hours of exposure, but in 6 h exposed sample, it was slightly upregulated (Fig. 2e). In the case of *daf-2* mutants, *daf-16* got upregulated. But both *age-1* and *utx-1* had a

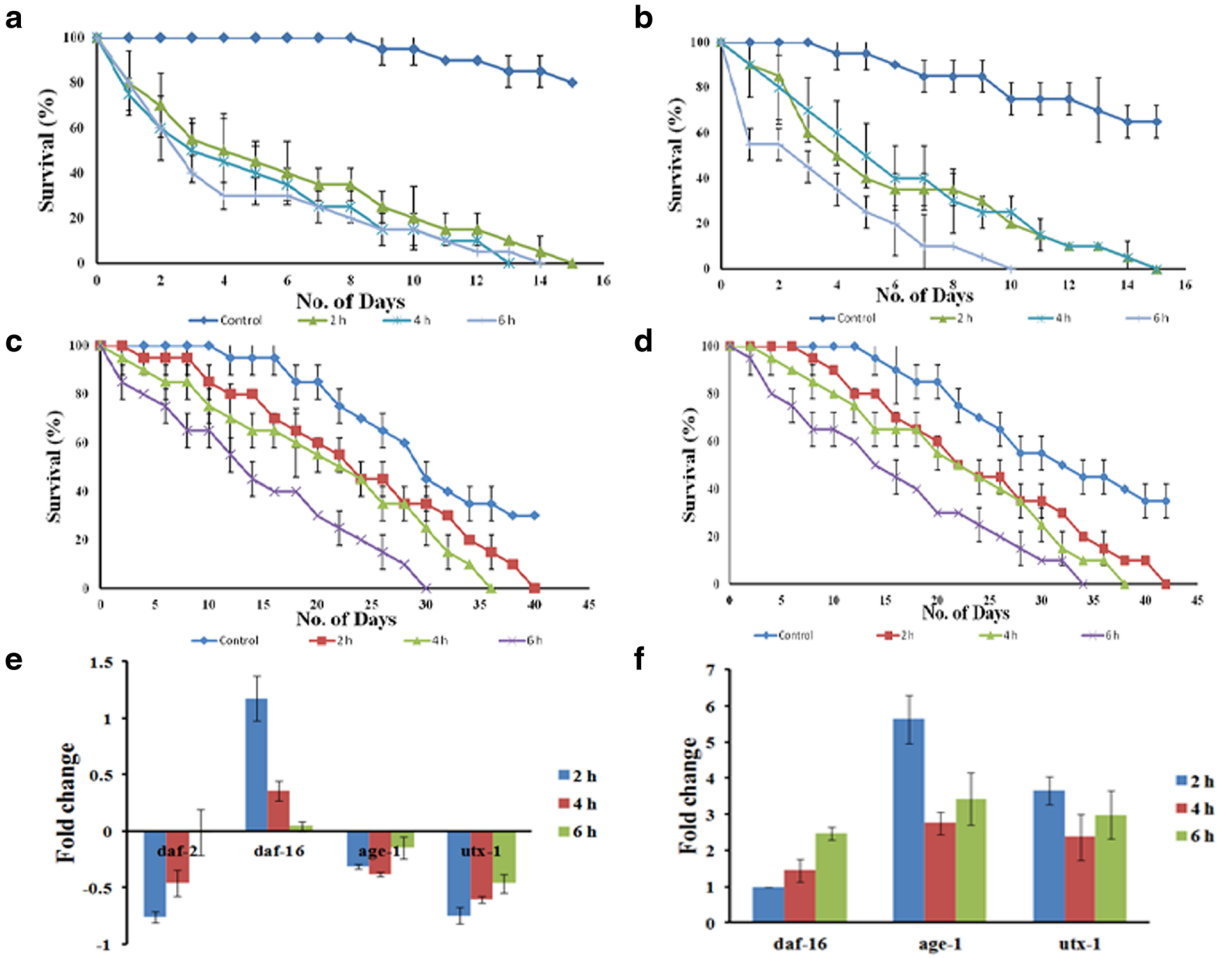

Fig. 2 Effect of UV-A on *C. elegans* lifespan. **a** Lifespan assay of wild type *C. elegans* in liquid media. Worms survived up to 15, 13, and 14 days when exposed to 2, 4, and 6 h of UV-A, respectively ($p < 0.05$). **b** Lifespan assay of wild type *C. elegans* in solid media. Worms survived up to 15, 15, and 10 days when exposed to 2, 4, and 6 h of UV-A, respectively ($p < 0.05$). **c** Lifespan assay of *daf-2* mutant worms in liquid media. Worms survived up to 40, 35, and 30 days when exposed to 2, 4, and 6 h of UV-A, respectively ($p < 0.05$). **d** Lifespan assay of *daf-2* mutant worms in solid media. Worms survived up to 42, 38, and 33 days when exposed to 2, 4, and 6 h of UV-A, respectively ($p < 0.05$). **e** qPCR expression in wild type worms showing upregulation of *daf-2* and *utx-1* and downregulation of *daf-16* (1.18, 0.36, and 0.04 folds in 2, 4, and 6 h, respectively) during the course of exposure. Altered regulation was expressed by *age-1*. **f** qPCR expression in *daf-2* mutant worms showing upregulation of *daf-16* (0.98, 1.46, and 2.48 folds in 2, 4, and 6 h, respectively). However, both *age-1* (5.63, 2.76, and 3.43 folds in 2, 4, and 6 h, respectively) and *utx-1* (3.67, 2.37, and 2.98 folds in 2, 4, and 6 h, respectively) are showing higher fold expression than *daf-16*

higher fold expression than *daf-16* (Fig. 2f). The altered regulation of these genes that regulate lifespan also supported our findings.

UV-A-mediated aging is independent of mitochondrial signaling and dietary restriction

Apart from the IIS pathway, the aging process is mediated by mitochondrial signaling (Lakowski and Hekimi 1996) and calorie restriction process (Yen and Mobbs 2010). Quantitative PCR analysis of *clk-1* and *eat-2* which are the key regulators of these above events were performed to confirm whether the aging process initiated by UV-A is dependent on any of these pathways. It was observed that the level of expression of *clk-1* was downregulated and that of *eat-2* was almost constant during the course of exposure (Fig. 3).

UV-A disrupts the normal healthspan of *C. elegans*

Further, pharyngeal pumping and egg laying were monitored to assess the healthspan of the nematode after

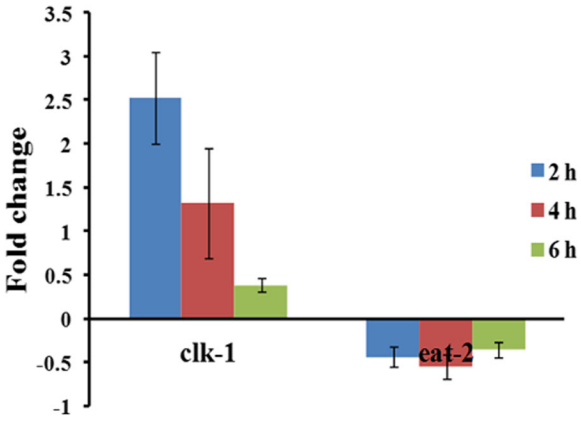

Fig. 3 Differential expression of *clk-1* and *eat-2* upon UV-A exposure in *C. elegans*. The expression of *clk-1* was downregulated during the course of exposure whereas *eat-2* was downregulated consistently

exposure to UV-A. The reduced rates of both parameters suggest that UV-A has a negative impact on the normal survival and health of *C. elegans*. The pharyngeal pumping almost completely seized within 15 days in 2 and 4 h exposed worms whereas in the case of 6 h exposed worms, complete seizure occurred within 7 days (Fig. 4a). In the case of egg laying, the brood size drastically reduced as 227, 166, and 118 in 2, 4, and 6 h exposed worms, respectively, as compared to control which laid 314 eggs (Fig. 4b). The genes necessary for normal healthspan, *egl-8*, *egl-30*, *dgk-1*, and *goa-1* were analyzed during exposure. It was observed that both *egl-8* and *egl-30* were downregulated during the initial hours of exposure, which later got slightly upregulated. In the case of *dgk-1* and *goa-1*, the expression was upregulated during the initial hours which later on got subsided. The expression pattern indicated that UV-A has damaged the normal healthspan of the worms (Fig. 4c).

UV-A damages the neuronal behavior

Since all the activities of the worm are mediated by the 302 neurons in the system, the neural network was analyzed for any damage during UV-A exposure, through drop and osmosensation assays. It was observed that worms exposed to UV-A had caused significant damage to the worm's neural network since the response of the worms towards chemical repellents was altered after exposure (Tables 2 and 3).

HSF-1 regulates the aging mechanism

Western blot analysis was done for HSF-1 to understand the role of DAF-16 pathway in the UV-A-mediated aging process. It was observed that HSF-1 was highly expressed in the 6 h exposed worms, whereas the expression was absent in 4 h exposed worms. In the case of 2 h exposed worms, the expression was meager as compared to control. The elevated expression suggests that UV-A radiation has altered the normal aging mechanism of the nematode (Fig. 5) which was further quantified.

Discussion

Persistent and continuous exposure to UV-A will cause photoaging and skin cancer (MacFarlane and Alonso 2009). Even though, UV-A is considered to be less carcinogenic as compared to UV-B, the former appears to induce photoaging by generating reactive oxygen species, superoxides and other free radicals that subsequently increases the chances of skin cancer (MacFarlane and Alonso 2009; Burke and Wei 2009; Shirai et al. 2015). Mortality rates due to skin-related diseases and cancer are on the rising side, even in this era of modern medicine (Juckett 2010). Almost all of the available sunscreens elicit protection against UV-B (Burke and Wei 2009), even though UV-A is known to reach earth predominantly than UV-B (Hung et al. 2015) and also cause detrimental effects. So there is a far cry need to find strategies to inhibit the action of UV-A on biological systems. *C. elegans* with its immense advantages appear to be a suitable model to study this phenomenon, though the lack of a thick epithelial layer may be considered as a hindrance. The *col-19*::GFP strain was used in order to understand the response of *C. elegans* against UV-A. In humans, collagen is necessary for the strength and elasticity of the skin. However, during aging, collagen probably get degraded and accumulated in the skin, which results in wrinkle formation. Many environmental stresses including UV radiation induces photoaging also appeared to accelerate the wrinkle formation (Chauhan and Shakya 2009) and eventually led to skin cancer (Chiarelli-Neto et al. 2014). In wild type *C. elegans*, the expression of *col-19* begins at late L4 stage at low levels (Hada et al.

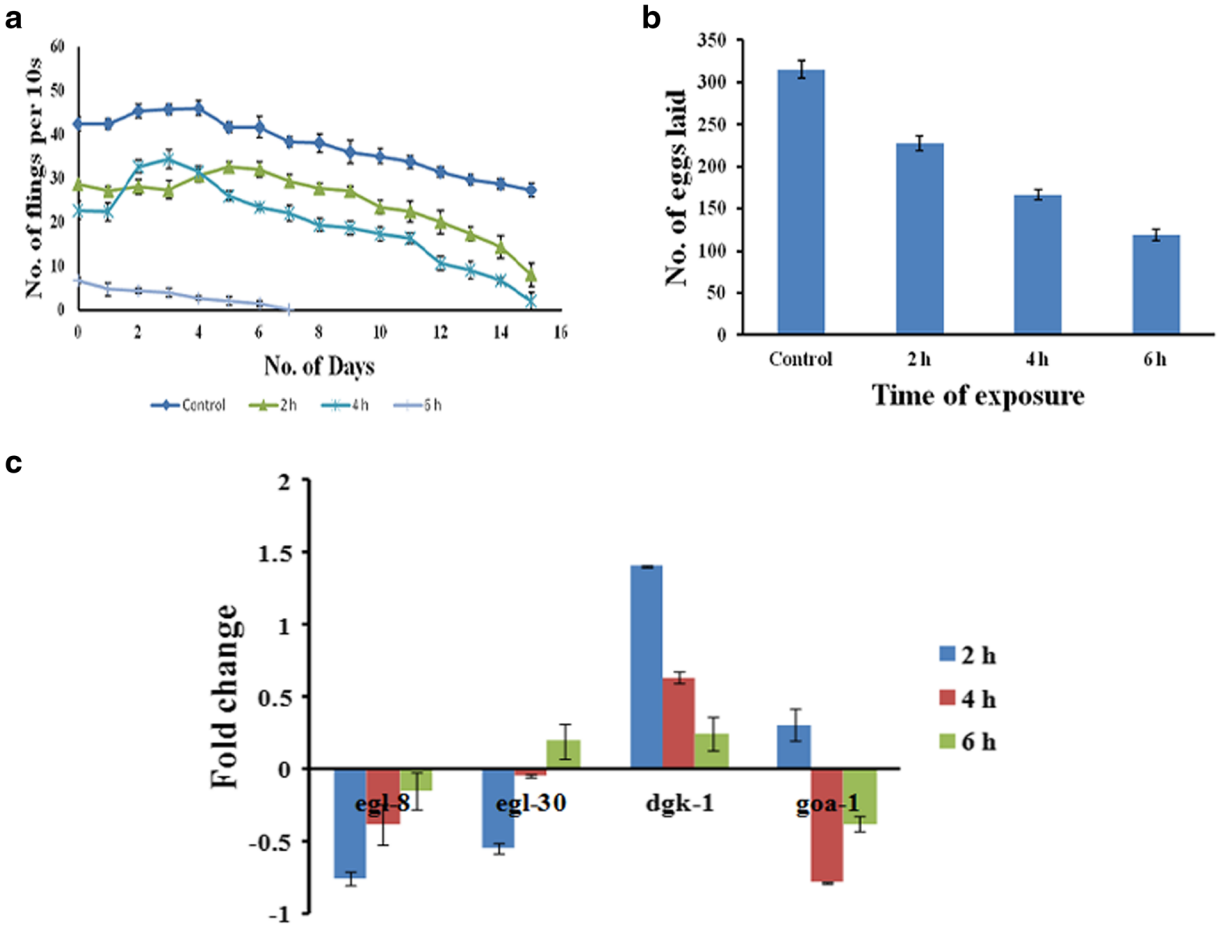

Fig. 4 Evaluation of healthspan in *C. elegans* exposed to UV-A. **a** Pharyngeal pumping assay in *C. elegans.* The pharyngeal movement was reduced in worms exposed to UV-A ($p < 0.05$). **b** Brood size assay in *C. elegans.* The total number of eggs laid was 227, 166, and 118 in 2, 4, and 6 h exposed worms, respectively, when compared to control (314) ($p < 0.05$). **c** qPCR expression of *egl-8* and *egl-30* was found to be downregulated from the initial hours of exposure which showed slight upregulation during the course of exposure. In the case of *dgk-1* and *goa-1*, it was upregulated during the initial hours which was later subsided

2010), and it acts like an adult specific marker (Thein et al. 2003; Li and Paik 2011). Further, it deciphers the accumulation of collagen inside the organism. The increasing accumulation of this gene directly implies that the worm has undergone photoaging upon exposure to UV-A. The qPCR analysis of this gene in wild type nematode has confirmed that UV-A has an impact on the outer epithelial layer of the model host. The collagen production assay, which estimates the level of collagen synthesis during a given period, also supported this view (Fig. 1). Since the worms had undergone aging, it might have lost its ability to synthesize collagen.

Table 2 Differential activity of worms exposed to UV-A against repellent glycerol

Time of exposure	Worms tested	Worms repelled	No response
Control	10	10	0
2 h	10	10	0
4 h	10	8	2
6 h	10	6	4

Any kind of environmental stress will have a potential impact on the lifespan of the nematode (Patananan et al. 2015). Moreover, lifespan analysis is the first and foremost indication in aging analysis (Tissenbaum 2012). The reduced survival of UV-A-exposed worms clearly indicated that worms were susceptible to continuous UV-A exposure and related induced photoaging. The DAF-2/DAF-16 pathway is otherwise called as

Table 3 Differential activity of worms exposed to UV-A when introduced into a high osmotic barrier

	Trial 1		Trial 2		Trial 3	
	Worms introduced	Worms escaped	Worms introduced	Worms escaped	Worms introduced	Worms escaped
Control	10	0	10	0	10	0
2 h	10	0	10	1	10	1
4 h	10	0	10	1	10	2
6 h	10	1	10	1	10	1

insulin-like signaling pathway in *C. elegans*. This regulates the lifespan, normal metabolism, and development (Evans et al. 2008). The altered regulation of the major players in this pathway, *daf-2*, *daf-16*, and *age-1* suggested that the changes in the lifespan of the host was under the influence of the above pathway. The genes *daf-2* and *age-1* are negative regulators of lifespan whereas *daf-16* positively regulates lifespan (Sonani et al. 2014). In the present study, as the time of exposure increased, the level of expression of *daf-2* and *age-1* were also increased; in turn, the expression of *daf-16* decreased. The above data clearly indicate that UV-A radiation had altered the aging mechanism of the host system (Fig. 2a–b, e).

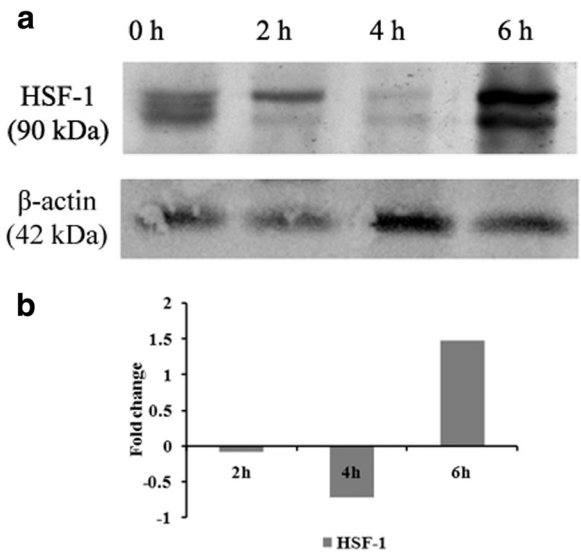

Fig. 5 Western blot analysis of HSF-1 in wild type *C. elegans.* **a** Maximum expression was observed in 6 h exposed worms, whereas the expression was absent in 4 h exposed worms. In the case of 2 h exposed worms, the expression was meager as compared to control. **b** Quantification of the expressed HSF-1 was done using the ImageLab software

To further confirm the role of this pathway, lifespan assay was carried out in *daf-2* mutants after exposure. DAF-2 is the major player which phosphorylates DAF-16 in cytoplasm and prevents its integration into nucleus. When DAF-2 is mutated, DAF-16 will integrate into the nucleus leading to the activation of many genes responsible for lifespan and stress resistance (Murphy and Hu 2013). The decreased survival in mutant worms due to exposure indicates that this pathway was also affected. The qPCR data suggested upregulation of *daf-16* which is expected since *daf-2* is mutated. But *age-1* had a higher fold expression than *daf-16* which suppressed the lifespan of the mutant worms (Fig. 2c–d, f).

UTX-1 constitutes one of the histone demethylases which is conserved in all mammalian species (Swigut and Wysocka 2007). Previous reports stated that RNAi of *utx-1* in *C. elegans* extends lifespan up to 30 % in a DAF-16 dependent manner. The expression of *utx-1* was followed by the increased expression of *daf-2* and other aging mechanisms (Jin et al. 2011) which subsequently regulate the aging process in somatic cells and independent of germ cells (Maures et al. 2011). The increased expression of *utx-1* in wild type worms after exposure suggests that the aging process was accelerated due to exposure whereas in the case of *daf-2* mutants, the regulation seems to be almost constant because of the absence of *daf-2* to mediate the aging process (Fig. 2e-f).

Apart from the IIS pathway, the mitochondrial genes (*clk-1*) (Lakowski and Hekimi 1996) and dietary restriction genes (*eat-2*) (Yen and Mobbs 2010) are known to play a role in the aging process. In *C. elegans*, mutation in *clk-1* gene causes extension of adult lifespan (Hekimi et al. 1998). Mutation of *eat-2* causes reduced pharyngeal pumping and food intake. This leads to calorie restriction and lifespan extension which is proved in

many mammalian systems (Yen and Mobbs 2010). When exposed to UV-A, the level of expression of *clk-1* was found to be decreasing and that of *eat-2* was almost constant during the course of exposure in wild type worms. These results suggested that the aging process accelerated by UV-A is not dependent on mitochondrial signaling or dietary restriction process (Fig. 3).

Similar to lifespan, it is necessary to know the healthspan of the worm during its survival. Healthspan can be defined as the healthy and productive time of an organism that will decline during aging (Tissenbaum 2012). In the current scenario, extending lifespan without extending health will not fulfill the goal of aging research. Some of the recent studies show that phycoeryhtrin isolated from a marine cyanobacterium (Sonani et al. 2014) and cranberry extract (Guha et al. 2013) can extend the healthspan of nematode along with extending lifespan. Here, we have used pharyngeal pumping and egg laying ability of *C. elegans* as the phenotypic readouts to measure healthspan. As the worms undergo aging, the rate of pharyngeal pumping reduced and consequently seized prior to its death (Collins et al. 2008). Here, upon UV-A exposure, the level of pharyngeal pumping has reduced considerably, which indicates that the radiation has accelerated the aging process inside the worms. Similarly, the movement of worms after exposure was altered (data not shown). This may be due to the degradation of muscles that help in movement, which is common during aging (Herndon et al. 2002). Already we have stated that UV-A radiation accumulates degraded collagen in the worm's outer layer. In addition, any kind of stress altered the reproductive ability of most of the living organisms including human (Cizmeli et al. 2013; Duan et al. 2015). In a recent study, it was proved that exposure of *C. elegans* to copper reduced the number of eggs laid (Song et al. 2014). In our study also, the rate of egg laying was reduced which implies that the UV-A irradiation has altered the normal functions taking place inside the model organism (Fig. 4).

Similarly, the expressions of orthologs of G_o ligands, (*egl-8*, *egl-30*, *goa-1*, and *dgk-1*) that are essential for chemotaxis apart from regulation of healthspan of the nematode (Hofer 2005) also substantiate our findings. An ortholog of the heterotrimeric G protein alpha subunit Gq, *egl-30*, (Wang and Wadsworth 2002) and its downstream player, *egl-8*, (Ziegler et al. 2009) in *C. elegans* plays a pivotal role in regulating pharyngeal pumping, egg laying and locomotion (Govorunova et al. 2010). Previous reports suggest that mutation in *goa-1* has a negative impact on egg laying, locomotion, and other normal behaviors of the worm (Matsuki et al. 2006). With the downstream activator *dgk-1*, GOA-1 will inhibit the *egl-30*-mediated DAG pathway (Matsuki et al. 2006; Avery and You 2012). During the initial hours of exposure (2 h), *egl-8*, *egl-30* were downregulated, and *dgk-1*, *goa-1* were upregulated. Alterations in these genes might have affected both pharyngeal pumping and brood size. Even though the regulation was altered in during the later hours of exposure (4 and 6 h), worms were not able to regain the normal health as UV-A had already triggered photoaging processes in *C. elegans* (Fig. 4).

C. elegans has 302 neurons that control, co-ordinate, and monitor the activities inside the system. The decreased level of insulin signaling via *daf-2* RNAi have shown to improve the mechanosensation and alter many key neuronal activities of the host, *C. elegans* (Scerbak et al. 2014). The present study found *daf-2* to be upregulated during UV-A exposure (Fig. 2e) and that led us to observe the changes in their neuronal behavior of the nematodes after UV-A-mediated photoaging. Chemotaxis is a behavior of the worm with which it responds to a particular chemical stimulus. In *C. elegans* benzaldehyde is usually used as a positive control for chemotaxis (Rabinowitch et al. 2014). In a recent study, it was proved that worms show no chemotaxis towards ethanol (Patananan et al. 2015). Our group has previously reported that *C. elegans* avoid *Vibrio alginolyticus* (Durai et al. 2011) whereas it gets attracted to *Cronobacter sakazakii* (Sivamaruthi et al. 2011), which may be by the alteration of serotonin transporter (Sivamaruthi et al. 2015a). When nematodes where exposed to UV-A, there was no significant change in the rate of chemotaxis behavior (data not shown). Hence, neural behaviors of the system were analyzed using drop and osmosensation assays.

ASH neurons in the amphid regions of *C. elegans* play a pivotal role against osmotic stress, volatile chemicals, and also mechanosensation by reversing its movement (Sambongi et al. 1999; Tobin and Bargmann 2004). Hilliard et al. (2002) stated that sensation of a repellent placed on the tail region of the worm and subsequent reversal be mediated by a head-to-tail spatial map. When the worms exposed to UV-A, this network system have partially damaged as the time of exposure increased. This was attributed to the depletion of the activity of ASH neurons (Sambongi et al. 1999) or

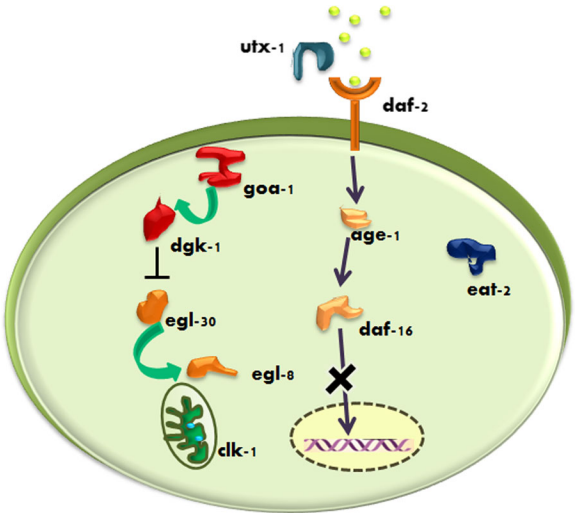

Fig. 6 Schematic representation of initiation of photoaging by UV-A. Exposure to UV-A regulates the pivotal players of the IIS pathway, such as *daf-2* (which is mediated by *utx-1*) along with *age-1*, which in turn mediates the phosphorylation and eventually blocking *daf-16* from integrating into the nucleus by which lifespan was reduced. Also, the candidate players of the DAG pathway were differentially regulated, by which a reduction in healthspan was also observed. However, the other mediators of lifespan regulation, mitochondrial gene *clk-1* and dietery restriction gene *eat-2*, were found unaffected during the course of exposure

the increased activity of PHA and PHB phasmid neurons (Hilliard et al. 2002). The osmosensation assay results also suggest the damage in neural network, since it is also chiefly mediated by ASH neurons (Srinivasan et al. 2008).

In nematodes, the transcription factor HSF-1 activates several proteins which are needed to maintain protein homeostasis during thermal stress (Kenyon 2010). Moreover, this DAF-16 dependent protein plays a pivotal role against oxidative stress (Honda and Honda 1999), invading pathogens and their subcellular components (Sivamaruthi et al. 2015b; JebaMercy et al. 2013), and heat shock (Volovik et al. 2014). All these factors help HSF-1 to play a role in the lifespan extension of the nematode (Kenyon et al. 1993; Antebi 2007). However, the activities of the DAF-16 were downregulated by the IIS pathway receptor, DAF-2 by phosphorylating DAF-16 (Henderson and Johnson 2001) and DDL-1 that is required by HSF-1 for its cellular localization (Chiang et al. 2012). Since both *daf-16* and *hsf-1* are important for the lifespan of the nematode, Western blot analysis of *hsf-1* was done. Both 2 and 4 h exposed worms showed significant downregulation that probably led the reduced survival of the same. The elevated expression suggests that DAF-2 appears to be upregulated during UV-A exposure, which probably suppressed both DAF-16 and HSF-1 (Fig. 5).

Conclusion

This is the first report that UV-A can cause detrimental effects on the model nematode. This was suggested by the expression of collagen inside the body of *C. elegans*. Our results emphasize that UV-A radiations not only decreases lifespan but also damages its healthspan. This response was independent of mitochondrial and dietary restriction pathways (Fig. 6). The damaged neural network system suggests the level of damage induced by the radiation. The studies using *daf*-2 mutants confirmed that IIS pathway appears to be regulated during exposure. Apart from this, the qPCR analysis of candidate genes and Western blot analysis of HSF-1 shows that heat shock factor was also regulated. More proteomic analyses are required to identify the players involved in the process which will pave the way for drug discovery against UV-A-mediated damages.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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