The assessment of the protective impact of spidroin extract against UV-A radiation damage by using earthworms (Aporrectodea caliginosa) as a robust human skin model via macroscopic and histological observations

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Abstract
Numerous studies have confirmed the damage caused by excessive exposure to ultraviolet-A rays. Malignant melanoma and skin cancer are two of the most serious health consequences. Thus, the UV-A protector is intended to protect the skin, especially the two primary layers of skin (epidermis that represents the interface between the body and its surroundings and dermis). Spider silk is the most powerful natural fibre due to its regeneration, biocompatibility, antimicrobial, wound healing, antiseptic, and blood clotting properties. This work targeted to determine the protective effect of spidroin extract against UV-A radiation damage. Earthworms Aporrectodea caliginosa were collected from Assiut University’s farm. Each set of ten earthworms was separated into six groups and placed in a plastic container. Webs of spiders collected from trees and old houses. Spidroin was extracted and utilised in this work to determine the potential effects of topical application on UV-A protection. The experiment is divided into two sections: (1) UV-A exposure and (2) the use of spidroin extract to protect the earthworms from ultraviolet radiation. Two control groups (1, 2) of worms were not received UV-A exposure, and four groups (3, 4, 5, 6) were exposed to UVR-A. In contrast, groups (5, 6) were received spidroin extract before exposure to UV-A. Each group from the groups (3, 4, 5, 6) was exposed for three consecutive days (¼ hour/day, ½ hour/day, and 1 h/day), using a UV-Lamp with a wavelength of 366 nm. The histopathological changes revealed that after 1⁄4 h of UV exposure, the cuticle was swollen with a slightly detached epithelium. The cuticle was down after 1⁄2 h of exposure, and the epidermis was totally damaged and necrosed. After 1 h, the exposure showed destruction of the epidermis in the circular muscle with a loss of muscle filament integrity, varying size, and altered nucleus form, along with mild disintegration of longitudinal muscle. Spidroin extract is critical for earthworm protection against UV-A radiation damage and able to regeneration. For the first time, morphological and histological analysis was established to detect the Spidroin extract evaluated for topical application on earthworms. Earthworms can be considered as a robust human skin model prior to UV-A exposure. It induces a complete protective effect against UV-A radiation damage in earthworms.

Keywords UV-A radiation · Earthworm · Aporrectodea caliginosa · Spidroin extract

Introduction
Undoubtedly, solar radiation is the main source of ultraviolet rays. The majority of those rays reach the Earth’s surface, causing tanning, skin ageing, eye damage, and even immunosuppression (Clydesdale et al. 2001).

Ultraviolet rays are a form of electromagnetic radiation that falls between visible light and x-rays; their wavelength ranges from 10 to 400 nm. Ultraviolet rays are typically classified into three categories based on their interaction with other biological materials: UV-A (longest wavelengths from...
Biologists are particularly taken with the earthworm’s capacity for rapid regeneration. However, some aspects of its rehabilitation remain a mystery (Shao et al. 2020). Numerous scientists are intrigued by the earthworms’ amazing capacity to regenerate (Abd Ellah et al. 2019). Thus, they have researched their regeneration (Allobophora caliginosa) (Rosi and Giovannini 1971; Koritsánszky 1974; Koritsánszky and Hartwig 1974; Galuszka et al. 2015; Abd Ellah et al. 2019).

Following a literature review using earthworms as a model for examining environmental risk factors, only a few studies on earthworms were established (Albro et al. 1997; Misra et al. 2005; Hirano and Tamae 2010; Abd Ellah et al. 2019). Earthworms were used in preclinical studies instead of higher laboratory animals because they are plain, inexpensive, and responsive animal models (Misra et al. 2005).

Spider silk (pre-silk) is a natural product, which is the strongest natural fiber with the greatest elasticity. It was previously utilised for its remarkable biomedical, mechanical, and antiinflammatory properties, clot formation, neural repair, ligament regeneration, and wound healing (Eisoldt et al. 2011; Mirgiani et al. 2012). Spider silk has a wide range of applications; it is tolerated in vitro, cell culture, and in vivo, such as pigs, without eliciting an inflammatory response or adverse body reaction. These findings suggest that it could be utilised in medicine without triggering biocompatibility issues, eventually paving the way for a slew of new opportunities in regenerative medicine and tissue engineering (Scheller et al. 2001). Spiders use their webs to shield their eggs from harmful rays and external stimuli, ensuring their safety and health (Humenik et al. 2011).

The skin is the body’s largest organ, consisting of two distinct layers: epidermis and dermis. The ectodermal epidermis serves as the interface between the body and the environment. Thus, epidermal biological and physical properties play a significant role in the resistance of the epidermis to environmental stressors such as infectious diseases, chemical agents, and UV resistance (Slominski and Wortmans 2000; Lowe 2006; Fuchs 2007; Slominski et al. 2012).

The primary objective of this analysis was to determine the efficacy of spidroin extract in protecting against the harmful effects of UV-A radiation. To the best of our knowledge, this is the first article to investigate the protective effect of spidroin extract against UV-A harm using earthworms as a human skin model.

Materials and methods

Earthworms’ collection

Earthworms were collected by digging and hand sorting soil from Assiut University’s farm grassland. Earthworms
were kept in perforated plastic boxes to allow ventilation. The collected samples were transferred to the laboratory of the Zoology Department, Faculty of Science, Assiut University. The earthworms were then identified and separated according to their species. According to keys, the earthworms (Aporrectodea caliginosa) were isolated from their substrates and other earthworm species (Brinkhurst 1966; Brinkhurst and Jamieson 1971; Zorn et al. 2008; Yousefi et al. 2009; Plisko 2010). They were kept under laboratory conditions (25–28 °C, 12 h light/12 h darkness) and fed daily cow manure and leaves for two weeks before the studies to acclimate to the laboratory setting after being placed in perforated small plastic boxes with substrate wet filter paper.

Earthworms regeneration

Similarly, earthworms (Aporrectodea caliginosa) were divided into six groups (each of 10 worms); groups (1, 3 & 5) were left without any intervention, and groups (2, 4 & 6) were split in the anterior part of the skin (front ten segments) with a disposable scalpel to inflict injury. Simultaneously, group 2 (Control cut) earthworms were left to natural healing (untreated control). Visual examination of wound healing over 12 days was used to determine the presence of residual worms in the control group.

Collection of spider webs and Spidroin extract

Spider (Araneus diadematus and Tegenaria domestica) webs or silks were collected from trees and old buildings using a stick, as previously performed (Barrion and Litsinger 1995). Species were identified by keys and articles that recognised spiders to avoid any trapped plants, insects, dust, and other extraneous items. We washed the gathered webs several times and then allowed them to dry at room temperature. These spider webs have been preserved in tightly sealed containers for further usage.

According to an updated procedure, spidroin was extracted using Sodium hydroxide solution (Lateef et al. 2016). In a nutshell, 1 g of spider silk was spread across 10 mL of 0.1 M NaOH. The dispersion was heated at 90 °C with continuous stirring for 1 h and then cooled (Abd Ellah et al. 2019). The resulting dispersion was centrifuged at 5000 rpm for 30 min to clear any residual spider silk. The supernatant was collected and centrifuged at 5000 rpm for an additional 30 min. The final supernatant containing the spider silk extract (more precisely, spidroin protein) was refrigerated for later use.

Spidroin protein quantitation

Bradford protein assay was used to quantify the total protein content of the extract (Bradford 1976). The unknown protein samples were combined with Bradford reagent (Coomassie Brilliant Blue 0.01% in 4.7% ethanol and 8.5% phosphoric acid, 2.5 ml) and incubated for 15 min at room temperature. The blue-coloured solutions were spectrophotometrically compared to a similarly treated blank at 595 nm. Triplicates of the samples were tested. The unknown samples’ content was measured using standard BSA solutions (5–50 g/100 l) to create a calibration curve.

Experimental setup and exposure to UV-A radiation

Earthworms were divided into six groups (10 Earthworms per set): Group 1 (control) and groups 3 and 5 were left without intervention, while the other three groups (2, 4, and 6) were subjected to a sterile-scalpel cut or amputation in the skin of the front part (the first ten body segments). Worms from group 2 (control cut) were left to regenerate naturally. Worms from Groups 3 and 4 were exposed to UV-A using a 366 nm peak emission UV-Lamp (ULTRA-VIOLET Products, Inc., Santa Gabriel, California, USA model UVL-56). The typical intensity is 750 µW per cm². Dose (J/cm²) = intensity (W/cm²) × times(s)% 1000 = 750 × 60 × 6 0%1000 = 2700 J/cm².

The UVR-A supply was installed 30 cm above the experimental area. Worms from Groups 5 and 6 were treated with spidroin extract prior to UV-A exposure. Spidroin extract was used for three consecutive days once a day. The remaining worms in group 2 had their wound healing assessed visually over 12 days. We applied the spidroin extract using a spray bottle by covering the whole worm body. Each group received irradiation for 15 min per day, 30 min per day, and 1 h per each group for three days and three container replicates per group (Kligman et al. 1983; Häkkinen and Oikari 2004). UVR-A effects were observed in plastic containers (20 cm * 20 cm) containing 2 mm buffered saline (0.01 M, pH 7.4) or moist filter paper with buffered saline.

Histological investigations

After three days, tissues were collected from three worms per group for histological examination, while wound healing was visually assessed for the remaining worms in Group 2 (control cut) over 12 days. Overnight fixation in 10% neutral buffer formalin was performed on the samples (pH 7.2). Following a thorough cleaning with water, the samples are dehydrated in increasing concentrations of ethanol, then embedded in methyl benzate and finally in melted paraffin wax. Tissue samples were carved into paraffin blocks using a microtome. The tissue section was 5 µm. A 5–6 µm thick paraffin piece was cut and put on glass slides before being dried in an incubator. Sections were stained with Eosin and Hematoxylin for general histological inspection by light microscope after deparaffinisation and rehydration (Drury and Wallington 1980).
The experiment of six groups (control and exposed groups) was repeated on three occasions. One-way ANOVA examined the significance of differences between the control groups and the treated groups. A $P < 0.05$ or 0.01 degree of significance was recognized as essential or highly relevant. Statistical prisms were used.

Results

Macroscopic observation of earthworms changes during regeneration

Regeneration was observed in the anterior earthworm (*Aporrectodea caliginosa*). Group 1 was left without any interference. Group 2 on zero h after haemorrhage, amputation, redness, oedema, and exudation around the wound region took more than 12 days for regeneration; these findings suggest that the worm's ability to survive and regenerate is harmed when a part of it is cut. Cutworms fared better than worms exposed to UV-A in terms of survival and regeneration in group 2 (Fig. 1). Groups 5 and 6 were received the spidroin extract before exposure to UV-A. Group (5) was not damaged. Group (6) coagulated the fastest and demonstrated complete wound closure in 5 days (Fig. 2B &C). Earthworms were divided into 6 groups. Group 1(control), group 2 (regenerated control), groups 3 and 4 were exposed to UV-A (Fig. 2A). While groups 5 and 6 were sprayed with spidroin extract before being exposed to UV-A (Figs. 3, 4, 5).
Histological study of earthworms

On the 12th day of worm regeneration, histological examination was performed at the amputation site without obtaining the spidroin extract. Histological observation of the cross-section of the body wall of earthworms in the control group demonstrated the normal earthworm architecture of the cuticle (C), the epidermis (Ep), circular muscle (CM), and longitudinal muscle (LM), as shown by hematoxylin and Eosin staining (Fig. 6A). For group (3), the histopathological changes after 1/4 h of UV exposure, the cuticle was swollen with a slightly detached epithelium. Further, the epidermis displayed cloudy swelling and progressive epidermis hypertrophy (Fig. 6B). Light epithelial layer desquamation was observed after 1/2 h of exposure. The cuticle was down after 1/2 h of exposure, and the epidermis was totally damaged and necrosed (Fig. 6C). Total epithelial necrosis and desquamation were detected after 1 h of exposure. After 1 h, the exposure showed destruction of the epidermis in the circular muscle with a loss of muscle filament integrity, varying size, and altered nucleus form, along with mild disintegration of longitudinal muscle (Fig. 6D).

Tissue structure of Aporrectodea caliginosa in the control group (2) showed the normal structure of earthworm: wall cuticle (C), the epidermis (Ep) arrow, circular muscle (CM), and longitudinal muscle (LM), after regeneration and wound healing (Fig. 7A). Histopathological changes in the group (4) after 1/4 hour of exposure revealed cloudy swelling and progressive hypertrophy of the epidermis (arrow) (Fig. 7B). After 1/2 hour of exposure, the cuticle showed a break down, and the epidermis was completely damaged and necrosed (Fig. 7C). The epidermis was destroyed, with necrosis to circular muscle and mild disintegration of longitudinal muscle after 1 h of exposure (Figs. 7A, D & J and 7D).

In the group (5) (Figs. 2A, E, and H) and group (6) (Figs. 2C, F, & I), the body wall revealed the intact structure of epidermal, circular, and longitudinal muscles, indicating that spidroin extract did not promote the protective effect against UV-A radiation damage in earthworms. It was thoroughly covered in spidroin extract before being exposed to UV-A. The skin of a poecilodromus caliginosa earthworms receiving the spidroin extract did not show any considerable differences from that of control groups (1 & 2).

Mortality of earthworms after exposed to UV-A

Findings confirm that exposure to UV-A radiation negatively affects worms and their ability to regenerate. Earthworms exposed to UV-A were about 83.34, 93.33, 56.67, 53.34% in groups (3, 4, 5 & 6) (Fig. 5); suggesting that the UV-A dosage exposure and the significant mortality of earthworms were significant (P < 0.01) and extremely significant (P < 0.0001).

Discussion

The outcomes of this research demonstrated that: (a) UV-A exposure can have detrimental effects on earthworms, and (b) spidroin extract protects against UV-A damage, but it is used in promoting wound healing.

Regarding the effects of phototoxicity, the contribution of different groups in the present study showed that exposed earthworms to UV-A caused the damage; this was the product of the work on earthworm Metaphire Posthuma (Chuang et al. 2004; Misra et al. 2005), who worked on three species of earthworms (Amynthas gracilis, Pontoscolex corethrurus, and Metaphire Posthuma) which was explained the destructive impact of ultraviolet radiation on earthworms. In addition, some researchers addressed the health effects of solar UV exposure, which is complex and dependent on different sociodemographic variables, including skin colour (Rendell et al. 2020). Earthworms (Lumbricus castaneus) were exposed to UV-C for 15 min each day showed slight alterations (Abd El-Aziz and Ali 2021). These changes are at their peak after 30 min each day. To determine the impact of UV-C radiation on earthworms, the complete absence of cell connections in earthworms with sloughed tissue was demonstrated using SEM examination. It was discovered that a long-term exposure to UV-C radiation resulted in serious damage and an increase in cancer risk. The death rate of both earthworms has risen.

Histological examination of the control group revealed normal earthworm skin composed of the cuticle, epidermis, circular and longitudinal muscles for groups that were left untreated with spidroin extract following wound induction (Groups 3 and 4). The effect of various doses of ultraviolet radiation on earthworms, resulting in pathogenesis, was investigated in this research. After 15 min of exposure to UV-A radiation, the cuticle swelled with a slightly detached epithelium. Additionally, they demonstrated cloudy swelling and progressive hypertrophy of the epidermis in this group; these findings corroborate previous evidence regarding the influence of UVR-A. This evidence is similar to (Albro et al. 1997; Chuang et al. 2004; Misra et al. 2005; Herndon et al. 2018). (Herndon et al. 2018) stated that UVR hardens the skin and causes it to lose its elasticity. After 30 min of exposure, we observed mild desquamation of the epithelial layer.

The cuticle was completely destroyed, and the epidermis was fully necrosed. After 60 min of exposure, complete necrosis and desquamation with epithelium separation were observed. The epidermis degraded, followed by necrosis in the circular muscle, characterised by irregular loss of muscular filament integrity and altered nucleic form with mild longitudinal disintegration. According to these findings, exposure to ultraviolet rays causes chronic ageing, damage to the immune system, and skin cancer (Matsumura
and Ananthaswamy 2004). (Lan 2019) confirmed that UVB chronic exposure (3 times a week for 8 weeks) has significant effects on the skin at high temperatures (i.e., dermal thickness). (Coelho et al. 2009) noted an improvement in human skin pigmentation following one or more UV exposures. UVB exposure has been shown to retard development and decrease survival rates in both the short and long terms (Alves et al. 2020). (Chen 2011) discovered that earthworms could regenerate immediately after a portion of the worm is cut off.

Nobody can deny that direct sunlight exposure is one of the most harmful causes for humans and living organisms since a variety of electromagnetic spectrums like ultraviolet rays are included in the sunlight. Since the skin is the largest organ in the human body and is most vulnerable to ultraviolet rays, it provides critical defence against environmental damage to the human body, leading to skin ageing, dryness, and carcinogenic changes in the dermal layer (Solano 2020). Consequently, sunscreens must be used to guard against ultraviolet damage. In this research, we used the earthworm
as a human skin model due to the existence of various triene and tetaene sterols in its skin that are similar to those found in humans, resulting in earthworm sensitivity to UV-R-A (Albro et al. 1997; Misra et al. 2005; Abd Ellah et al. 2019; Abd El-Aziz and Ali 2021). In the present work, the mortality rate was 83.34, 93.33, 56.67, and 53.34% in groups 3, 4, and 6, respectively.

Fig. 4 Macroscopic observation of (A): the earthworms of group(4) after UV-A exposure,(B), the earthworms of group(5) after UV-A exposure,(C): the earthworms of group(6) after UV-A exposure. Photomicrograph of: Tissue structure of (Aporrectodea caliginosa) (D,E), group(4)(E), group(5) and (F), group(6): cuticle (C), epidermis (Ep) arrow, circular muscle (C.M.) and longitudinal muscle (L.M.). H&E. bar = 50 µm

Fig. 5 Mortality of earthworms (Aporrectodea caliginosa) after UV-A exposure: (A, B, C) group(3) and (D, E and F) group(4). Shows significant mortality after exposure to UV-A (one-way ANOVA, N = 10, P < 0.001)
According to the findings, spidroin extract protects against UV-A damage and spider silk proteins, which exhibit substantial antimicrobial activity, can contribute to wound healing. (Abd Ellah et al. 2019) worked on spidroin gel (0.6% CP934), which can wound healing and effectively inhibit the growth of isolated methicillin-sensitive clinical bacteria. It is worth noting that the ultraviolet defence was shown to prevent high levels of UV radiation from the skin and other organisms (Lucas et al. 2006). In contrast, vitamin D production essential for bone health requires a moderate degree of UV exposure, but not for a long time.

**Conclusion**

Regarding the results of the present investigation, it can be concluded that spidroin extract can be used for topical application on earthworms *A. caliginosa* before exposure to UV-A. The spidroin extract is a complete protective impact against UV-A radiation damage in earthworms. Furthermore, the histological study showed that the inflammatory cells vanished. Exposure to UV –A caused the mortality to be about 83.34%, 93.33%, 56.67%, and 53.34% in groups (3, 4, 5, and 6), respectively. These findings documented the efficacy of spidroin extract in protecting against UV-A exposure and promoting wound healing; however, future research using mammalian experimental models will be required to use these findings.

**Significance statement**

In the present investigation, the results corroborate previous evidence on the influence of UV-A radiation damage that can be beneficial for a modifiable risk factor for ageing, immune system damage, and melanoma. This study helps the researcher uncover the critical role of UV-A exposure in causing previously unknown health risks, and the protective impact of spidroin extract against UV-A radiation damage.

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Author contribution FA and MS conceived and designed research. AE1 and AE2 revised the manuscript. FA, MS, and AT wrote the manuscript. All authors read and approved the manuscript.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval This chapter does not contain any experiments involving human participants or animals performed by any of the authors.

Consent for publication Not applicable.

Consent to participate Not applicable.

Informed consent This study does not include any studies that involve informed consent.

Conflict of interest The authors declare no competing interests.

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