MEDICAL SCIENCE

Beneficial role of Vitamin C on radiation-induced skin cellular injury

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ABSTRACT
Maintaining the health of your skin is greatly aided by vitamin C. Along with other supplements, vitamin C extract has been added as a dietary supplement because it can lessen the harmful compounds' tendency to oxidize. In this work, the potential protective effects of vitamin C were investigated in relation to the histological and histochemical alterations in the skin of rats following exposure to gamma radiation. 33 mature male albino rats served as the subjects for the current study. There were three equal groups formed from them. The control group received no medical treatment. Animals in the radiotherapy group were given one dose of gamma radiation. Rats in the third group were administered Vitamin C extract daily at a dose of 20 mg/kg - body weight one week prior to and one week following irradiation. Various stains were used to examine the histopathological and histochemical alterations in skin tissue. Gamma radiation-exposed rats' skin underwent a number of histological and histochemical alterations. Administering vitamin C extract helped to improve these changes. The current study demonstrated Vitamin C's curative and preventive effects on albino rats' skin against radiation-induced skin damage.

Keywords: Vitamin C, Skin injury, Radiation.

1. INTRODUCTION
For humans, vitamin C is a necessary nutrient. In biological fluids, vitamin C functions as a strong water-soluble antioxidant. Ascorbic is a name formed from a scorbutic. Ancient Egyptians, Greeks and Romans as well as people involved in the 13th century Crusade all described this disease (Humbert et al., 2018). The skin's distinctive form reflects the fact that its main purpose is to protect the body from the environment's antigens. The skin is made up of

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two layers: The inner dermal layer, which assures strength and suppleness and supplies the epidermis with nutrients and the outer epidermal layer, which is highly cellular and serves as a barrier. Vitamin C is found in high concentrations in normal skin, where it supports a number of well-known and significant functions, including promoting collagen formation and aiding in antioxidant defense against UV-induced photo damage (Pullar et al., 2017).

One of the often-utilized forms of cancer treatment is radiotherapy, although ionizing radiation frequently causes damage to the nearby healthy tissues. The most frequent side effect of radiotherapy is radiation-induced skin damage. The orbital electrons that surround the nucleus can be displaced by ionizing radiation because it has enough energy. DNA damage is caused by this displacement action in living tissues both directly and indirectly (Tabarraei et al., 2014). DNA strand breakage causes direct damage, while the production of ROS (reactive oxygen species) or free radicals causes indirect damage. Ionizing radiation exposure causes cellular and metabolic damage in the beginning thanks to ROS. Then, subsequently, the morphological manifestation and organ failure show themselves (Alizadeh et al., 2015).

Utilizing antioxidants can prevent or lessen the toxicity of free radicals, which protects against radiation. Carbohydrates, proteins, lipids and nucleic acids are examples of essential components in the body that vitamin C can guard against harm from free radicals and ROS that can be produced during normal metabolism and from exposure to toxins (Keser et al., 2012). Ascorbic acid appears to have radio protective properties due to its interactions with radiation-induced free radicals. After receiving whole-body gamma radiation, ascorbic acid helped the mice repair their wounds faster and prevented radiation sickness. It also reduced mortality (Duchesne et al., 1975).

Despite the fact that there have been an increasing number of studies on the protective mechanisms for radiation-induced skin damage, clinical treatment currently focuses primarily on antioxidant, anti-inflammatory and cyto-protective measures, including the use of vitamins, hormones, Chinese medicine, as well as the elimination of free radicals (El-Missiry et al., 2007; Sinha et al., 2011; Salam & Shaffie, 2023). This research of vitamin C's radio protective properties on radiation-induced skin damage in rats was the goal of the study.

2. METHODS

The PSA University Ethical Committee accepted the Animal Research Guideline for the Use and Care of Animals in Research, which we adhered to in our study, Al-Kharj (SCBR-045-2023). The study, which lasted from April 2022 to February 2023, was experimental. We bought vitamin C extract from Aldrich- Sigma Inc. in the USA. The needed quantity of the crushed tablets was then dissolved in distilled water. Then, it was given orally to rats for a period of one week prior to and one week following their exposure to radiation at a dose of 20 mg/kg- body weight each day. Biological Products and Vaccines in Egypt provided a total of 33 (130 ± 5 gm) male Swiss albino rats. They were monitored for 15 days while they acclimated in the lab. They were kept in plastic cages in a group setting under normal lighting, temperature, ventilation and humidity conditions.

Gamma Cell-40 was used in the irradiation process, which was witnessed by Radiation Research and Technology Center in Egypt. The Canadian Atomic Energy Commission produces the caesium-137 irradiation device known as the gamma cell-40. The apparatus offers total operator protection while enabling uniform Gamma-irradiation of small animals or biological samples. 3Gy of radiation was administered as a single dose at a rate of 0.54 Gy/min. At an 80 cm distance from the source to the surface, the radiation dose level was 3Gy (Wang et al., 2013).

Three groups of the test animals were created at random; 11 animals in each group. Normal, healthy rats in the control group received no therapy. Rats in the radiotherapy group were given a single dosage of 3 Gy of gamma radiation. One week prior to and one week following irradiation, the last group of rats in this group received daily treatment with Vitamin C at a level of 20 mg/kg body weight. The animals were kept on a strict fast throughout the administration, given water as needed and then put to sleep with pentobarbital sodium (35 mg/kg, i.p.) seven days after the radiation treatment. The animals were subsequently put to death by cervical dislocation. Skin samples that had been extracted were fixed in 10% neutral formalin solution.

Standard processing was followed by the creation of 4-m-thick paraffin slices for the histological and histochemical examinations using hematoxylin and eosin. Conversely, polysaccharides and collagen fibers were identified using periodic acid Schiff's (PAS) reagent and Mallory's trichrome stain, respectively (Chang et al., 2013). Additionally, the mercury bromophenol blue technique and toluidine blue stain was employed to determine total proteins and mast cell infiltration, respectively. Finally, using Feulgen's approach, DNA material was found. Using the micro image analyzer, the optical density (Pexil) of total protein was examined. Optically Transparent is a MOT. The mean and standard deviations were used to express all values (SD). The statistical analysis was performed using a student's t-test and the statistics package SPSS 13.0.

3. RESULTS

The skin sections from the control group exhibited normal histological characteristics of the epidermis and dermis, including thin epithelium, evenly spaced glands and undamaged hair follicles in the dermis. Four layers of keratinocytes made up the epidermis. There were many connective tissue cells capillaries and, in the dermis' papillary layer. In contrast, a dense fibrous connective tissue made up the inner reticular layer. The arrector pili muscle encircled the sebaceous, sweat and hair follicles in the dermis (Figure 3A, 4A, 5A).

The skin samples from the radiation group displayed a wide range of pathological conditions. Epidermal cells stopped growing, sebaceous glands and hair follicles disappeared, dermal cells swelled and collagen fibers became edematous (Figure 3B, 4B, 5B). Epidermal cells had nuclear pyknosis and karyolysis, corneum separation and an unorganized papillary layer. Skin samples from the Vitamin C Group and radiotherapy showed some epidermal and dermal structure that had been restored to its normal tissue pattern.

Images of the toluidine blue stained slice in the control group showed some mast cell infiltration (Figure 4A). However, the infiltration in the radiation group was minimal (Figure 3C, 4C). Compared to the radiation group, a moderate mast cell infiltration was found in the third group (Figure 4C, 5C). The examination of the skin sections treated with periodic acid and the histochemical analysis of the skin in the control group; PAS stain revealed a consistent distribution of PAS +ve materials. The basal lamina of the epidermis and the papillary and reticular layers of the dermis had a moderate staining affinity (Figure 1, 2, 4A, 4B) (Table 1).

The basal lamina of the epidermis, as well as the dermal papillary and reticular layers, displayed a modest PAS reaction in the radiotherapy group (Figure 4A, 4B). Compared to the Radiotherapy group, the staining affinity of the epidermal basal lamina and the dermal reticular and papillary layers was mild in the third group of animals (Figure 4B, 4D) (Table 1). In the skin slices from the control group that had been stained with the Feulgen stain, DNA granules of a magenta hue indicated a normal distribution of DNA content in the nuclei of dermal and epidermal layer cells (Figure 5A). DNA content was found to have significantly increased in the epidermal and dermal layer nuclei of radiotherapy group animal tissues (Figure 5B). DNA content in epidermal and dermal cell nuclei appeared to be more or less normal in the vitamin C-treated group (Figure 5D) (Table 1).

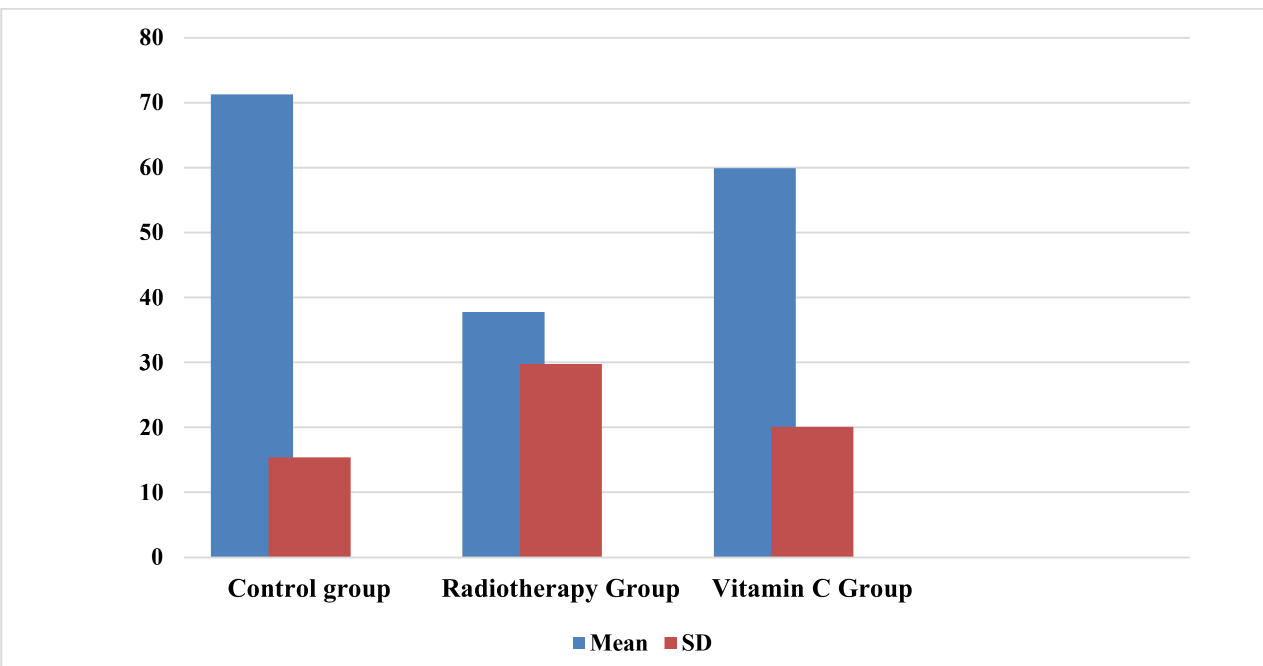

Figure 1 Displaying DNA content measured by MOT in the skin of different groups

Table 1 Demonstrating DNA content in the skin of the control and treatment groups and MOT values of PAS +ve materials

		Control Group	Radiotherapy Group	Vitamin C Group
DNA content	Mean	71.3	37.8	59.88
	SD	15.38	29.77	20.11
	%	4	3	-13.90
	t Test		0.001	0.002
PAS +ve	Mean	89.99	87.43	89.14
	SD	19.23	34.87	15.77
	%		-19.33	-633
	Mean	97.34	98.9	95.04

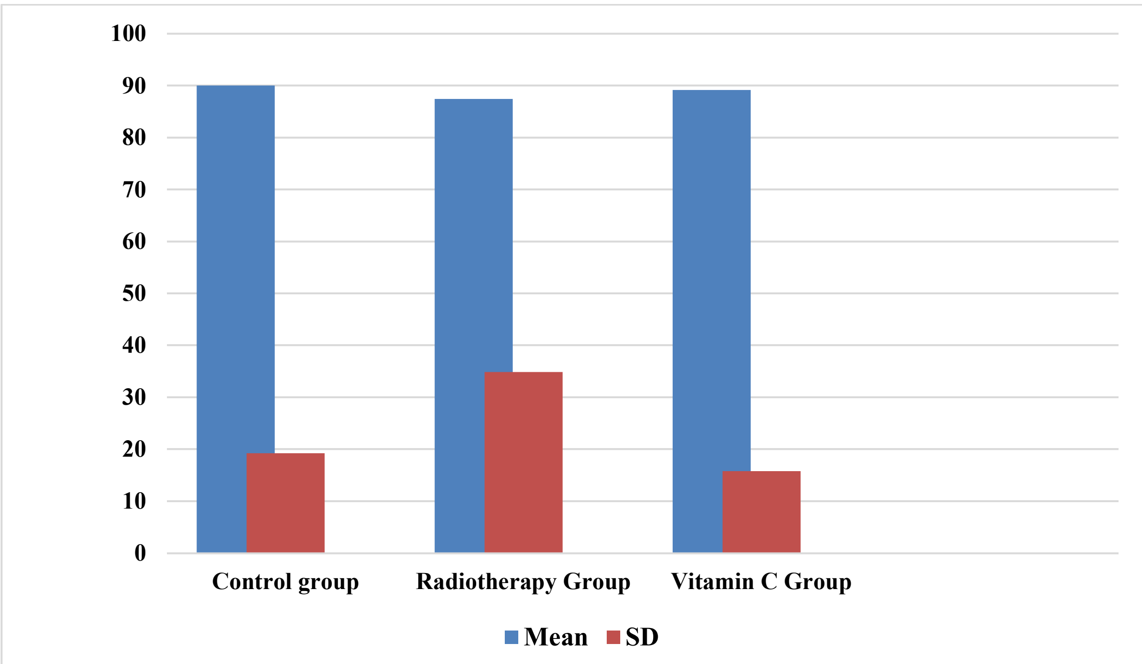

Figure 2 Displaying MOT values of PAS+ve materials in the treated and control groups' skin

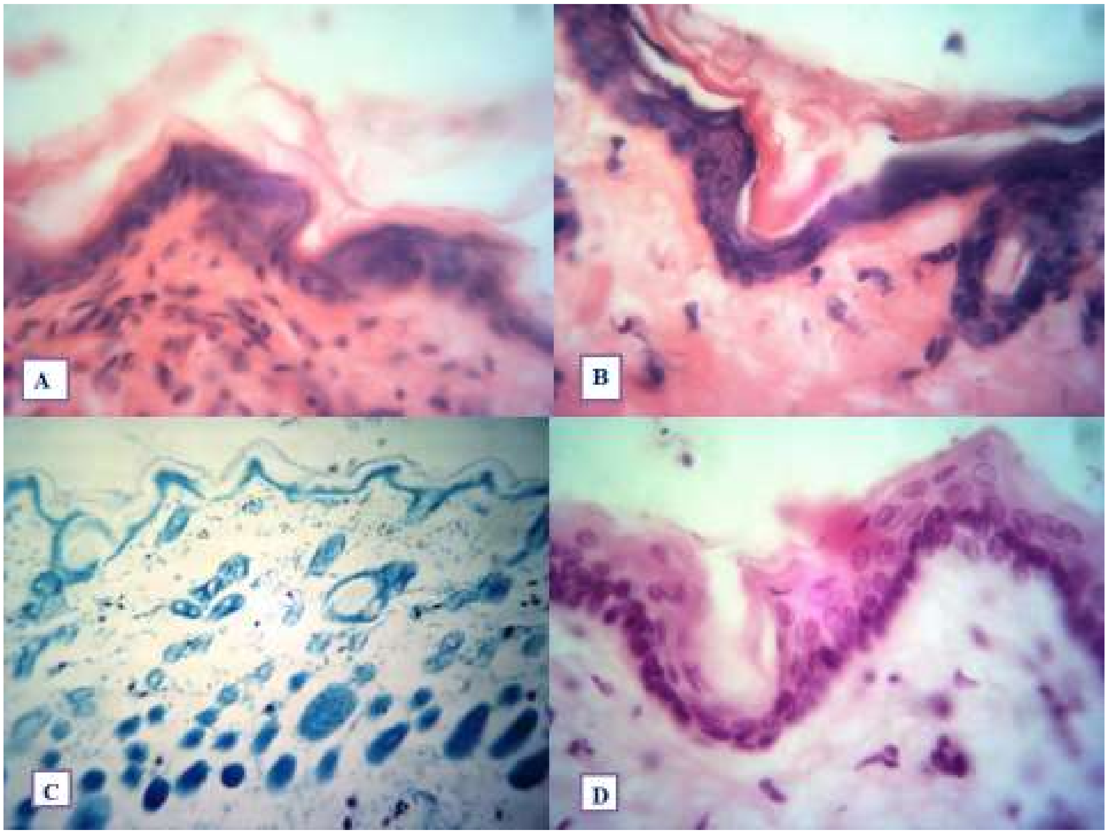

Figure 3 A) The histopathology results of the control group were normal using HE stain. Dermal and epidermal tissues were preserved. B) HE stain showing skin damage caused by radiation in the radiation group. It was possible to find swelling of the epithelial cells of the epidermis and hair follicles, led to organization disorder. C) TB stain of thin skin displaying a considerable mast cell infiltration D). HE stain image, shows a radioprotection of Vitamin C treated group against radiation induced skin damage

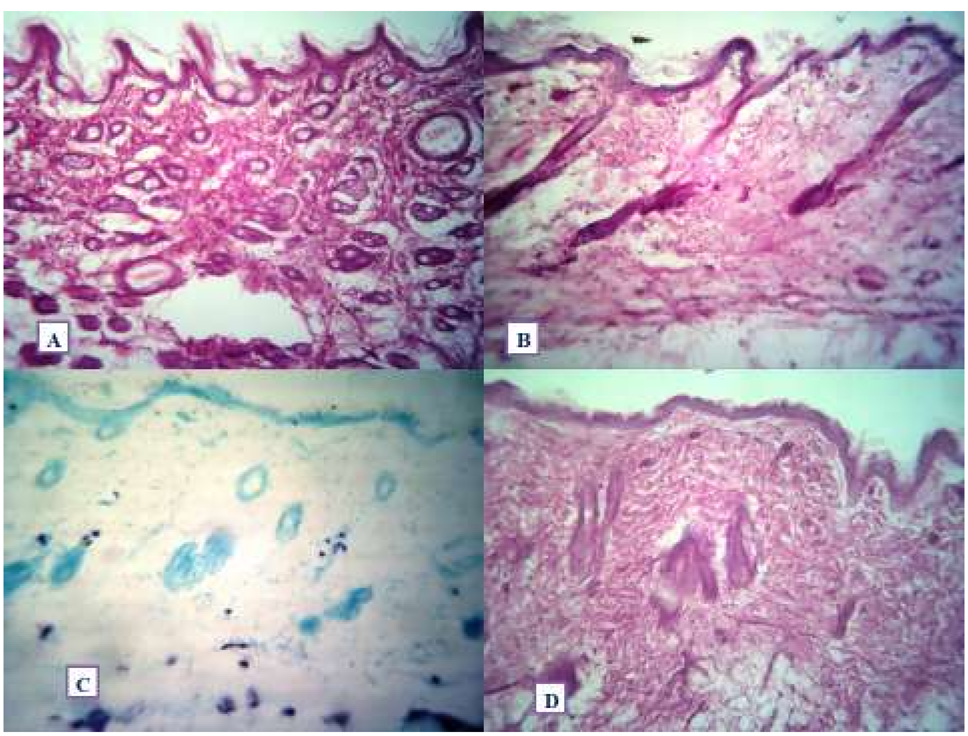

Figure 4 A) PAS stain, in the control group shows moderate PAS responses (magenta red) in the superficial layers, as well as the basal lamina of the epidermis. B) PAS stain, in the irradiation group; the dermal papillary, reticular and basal lamina of the epidermis, the irradiation group has a weak PAS reaction. C) TB stain of irradiated group the thin skin is mildly infiltrated by mast cells. D) The basal lamina of the epidermis, dermal papillary layers and reticular layers may be seen in the image of the vitamin C-treated group as having a moderate PAS reaction.

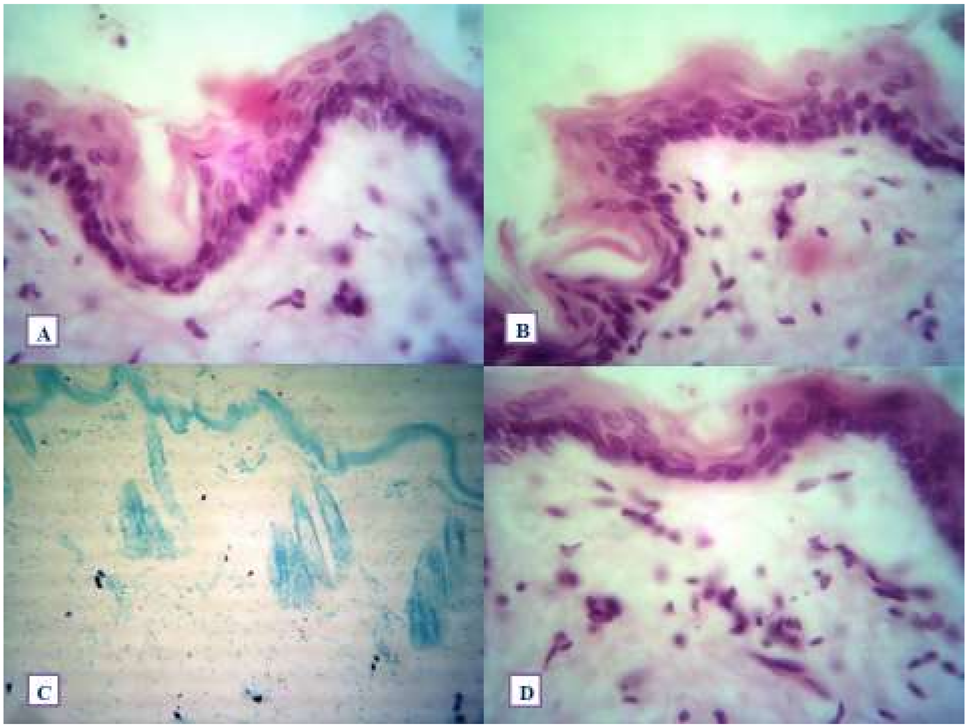

Figure 5 A) Feulgen stain of control group shows the nuclei DNA content in of the epidermal and dermal cells is distributed normally in the control group's skin, as seen by the histopathological images, which are granules of magenta color. B) Feulgen stain of irradiated group demonstrates DNA content in the dermal and epidermal cell nuclei is much higher in the group that received radiation treatment. C) TB stain of the vitamin C-treated group displayed a modest infiltration of mast cells. D) Feulgen stain of the group that received vitamin C therapy; DNA content in the dermal and epidermal cell nuclei appears normal

4. DISCUSSION

This investigation on the histological and histochemical changes in the skin of albino rats exposed to gamma radiation, as well as the potential therapeutic and preventive effects of vitamin C, was done in order to better understand these changes. Our current research demonstrates that ionizing radiation led to oxidative stress, which in turn caused tissues to become harmed by the release of organelle-specific enzymes. One of the few species, humans is dependent on dietary ascorbic acid supplements to survive (Humbert et al., 2018).

Numerous studies have demonstrated the antioxidants role in tissue healing following radiation-induced intestinal injury (Linard et al., 2013), lung injury (Wang et al., 2013), salivary gland impairment (Lim et al., 2013) and mixed radiation burn damage (Hao et al., 2009). On the other hand, ascorbic acid can be applied topically for cosmetic purposes and is a powerful skin-whitening agent (Chen et al., 2021). The skin damage following irradiation was visible using hematoxylin and eosin staining, which was consistent with earlier observations (Ertekin et al., 2004).

In our study, vitamin C was dissolved in water and given orally one day prior to radiation exposure so that it would be present at the tissue in safe and effective amounts before the production of free radicals by radiation. Our research revealed bigger and atypical epidermal cells as a result of radiation damage. This is in accordance to the work of Jang et al., (2016). According to the current study, ionizing radiation caused a degenerative effect and oxidative stress on the cells of tissues that release enzymes from organelles in a previous study (Kinoshita et al., 2014). While short-term exposure to ionizing radiation caused severe acute injury to the skin with depigmentation of hairs, which may lead to further depletion of tissue endothelial and stem cells.

Ionizing radiation harms organisms; hence the most effective way to protect humans or lessen its negative effects may be through pharmacological intervention or the use of medicinal herbs (Kumar and Tiku, 2016). In the present study, radiation-exposed rats' skin showed recovery in dermal and epidermal components, including their hair follicles and sebaceous glands, after being treated with Vitamin C extract. These findings are in line with the findings of Pazyar et al., (2014) who observed that olive oil, ginseng, chamomile and green tea were effective in treating wounds of skin.

In our findings, vitamin C extract treatment led to an improvement in DNA and total protein levels. This improvement may be the result of vitamin C's ability to strengthen protein synthesis and repair DNA damage in skin tissue. However, the antioxidant properties of green tea, where oleuropein promotes the creation of endothelium as well as the synthesis of protein and mRNA, may also be to blame for this improvement (Carluccio et al., 2003). The histology results in the vitamin C-treated rats compared to the radiation group thus imply that vitamin C has a radio protective impact on the skin.

5. CONCLUSION

The results of the current research show that vitamin C treatment has a positive therapeutic effect against the histological and histochemical changes caused by gamma radiation in the skin of albino rats. These have an action that prevents damage to skin tissue, which may aid in reducing the chance of acquiring new illnesses.

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Authors' Contributions

All authors contributed to the research and/or preparation of the manuscript. Salman Bin Dayel, Ali Hassan A Ali, Mohammed Saad Alqahtani, Khaled W. Alsaygh and Tareq A. Althubiti participated in the study design and wrote the first draft of the manuscript. Hamoud M. Almubki, Fahad A. Alzahran, Saud S Alanazi, Meshari Mahud Alshammari, and Faisal Mubarak Almuqbel collected and processed the sample. Nasser Hassan Al-swedan, Abdulelah Eyad Alqudaimi, Saud Mohammed Alzahrani, Wujud Khalid Alanazi, and Alanoud Abdulqader Ahmad participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

Ethical approval

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam Bin Abdulaziz University Institutional Review Board (SCBR-045-2023).

Informed consent

Not applicable.

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This study has not received any external funding.

Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

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