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VITAMIN C AND SKIN PROTECTION

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ABSTRACT

Background: Vitamin C has a major role in maintenance of skin health. Vitamin C extract has been included as dietary supplement along with other supplements and can reduce the oxidizing effects of toxic substances. **Aim of the work:** This study aimed to explore the histological and histochemical changes in the skin of male albino rats after exposure to gamma radiation and the potential protective effect of Vitamin C. **Methods:** The current study was conducted on 30 adult male albino rats. They were divided into 3 equal groups. Control group (**C**) did not receive any treatment. Radiotherapy group (**R**) rats exposed to a single dose of gamma-radiation. Radiotherapy + Vitamin C Group (**R+V**) had rats treated with Vitamin C extract in a dose of 20 mg/kg body weight daily one week before and one week after irradiation. Histopathological and histochemical changes in skin were studied by different stains. **Results:** Rats exposed to gamma radiation showed several histological and histochemical changes in their skin. These changes were improved by using Vitamin C extract. **Conclusion:** The present work showed Vitamin C had preventive and therapeutic effect upon skins of albino rats against radiation induced skin damage.

KEYWORDS: Vitamin C, Albino rats, Radiation, Skin injury.

BACKGROUND

Vitamin C (ascorbic acid, ascorbate) is a simple low-molecular-weight carbohydrate. It is a water-soluble essential vitamin and plays important roles in the skin. It is involved in the formation of collagen and the skin barrier in the dermis, the ability to counteract skin oxidation, and the modulation of cell signal pathways.[1]

The skin is the largest multifunctional organ on the surface of the human body and consists of three layers: the epidermis, dermis, and subcutaneous tissue, which form the body's first line of defense against harmful external factors.[2] The epidermis (an external stratified, non-vascularised epithelium) is composed of keratinocytes and dendritic cells, and the stratum corneum can prevent both harmful substances and skin moisture loss.[3] the dermis is the underlying connective tissue, consisting largely of dense fibrous components produced by fibroblasts. It provides nutrition for the skin and is rich in blood vessels and nerve endings.[4]

Ionizing radiation damages, the biological systems in a major way by generating reactive oxygen species (ROS). These ROS interact with biological molecules to produce toxic free radicals which lead to lipid peroxidation and DNA damage.[5,6]

The use of antioxidant may inhibit or reduce the toxicity of free radicals and thus provides protection against radiation.[7,8]

Vitamin C can protect indispensable molecules in the body, such as proteins, fats, carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins. The radio protective effect of ascorbic acid seems to be due to its interactions with radiation-induced free radicals.[9] Ascorbic acid protected the mice against radiation-induced sickness, decreased the mortality and improved the healing of wounds after exposure to whole body gamma-radiation.[10,11]

Radiotherapy is one of the most commonly used methods of cancer treatment. However, it is usually associated with surrounding natural tissue injuries.[12,13] In fact, skin injury caused by radiation is the most common complication of radiotherapy.[12,13] Ionizing radiation has enough energy to unsettle the orbital electrons surrounding the nucleus. This procedure resulted in the displacement of living tissues results in DNA damage through direct and indirect effects.[14] These toxic products hinder the balance of antioxidant defense systems of the body.[15,16]

Recently, although there are more and more researches of the protective mechanism of radiation-induced skin injury, the measures of clinical treatment depend mainly on anti-inflammatory, antioxidant,[17-18] and cyto-protective, including using hormones, vitamins, Chinese medicine and removal of free radicals.

This study was conducted to investigate the radio-protective effects of vitamin C on radiation-induced skin injury in rats.

METHODS

Experimental animals

A total of 30 male Swiss albino rats (Sprague dawely strain weighting 130 ± 5 gm) were obtained from the Egyptian Organization for Biological Products and Vaccines. They were kept in the laboratory for 15 days under observation to acclimatize. They were housed collectively in plastic cages, maintained under standard conditions of light, ventilation, temperature and humidity. Animals were provided free access to food and water *ad libitum*. All efforts have been made to reduce the suffering animals and to keep the numbers of animals used to a minimum. All animal procedures were approved by the National Research Centre (NRC), Cairo, Egypt Local Ethical Review Committee and has been implemented in accordance with the "Guide for the Care and Use of Laboratory Animals".

Gamma –irradiation procedure: Irradiation process was performed using Gamma Cell-40 as observed by Egypt's National Center for Radiation Research and Technology, Cairo. The gamma cell–40 is a caesium-137 irradiation unit manufactured by Canadian Atomic Energy commission. The unit provides means for uniform Gamma-irradiation to small animals or biological samples while providing complete protection for operating personnel. The radiation dose level given was 3Gy as a single dose provided at rate of 0.54 Gy/min. The radiation dose level was 3Gy at a source-surface distance of 80 cm.[19]

Vitamin C extract was purchased from Sigma-Aldrich Inc., USA. The tablets were crushed and the required amount was dissolved in distilled water. It was administrated orally in a dose of 20 mg/kg body weight daily from one week before to one week after the irradiation of rats.[20]

Experimental design

The experimental animals were randomly divided into 3 groups (10 animals in each group). Control group (**C**): normal healthy rats didn't receive any treatment. Radiotherapy group (**R**): rats exposed to a single dose of gamma-radiation, 3 Gy. Radiotherapy + Vitamin C Group (**R+V**): rats of this group were treated with Vitamin C in a dose of 20 mg /kg body weight daily one week before to one week after irradiation.

At the end of the administration, the animals were fasted overnight with water *ad libitum*, anesthetized with pentobarbital sodium (35mg/ kg, i.p.) and sacrificed by cervical dislocation on 7 days' after irradiation.

Histological and histochemical techniques

Skin samples were excised and fixed in 10% neutral formalin. After routine processing, paraffin sections (4µm in thickness) were prepared for processing the histological and histochemical studies with hematoxylin and eosin.[21] On the other hand, collagen fibres were detected by using Mallory's trichrome stain and polysaccharides were detected by using periodic acid Schiff's (PAS) reagent.[22] In addition, Toluidine blue stain was used for detection of mast cells infiltration and total proteins were detected by using the mercury bromophenol blue method. Finally, DNA material was detected by using Feulgen's method.[21]

Image analysis: The optical density (Pexil) of total protein and PAS+ve materials were analyzed by using micro image analyzer, software for microscopy ver 2.3. MOT mean optical transparency.

Statistical analysis

Statistical differences between experimental groups were assessed using one-way analysis of variance (ANOVA) and $p<0.05$ indicated statistically significant difference. All values were expressed as the mean ± standard deviations (SD). Statistical analysis was performed with student's t-test using the statistical software SPSS 13.0.

RESULTS

Hematoxylin & Eosin stained section

In Control group (**C**), the skin sections stained with H & E showed normal histological appearance of epidermis and dermis i.e thin epithelium, regularly distributed glands and intact hair follicles in dermis **(Fig.1 A)**. Epidermis had four layers of keratinocytes. The papillary layer of dermis had numerous capillaries and connective tissue cells. In addition, the inner reticular layer was composed of a dense fibrous connective tissue. Dermis contained sebaceous and sweat glands and hair follicles surrounded by arrector pili muscle **(Fig.1A)**.

The skin sections from Radiotherapy group (**R**) showed many pathological disorders. There was discontinuation of epidermal cells, loss of hair follicles and sebaceous glands, dermal cell swelling as well as collagen fiber edema **(Fig. 1B).** There was nuclear pycnosis and karyolysis of epidermal cells, corneum detachment and disorganized papillary layer.

Radiotherapy + Vitamin C Group (**R+V**) skin sections revealed partial restoration of the epidermal and dermal structure to normal tissue pattern. in **(Fig. 1C)**.

Toluidine blue stained section

In Control group (**C**), images revealed moderate infiltration of mast cells **(Fig. 2A)**. In Radiotherapy group (**R**) the infiltration was mild **(Fig. 2B)**.

In (**R+V**) and (**R+S)** Groups a moderate infiltration with mast cells was detected in comparison to (**R**) Group **(Fig. 2C)**.

Histochemical observations of the skin

Polysaccharides

In Control group (**C**), observation of the skin sections stained with periodic acid Schiff's (PAS) stain showed normal distribution of PAS +ve materials (magenta color). Also, there was a moderate staining affinity of the basal lamina of epidermis as well as of reticular and papillary layers of dermis **(Fig. 3A) (table.1)**.

Radiotherapy group (**R**) showed a weak PAS reaction in the basal lamina of epidermis and dermal papillary and reticular layers **(Fig. 3B)**. In (**R+V**) and (**R+S)** Group animals the staining affinity of epidermal basal lamina and dermal papillary and reticular layers was moderate as compared Group 2 **(Fig. 3C, table.1).**

DNA

In Control group (**C**), observation of the skin sections stained with Feulgen stain showed normal distribution of DNA content in the nuclei of cells of epidermis and dermal layers in the form of magenta color granules **(Fig. 4A)**. Radiotherapy group (**R**) animals a noticeable increase in DNA content was detected in the nuclei of the epidermis and dermal layers **(Fig. 4B)**. In (**R+V**) and (**R+S)** Groups animals a more or less normal appearance of DNA content was seen in the nuclei of epidermal and dermal cells **(Fig. 4C, table.1)**.

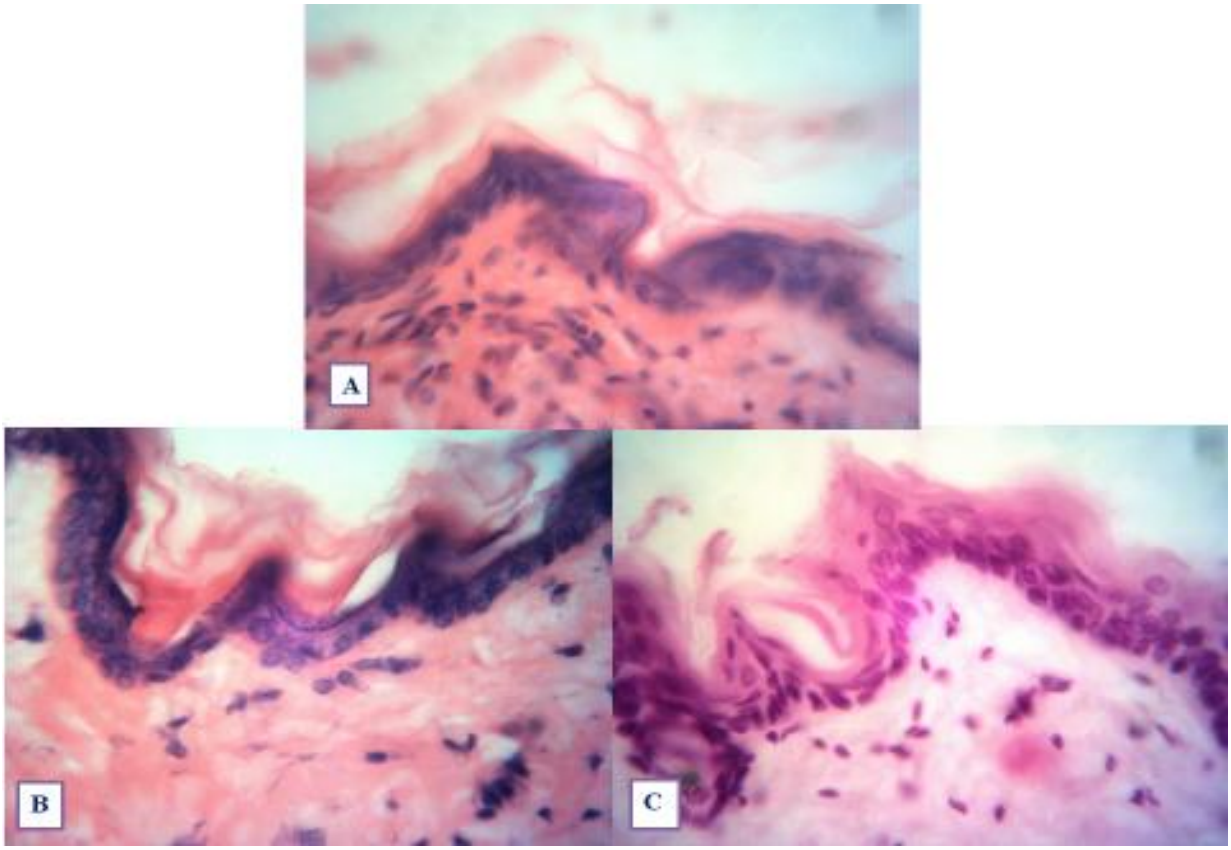

Fig.1: The histopathological images of the skin of the current study. A) Normal histopathological images in the C group. Epidermal and dermal structures were intact; B) Injury of the skin in the R group due to radiation. Epidermal cells and hair follicle epithelial cells swelling, nucleus pycnosis, collagen fiber edema, lead to arrangement disorder, together with congestive vascular reactivity and inflammatory cell infiltration could be found. C). Images of R + V group, shows a radioprotection against radiation induced skin damage in the form of epidermal cells swelling, hair follicle epithelial cells swelling, collagen fiber edema, and nucleus pycnosis. (HE X 400).

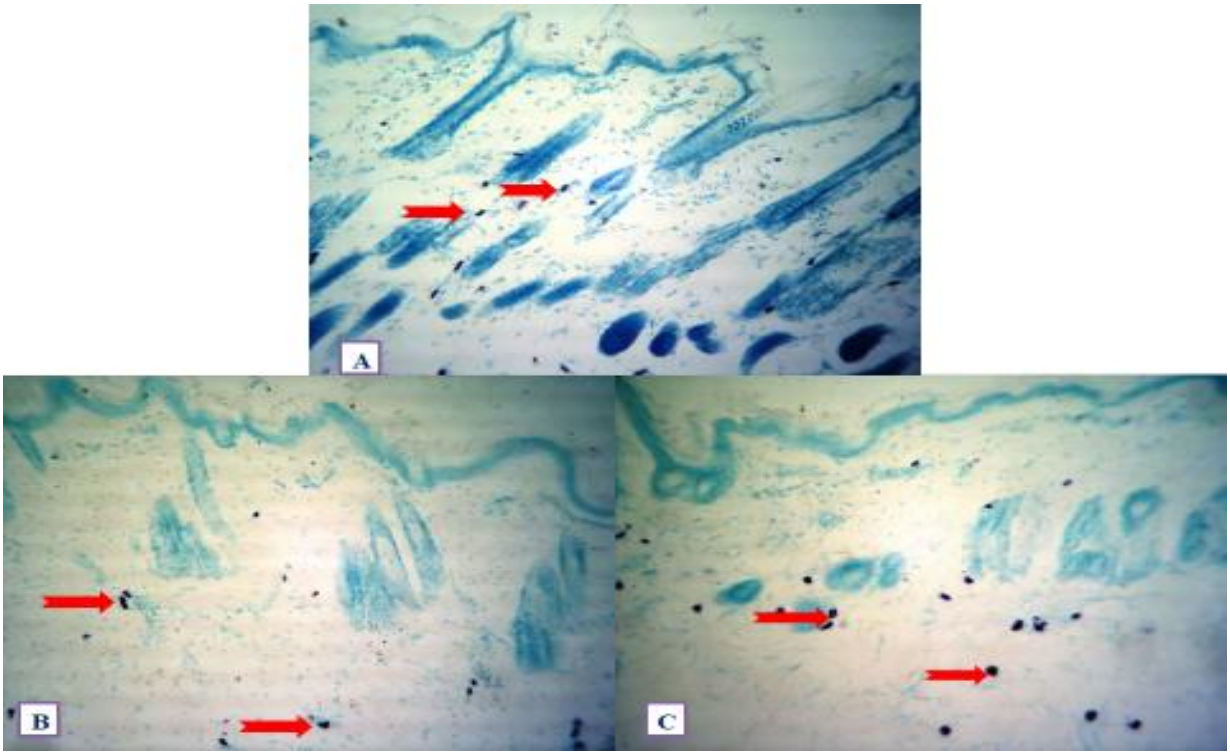

Fig.2: A) Thin skin of adult albino rat showing moderate infiltration with mast cells (red arrows). B) The irradiated thin skin showing mild infiltration with mast cells (red arrows). C). The group treated with Vitamin C showing moderate infiltration with mast cells (red arrows) (T.B. X200).

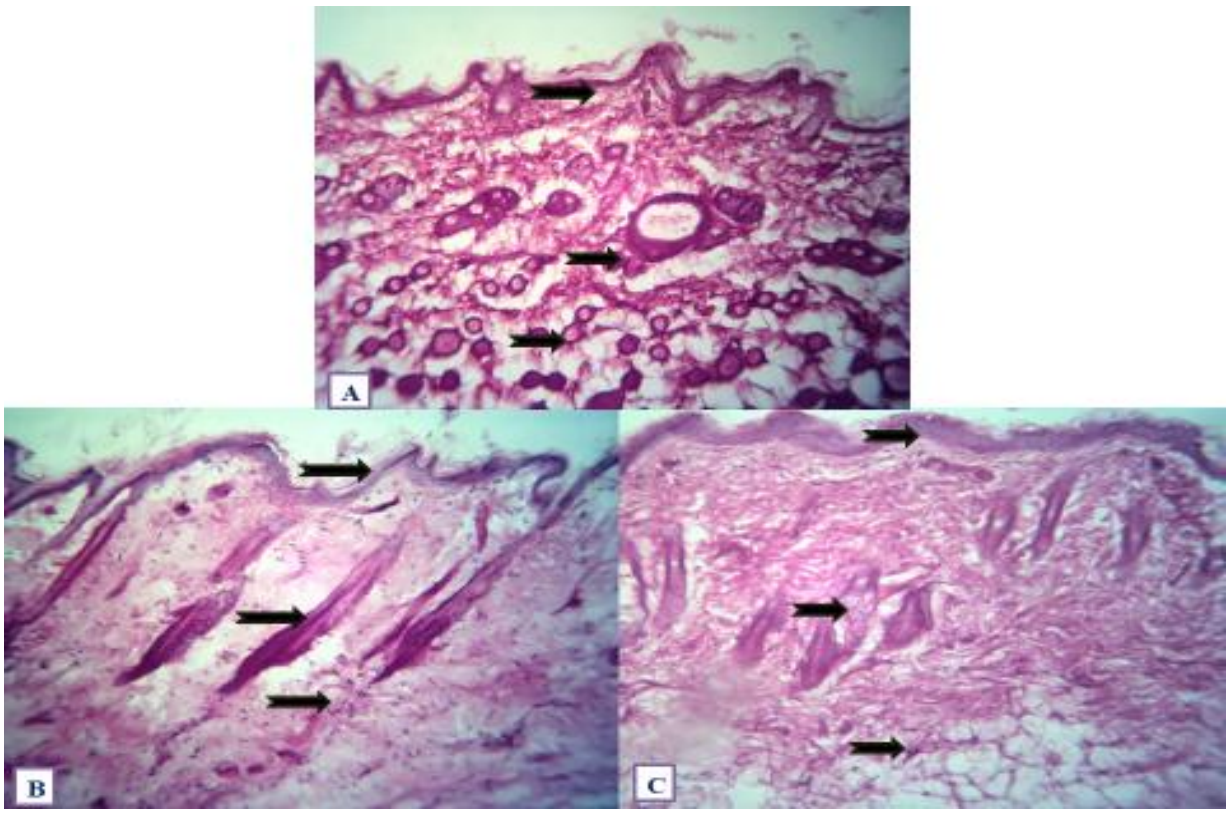

Fig.3: A) Moderate PAS reaction (magenta red) in the basal lamina of the epidermis, dermal papillary and reticular layers (black arrows) in the control group. B) The irradiated R group shows weak PAS reaction in the basal lamina of the epidermis, dermal papillary and reticular layers (black arrows). C). Images of R + V group showing moderate PAS reaction in the basal lamina of the epidermis, dermal papillary and reticular layers (black arrows). (PAS. X200).

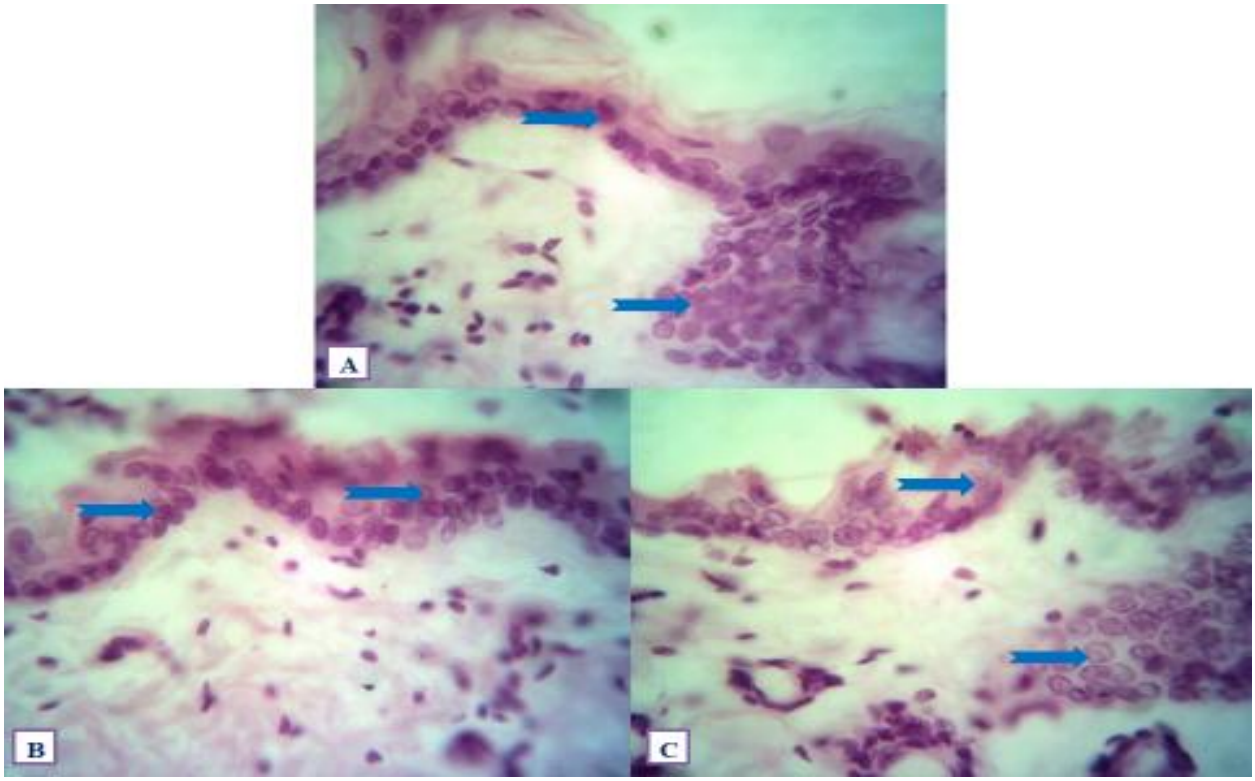

Fig.4: A) The histopathological images of the skin of the control group showing normal distribution of the DNA content in nuclei of the epidermal and dermal cells in the form of magenta color granules (blue arrows). B) The irradiated R group shows marked increase of the DNA content in nuclei of the epidermal and dermal cells (blue arrows). C). Images of R + V group showing more or less normal appearance of DNA content in nuclei of the epidermal and dermal cells (blue arrows). (Feulgen stain. X400).

Table 1: Showing MOT values of PAS +ve materials and DNA content in skin of the control and treated groups.

		Control	R group	R+V group	R+S Group
PAS +ve	Mean	96.12	78.6	92.05	83.3
	S.D.	18.11	23.99	12.99	30.90
	%		-21.01	-733	-16.11
	t Test		0.03*	0.04*	0.38
DNA content	Mean	65.1	39.6	55.77	99
	S.D.	13.29	31.99	19.29	17.90
	%		-36.51	-14.70	-18.11
	t Test		0.001*	0.002**	0.21

*Significant (P< 0.05), ** Highly significant (P<0.01)

DISCUSSION

This study was undertaken to investigate the histological and histochemical changes in the skin of male albino rats after exposure to gamma radiation in addition to the likely preventive and therapeutic effect of Vitamin C. Present study of ours also shows that the ionizing radiation caused oxidative stress and therefore the destructive effect on tissues with release of enzymes from organelles.

Many studies have shown that using antioxidants are involved in repair of tissue in intestinal injury caused by radiation.[22,23] lung injury,[24,25] salivary gland damage.[26] and combined radiation burn injury.[27]

Hematoxylin and Eosin staining showed the skin damage after irradiation, which were in agreement with previous reports.[28,29]

Many studies explain an increased collagen fiber content after irradiation as Alkaabi who disclosed increased collagen fibers and glycogen content in skins of rats after radiation.[30]

Our study results showed larger and irregular epidermal cells due to irradiation injury. This is in accordance to the work of Won et al.[31]

The current study the rat skin exposed to radiation and then treated with Vitamin C extract exhibit an improvement in epidermal and dermal components with

their hair follicles and sebaceous glands. These results are in concordant to the work of Pazyar et al.[32] who reported beneficial effects of olive oil, ginseng, green tea and chamomile in the management of skin wounds.

An improvement of both DNA and total protein contents were noted in our results following Vitamin C extract administration. This amelioration may be due to the action of Vitamin C on the skin tissue via DNA repairing system and reinforce protein synthesis. On the other hand, this improvement may also be due to the antioxidant activity of green tea where oleuropein stimulates endothelium formation as well as synthesis of mRNA and protein.[33]

In our work, the vitamin C dissolved in water, were administered orally as, one day before irradiation, so the vitamin C was present in the tissue with nontoxic and appropriate concentrations before production of free radicals by irradiation. Lipid peroxidation takes place after irradiation or free radical attack.[34] Vitamin C is an antioxidant molecule and prevents lipid peroxidation in plasma and inside the cell.[35,36] Thus, the histological findings in rat treated with vitamin C in comparison with the radiation group suggest that vitamin C exert its radioprotective effect on the skin. Therefore, also it was shown that vitamin C could have a slight radioprotective effect on rat skin.

CONCLUSION

According to the results obtained in the current study administration of Vitamin C provides good therapeutic effect against gamma radiation induced histological and histochemical alterations in skin of male albino rats. These have a protective effect against skin tissue damage which may contribute to decrease in the risk for further skin disorders.

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