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Exposure to gamma rays induces early alterations in skin in rodents: Mechanical, biochemical and structural responses

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ABSTRACT

In this study, the effect of gamma rays has been investigated on the normal rat skin using biomechanical, biochemical and histological techniques. Seventeen male Wistar albino rats were divided into two groups (control ($n=7$) and irradiated ($n=10$)). The irradiated group was treated with a ^{60}Co gamma source at a dose of 10 Gy at room temperature. Skin biomechanics were measured with tensile test using biomaterial testing machine and maximum load, stiffness, energy absorption capacity, ultimate stress, ultimate strain and elastic modulus were calculated. In the irradiated group, energy, strain and toughness were significantly lower than in the control group ($p<0.05$). However, strength, displacement, stiffness, stress and elastic modulus were similar to that of the control group ($p>0.05$). Catalase (CAT) activities and the levels of malondialdehyde (MDA) in the skin of rats were measured using the biochemical methods. MDA levels significantly increased whereas CAT activities decreased in the irradiated group as compared with the control group ($p<0.05$). Diameters of collagen fibers were measured by transmission electron microscopy. There was no significant difference ($p>0.05$) between control and irradiated groups for collagen fiber diameter. Thickness of epidermis was significantly lower than the control group. There were no changes in the epidermis between the irradiated group and the control group ultrastructurally. The results of this study show that the gamma irradiation has a significant effect on normal healthy skin

1. Introduction

The skin is an organ functioning as a physical barrier to protect the body against hazards of the environment. The skin's complex mechanical properties include the elastic properties of solid materials and the viscous properties of fluids. Many physiologic processes change skin properties significantly. Pathologic changes are generally correlated with changes in skin elasticity (Tilleman et al., 2004).

The increasing use of radioactive materials in industry, medicine, science, military and in localized areas of high radiation within nuclear facilities have significantly increased the potential of large-scale, uncontrolled exposure to radiation. Gamma rays are a form of electromagnetic radiation or light emission of frequencies produced by sub-atomic particle interactions, such as electron–positron annihilation or radioactive decay. Due to their tissue penetrating property, gamma rays/X-rays have a wide variety of medical uses such as in CT scans and radiation therapy (Anber and Spangler, 1985)

Radiation is a known producer of reactive oxygen species (ROS). When water, the most abundant intra- and extracellular material, is exposed to ionizing radiation, decomposition occurs, through which a variety of reactive oxygen species, such as $^{\bullet}OH$, $O_2^{\bullet-}$, $^{\bullet}OH_2$ and H_2O_2, is generated (Ewing and Jones, 1987). These reactive oxygen species cause oxidative damage in lipids, DNA and proteins (Davies and Truscott, 2001; Girotti, 2001; Ravanat et al., 2001). It is known that antioxidant enzymes such as superoxide dismutase (SOD) and catalase play important defensive roles by scavenging $O_2^{\bullet-}$ or H_2O_2 (Dreher and Maibach, 2001; Shindo and Hashimoto, 1998). When ROS generation increases and exceeds the defense capabilities of the organism, oxidative stress occurs. Skin is one of the major target organs for oxidative stress (Kohen, 1999; Kohen and Gati 2000). Skin is rich in lipids, proteins and DNA, all of which are extremely sensitive to the oxidation process.

ROS react with polyunsatured fatty acids to form lipid peroxides, which decompose to yield a cascade of reactions including the formation of the known mutagen malondialdehyde

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(MDA). Lipid peroxides have been shown to produce an irreversible impairment of membrane fluidity and elasticity, which can lead to the rupture of the cell (Wickens, 2001).

Radiation is one of the hazardous matters and pathophysiological changes in the skin caused by ionizing radiation include erythema, desquamation for early changes occurring within hours and weeks as well as dermal atrophy and telangiectasia for later effects (Chi et al., 2005). The effects of ionizing radiation on the skin have been the subject of extensive research (Muller et al., 2006; Franchi et al., 2006; Donetti et al., 2005; Fangman and Cook, 2005; Franco et al., 2005; Lyng et al., 2004). However, there is limited study investigating the acute effects of gamma radiation on the skin (Hoashi et al., 2008). In addition, very few studies have addressed the effect of ionizing radiation on skin biomechanics and structure (Ranu et al., 1975; Ranu, 1991).

To investigate the relationship between a single dose of 10 Gy gamma irradiation-induced mechanical and structural damage, oxidative stress, it might be important to explain the mechanism of action of ionizing radiation on skin mechanics. Therefore, we measured strength, stiffness, absorbed energy, stress, strain, toughness and elastic modulus, structure of epidermis and collagen, MDA concentration and catalase activity.

2. Material and methods

Seventeen healthy adult male Swiss albino Wistar rats (6–8 weeks of age and average body weight 180–200 g) were used in this study. Rats were obtained from the Experimental Animal Center, University of Mersin, Turkey. The study was approved by the research and ethical committee of the Mersin University. The rats were housed in polycarbonate boxes (two or three rats per box) with steel wire tops and rice husk bedding. They were maintained in a controlled atmosphere of 12 h dark/light cycle, 22 ± 2 °C temperature, and 50–70% humidity, with free access to pelleted feed and fresh tap water. The animals were randomly assigned into two groups: the control group ($n = 7$) and the irradiated group ($n = 10$).

All the rats were irradiated under general anesthesia and provided with 10 mg/kg body weight xylazine hydrochlorate and 15 mg/kg body weight ketamine hydrochloride diluted in sodium chloride. Irradiation was delivered by a Picker® C-9 ^{60}Co teletherapy unit at a dose rate of 82 cGy/MU. Legs of rats were irradiated in supine position individually using an anterior 30 × 30 cm single field with a depth of 0.5 cm with a single 10 Gy fraction dose. The radiation field was shielded with lead blocks.

The animals were sacrificed at 10 days after irradiation by high-dose anesthetization. The skin samples were excised. Tissue samples were used for biomechanical, biochemical and histological analyses.

2.1. Measurement of skin biomechanics

The biomechanical properties of skin were investigated using a tensile testing machine (MAY03, USA) equipped with a 50 kg load cell. The tensile loading speed in all tests was 1 mm/min. Data were transferred to the computers translating to the numerical signals by a 16-bit A/D converter for off-line analysis. The sampling rate was chosen as 1000 sample/s. Each specimen was subjected to a small initial preload (0.1 N) before actual testing. Load–displacement data were recorded using BIOPAC MP 100 Acquisition System Version 3.5.7 (Santa Barbara, USA). Strength represents the maximum tensile force applied until breaking occurred. The slope of the linear portion of the load–displacement curve defines stiffness and the area under the load–displacement curve is defined as the energy absorption capacity. Load–displacement recordings were normalized by cross-sectional area and this curve was converted to a stress–strain curve. Stress–strain curves for each specimen were generated and the ultimate stress, ultimate strain and toughness were determined. The ultimate stress was calculated from the following equation (Nigg and Herzog, 1999):

$$\sigma = F/A$$

where σ is the ultimate stress (MPa), F is the failure load (N) and A is the cortical area of the specimen (m^2).

The ultimate strain was calculated from the following equation (Nigg and Herzog, 1999):

$$\varepsilon = \Delta L/L_0$$

where ε is the strain, ΔL is the change in the length (mm) and L_0 is the original length.

Elastic modulus was calculated from the following equation:

$$E = \sigma/\varepsilon$$

2.2. Biochemical evaluation

After excision, fresh skin samples were homogenized with 50 mM phosphate buffer (pH 7.4). Then, homogenates were centrifuged at 10,000*g* for 15 min at 4 °C. Supernatants were separated and kept at −20 °C until enzyme activity and malondialdehyde measurements were performed.

Protein determination in supernatants was made according to Lowry et al. (1951) using bovine serum albumin as standard. All chemicals were obtained from Sigma®.

Tissue MDA levels were determined by thiobarbituric acid (TBA) reaction. A Carry 50 spectrophotometer (Varian, Inc.®, USA) was used, at a wavelength of 532 nm. The principle of the method depended on the measurement of the pink color produced by the interaction of barbituric acid with malondialdehyde elaborated as a result of lipid peroxidation. The colored reaction with 1,1,3,3-tetraethoxy propane was used as the primary standard. The determination of MDA levels was performed by the method of Yagi (1998). MDA levels were expressed as a nanomol per milligram of protein (nmol/mg protein).

Tissue catalase (CAT) activity was measured in supernatants by the method of Aebi (1984). The decomposition of the substrate (H_2O_2) was monitored spectrophotometrically at 240 nm. Specific activity was defined as micromole substrate decomposed per minute per milligram of protein (i.e. U/mg protein).

2.3. Histological evaluation

Skin samples were placed in formaline 10%. Routine tissue processing for light microscopy was performed on all specimens. The skin samples were embedded in paraffin. Sections (5 μm) were cut by microtome and stained with Masson's trichrome. These sections were examined with an Olympus BX50 light microscope. Five different areas were photographed by a digital camera (Nikon Coolpix 5000, Japan) for all samples. Randomly selected, ten areas were used for epidermal thickness measurement. Measurement was performed using a commercially available software (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

For electron microscopic investigations, skin samples were fixed with 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, dehydrated in graded alcohol series, cleared with propylene oxide and embedded in expoxy resin. Thin sections (50–70 nm) were cut by ultramicrotome (Leica UCT-125, Leica Microsystems GmbH, Wien, Austria) and contrasted with uranyl acetate and lead citrate. Sections were examined and photographed by an electron microscope (JEOL JEM-1011, Jeol Ltd., Tokyo, Japan). Randomly distributed collagen fibers were examined and cross-sections of them were selected for measurement. These areas were photographed by a digital camera attached to the electron microscope. Photographs were transferred to a commercially available software (Olympus Soft Imaging Solutions GmbH, Münster, Germany) and diameters of 100 collagen fibers of each animal were measured by this software.

2.4. Statistical analysis

Statistical analysis was performed using a commercially available software (SPSS v. 10.0 SPSS Inc., Chicago, IL, USA). After documenting normal distribution (Kalmogorov-Smirnov), data were expressed as mean ± SD and analyzed using the Student *t*-test. The significance level was set at $p \leqslant 0.05$.

3. Results

The results of biomechanical tests for irradiated skin are shown in Table 1. In the irradiated group, energy ($p = 0.042$), strain ($p = 0.05$) and toughness ($p = 0.039$) were significantly lower

Table 1
Mechanical parameters of skin in control and irradiated rats

Variables	Control ($n = 7$)	Irradiated ($n = 10$)	p
Strength (N)	38.25 ± 7.37	36.16 ± 8.17	0.597
Displacement (mm)	8.16 ± 1.67	7.01 ± 1.06	0.102
Energy (mJ)	153.95 ± 29.24	124.65 ± 25.01	0.042*
Stiffness (N/mm)	5.32 ± 1.13	4.88 ± 1.60	0.569
Stress (MPa)	5.98 ± 1.13	5.75 ± 1.33	0.715
Strain	0.50 ± 0.07	0.43 ± 0.076	0.05*
Toughness (MPa)	1.48 ± 0.17	1.22 ± 0.33	0.039*
Elastic modulus (MPa)	13.90 ± 3.63	12.24 ± 4.43	0.457

*Significantly different from control at $p < 0.05$.

than in the control group. Strength, displacement, stiffness, stress and elastic modulus were not different than that of the control ($p>0.05$). The level of MDA and the activity of CAT in skin are presented in Table 2. It was found that MDA levels significantly increased, whereas CAT activities decreased in the irradiated group as compared with the control group ($p<0.05$).

Epidermal thickness was significantly lower in the irradiated group than that of the control group ($p<0.05$) (Figs. 1–3). There were no changes in epidermis between the irradiated group and the control group ultrastructurally (Figs. 4 and 5). Ultrastructurally, normal collagen fiber organization was observed in the control group and in the irradiated group in dermis (Figs. 6 and 7). Cross-striations of collagen fibers were observed clearly in longitudinal sections. No significant differences ($p>0.05$) were observed between control and irradiated groups for collagen fiber diameter (Fig. 8).

Table 2
MDA concentration and CAT activity in the control group and the irradiated group

Variables	Control ($n=7$)	Irradiated ($n=10$)
MDA (nmol/mg protein)	0.51 ± 0.10	$1.83\pm0.61^*$
CAT (U/mg protein)	182.21 ± 30.49	$90.13\pm20.61^*$

*Significantly different from the control at $p<0.05$.

4. Discussion

In the present study, we investigated the effects of biomechanical, biochemical and histological changes on the tenth day post irradiation after a single dose of 10 Gy gamma radiation in the normal rat skin. Many researchers used different single doses (1–50 Gy) and intervals to observe the acute effects of gamma irradiation on the skin (Ran et al., 2004). For example, Hebbar et al. (2002) reported that acute skin reactions started developing on the fourth day post irradiation after a single dose of 35 Gy and increased with time in the treatment groups. Chen et al. (1999) reported that, at a single dose of 40 Gy by day 34, radiodermatitis scores progressed to moist desquamation in rats in the radiation

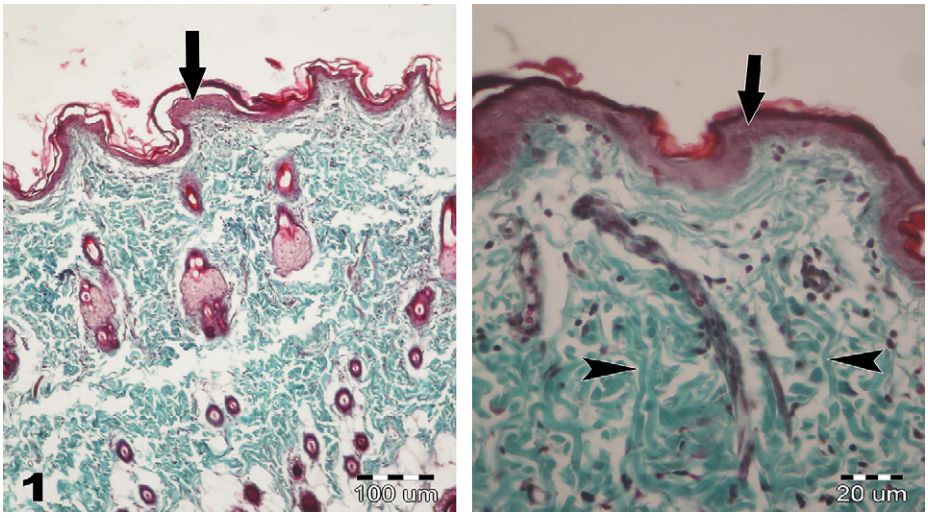

Fig. 1. Epidermal thickness in the control group. Epidermis (arrow) and collagen fiber (arrow head).

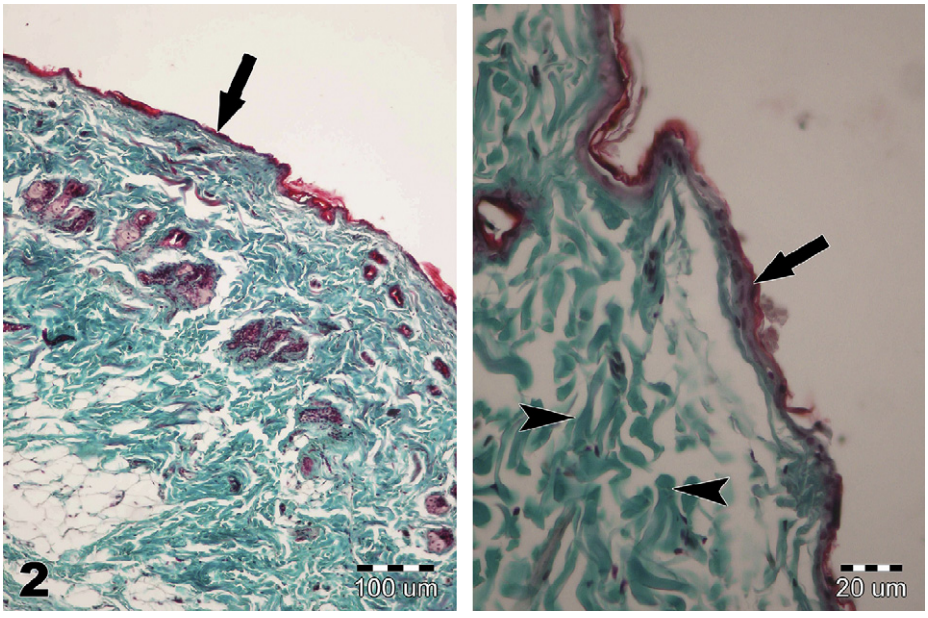

Fig. 2. Epidermal thickness in the irradiated group. Epidermis (arrow) and collagen fiber (arrow head).

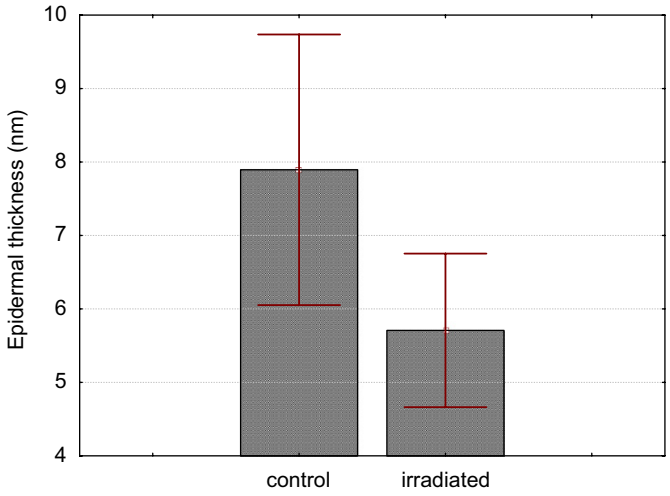

Fig. 3. Epidermis thickness of skin in control and irradiated rats.

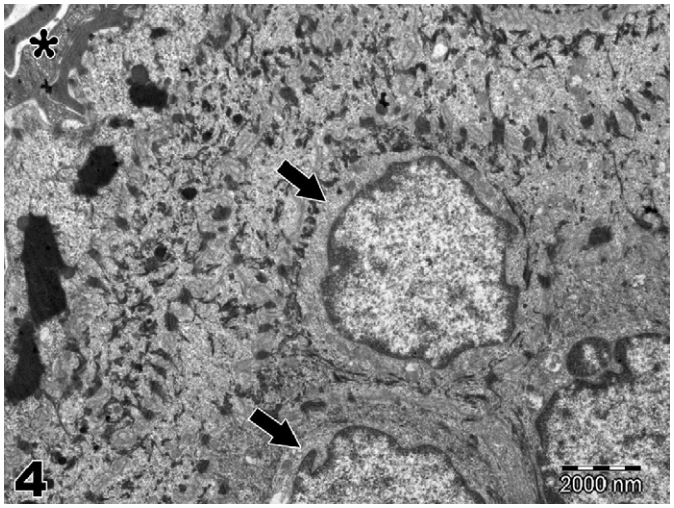

Fig. 4. Ultrastructure of epidermis in the control group. Stratum corneum (asterisk) and keratinocyte (arrow).

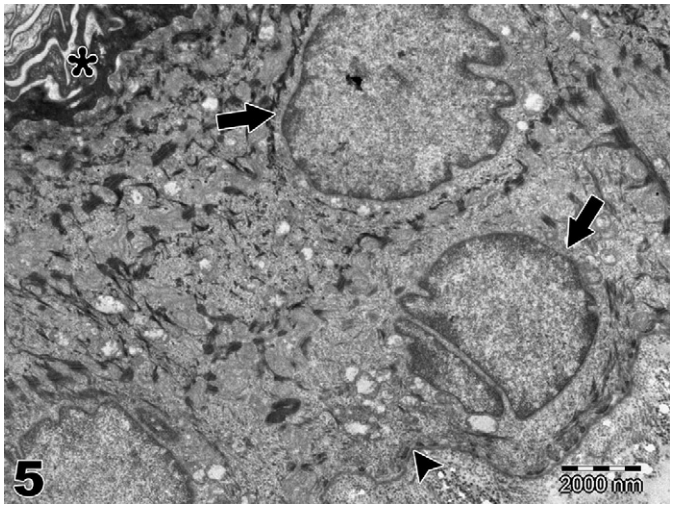

Fig. 5. Ultrastructure of epidermis in the irradiated group. Stratum corneum (asterisk), keratinocyte (arrow) and basal lamina (arrow head).

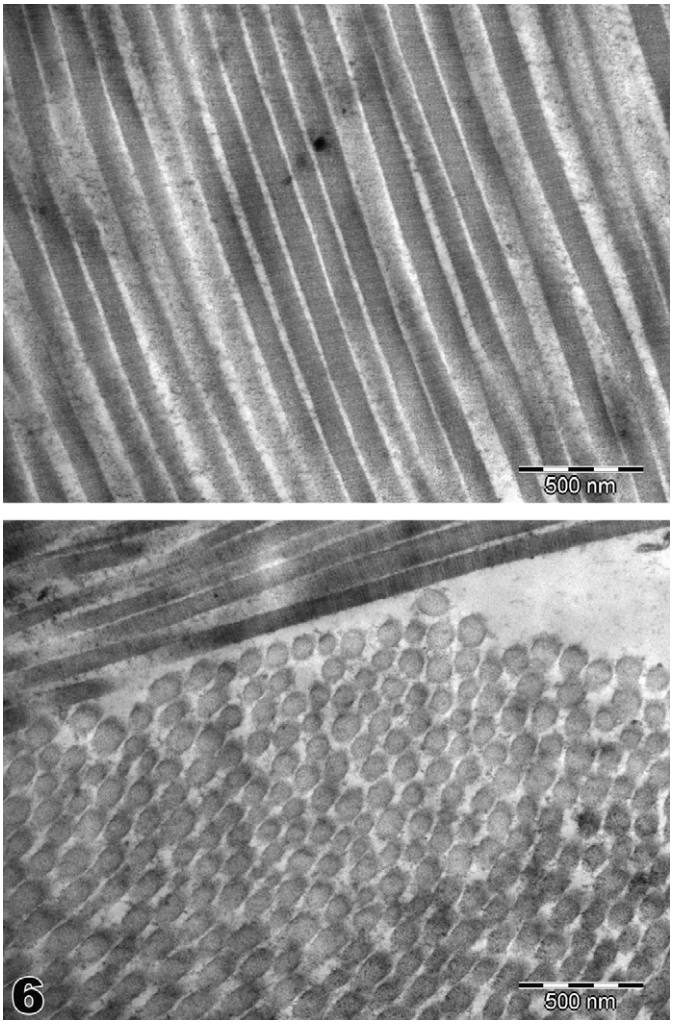

Fig. 6. Collagen fiber organization in the control group.

group. Our data demonstrated that a single fractional dose of 10 Gy gamma radiation for 10 days induced biomechanical, biochemical and histological changes in the normal rat skin.

It is well known that the mechanical properties of the skin change with environmental factors and disease. Objective functional assessment of skin mechanics was necessary in order to correlate mechanical properties with clinical, histological and biochemical findings (Ridge and Wright, 1966).

To gain an understanding of the biomechanical behavior of the irradiated skin, we measured strength, stiffness, absorbed energy, stress, strain, toughness and elastic modulus. Absorbed energy, strain and toughness were reduced by 20%, 14% and 18%, respectively, in the irradiated group in comparison to the control group. The mechanical properties of skin are largely associated with the collagen fibers (Lu et al., 2000; Ranu et al., 1975). Therefore we thought that these decreases may be related to skin collagen. In order to ascertain whether the mechanical properties were associated with corresponding alterations in morphological signs of skin collagen, ultrastructural examinations were carried out. We determined collagen fibril architecture and diameter at the ultrastructural level in skin. The mean diameter values of collagen fibrils and fibril ultrastructure in the irradiated group were not different from the control group. This suggests that the collagen morphology does not change after radiation. Reduced mechanical properties may be related to the decrease of collagen synthesis or functional properties of collagen fibers. Ranu et al.

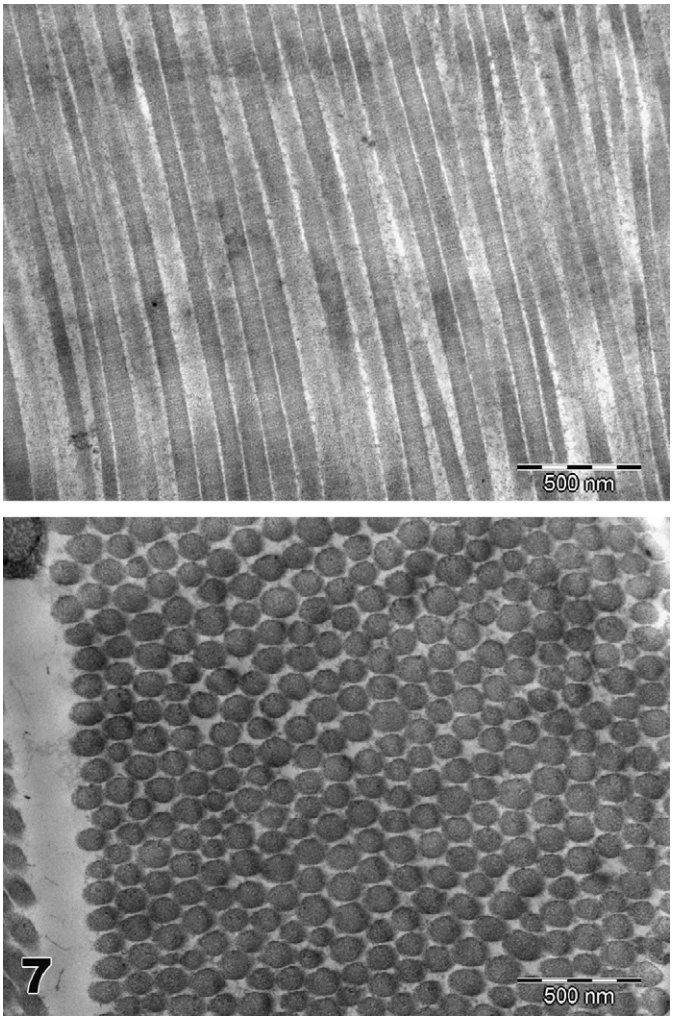

Fig. 7. Collagen fiber organization in the irradiated group.

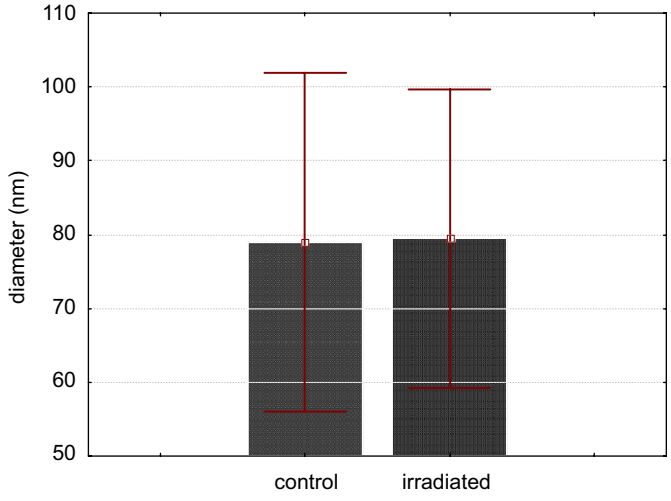

Fig. 8. Collagen fiber diameters of skin in control and irradiated rats.

(1975) measured the effects of X-irradiation on the mechanical properties of rat skin and found that the elastic properties associated with fiber alignment were little affected in the radiation range of 1000–3000 rad and the stiffness of the collagen decreased by doses. In this study we found that epidermal thickness was reduced after irradiation. The decrease of the epidermal thickness may be related to the decreasing effect of mitotic activity of radiation. The thinning of epidermis may affect the epidermal functions (barrier, lining, protecting). Variations in epidermal thickness may influence the penetrations of the gamma rays into the dermal layers. The amount of radiation passing through a specific area is inversely proportional to the square of the distance of that area from the energy source (Brown et al., 1999). Therefore, reduced epidermal thickness increases the amount of radiation passing from epidermis to dermis. This condition may affect the mechanical behavior of dermis. The mechanical behavior of the dermis dominates the mechanical behavior of skin in normal conditions (Silver et al., 2003).

In the present study, our data showed that the level of MDA in skin was increased by irradiation, which suggested that the large amount of oxygen radicals was generated from irradiation. Jagetia et al. (2003) demonstrated that lipid peroxidation increased in the skin of mice exposed to gamma radiation.

Irradiation of rat skin to gamma radiation resulted in the decline of CAT activity. A similar kind of decline in the skin CAT activity has been observed in the studies of Chang and Zheng (2003) after radiation. The depletion in CAT activity after radiation may be due to its consumption in the removal of peroxides. As a result, radiation-induced decline in CAT activity promotes the formation of free radicals, and the initiation and propogation of lipid peroxidation.

5. Conclusion

Our results have shown that gamma radiation induced the generation of reactive oxygen species in skin accompained by a decrease in CAT activity. We may suggest that reactive oxygen species damaged to the connective tissue components of the dermis, which likely influence cell behavior via cell–matrix interactions. Therefore biomechanical properties of skin, especially absorbed energy, strain and toughness were changed. It can be suggested that the therapeutic use of some antioxidants may be beneficial, as antioxidant supplementation may support reduced free radical levels for patients undergoing radiotherapy.

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Protection of human subjects and animal welfare

The study was approved by the research and ethical committee of the Mersin University.

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