

Role of Green Tea or Bone Marrow Mesenchymal Stem Cells against Skin Damage Induced in Male Albino Rats Exposed to Gamma Radiation

Keywords: Green tea; Albino rats; Skin; Bone marrow mesenchymal stem cells (BMSCs)

Abstract

Background: Green tea intake is accompanied with a lower incidence of cancer, cardiovascular disease and neurodegenerative disorders; hence green tea extract has been included as dietary supplement along with other supplements and multivitamins.

Aim of the work: This study aimed to investigate the histological and histochemical changes in the skin of male albino rats after exposure to gamma radiation and the possible preventive and therapeutic effect of green tea and Bone Marrow Mesenchymal Stem Cells (BMSCs).

Material and methods: The current study was carried out on 40 adult male albino rats. They were divided into 4 equal groups. Group one (G1) comprised control rats. Group two (G2) comprised of rats exposed to a single dose of gamma-radiation. Group three (G3) had rats treated with green tea extract in a dose of 35 mg/kg body weight daily one week before and one week after irradiation. Group four (G4) contained rats treated with BMSCs 5 hours after they were irradiated with gamma-radiation.

Histopathological and histochemical changes in skin of albino rats were studied.

Results: Rats exposed to gamma radiation showed several histological and histochemical changes in their skin. These changes were improved by using either green tea or bone marrow mesenchymal stem cells. BMSCs showed more obvious therapeutic effect than green tea extract.

Conclusion: The present work showed that both green tea and BMSCs had preventive and therapeutic effect upon skins of albino rats after they were irradiated with gamma radiation.

Introduction

The skin is the largest organ of the body, comprising between 15 and 20% of the total body weight. It performs numerous functions, including thermo regulation, sensory perception, excretion and absorption as well as protection from insults, dehydration and infection. It consists of two tissue types; the epidermis (an external stratified, non-vascularised epithelium) and dermis (underlying connective tissue, consisting largely of dense fibrous components produced by fibroblasts) [1].

Radiotherapy is one of the commonly used modality for cancer therapy. However it is usually associated with injures of the surrounding normal tissues [2,3]. In fact, radiation-induced skin injury is the most common complication of radiotherapy [2,3]. Ionizing radiation has sufficient energy to displace the orbital



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Submission: 05 October, 2017

Accepted: 30 October, 2017

Published: 10 November, 2017

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electrons surrounding the nucleus. This displacing action in living tissues results in DNA damage through direct and indirect effects [4]. Direct damage occurs due to DNA strand breakdown while indirect effect occurs via generating reactive oxygen species (ROS) or free radicals [4]. The major targets for ROS include proteins, lipids, nucleic acids and DNA–protein cross-linking. ROS also induce lipid peroxide production [5,6]. These toxic products disturb the balance of antioxidant defence systems of the body [7,8]. Mesenchymal stem cells are a population of adult stem cells which are a promising source for therapeutic application. These cells can be isolated from the bone marrow. They can be easily separated from the Hematopoietic Stem Cells (HSCs) due to their plastic adherence [9]. Adult bone marrow MSCs can differentiate into many mesenchymal cell types such as osteocytes, chondrocytes and adipocytes [10]. They can also differentiate into some non-mesenchymal cells such as neural cell under appropriate experimental conditions [10].

Camellia sinensis O. Kuntze belonging to Theaceae family (commonly Known as green tea in English) has antioxidant, anticarcinogenic, antiviral, and bactericidal properties [11]. Green tea is a rich source of polyphenols (consisting of avanol monomers (avan-3-ols) also referred to as catechins) which are antioxidants in nature [12]. These natural antioxidants from green tea extracts have recently attracted considerable attention for preventing oxidative-stress related diseases such as cancer, cardiovascular and degenerative conditions [13]. The exact mechanism of protection exerted by green tea is unclear but it has been suggested that it might be due to the antioxidant effect of its polyphenols and catechins [14-16].

Many studies have shown that the polyphenolic fractions isolated from green tea inhibit oxidant stress and possess anti-inflammatory activity [17].

This study aimed to investigate the histochemical and histological changes in the skin tissue of male albino rats which exposed to gamma radiation and the possible protective role of both green tea and BMSCs.

Material and Methods

Experimental animals: A total of 40 male Swiss albino rats (Sprague dawley 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 and allowed free access to standard pellet diet and tap water.

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 Atomic Energy commission of Canada. 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 single dose.

Green tea extract: was obtained as 300 mg tablets synthesized by MEPACO-Egypt with the name of Multi-Treat. The tablets were crushed and the required amount was dissolved in distilled water. It was given orally in a dose of 35mg/kg body weight daily from one week before to one week after the irradiation of rats.

Mesenchymal Stem Cells (BMSCs) Transplantation: Bone marrow mesenchymal cells were obtained from donor rats which were selected from the same breed and strain as the experimental rats. The donor rats were sacrificed, their femur bones dissected out and cleaned. Both ends of these bones were chipped by bone nibbling forceps. The marrow was blown off of the femur into saline solution under sterile conditions. The solution was thoroughly mixed by drawing and expelling it several times by the syringe without needle.

Experimental design

The experimental animals were randomly divided into 4 groups (n=10) as following.

Group 1: Control rats (G1): normal healthy rats left without any treatment.

Group 2: Irradiated group (G2): rats exposed to a single dose of gamma-radiation, 3 Gy.

Group 3: Irradiated rats+green tea treatment (G3): rats of this group were treated with green tea in a dose of 35 mg /kg body weight daily one week before to one week after irradiation.

Group 4: Irradiated rats+BMSCs injection (G4): rats of this group were irradiated with a single dose of gamma-radiation 3Gy and then treated with transplanted (BMSCs) 3×10^6 cells/ml suspension through caudal vein about 5 after radiation exposure.

The experimental rats were sacrificed at 7 days post irradiation.

Histological and histochemical techniques: Skin tissue were immediately excised and fixed in 10% neutral formalin. Paraffin sections (5 μ m in thickness) were prepared for processing the histological and histochemical studies. For general histology, sections were stained with Harris' hematoxylin and eosin [18]. Collagen fibers were detected by using Mallory's trichrome stain [18].

Polysaccharides were detected by using periodic acid Schiff's (PAS) reagent [18]. Toluidine blue stain was used for detection of mast cells infiltration [18]. Total proteins were detected by using the mercury bromophenol blue method and DNA material was detected by using Feulgen's method [18].

Results

Histopathological observations of the skin

Hematoxylin & Eosin stained section: In Group 1 animals (G1 control group), observation of the skin sections stained with H & E revealed normal histological appearance of epidermis and dermis i.e. thin epithelium, regularly distributed hair follicles and glands in dermis (Figures 1A and 1B). Epidermis was composed of stratified squamous epithelium having four layers of keratinocytes. The underlying papillary layer of dermis had abundant capillaries and connective tissue cells whereas the inner reticular layer was composed of a denser connective tissue rich in fibers. Dermis contained sweat and sebaceous glands and hair follicles surrounded with arrector pili muscle (Figures 1A and 1B).

Observation of skin sections from group 2 animals (G2 irradiated group) showed many pathological changes. There was discontinuation of epidermal cells, loss of hair follicles and sebaceous glands, dermal cell swelling as well as collagen fiber edema (Figure 1B). There was pyknosis and karyolysis of epidermal cells, corneum detachment and disorganized papillary layer (Figures 2A and 2B).

In Group 3 (G3 irradiated+green tea treatment) animals observation of skin sections revealed partial return of the epidermal and dermal structure to normal histological pattern in (Figures 1C and 2C).

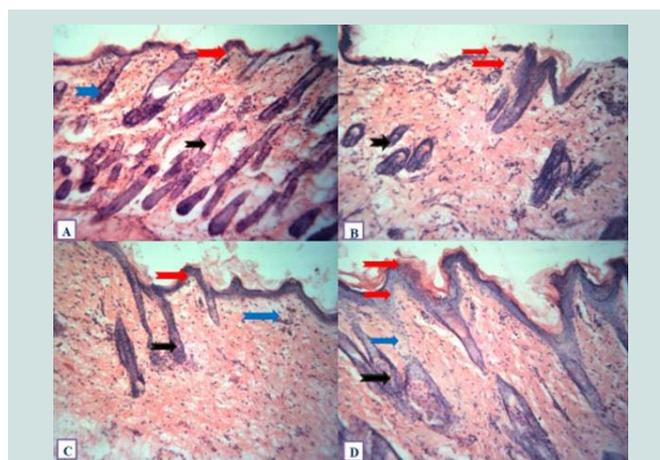


Figure 1: **A)** A photomicrograph of a control thin skin of adult albino rat showing normal histological appearance of epidermis (red arrows), dermis (blue arrow) and hair follicles (black arrows). **B)** A photomicrograph of the irradiated thin skin of adult albino rat showing arrangement disorder in the form of discontinuous epidermal and dermal cells (red arrows), degenerated hair follicle epithelial cells (black arrow). **C)** A photomicrograph of the irradiated thin skin of adult albino rat treated with green tea showing partial return of the epidermal and dermal structures to normal histological appearance in comparison to control group. **D)** A photomicrograph of the irradiated thin skin of adult albino rat treated with BMSCs injection showing marked return of the epidermal and dermal structures to normal histological appearance in comparison to control group. Invasion of migratory stem cells for dermal layers is also detected. (Hx. & E. x200).

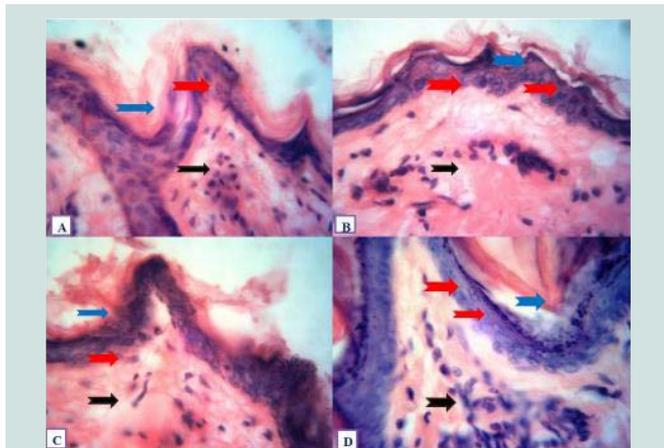


Figure 2: **A)** A photomicrograph of a control thin skin of adult albino rat showing normal well organized histological appearance of epidermis (blue and red arrows) and dermal papillary layer (black arrow). **B)** A photomicrograph of the irradiated thin skin of adult albino rat showing corneum detachment (blue arrow), epidermal condensed cells with cellular pyknosis and karyolysis (red arrows) and disorganized dermal papillary layer (black arrow). **C)** A photomicrograph of the irradiated thin skin of adult albino rat treated with green tea showing partial return of the epidermal and dermal cellular structures to normal histological appearance in comparison to control group. **D)** A photomicrograph of the irradiated thin skin of adult albino rat treated with BMSCs injection showing marked return of the epidermal and dermal cellular structures to normal histological appearance in comparison to control group. Invasion of migratory stem cells for basal epidermal and dermal layers is also detected. (Hx. & E. x400).

With Group 4 (G4, irradiated + BMSCs injection) animals similar observation indicated a marked return of the epidermal and dermal cellular structures to normal histological appearance (Figures 1D and 2D). Invasion of migratory stem cells into basal epidermal and dermal layers was also detected).

Mallory's trichrome stained section: In Group 1 animals (G1 control group), observation of the skin sections stained with Mallory's trichrome revealed normal appearance and arrangement of collagen fibers. Small collagen fiber aggregates were seen just below the basal lamina of the epidermis. The fibers appeared as fine loosely arranged network in papillary layer of dermis while in its reticular layer the fibers became more abundant and united into thick irregular bundles (Figure 3A).

Observation of skin sections from group 2 animals (G2 irradiated group) showed many irregularly arranged dense collagen fibers below the basal lamina of the epidermis as well as in dermal papillary and reticular layers (Figure 3B).

In Group 3 (G3 irradiated+green tea treatment) animals observation of skin sections revealed a moderate decrease in collagen fiber content as compared to irradiated group (Figure 3C).

In Group 4 (G4 irradiated+BMSCs injection) animals the collagen fiber content showed a marked decrease in comparison to the irradiated group (Figure 3D).

Toluidine blue stained section

In Group 1 animals (G1 control group), observation of the skin sections stained with Toluidine blue revealed moderate infiltration of mast cells (Figure 4A).

In skin sections from Group 2 animals (G2 irradiated group) the infiltration was mild (Figure 4B).

In Groups 3 (G3 irradiated+green tea treatment) & 4 (G4 irradiated+BMSCs injection) a moderate infiltration with mast cells was detected comparison to Group 2 (Figures 4C and 4D).

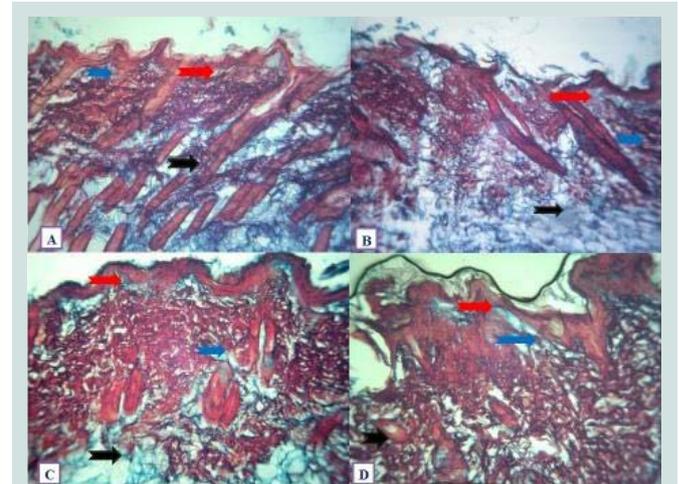


Figure 3: **A)** A photomicrograph of a control thin skin of adult albino rat showing small collagen fibers aggregates below the basal lamina of the epidermis (red arrow), loosely arranged fibers in the dermal papillary layer (blue arrow) and thick irregular bundles in the dermal reticular layer (black arrow). **B)** A photomicrograph of the irradiated thin skin of adult albino rat showing irregular densely arranged collagen fibers below the basal lamina of the epidermis (red arrow), in the dermal papillary and reticular layers (blue and black arrows respectively). **C)** A photomicrograph of the irradiated thin skin of adult albino rat treated with Green tea showing moderate decrease in the collagen fibers content in comparison to the irradiated group. **D)** A photomicrograph of the irradiated thin skin of adult albino rat treated with BMSCs injection showing marked decrease in the collagen fibres content in comparison to the irradiated group. (Mallory's trichrome. X200).

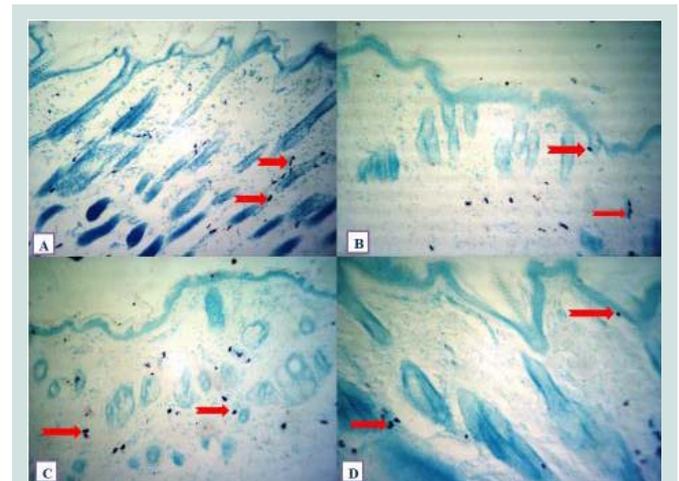


Figure 4: **A)** A photomicrograph of a control thin skin of adult albino rats showing moderate infiltration with mast cells (red arrows). **B)** A photomicrograph of the irradiated thin skin of adult albino rat showing mild infiltration with mast cells (red arrows). **C)** A photomicrograph of the irradiated thin skin of adult albino rat treated with Green tea showing moderate infiltration with mast cells (red arrows). **D)** A photomicrograph of the irradiated thin skin of adult albino rat treated with stem cell injection showing moderate infiltration with mast cells (red arrows). (T.B. X200).

Histochemical observations of the skin

Polysaccharides: In Group 1 animals (G1 control group), observation of the skin sections stained with periodic acid Schiff's (PAS) stain showed normal distribution of PAS +ve materials (magenta color). There was a moderate staining affinity of the basal lamina of epidermis as well as of papillary and reticular layers of dermis (Figure 5A).

The same observation in Group 2 (G2 irradiated group) animals revealed a weak PAS reaction in the basal lamina of epidermis and dermal papillary and reticular layers (Figure 5B).

In Groups 3 (G3 irradiated+green tea treatment) & 4 (G4 irradiated+BMSCs injection) animals the staining affinity of epidermal basal lamina and dermal papillary and reticular layers was moderate as compared Group 2 (Figures 5C and 5D).

Total protein: In Group 1 animals (G1 control group), observation of the skin sections stained with mercury bromophenol blue stain showed normal distribution of total protein content. This was demonstrated by deep to moderately stained cells in epidermal basal lamina and the two layers of dermis (Figure 6A).

In Group 2 (G2 irradiated group) animals the observation of skin sections stained with mercury bromophenol blue revealed a marked increase in amount of total proteins indicated by very deeply stained cells in the basal lamina of epidermis and dermal papillary and reticular layers (Figure 6B).

In Groups 3 (G3 irradiated+green tea treatment) & 4 (G4 irradiated+BMSCs injection) animals the protein content was increased in basal lamina of epidermis and decreased in the papillary and reticular layers of dermis (Figures 6C and 6D).

DNA: In Group 1 animals (G1 control group), observation of the skin sections stained with c showed normal distribution of DNA

content in the nuclei of cells of epidermis and dermal layers in the form of magenta color granules (Figure 7A).

In Group 2 (G2 irradiated group) animals a noticeable increase in DNA content was detected in the nuclei of the epidermis and dermal layers (Figure 7B).

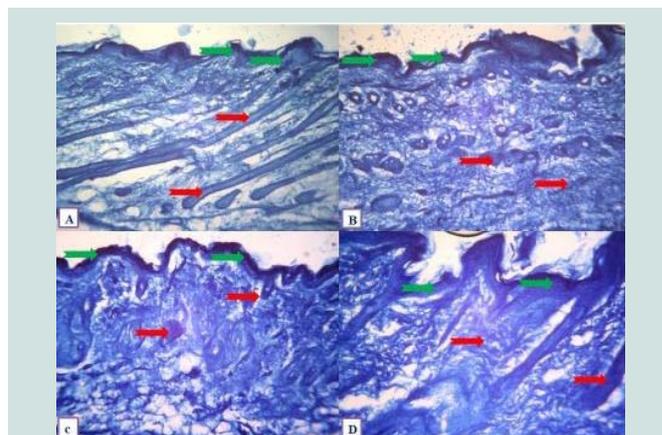


Figure 6: A) A photomicrograph of a control thin skin of adult albino rat showing normal distribution of total protein in the in the basal lamina of the epidermis (green arrows), dermal papillary and reticular layers (red arrows). B) A photomicrograph of the irradiated thin skin of adult albino rat showing marked increase in amount of total protein (very deep staining) in the basal lamina of the epidermis (green arrows), while it was decreased in the dermal papillary and reticular layers (red arrows). C) A photomicrograph of the irradiated thin skin of adult albino rat treated with Green tea showing increased amount of total protein (deep staining) in the basal lamina of the epidermis (green arrows), while it was decreased in the dermal papillary and reticular layers (red arrows). D) A photomicrograph of the irradiated thin skin of adult albino rat treated with BMSCs injection showing increased amount of total protein (deep staining) in the basal lamina of the epidermis (green arrows), while it was decreased in the dermal papillary and reticular layers (red arrows). (Mercuric bromophenol blue. X200).

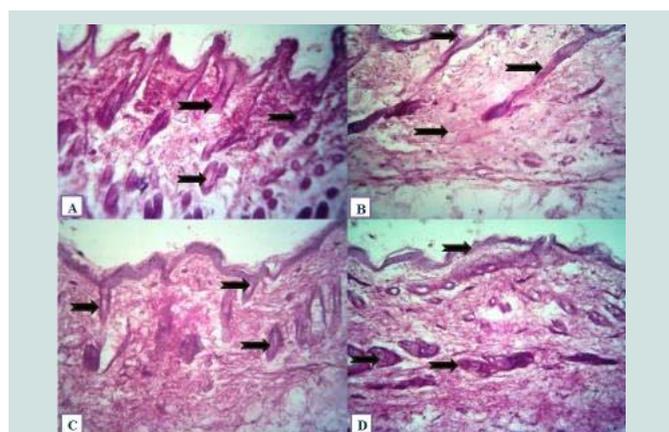


Figure 5: A) A photomicrograph of a control thin skin of adult albino rat shows moderate PAS reaction (magenta red) in the basal lamina of the epidermis, dermal papillary and reticular layers (black arrows). B) A photomicrograph of the irradiated thin skin of adult albino rat showing weak PAS reaction in the basal lamina of the epidermis, dermal papillary and reticular layers (black arrows). C) A photomicrograph of the irradiated thin skin of adult albino rat treated with Green tea showing moderate PAS reaction in the basal lamina of the epidermis, dermal papillary and reticular layers (black arrows). D) A photomicrograph of the irradiated thin skin of adult albino rat treated with BMSCs injection showing moderate PAS reaction in the basal lamina of the epidermis, dermal papillary and reticular layers (black arrows) (PAS. X200).

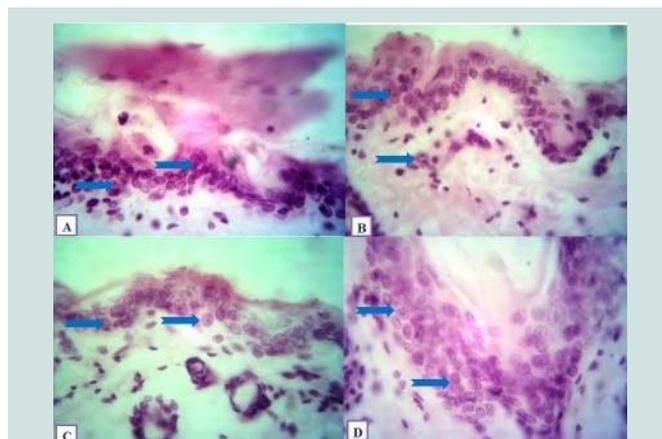


Figure 7: A) A photomicrograph of a control thin skin of adult albino rat showing normal distribution of the DNA content in nuclei of the epidermal and dermal cells in the form of magenta color granules (blue arrows). B) A photomicrograph of the irradiated thin skin of adult albino rat showing marked increase of the DNA content in nuclei of the epidermal and dermal cells (blue arrows). C) A photomicrograph of the irradiated thin skin of adult albino rat treated with Green tea showing more or less normal appearance of DNA content in nuclei of the epidermal and dermal cells (blue arrows). D) A photomicrograph of the irradiated thin skin of adult albino rat treated with BMSCs injection showing more or less normal appearance of DNA content in nuclei of the epidermal and dermal cells (blue arrows). (Feulgen stain. X400).

In Groups 3 (G3 irradiated+green tea treatment) & 4 (G4 irradiated+BMSCs injection) animals a more or less normal appearance of DNA content was seen in the nuclei of epidermal and dermal cells (Figures 7C and 7D).

Discussion

Experimental studies on animals have shown that exposure to ionizing radiation induces oxidative stress in different tissues [19-21]. Many investigators have suggested that the generation of reactive oxygen species (ROS) after irradiation results in cyclic and long lasting up regulation of inflammatory cytokines. It leads to the recruitment of inflammatory cells such as neutrophils and macrophages. These cells are responsible for the damage of tissues seen after radiation [22-25]. Long term exposure to ionizing radiation induced capillary reduction and severe atrophy in the dermis where as a short term exposure caused depigmentation of hairs and depletion of tissue stem cells [26].

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.

Our results shows an increased collagen fiber content after irradiation which is in accordance to the study of Alkaabi who also detected increased collagen fibers and glycogen content in skins of rats and those of their pups after radiation [27].

Our study results showed larger and irregular epidermal cells due to irradiation injury. This is in accordance to the work of Won et al. [28].

In our study the skins of the rats exposed to radiation and subsequently treated with green tea extract showed an improvement in the architecture of epidermal and dermal components with their appendages i.e. the hair follicles and sebaceous glands. These results are in agreement to the work of Pazyar et al. who reported beneficial effects of olive oil, ginseng, green tea and chamomile in the management of skin wounds [29].

An improvement of both DNA and total protein contents were noted in our results following green tea extract administration. This improvement may be due to the action of green tea on the skin tissue via DNA repairing system and enhancing protein synthesis. In addition, 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 [30].

Attenuation of abnormal histological appearance of tissues following BMSCs injection as seen in our study was also reported by many authors. They have attributed such recovery to the radical scavenging activities of BMSCs which prevent the accumulation of hydroxyproline in tissues [19,20,31].

In our study Bone Marrow Mesenchymal Stem Cells (BMSCs) treatment after irradiation improved PAS +ve materials, DNA and total protein content in the skin tissue compared to irradiated group. Similar results were reported in other studies as well [31].

Skin tissue restoration following gamma radiation exposure could be due to the therapeutic effect of BMSCs. As the stem cells can be transplanted to replace non functional or lost stem cells in tissues to enhance tissue healing and restore their original functions [21].

Conclusion

According to the results obtained in the current study administration of green tea or Bone Marrow Mesenchymal Stem Cells (BMSCs) 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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ISSN: 2575-7563

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