The Protective Effect of Olive leaves Extract or Bone Marrow Mesenchymal Stem Cells on Skin Tissue Damage Induced in Gamma Irradiated Rats

Nahed Mohamed Mansour Emam*and Khadija Abdul jalil Fadladdeen**
* Faculty of Sciences, Al- Arish University, Egypt
** Faculty of Science, University of King Abdel-Aziz University, Saudi Arabia
Corresponding author email: mansour.nahed@yahoo.com & k_jalil1@yahoo.com

ABSTRACT

Aim of the work: this study aimed to examine the histological and histochemical changes in the skin tissue of male rats after exposure to gamma radiation and the possible therapeutic effect of either olive leaf extract or bone marrow mesenchymal stem cells.

Material and methods: the present study was applied on forty adult male albino rats (*Sprague Dawely* strain). The rats were categorized equally into five groups (1-C group: control rats; 2- O group: rats treated with plant leaves extract (15 mg/kg b. wt. /daily); 3-R group: rats exposed to a single dose of gamma-radiation (3 Gy); 4-RO group: rats of this group treated with olive leaf extract 15 mg /kg b.wt./daily one week prior to irradiation and one week post irradiation; 5- RS group: rats of this group were irradiated with 3Gy then treated with bone marrow mesenchymal stem cells (3×10^6 cells / ml suspension) through the caudal vein about 5 hours post radiation exposure. Histopathological and some common histochemical changes were studied.

Results: rats exposed to gamma radiation showed several histological and histochemical changes, these changes were improved by using either olive extract or bone marrow mesenchymal stem cells. The bone marrow mesenchymal stem cells (BMSCs) showed more obvious curative effect than olive leaf extract.

Conclusion: the present work showed that both olive leaf extract and bone marrow mesenchymal stem cells have skin tissue radiotherapeutic effects against gamma radiation in male albino rats.

Keywords: γ-radiation - Albino rats - skin - bone marrow mesenchymal stem cells (BMSCs)– Histopathology- Histochemistry.

INTRODUCTION

The skin is the largest organ of the body, comprising between 15 and 20% of the total body weight and 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). Radiation damage to the skin can be described at all levels of organization from the cellular up to the organ level. The damaging effects of ionizing radiation lead to cell death and increased risk for diseases. Ionizing radiations cause formation of free radicals which in turn destroy DNA and other cellular components [1].

Ionizing radiation is that type which contains sufficient energy to displace an orbital electron around the nucleus. The most important effect of this displaced electron on living tissue is the potential damage of cell's DNA, which may occur directly or indirectly [2]. Direct damage occurs when the displaced electron breaks a DNA strand. Indirect damage occurs when electron reacts with water molecule, creating a powerful hydroxyl radical which then damages the cell's DNA. Ionizing radiation absorption causes immediate biochemical, sub cellular and cellular damage, while its morphological expression and organ dysfunction are often considerably delayed [3]. Accidental exposure and the therapeutic application of gamma radiation are the main triggers for the production of reactive oxygen species (ROS) in cells [4]. Superoxide anions, hydrogen peroxide and hydroxyl radicals are the most important types of ROS that react with macromolecules, resulting in cell dysfunction and tissue damage [5]. The major targets for ROS include proteins, lipids and nucleic acids, generating DNA strand breakage, DNA– protein cross linking and lipid peroxide production [6]. These toxic products affect the balance of antioxidant systems such as glutathione and enzymatic antioxidant defense systems [7]. Skin damage post-irradiation was detected in rats exposed to a single dose of radiation (45Gy) and

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there were many histological changes including inflammation and fibrosis in the skin tissue [8]. The same authors also noticed that bone marrow mesenchymal stem cells (BMSCs) can effectively reduce inflammation and fibrosis in the wounded skin and influence the repair of acute radioactive skin injury.

Olive leaves **(*Olea europaea*)** are traditionally used in many medical conditions for its potent antioxidant activity [9]. Recently, olive leaf has been reported as an anti-inflammatory, antioxidant and anti diabetic agent [10] .The main component of the olive leaves is oleuropein, which is thought to be responsible for pharmacological effects. Oleuropein is the molecule that provides olive oil with its multitude of good health and life-extending benefits, it is the polyphenol that can help lower bad cholesterol and blood pressure, prevent cancer, protect against oxidative damage and help guard against cognitive decline [11]. Oleuropein is reliable for most of olive oil's antioxidant, anti-inflammatory and disease-fighting characteristics [12]. ·Oleuropein completely regressed and inhibited different types of mice tumors within 9 to 12 days of its administration [13]. Oleuropein has also been reported to have hypoglycemic and antioxidant effects [14]. Furthermore, olive leaves contain, flavonoides (e.g.,luteolin, apigenine, rutin), triterpenes(oleanolic and maslinic acid) and chalcones such as olivin,olivindiglucoside [15].

Mesenchymal stem cells are a population of adult stem cells and they are promising sources for therapeutic applications. Stem cells are self-renewal and give rise to all the differentiated cell types of adult body. They are classified as toti-, pluri- or multi-potent stem cells based on number of the different cell types they can give rise to it. Stem cell response can be influenced by the chemical, topological, mechanical and physiochemical factors [16]. Mesenchymal stem cells are multipotent stem cells and had strong immunoregulatory effects and play a special role in inhibiting inflammatory reactions and promoting tissue repair [17]. Moreover, mesenchymal stem cells can reduce the expression of various inflammatory factors and promote the repair of various tissues and organs injury [18]. Mesenchymal stem cells post-irradiation showed a marked ability to overcome radiation injuries or tissue damages from the biochemical histological and histochemical view in rats [19 &20].

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 olive leaf extract or bone marrow mesenchymal stem cells against the induced skin tissue damage.

MATERIAL AND METHODS

Experimental animals:

Forty male Swiss albino rats, Sprague - Dawely strain (130 ± 5 g were obtained from Egyptian Organization for Biological Products and Vaccines. They were kept for about 15 days, before the onset of the experiment under observation to acclimatize the laboratory conditions. Rats were housed in several plastic cages, kept under the slandered conditions of light, ventilation, temperature and humidity and allowed the standard pellet diet and tap water.

Gamma irradiation procedure:

The process of irradiation was performed using Gamma Cell-40 achieved by Egypt's National Center for Radiation Research and Technology, Cairo, Egypt. The gamma cell–40 is a caesium-137 irradiation unit manufactured by Atomic Energy of Canada Limited. The unit provides means for uniform Gamma-irradiation of small animals or biological samples whereas providing the complete protection and safety for operating personnel. The dose rate was 0.54 Gy/min at the time of the experiment. The radiation dose level was 3Gy single dose.

Olive leaves *(Olea europaea*) Extraction:

Olive (*Olea europaea*)) leaves were weighed and ground to a fine powder in an electric mixer. The powdered plant material was extracted twice (24h each time) in70% ethanol by soxhlet apparatus. The extract was supplied to the groups of animals as a single dose (15 mg/kg b.w.) via intragastric gavages daily for 2 weeks [21].

Mesenchymal stem cells Transplantation:

Bone marrow transplantation rat donors and recipients were chosen of the same inbred strain, sisters to sisters. The donors were sacrificed and femur bones were dissected out, cleaned and both ends of the femur bones were chipped by bone nibbling forceps. The marrow was pushed from the femur bone into saline solution under sterilized conditions. The bone marrow solution

was surrounded by some ice cubes, mixed by drawing and expelling it several times from the syringe without needle to avoid any mechanical damage to the cells.

Concentration of mesenchymal stem cells transplantation was 3×10^6 cells/ml. The bone marrow suspension was transplanted into the irradiated rats through the caudal veins according to the method of Abdel Aziz *et al.* [22]. Ten animals received 100 µL cell suspension.

Experimental design:

The experimental animals were randomly categorized into 5groups (n=10) as following.

Group 1: control rats: normal healthy rats left without any treatment (**C**).

Group 2: rats were treated with olive leaves extract (**O**) for 2 weeks (15 mg /kg body weight/daily).

Group 3: the irradiated group: rats were exposed to a single dose of gamma-radiation, 3 Gy (**R**).

Group 4: olive irradiated rats (**RO**): rats of this group were treated with olive leaf extract at 15 mg /kg body weight/daily one week before and one week after irradiation.

Group 5: stem cell-irradiated animals(**RS)**: rats of this group were irradiated with 3Gy then treated with transplanted (MSCS) 3×10^6 cells/ml suspension through the caudal veins of rats about 5 hours post radiation exposure.

The experimental rats were sacrificed at 7 days post irradiation.

Histological and histochemical techniques

Skin tissues from the dorsal part were fixed in 10% neutral formalin solution. The pparaffin sections (5µm) of the skin sections were stained with Harris' hematoxylin and eosin [23]. Collagen fibres were stained by using Mallory's trichrome stain[24]. Polysaccharides of all skin tissues were detected by using the periodic acid Schiff's (PAS) reagent[25].Total proteins were detected by using the mercury bromophenol blue method [26] and DNA materials were detected by using Feulgen's method. [27]. Mast cells were detected by using toluidine blue stain **[28].**

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

RESULTS

Histopathological observations of the skin

Control group (**C**): hematoxylin and eosin stained sections of the rat's skin showed normal structure of control rat skin and well developed epidermal and dermal layers which contain normal hair follicles and their sebaceous glands **Fig. 1.** Mallory's trichrome stained sections of the skin tissue of the control group showed normal distribution of the collagen fibers since thin collagen are distributed through the epidermal and dermal layers **Fig. 8**.

Olive leaves extract treated group (**O**): there were no detectable histological or histochemical changes in the skin tissue of this group compared with the control group.

Irradiated group (**R**): skin of 3 Gy gamma irradiated rats showed many histopathological changes represented by highly corrugated, atrophied, ruptured and distorted epidermal layer, highly thickened keratin layer, numerous degenerated areas in the widened dermal layer. Some hair follicles are atrophied and damaged **Figs. 2-4**. Mallory's trichrome stained sections demonstrated highly increased collagen fibers in the distorted epidermal and dermal layers **Fig. 9**.

Olive irradiated group (**RO**): skin of rats exposed to gamma radiation and treated with olive extract showed some signs of improvement in the architecture of the dermal and epidermal layers and hair follicles with their sebaceous glands. **Fig.5.** Moderate or slight increase in staining affinity of collagen bundles deposition was detected in the dermal and epidermal layers in comparison with the control group **Fig.10**.

Stem cells irradiated group (**RS**): skin of rats exposed to gamma radiation and treated with stem cells showed well developed histological architecture of the epidermal and dermal layers and the hair follicles **Figs.6 &7**. A slight increase of collagen fibers was demonstrated in the epidermal and dermal layers of this group compared to the control group **Fig.11**.

Histochemical observations of the skin

Polysaccharides

Table1 , **histogram 1** and **figures 12-15** illustrated the changes in PAS +ve materials in sections of the skin tissue of the control and treated groups .Normal distribution of PAS +ve

materials (magenta color) was seen in skin tissue of the control rat(**Fig. 12)** which was indicated by moderate staining affinity of the skin epidermal and dermal layers. Decreased staining affinity of PAS +ve materials was detected in the dermal and epidermal layers of **R** group **(77.7)** compared to the control one **(97.16).** The degenerated areas were poorly or negatively stained **Fig.13.** However, a noticeable increase of PAS +ve materials was demonstrated in skin tissue of **RO (90.06)** group (**Fig.14)** and **RS** group (**82.49**) (**Fig.15)** compared to skin of rats of **R** group.

Total protein

Table 2, histogram 2 and **figures 16-19** illustrated the changes in total protein in sections of the skin tissue of the control and treated groups. Skin tissue of rats of the control group showed normal distribution of the total protein content represented by densely stained total protein in the epidermal layer and hair follicles with less stained dermal layer **(112)(Fig.16)**. Highly increased staining affinity of total protein was realized in the thickened keratin layer in **R** group in addition to decreased staining affinity in the dermal layer especially in the damaged hair follicles **(92.76)(Fig17)**. Increased staining affinity of total protein was detected in skin tissue of rats of **RO** group **(99)** (**Fig. 18)** and dermal and epidermal layers tissues of **RS** group **(98.6) (Fig. 19)** compared to **R** group.

DNA

Table 3, histogram 3 illustrated the changes in DNA content in sections of the skin tissue of the control and treated groups. DNA of skin tissue of the control reached **64.80 (Table 3& histogram3).** A noticeable decrease in DNA content was detected in skin of rats of group **R** and reached **40.56 (Table 3& histogram3).** Skin tissue nuclei of **RO** group and **RS** group revealed an increase of DNA materials **(Table 3& histogram3)** and their densities reached **54.73**, **53.43** respectively.

Mast cells

Figures 20-24 illustrated the changes in mast cells count in sections of the skin tissue of the control and treated groups. Skin tissue of the control rats showed normal distribution of mast cells **(Fig.20).** Skin sections of **R** group showed a significant increase (**33)** in the mast cells count in the dermal layer **(Figs. 21&22). RO** group showed decreased number of mast cells count (**15)** in the skin tissue in comparison with the irradiated group **(Fig. 23).** Also **RS** group exhibited highly decreased mast cells count (**7)** in the dermal layer **(Fig. 24).**

DISCUSSION

Skin is one of the most radiosensitive organs of the body. The early skin reactions to radiation were commonly recorded and cause the majority of human skin cancers **[29].**

Gamma radiation is absorbed by the epidermis and induced skin tissue damage; it is the major cause of various skin disorders, including skin cancers. The increased reactive oxygen species (ROS) level may be an important factor in sensitization to tumor therapy [30]. Oxidative damage induced by radiation leads to alteration in both lipid bilayer fluidity and permeability properties So**,** membrane lipids are easily affected by reactive oxygen species (ROS) produced by ionizing radiation, causing structural and functional impairment, also the cell membrane permeability is disturbed following irradiation as exhibited by changes in tissue ionic contents of Na^+ and K^+ [31&32].

Various intrinsic and extrinsic circumstances and the biochemical activity of the cell can make it lose control over the formation and management of free radicals. This imbalance in the formation and use of free radicals in tissue is known as "oxidative stress". It results from a disturbance of the balance between the formation of reactive oxygen species and the defense provided by cell antioxidants **[33].**

Many dystrophic changes were observed in the skin of gamma irradiated rats in the present study including highly corrugated, atrophied, ruptured and distorted epidermal layer, thickened keratin layer, numerous degenerated areas in the widened dermal layer. Some hair follicles were atrophied and damaged. Similar results of other invistigators suggested that generation of ROS immediately after irradiation, with chronic and cyclic up-regulation of inflammatory cytokines and the recruitment of inflammatory cells such as neutrophils and macrophages are responsible for the damage seen in the tissues after irradiation [34-37].

Moreover, ROS-induced mitochondrial DNA

deletions compromise mitochondrial function; these factors together with direct protein oxidation due to irradiation contribute to accelerate degradation of extracellular matrix of tissues **[38].**

In accordance with the present study, ionizing radiation caused oxidative stress and destructive effect on the cells of tissues which release enzymes from organelles whereas, the long term exposure to ionizing radiation induced capillary reduction and severe atrophy in the dermis while, the short term exposure to ionizing radiation induced severe acute injury to the skin with depigmentation of hairs, that may cause more depletion of tissue stem cells and endothelial cells in the tissue [39]. Ionizing radiation produces harmful effects on the organisms therefore, pharmacological intervention or medicinal plants could be most potent strategy to protect human or ameliorates the deleterious effect of ionizing radiation [40].

Moreover, free radicals formed during irradiation can cause a variety of membrane changes including, lipid peroxidation, hydrolysis of phospholipids, hydrolysis of disulfide bridge, damage in membrane proteins and lipid protein cross links [41]. Moreover, another study proved that low dose ionizing radiation was able to induce specific transcriptional responses in human keratinocytes [42] and UV irradiation-induced methionine oxidation in human skin keratins [43]. Also**,** gamma-irradiation of animals at the sub lethal and lethal dose levels alters the metabolism of various organs and causes a series of biochemical and physiological disturbances in the different biological tissues [44]. Moreover, ionizing radiation induced delayed destabilization of the genome in the progenies of surviving cells; this induced genomic instability is manifested by delayed induction of radiation effects, such as chromosomal aberrations, mutation and cell death [45]. In accordance with the present study, increased collagen fibres post-exposure to radiation was detected also by **Alkaabi** [46] **and Salem**[47] who observed increased glycogen content in skin tissue of irradiated rats and their fetuses.

Highly atrophied, ruptured and distorted epidermal layer of the exposed group in the present study showed decreased PAS +ve materials in the dermal and epidermal layers and atrophied hair follicles. Dcreased polysaccharides were observed in skin tissue post exposure to gamma rays [48].

Decreased staining affinity of both DNA and total protein in the present study may be due to the generation of ROS and consequent oxidative stress [49]. Gamma radiation can induce DNA damage by the reactive oxygen species (ROS) directly or indirectly. Whereas, such damage can occurs due to direct ionization of the DNA molecule itself or indirectly through the formation of toxic products, such as free radicals, hydroperoxy radicals, hydrogen peroxide and ions that diffuse from the site of formation and interact with any molecules in their path [30]. If normal cells failed to repair such damage, it can lead to the cell cycle inhibiting even premature senescence and cell apoptosis [50]. Free radicals could also react with DNA bases, impairing their structure and leading to mutations. Another authors added that this hypostainability may be due to damaged DNA [51], increased action of lytic enzymes or ruptured cytoplasmic organoids such as mitochondria, ribosomes, RER and cellular membranes [52,53]. Increased mast cells in skin tissue of the exposed rats in this study may be due to damaging tissue and cell structures.

In the present study, skin of rats exposed to gamma radiation and treated with olive extract showed some signs of improvement in the architecture of the dermal and epidermal layers and hair follicles with their sebaceous glands and also improved results of the histochemical analysis were realized compared to the irradiated group. Our results are in agreement with the results of **Pazyar *et al*.** [54] who reported the beneficial effects of olive oil, ginseng, green tea and chamomile in the management of skin wounds. These medicinal plants possess significant pharmacological effects and considered potent phytotherapeutic agents that have been largely used for cutaneous wound healing. Moreover, supplementation with olive oil improved cutaneous wound healing in chronically stressed mice and olive oil treatment reversed the reduction in fibroblast migration and collagen deposition and reduced lipid peroxidation [55]**.** This improvement in the present study may be related to the action of oleuropein, the most abundant phenolic compound in olive leaves that showed healing effects on

wounded skin by accelerating the epithelial formation process, enhancing collagen fibres generation and increasing the blood supply to the wounded area [56].

Improvement of PAS +ve materials ,total protein, DNA and mast cells contents were noted in the present results following olive extract application compared to the irradiated group. Such improvement may be due to the action of olive leaf extract on the tissue by DNA repairing system and enhancing protein synthesis .Also oleuropein acts as a free radicals scavenger, since DNA materials are the main target of them. In addition, this improvement may also be due to the antioxidant activity of olive leaf extract where oleuropein stimulates endothelium formation as well as synthesis of mRNA and protein [57].

Mesenchymal stem cells injection of irradiated rats at the present study exhibited somewhat normal architecture of the skin tissues with more or less normal distribution of collagen fibres and improved the levels of histochemical analysis compared to the irradiated group. Attenuation of abnormal histological appearance of some tissue following BMSCs injection was reported by many authors attributed such recovery to their radical scavenging activities, which prevent the accumulation of hydroxyproline in the tissues [17, 18, 58]

Results of the present study showed that BMSCs can effectively reduce inflammation and fibrosis in the wounded skin and promote the repair of acute radioactive skin injury and these results are in agreement with the results of **Zheng *et al.*** [8] , thus BMSCs may be developed as a novel treatment for wound healing or skin tissue damage.

Bone marrow mesenchymal stem cells treatments improved PAS +ve materials, DNA and total protein content in the skin tissue compared to irradiated group. Somewhat normal total protein content was reported in rat treated with the bone marrow cells post-irradiation [17] .

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 [59].

CONCLUSION

According to the results obtained in the current study, administration of oil leaf extract or bone marrow mesenchymal stem cells (BMSCs) provided therapeutic effects against gamma radiation induced histological and histochemical alterations in skin of rats. A better ameliorative effect was obvious in BMSCs treatment; it has a protective effect against skin tissue damage which may contribute to decrease the risk for further skin disorders.

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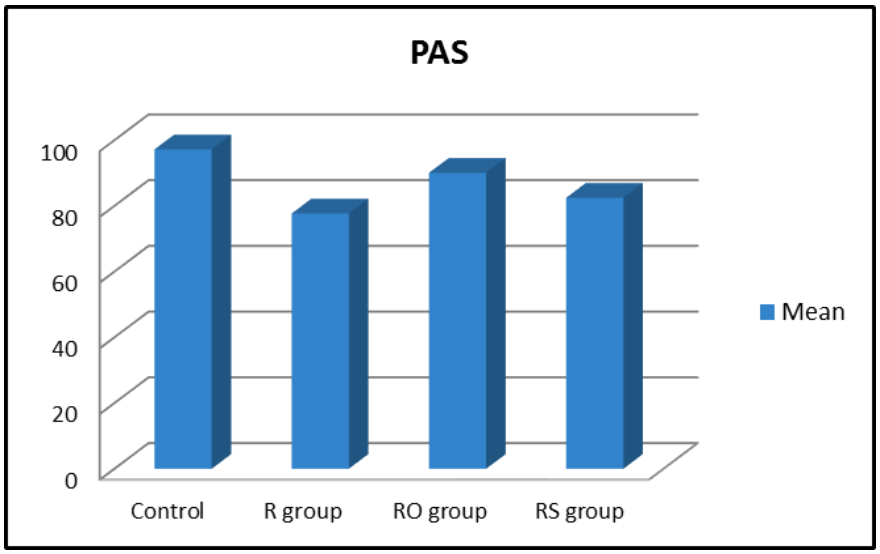
Table 1: showing MOT values of PAS +ve materials in skin of the control and treated groups

	Control	R group	RO group	RS group
Mean	**97.16**	**77.7**	**90.06**	**82.49**
S.D.	**17.24**	**24.95**	**13.25**	**30.96**
%		**-20.03**	**-7.31**	**-15.10**
t Test		**0.03***	**0.04***	**0.37**

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

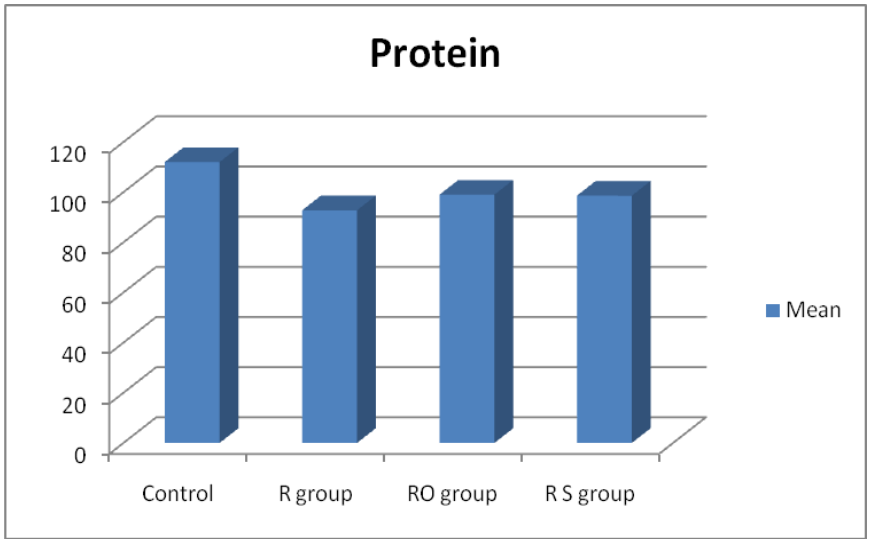


Histogram 1: revealing MOT values of PAS +ve materials in skin of the control and treated groups

Table 2: showing MOT values of protein in the skin of the control and treated group.

	Control	R group	RO group	R S group
Mean	**112**	**92.76**	**99**	**98.6**
S.D.	**15.35**	**16.08**	**20.05**	**11.50**
%		**-17.17**	**-11.60**	**-11.96**
t Test		**0.18**	**0.006****	**0.003****

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

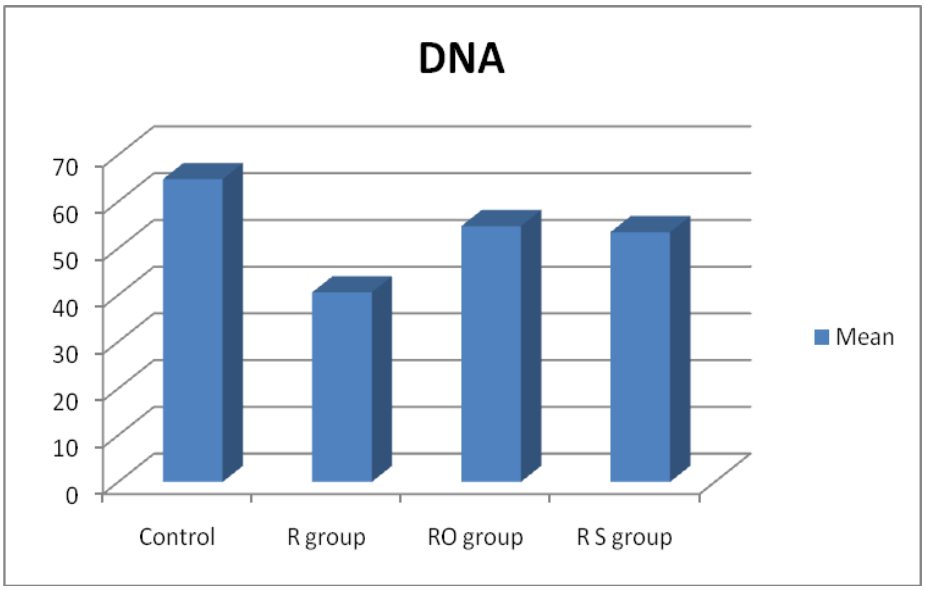


Histogram 2: Revealing MOT values of protein in skin of control and treated groups

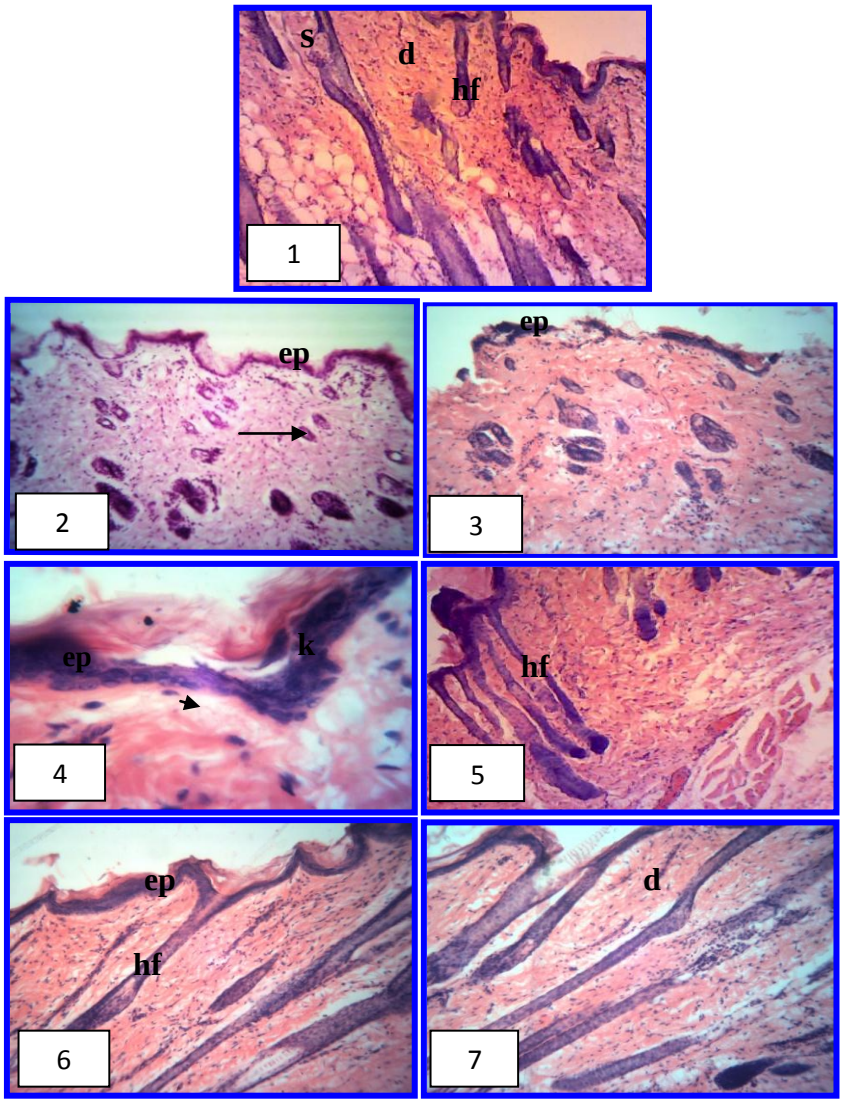
Table 3: showing MOT values of DNA content in the skin of the control and treated group.

	Control	R group	RO group	R S group
Mean	**64.80**	**40.56**	**54.73**	**53.43**
S.D.	**12.88**	**31.78**	**19.36**	**18.94**
%		**-37.40**	**-15.54**	**-17.54**
t Test		**0.01****	**0.001****	**0.20**

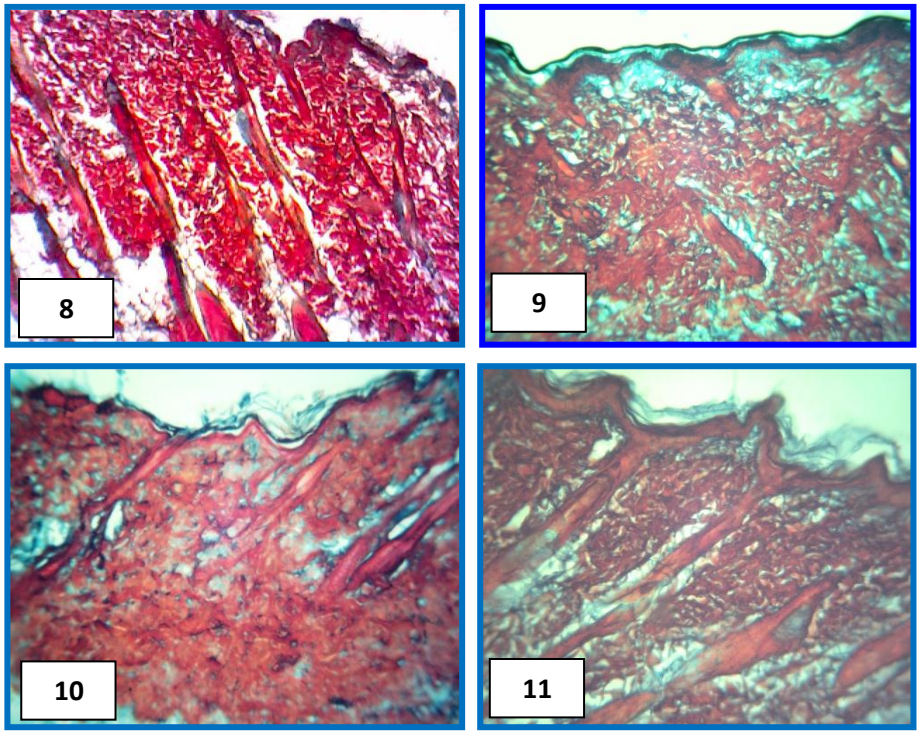
*Significant (P< 0.05), ** Highly significant (P<0.01)



Histogram 3: Revealing MOT values of DNA content in skin of the control and treated groups



Figures 1-7: photomicrographs of skin tissues of the control and treated groups
Fig.1:a photograph showing normal structure of control rat skin. Note well developed epidermal (ep) and dermal (d) layer which contain hair follicles (hf) and their sebaceous glands (s) (X200).
Figs.2-4- Skin tissues of R group showing highly corrugated, atrophied, ruptured and distorted epidermal layer (ep). Some hair follicles are atrophied and damaged (arrow) (X 200).
Fig. 4- Notice: highly thickened keratin layer (k), and numerous degenerated areas in the widened dermal layer (arrow head) (X400).
Fig. 5-Skin tissues of RO group showing well developed architecture of the dermal and epidermal layers and hair follicles (hf) with their sebaceous glands (X 200).
Figs.6- 7- Skin tissues of RS group showing well developed histological architecture of the epidermal (ep) and dermal layers (d) and so the hair follicles (hf) (X 200).



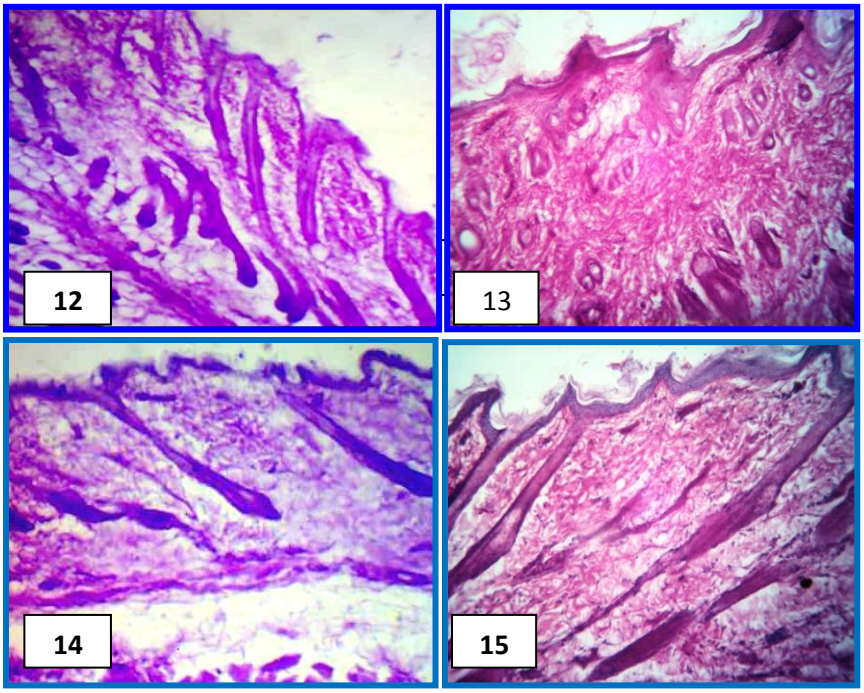
Figures 8-11- Photomicrographs of skin tissue showing distribution of the collagen fibres in the rats of control and treated groups (Mallory's trichrome stain X 200)

Fig.8: skin tissue of a control rat showing thin collagen fibers in the dermal and epidermal layers **(X 200).**

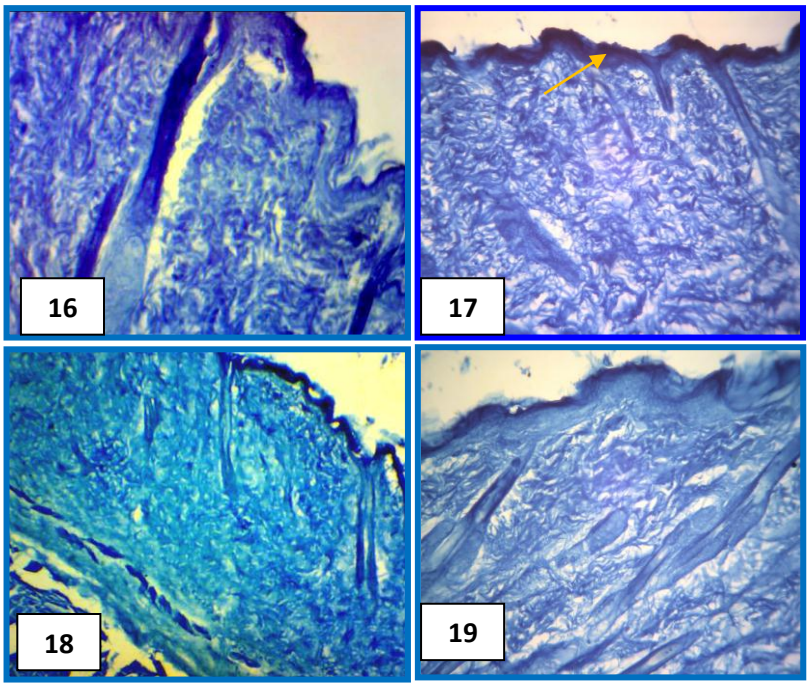
Fig. 9: skin tissue of R group showing highly increased collagen fibers in the distorted epidermal and dermal layers (X 200).

Fig.10: skin tissue of RO group showing decreased collagen fibers in the epidermal and dermal layers in comparison with **R** group (X 200).

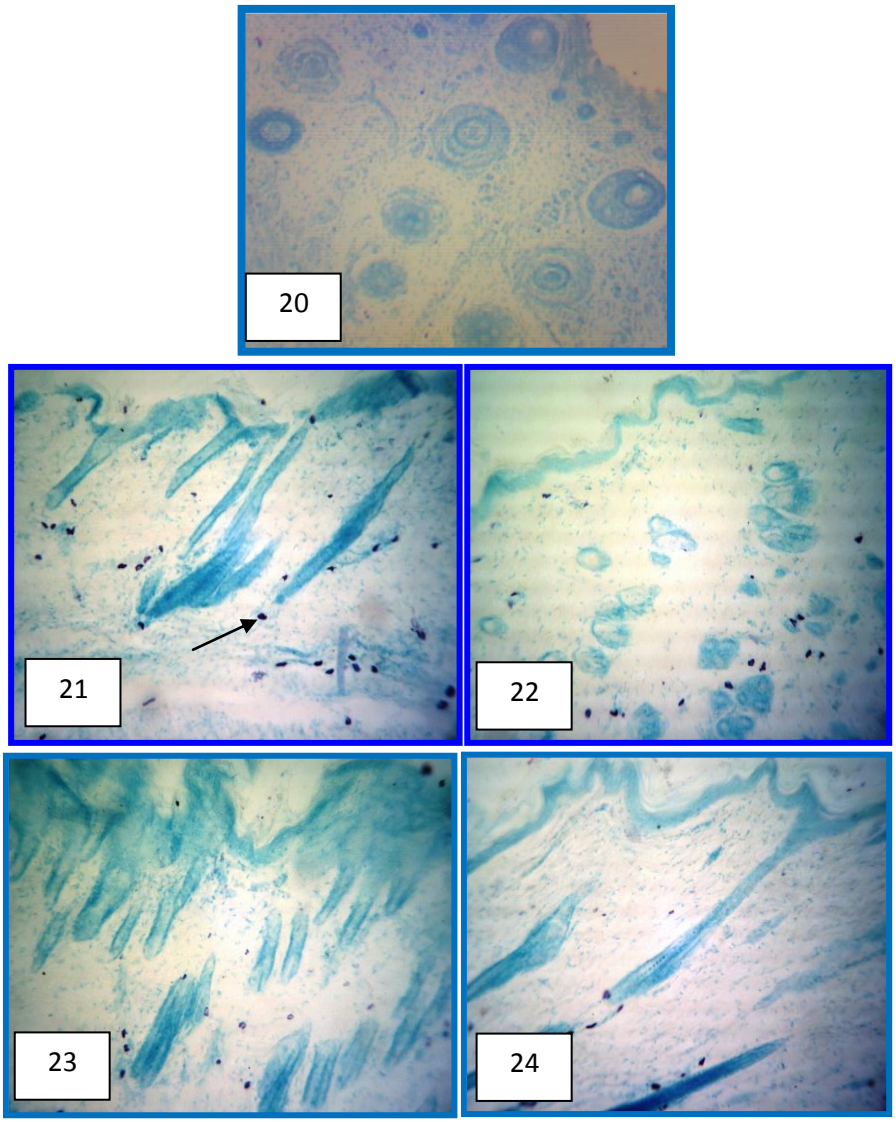
Fig. 11: skin tissue of RS group showing decreased collagen fibers in the epidermal and dermal layers compared to **R** group (X 200).



Figures12-15-Photomicrographs of rats skin tissues showing distribution of PAS +ve materials in the control and treated groups (PAS X 200)
Fig.12: skin tissue of a control rat showing moderately stained PAS +ve materials in the dermal and epidermal layers **(X 200).**
Fig13: skin tissue of R group showing decreased PAS +ve materials in the dermal and epidermal layers and atrophied hair follicles. Notice the degenerated areas are poorly or negatively stained. (X 200).
Fig. 14: skin tissue of RO group showing increased PAS +ve materials compared to **R** group (X 200).
Fig. 15: skin tissues of RS group showing increased PAS +ve materials in comparison with **R** group (X 200).



Figures16-19-Photomicrographs of rat skin tissue showing total protein distribution in the skin tissue of the control and treated groups (Mercury bromophenol blue X 200)
Fig.16: skin tissue of a control rat showing densely stained total protein in the epidermal layer and hair follicles with less stained dermal layer. (X 200).
Fig. 17: skin tissue of R group showing increased total protein in the thickened keratin layer (arrow) with decreased staining affinity in the dermal layer especially in the damaged hair follicles (X 200).
Fig.18: skin tissue of RO group showing increased total protein in the dermal and epidermal layers in comparison with **R** group (X 200).
Fig. 19: skin tissue of RS group showing increased total protein in the epidermal and dermal layers compared to **R** group (X 200).



Figures 20-24: Photomicrographs of skin tissue showing distribution of mast cells in the control and treated groups (Toluidine blue X 200)

Fig 20: photomicrograph of a section of the skin tissue of the control group showing few mast cells in the dermal layer

Figs.21-22: skin tissue of R group showing highly increased number of mast cells in the skin tissue (→).

Fig 23: skin tissue of RO group showing decreased number of mast cells in the skin tissue in comparison with the irradiated group.

Fig 24: skin tissues of RS group showing highly decreased number of mast cells in the skin tissue in comparison with the irradiated group.