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The Positive Role Of Laser Radiation Helium Neon With A Wavelength Of 632.8 Nm On Wound Healing Of Laboratory Mice (*Mus musculus*)

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Abstract:

The present study was designed in laboratory mice Mus musculus to determination role laser Helium-Neon (He-Ne) with a wavelength of 632.8nm in wound healing. Thirty mice male and female were divided into five groups. First group considered as the control group while the other groups were exposed to the Laser at (one day, three days, five days and ten days) respectively. Then showing results collagen fibers and fibroblasts have widespread in the tenth day of the experiment.

Keywords: laser helium neon, skin, collagen fibers, fibroblasts.

Introduction

A laser is an optical device that emits coherent light (electromagnetic radiation). The term LASER is an acronym for Light Amplification by Stimulated Emission of Radiation, Some of the Medicine applications of laser include, laser healing, surgical treatment, kidney stone treatment, eye treatment, dentistry, acne treatment, and hair removal. etc. over the last decade, interest in phototherapy has emerged, including low-energy laser therapy, which has been developed and became more widely for the treatment of various conditions including wound healing and pain relief [1]. Any exposure to laser light may stimulate or inhibit cellular function and may lead to beneficial effects. This technique is called phototherapy or laser therapy [2]. The study was proved that radiation is good enough to regenerate skin cells and increase collagen fibers inside the dermis compared to the control group [3]. Laser irradiation stimulates the growth of fibroblasts in the dermis and also facilitates the healing of ulcers, The efficiency of Laser is depends on exposure time and energy density, and the laser can either stimulate or inhibit the growth of fibroblasts [4]. In the laser therapy, a laser beam is directed toward the sample. Therefore, the observation of the effects on the sample depends on several variables, energy intensity, duration of laser exposure and wavelength. The duration of laser exposure is an important variable in controlling desired and unwanted effects on body tissues [5].

The penetration depth of a few microns can be considered as a treatment for skin lesions because most of the target cells such as fibroblasts, keratinocytes, macrophages and endothelial cells are located within the upper epidermis [6]. The study showed that the bio-stimulation of light promotes tissue repair by accelerating the production of collagen and promotes the stability of the total connective tissue in the healing process of male laboratory rats infected with streptozotocin-induced diabetes. by increasing collagen in the treated group by laser compared with the control group [7].

The study showed that wound healing consists of several types of cells, including fibroblasts, lymphocytes, monocytes, macrophages and epithelium cells, A number of factors were studied, including laser type, wavelength, irradiation time, irradiation and distance of penetration [8]. Laser treatment in laboratory studies has benefits such as it speeds up biochemical reactions, activates fibroblasts, accelerates the metabolism of collagen, increases blood vessel expansion, activates lymphocytes and produces mitochondrial adenosine triphosphate (ATP). When laser therapy is applied at an appropriate, it can act on the biochemical stimulation of the tissues by stimulating the cells on the cellular divide, which is a vital step in the process of tissue repair [9]. The study using photodynamic therapy to explain the effect of skin lesions on mice and the results showed a buildup of epithelial cells in wound margins and deposition of higher amounts of collagen fibers [10]. The study showed that Laser therapy was more effective in promoting cellular responses involving inflammation reduction, and reorganization of collagen fibers in damaged tissues [11]. Helium-Neon is one of the most types of lasers used in laboratory experiments, the laser emits visible red light in the form of a continuous beam of the wavelength of 632.8 nm and produces medium energy typically between (1-10 mw), the effects of low-level laser therapy (LLL) are photochemical rather than thermal [12].

Materials and Methods:

The Experimental Animals

In the current experiment, 30 male and female laboratory mice of age 8-10 weeks were used. The weights were measured by the sensitive balance and the weight of the mice was (25 ±5) gm. The mice were placed in plastic cages and divided into five groups each group of six mice. The first group is the control group, but the test of the groups was exposed to Helium-Neon laser with the wavelength of 632.8 nm for different periods of time, group A for one day, group B for three days, group C for five days and group D for ten days. The duration of laser exposure is 60 seconds. All animals were left throughout the study period in the animal house of the college of education for pure sciences.

Laser radiation

Laboratory mice were irradiated with Helium-Neon laser at a wavelength 632.8 nm and a power density of 4 joule /cm² for ten days except the control group which was not exposed to laser radiation.

Histological Study

Ten days after exposure the mice to the laser, all animals were anesthetized by chloroform to obtain samples from the skin. The samples were placed in a formalin solution at a concentration of 10% for 48 hours. The samples were then washed with running water for one hour, Samples were transferred to a series of alcohol ethanol with a rising concentration 30%,50%,70%, 80%, 90%,100%,100% for two hours. The samples were placed in xylol for 15 minutes for clearing and to be transparent. The samples were placed in a convection oven containing three groups of pure paraffin wax with a temperature of (58-60) C° for one hour for each group. Paraffin wax was poured into special templates. Then the samples were moved to those molds and left to harden to ready for cutting and then kept in the refrigerator. After the pruning of the templates, it was fixed on the rotary microtome for the purpose of cutting with a thickness of 5 micrometers, were put into a water bath with a temperature of (37- 40) C° for spreading them. The sections were placed on clean glass slides left to dry for 24 hours. Slides were placed in the xylol in two stages of 15 minutes. The slides were transferred to a descending chain of concentration of ethanol alcohol for 2 minutes per concentration. The slides were transferred to distilled water for 2 minutes and placed in the hematoxylin dye for five minutes and washed with tap water for five minutes and transferred to the splitting solution for two seconds. Washed with tap water for five minutes, put in eosin dye for two minutes and were passed through a series of ascending concentration of ethanol alcohol for two minutes for each concentration. The slides were transferred to the xylol twice for each time 5 minutes. P.D.X substance was used for mounting the sections for the purpose of installing the slide cover and left to dry at room temperature. Histological slices were examined using an optical microscope type (Micros). The slides were photographed using a camera connected to the optical microscope and these slides were photographed under different magnification forces to detect tissue changes [13]. The statistical analysis was performed using a variance analysis (ANOVA) In analyzing the results statistically using a program (SPPS) [14].

Results and Discussion

Results of histological sections of the animal's skin of the control group showed the basic structures of the skin which included the surface layer Epidermis which contains several layers of stratified squamous epithelium cells externally covered with keratin and to the inside of the skin layer is the layer of dermis where the spread of collagen fibers can be observed, fibroblasts, blood vessels as well as the structures

attached to the skin (hair, sweat glands, sebaceous glands and their ducts). Histological changes of the skin of exposed groups included re-spread epithelium cells in the wound area with the proliferation of collagen fibers and blood vessels near the wound area. Especially in group shown in the figures (1,2,3,4,5 and 6).

The reason for an increase in the distribution and spread of collagen fiber and epithelium cells in the skin may be due to laser radiation. collagen is an important factor in wound healing, where a lot of collagen fibers are formed in the wound area, collagen creates a new structure that gives the texture strength to restore its normal state, this is consistent with what has been suggested [15] that the effectiveness of low-level laser therapy (LLLT) accelerates wound healing. It also agrees with [16] those who have indicated that the wound healing process is progressively accelerated in a group of patients who have been treated with low-level laser therapy. The result is agrees with [17] is that LLLT work to improve the spread of fibroblasts in the wound area at the time of laser exposure, and agree with [18] those who have shown that LLLT is effective for wound healing and for varying periods of time for a group of laboratory rats in terms of collagen and mitochondrial fibers forming higher than the control group. It agrees with [19-20] that LLLT promotes healing of skin wounds in laboratory mice. The results also showed a significant improvement in wound healing. It agrees with [21] that the LLLT effect can be observed on fibroblasts and increased collagen. In addition, it contributes to the increase of movement of epithelium cells. The results of the current study also agree with [22] that LLLT treatment showed positive results in accelerating the healing of wounds in laboratory mice. It also agrees with [23] that LLLT irradiation has stimulated the spread of collagen.

Low-level laser therapy (LLLT)also had a positive effect in accelerating wound healing due to the proliferation of fibroblasts, collagen fibers and blood vessels near the wound area. These findings are consistent with [24] those who point out that the enhancement of wound healing due to LLLT exposure is the result of the increased proliferation of fibroblasts in the wound area. They also agree with [25] that LLLT supports the proliferation of fibroblasts. They also agree with [26] LLLT is used to accelerate wound healing in laboratory mice. The results of the current study are consistent with [27] those who have shown that LLLT can enhance epithelial cell reshaping in seven days significantly during the wound healing period, thus accelerating wound healing in laboratory mice, and agrees with [28] those who pointed out that exposure to laser radiation contributes to the process of tissue repair by increasing collagen fiber and the number of muscle fibers in the irradiation area. It also agrees with [29] those who explained that laser therapy accelerates wound healing in laboratory mice by stimulating the growth and proliferation of fibroblasts. The wound is a condition that causes skin tissue to break down due to an external effect. Accelerating wound healing also indicates a healing rate, i.e., reduced infection of the bacteria that infect the skin and thus inhibition of wound infection and these results are consistent with [30] that Helium- Neon laser therapy has contributed to the proliferation of epithelial cells and wound healing in laboratory mice. It also reduced the number of inflammatory cells, which accelerated the healing process. It also agrees with [31] that histopathological tests showed increased epithelial cell proliferation near lesions and a reduction in inflammatory cells in laser irradiation areas.

It also agrees with [32] that the effect of the Helium-Neon laser is determined by two factors: wavelength and energy density. They also play a major role in stimulating or inhibiting cellular metabolism. It also agrees with [33] that laser beam penetration is effective at wavelengths ranging from 600-1100 nm. It also agrees with [34] that the depth of laser penetration is as low as a few microns and is sufficient to treat skin lesions because most target cells, such as fibroblasts, keratinocytes and endothelial cells, are located inside the epidermis and upper end of the dermis. They also agree with [35] those who point out that the most

effective laser treatment is a low level of wound healing in energy density doses ranging from 3 - 6 joule /cm² and the wavelengths range from 632.8 -1000 nm, they are safe and give good results in wound healing. It also agrees with [36] that the laser affects biological tissues and depends on the determination of wavelength of laser, energy and irradiation time for bioconcentration purposes because the process of bioconcentration generally promotes cell proliferation.

Those who indicated that a Helium Neon laser with a wavelength of 632.8 nm showed that it had good therapeutic effects in living tissue .

It is also agreed with [37] that the effect of laser therapy on wound reduction in laboratory mice using Helium-Neon laser radiation with the presence of many numbers of fibroblasts in wound areas. It agrees with [38] the fact that the helium neon fluorescence stimulates the proliferation of much higher cells compared to the control group as it achieves the repair of laser exposed tissue. It also agrees with [39] those who indicated accelerated wound healing in laboratory mice after exposure to Helium- Neon light at a wavelength of 632.8 nm and a 4 Joule energy density. It is also consistent with [40] those who indicated that exposure to laser significantly enhances cell proliferation and migration, which may accelerate the re-formation of wound tissues by low-level laser therapy. The current study does not agree with [41] those who showed that the use of Helium-Neon laser at a wavelength of 632.8 nm for five days had no effect on cell proliferation and migration and the concentration of collagen fibers after irradiation.

Table (1): shows the density of collagen fibers and epithelial cells of male and female skin lesions for control groups and exposure groups for Helium-Neon wavelength 632.8 nm.

Days	The density of collagen fibers and epithelium cells of male by micrometer.		The density of collagen fibers and epithelium cells of female by micrometer.	
	Control	Exposed	Control	Exposed
First day	**95±0.365a**	**100±0.575aC**	**95±0.261a**	**100±0.677aD**
Third day	**95±0.792a**	**105±0.195bC**	**95±0.889a**	**110±0.538bC**
Fifth day	**100±0.424a**	**120±0.328bB**	**100±0.220a**	**120±0.344bB**
Tenth day	**95±0.883a**	**130±0.346bA**	**100±0.359a**	**135±0.413bA**
L.S. D	**LSD=59.55 df=5 Sig. (0.00)****		**LSD=63.48 df=5 Sig. (0.00)****	

- The numbers in the table Express values Mean (S.D) and L.S.D values.
- The small letters indicate a significant difference under the level probable ($p \leq 0.05$) When comparing horizontally the control and exposure groups to each factor.
- The capital letters indicate a significant difference under the level probable ($p \leq 0.05$) When comparing vertically the control and exposure groups to each factor.

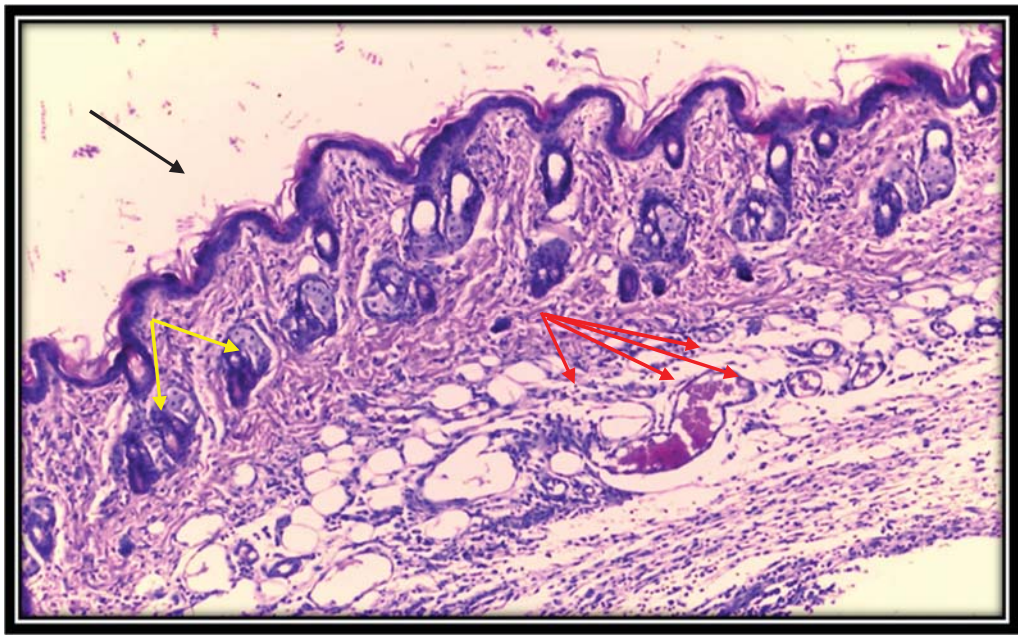


Figure (1): Longitudinal section of the male skin of the laboratory mice (control group) showing epithelium cells (black arrow), collagen fibers (yellow arrow) and blood vessels (red arrow) (H & E) ,(100X).

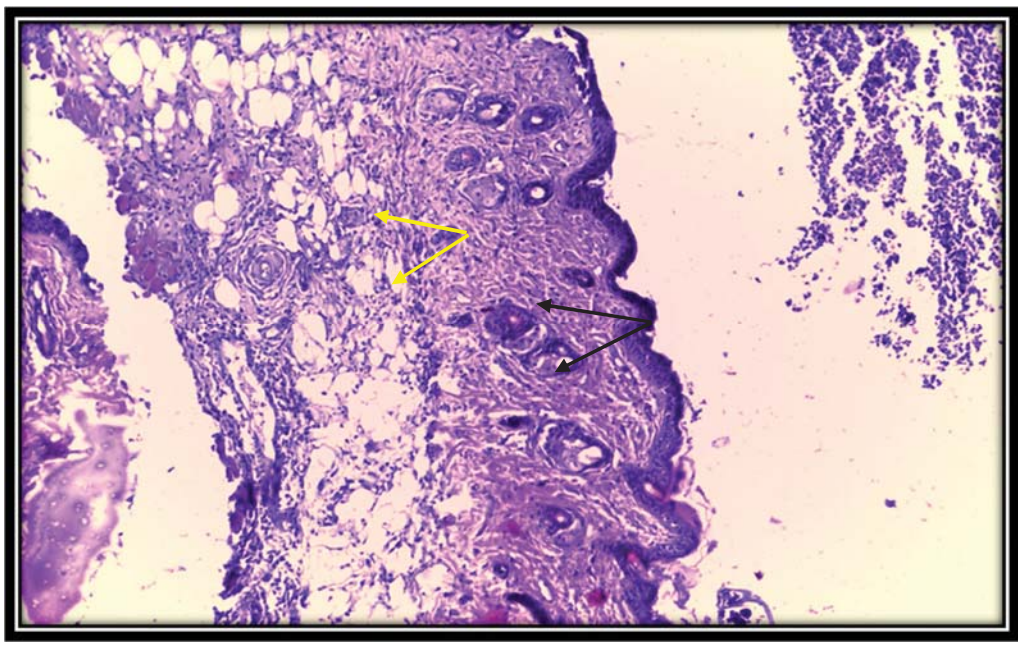


Figure (2): Longitudinal section of the male skin the laboratory mice increased the epithelium cells of the skin (black arrow) and collagen fibers (yellow arrow), He-Ne irradiation for five days (H & E), (100X).

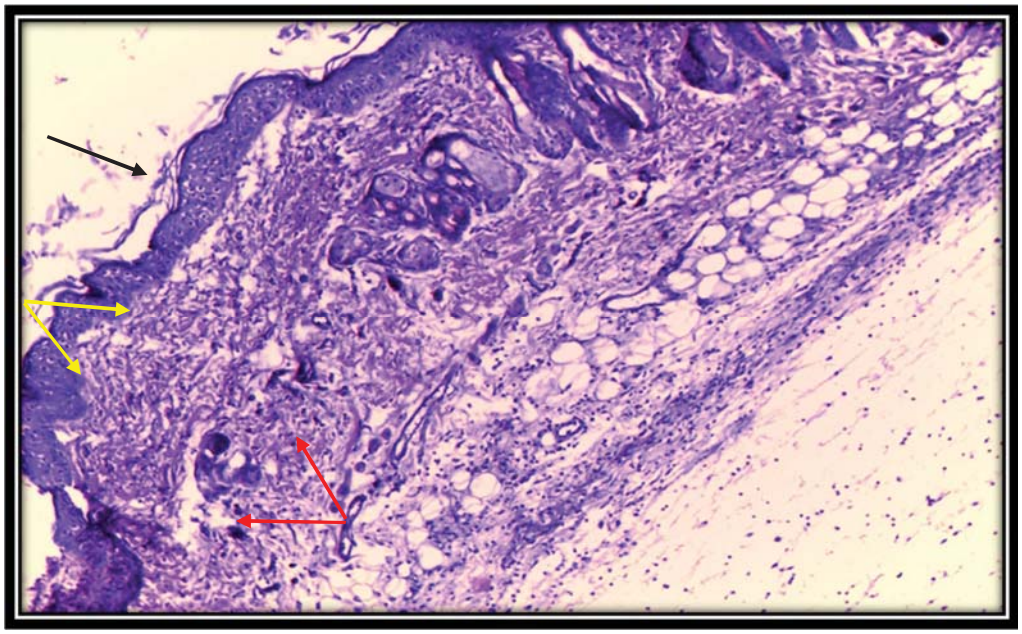


Figure (3): Longitudinal section of female skin the laboratory mice show increase in epithelium cells (black arrow), collagen fibers (yellow arrows) and blood vessels (red arrows), He-Ne irradiation for ten days (H & E), (100X).

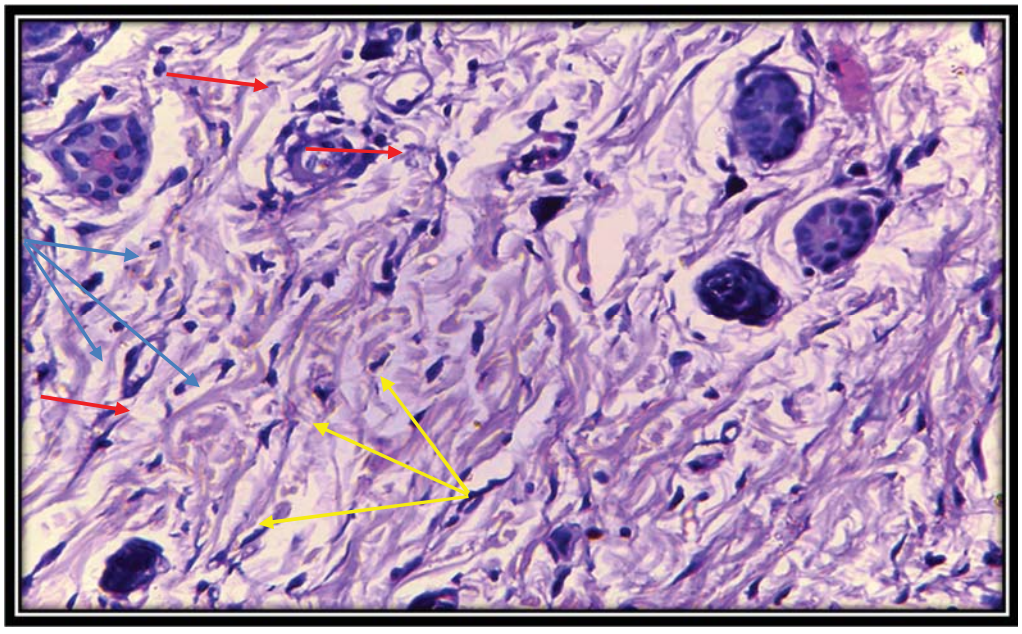
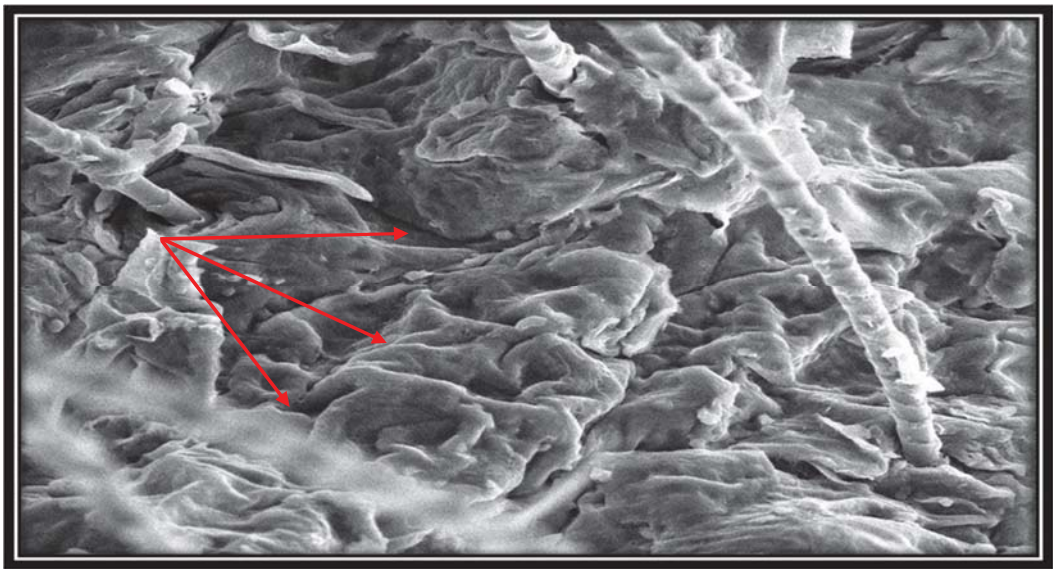
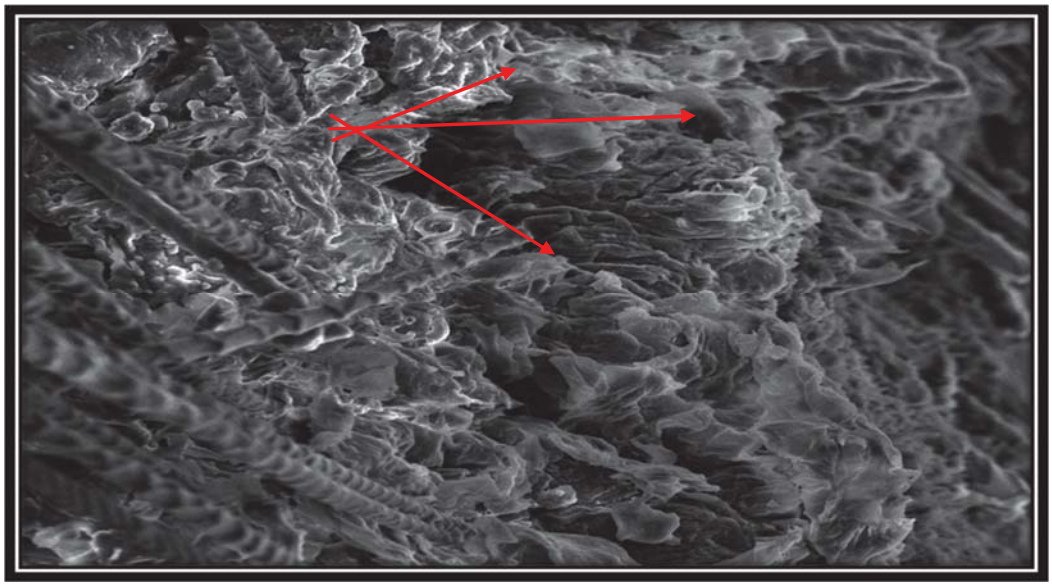


Figure (4): Longitudinal section of female skin the laboratory mice show the proliferation of collagen fibers (yellow arrows), inflammatory cells (blue arrow) and blood vessels (red arrows), He-Ne irradiation for ten days (H & E), (400X).



-----50µm------

Figure (5): Male skin laboratory mice from outside, control group showing the stratified squamous epithelium cells (red arrow), scanning electron microscopy microscope, 50 nanometer magnification power.



-------100µm--------

Figure (6): Female skin the laboratory mice from the outside shows a clear increase in the stratified squamous epithelium cells (red arrow), He-Ne irradiation for ten days, imaging electron microscopy scanner, 100 nanometer magnification power.

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