

**Assessment of Silver Nanoparticles for Burn Injury Healing in Rats**Mustafa Isam Alsarhan¹, Abbas Ali Hussein^{1*}¹Department of Surgery and Obstetric, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq*Corresponding Author Email: Abbas.Ali@qu.edu.iq**Abstract**

The present research aims to assess the histological and clinical effects of applying silver nanoparticles (AgNPs) to rats with superficial second-degree burns. A total of 20 male Wistar rats weighing (240 ± 20 gm) have been used, and they were split in a random way into two equal groups: the G1/control group, which received no treatment, and the rest, while the G2/silver group (AgNPs) received treatment with topical application of AgNPs for consequent 7 days. Following general anesthesia, a stainless-steel bar was placed in a boiling water for 15 minutes, creating one circle site on the animal's body to cause second-degree superficial burns. Histological and clinical assessments regarding the wound healing were conducted. According to macroscopic findings, group2(G2) experienced a substantial ($p \leq 0.05$) increase in wound contraction compared to G1 in the two groups. Microscopic cells in group1(G1), at day 7, demonstrate the existence of a thick crust over the skin's surface and a sparse amount of stratum basale hyperplasia, along with inflammatory cell infiltration and profuse collagen in the dermis as in addition to hemorrhage and profuse irregular thick collagen. On the fourteenth day, there was a broad incision, sloughing of the epidermal layers, infiltration of the inflammatory cells, and profuse collagen. Mild hyperplasia regarding the epidermal layers in surrounding area, along with the existence of granulation tissue that is characterized through the fibrosis with a thick collagen network, growth of new blood vessels, infiltration of inflammatory cells, primarily macrophages, and fibroblast proliferation. At 21, the epidermal layers reveal incomplete hyperplasia, a narrow incision with profuse granulation tissue, profuse fibrosis with few collagens, and significant infiltration of the inflammatory cells, primarily lymphocytes and macrophages. Stratum basale had undergone certain stratum basale hyperplasia, the epidermal layer has entirely shed, and G2 at seven days exhibits a crusty layer above skin's surface. The tissue is infiltrated by inflammatory cells, notably lymphocytes and macrophages, although little granulation tissue is produced. At 14 days, there is a purulent discharge and a thick crust above the skin's surface, and the stratum basale has only incompletely and mildly hyperplized. Hemorrhage, the appearance of granulation tissue, and the creation of a new B.V. are also present, as well as an infiltration of inflammatory cells, primarily lymphocytes and macrophages, a thin collagen network, and fibroblast growth in the dermis. The epidermal layer had completely epithelized and developed a keratinized layer following 21 days. The growth of new blood vessels, RBCs, hair follicles, a thin collagen network, and fibroblast proliferation are also present, along with abundant and frequent fibrosis. The use of AgNP in treating rats with superficial 2nd-degree burns was observed to enhance the healing process and speed up proliferation, wound contraction, and complete healing in less time.

Keywords: Silver, Nanoparticles, Second Degree Burn, Rat**Introduction**

Living beings may survive in various ecological conditions thanks to their skin and related structural components. The first and most crucial role is to provide a barrier between the body and its surroundings in

order to preserve internal meostasis, lessen friction from the environment, and guard against predator scratches and bites. Sexual and social communication is made possible by the skin glands' pigmentation and odor (1,2). The primary function of the skin is to shield the mammalian body from external threats



such as UV radiation, chemicals, temperature changes, and microorganisms. Mammals' skin serves as a physical barrier that protects them from their surroundings. Skin's protective function is connected to highly developed signaling and sensory systems that may respond to environmental changes and connect with organs and body cells (3). Burn injuries continue to pose a severe threat to public health, with an annual rise in avoidable fatalities and impairments. When skin cells are exposed to direct flame, heated solid objects, or boiling liquids, thermal burns result. As a result of the extended hospital admissions and rehabilitations with possibly infectious illnesses which might lead to sepsis mortality, burn injuries are a global health issue which is made worse in the developing countries. They are also associated with large costs for public health systems. World Health Organization (WHO) have estimated that 180000 burn-related deaths occurred in 2017, with two-thirds of these deaths that occur in Southeast Asia and in Africa. Nearly a million people in India have burns that range from mild to severe each year (4).

The microbicidal activity of silver nanoparticles (AgNPs) is mediated by newly elucidated mechanisms. Sondi and Salopek-Sondi used *E. coli* to describe the bactericidal activity regarding AgNPs against Gram-negative bacteria initially. They learned that Nanoparticles (NPs) accumulate in "pits" which form in cell wall. Subsequently, AgNPs produce free radicals that injure the cell and kill bacterium (5). In addition, redox process by which the silver ions are released from AgNPs is one of the factors that damages bacteria (6). It is thought that such inhibition of bacterial metabolic enzymes takes place since the thiol group regarding cysteine specifically react with silver ions. Furthermore, the silver ions could replace other metal ions that have been previously connected to the cysteine in the peptide or form new connections with cysteine residues to kill the bacteria. AgNPs' antibacterial effect and diameter are correlated, and studies have demonstrated that

a smaller diameter is linked to higher bactericidal activity (7).

In the medical field, AgNPs were used for a number of applications, including medical devices, drug delivery systems, and biosensors (8,9). The powerful antibacterial properties regarding the AgNPs were thoroughly studied, and the findings provide important insight for future antibacterial medications (10). AgNPs have been effective bactericides against *P. aeruginosa*, *Salmonella Choleraesuis*, *Bacillus cereus*, *E. coli*, and *S. aureus* (11). Evaluation of the AgNPs' antiviral activity demonstrated a significant decrease in influenza virus proliferation both in vivo and in vitro. As a result, mice survived the H₃N₂ influenza virus challenge for a significantly longer period of time. The findings revealed modest pathologic changes accompanied with a decline in virus's titer in lung tissue of mice given AgNPs treatment. Those findings suggested that the Ag NPs may be used as effective agent in control of outbreaks of influenza and that Ag NPs may be helpful in the prevention of H₃N₂ influenza virus infection (12).

The anti-inflammatory properties of AgNPs have been demonstrated through peritoneal adhesion prevention using sutures coated with AgNPs. Those sutures reduced swelling without having a cytotoxic effect at the surgical site. Also, the mice's intestinal anastomosis showed improved mechanical strength and decreased inflammation when AgNP-coated sutures were used (13). AgNPs made through biosynthesis were tested in vitro for their ability to scavenge free radicals. According to the research, AgNPs could serve as potent antioxidants and aid to the defense of health against degenerative diseases such as cancer (14).

Materials & Methods

Twenty adult male Wistar rats were obtained from the animal house in the



veterinary collage/University of Al-Qadisiyah, weighing (200±30) with average age of (8-10 weeks) were used and housed under standard conditions in room temperature and ventilation, the animals have been allowed to get free access to water and food ad libitum.

All animals have been divided randomly into two groups (n=10). After general anesthesia with mixture of xylazine (10mg/kg B.W) and ketamine (50mg/ kg B.W) administered intramuscularly (15).

The dorsal portion of all animals were prepared for aseptic surgery. A second degree (1cm) diameter burn wound was caused by a hot cylindrical stainless steel (rod) with insulated rubber handle warmed five minutes within boiling water and put for ten seconds on the skin with its own weight (160 gm) and pressure for infliction of burns (16) the rod has been lifted of boiled water through the pinching of insulating handle with the middle fingers and thumb. The rod has been placed perpendicular onto skin; it rests on its own weight. Animals were individually kept in cages in same normal laboratory conditions. Group One (G1) control group in which all rats did not receive any treatments.

Group two (G2) Silver nanoparticles: In this group the burned site of animals was topically applied with 1 ml(10mg/ml) silver nanoparticles (US Research Nanomaterials, Inc, Houston, USA) suspended in 1ml PBS once daily for 7 days (17).

Characterization of nanoparticles

Particle size of materials that used in this study was measured before using material in the experiment, silver was examined using Ultra violet analysis (UV), The x-ray diffraction analysis (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Electron microscopy analysis (SEM) and determine the particle sizes, the data is recorded is an electronic program arranged for this aim that contains a cure graph and an images of particle size average.

Morphometric evaluation of burn healing:

Direct measurement of burn dimensions was calculated at 7th, 14th and 21st days post injury using a graduated millimeter measurement ruler (Fig 3-9).

Firstly, the diameter of circulation burn (zero day & 7th) were obtained and when the shape of injury was changed and not remain circular at (14th & 21st day) the outer dimensions of wound length (L=along the longest axis) and width (w=smaller axis perpendicular to length) were obtained. The same instrument (ruler) was used always and strict conditions of asepsis were concerned to prevent infection (18).

Measurement of wound area (size): progress changes in burnt area were noticed by direct measurement of wound dimension at 7th,14th and 21st days post injury. When the burn is circular, the circular surface area = (half the diameter)² x 3.14. when the shape of burn is changed the surface area = length x width (19).

Measurement of burn contraction: Burn contraction at 7th,14th & 21st days post injury was measured as percentage of reduction in original burn size by using the following formula: % burn contraction= (burn area on day 0-burn area on day n/burn area on area on day 0 x100) (19).

Epithelialization period measurement: Full epithelization time was measured as the number of days needed for dropping of dead tissue remnants without any residual raw burn. (20).

Histopathological evaluation of burn healing:

The assessment of histopathological slides stained with Hematoxylin and Eosin stains were contained: edema (presence or not)vascular proliferation and/or congestion, neutrophil infiltration, macrophage and/or lymphocyte infiltration, fibrosis, proliferation of fibroblast, presence of granulation tissue.(21).



The recent study has been achieved based on national guidelines for care and use of lab animals. All procedures of experimental protocol were approved by high committee for review and approval of the research proposals of the faculty in the university of Al-Qadisiyah college of veterinary medicine.

Results

Morphometric Assessment of the Burn Healing

All the animals have been clinically well noticing normal behavior and ingestion of food and water during the time of experiment (21 days), there was no any noticeable evidence of skin irritation, such as erythema, inflammation and edema during the period of study (Fig.1). The effect of burn injury on losing weight of rats was shown in (Table 1, Fig.2) No bleeding throughout the process of the surgery. Neither rats under treatment groups nor control one recorded any statistically significant variations on weight during the experiment.

The primary wound surface area (mm²) on first day of experiment was (70.85). Skin burns were measured on days 0,7,14 and 21 post burn injury. The average area of burn on 7th day in G1 and G2 were 60.91, 36.73 respectively. there were significant differences between G2 when compared with G1

On day 14 the G1, was 20.23, the difference was significant between G1 and G2 was noticed regarding the wound size between G2(8.88) when compared with G1 recorded lesser value of surface area after 14 days post burn injury. On day 21st the G1 and G2 were 10.12 and 7.2 respectively.

The contraction of wound is a parameter that is utilized for the assessment of the wound

healing, in which lesion area gradually decreased with the progress of healing time.

The contraction of wound on 7days the G1 and G2 were recorded 14, 48.1 respectively was increased significantly ($p < 0.05$) in G2 when compared with G1.

On 14 days the percentage of wound contraction of G1 and G2 was recorded 58.12, 89.35 respectively, where the G2 increased with G1.

On day 21st the G1 and G2 were recorded 78.2, 90.42 respectively it is clear that wound contraction was faster in G2 which was significant difference when compared with G1 (Table -2, Fig.2)

The epithelization period in treatment groups were shown between (14-20) days which it was increased then that of control group ("20) days. Table (3)

Table 1: Mean weight (gm) of the rats after burn injury

Groups	1 day	7 th days	14 th days	21 st days
G1	200±15Aa	215±18Ab	228±20Ac	235±21ABc
G2	198±13Aa	212±13Ab	224±12Ab	232±12Ac

Values represent Mean ± SE for 10 animals (Rats)

- Small letters represent horizontal statistical reading
- Capital letters represent vertical statistical reading
- Different letters denote to the significant difference, whereas similar letters refer to no significant difference at $P < 0.05$



Table 2: Surface area and wound contraction

Periods Groups	Surface area (mm ²)				Wound contraction %			
	0 day	7 days	14 days	21 days	0 day	7 days	14 days	21 days
G1	70.85 Aa	60.91 Ab	20.23 Ac	10.12 Aa	0 Aa	14 Ab	58.12 Ac	78.02 ABd
G2	70.85 Aa	36.73 Bb	8.88 Bc	7.22 ABd	0 Aa	48.1 Bb	89.35 Bc	92.42 Bc

- Small letters represent horizontal statistical reading
- Capital letters represent vertical statistical reading
- Different letters denote to the significant difference, whereas similar letters refer to the no significant difference at P<0.050

Table3: Epithelialization period per days in control and treated groups

Groups	Epithelialization
G1	20
G2	14



Figure 1: Photographs of control (G1) and treatment group (G2) burned skin circular injury of Wister rat show different phases of burn healing at 1, 7, 14 and 21 days

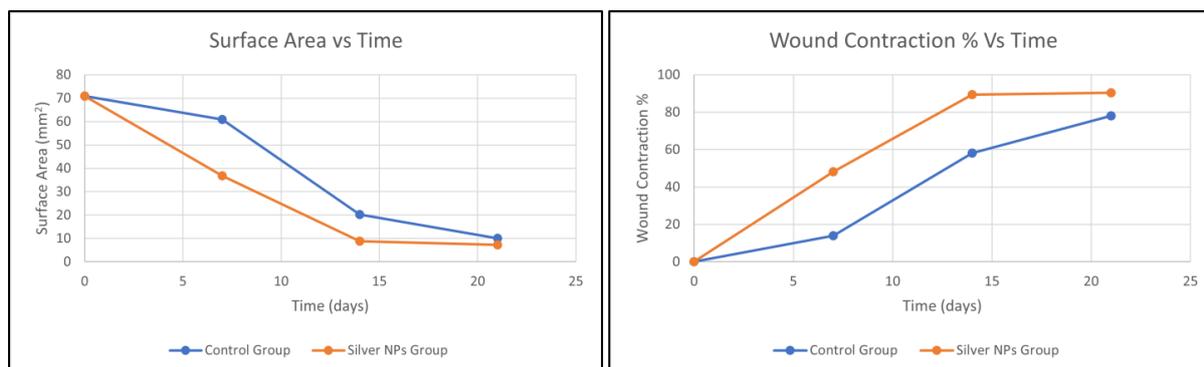


Figure 2: Surface area (size of wound) and wound contraction for G1 & G2

Histopathological Assessment of the Burn Healing

In G1, superficial thickness burns are injuries which result in a partial destruction of hair follicles, nerve endings, and sweat glands 7 days after the burn injury. On the seventh day after a burn injury, histological abnormalities were seen in the control group. There was a thick crust on the skin's surface, a little amount of stratum basale hyperplasia, inflammatory cells, and profuse, irregular thick collagen in the dermis (Fig.3-a). Higher magnification revealed hemorrhage, thick, uneven collagen, and moderate stratum basale hyperplasia (Fig.3-b). After 14 days following the burn injury, there was a large incision, extensive collagen production, and infiltration of inflammatory cells. there was also a slight hyperplasia of the epidermal and layers in the nearby area (Fig. 3-c).

In higher magnification, there were hyperplasia of stratum basale and few infiltrations of inflammatory cells mainly macrophages also noticeable new blood vessels formation and proliferation of fibroblasts (Fig.3-d).

After 21st days post burn injury, epidermal layers showed moderate incomplete hyperplasia with narrow incision (Fig.3-e).

In higher magnification, there were hyperplasia and proliferation of stratum basale, infiltration of inflammatory cells mainly macrophage and lymphocytes (Fig.3-f).

Silver Nanoparticle group (G2), After 7 days post burn injury the surface of skin illustrated presence of thick crust attached closely and mild hyperplasia of stratum basale with high infiltration of inflammatory cells with irregular thick network of collagen and dermis showed thick network of collagen with presence of adipose tissue (Fig.4-a&b)

After 14 days post burn injury, epidermal layers exhibited hyperplasia with presence of narrow incision, granulation tissue was marked and profuse with hemorrhage in dermis and presence of collagen fibers with inflammation cells and new vascularization and dermis showed inflammation of microphages in the dermis (Fig.4 c &d).

After 21st days post burn injury, the site of injury showed complete healing in which complete epithelization with formation of outer keratinized layer above the epidermis, there were hemorrhage and few infiltration of inflammatory cells with new vascularization and fibrosis in dermis (Fig.4-e) in higher magnification, there was formation of new vessels contains RBCs and inflammation cells mainly macrophages (Fig.4-f).

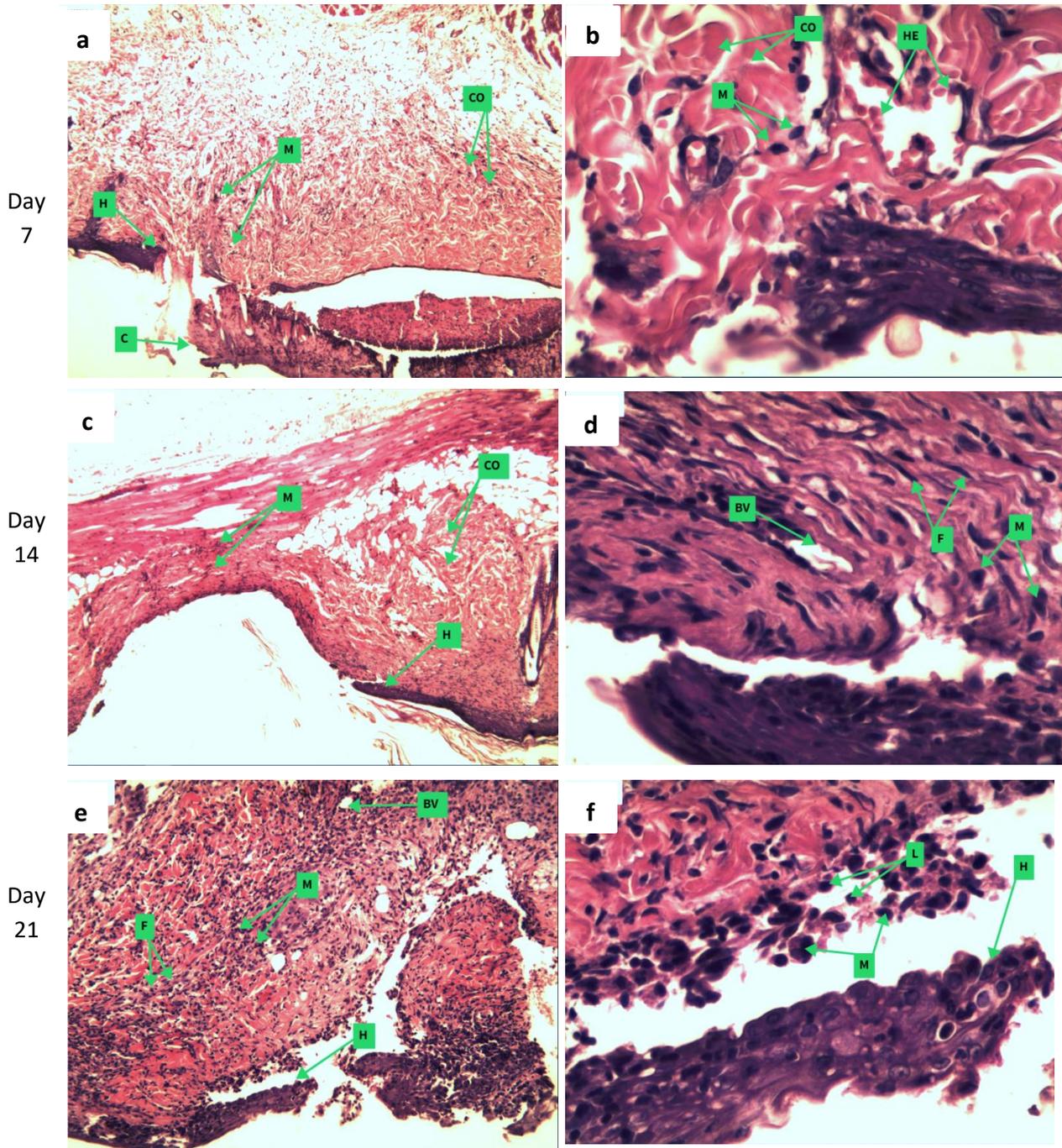


Figure 3: Epithelial tissue of rats in group 1 subjected to second-degree thermal burns, H&E staining **a-**There is presence of thick crust (C) above the surface of skin and few hyperplasia of stratum basale(20x) (H), with infiltration of inflammatory cells (M) and profuse collagen in the dermis (CO), **b-**Higher magnification(200x). there is hemorrhage (HE) and irregular and thick collagen and infiltration of macrophages and lymphocytes, **c-**There is wide incision and sloughing of epidermal layers, profuse collagen with infiltration of inflammatory cells and mild hyperplasia of epidermal layers in the neighboring region(20x), **d-**Higher magnification(200x).Note hyperplasia of stratum basale and few infiltration of inflammatory cells mainly Macrophages, new blood vessel formation (BV) and proliferation of Fibroblasts

(F), e-Moderate and incomplete hyperplasia of stratum basale with narrow surgical incision, high infiltration of inflammatory cells and presence of new blood vessel and fibrosis(50x), f- Higher magnification(200x). Hyperplasia and proliferation of stratum basale, infiltration of inflammatory cells mainly macrophages and lymphocytes (L).

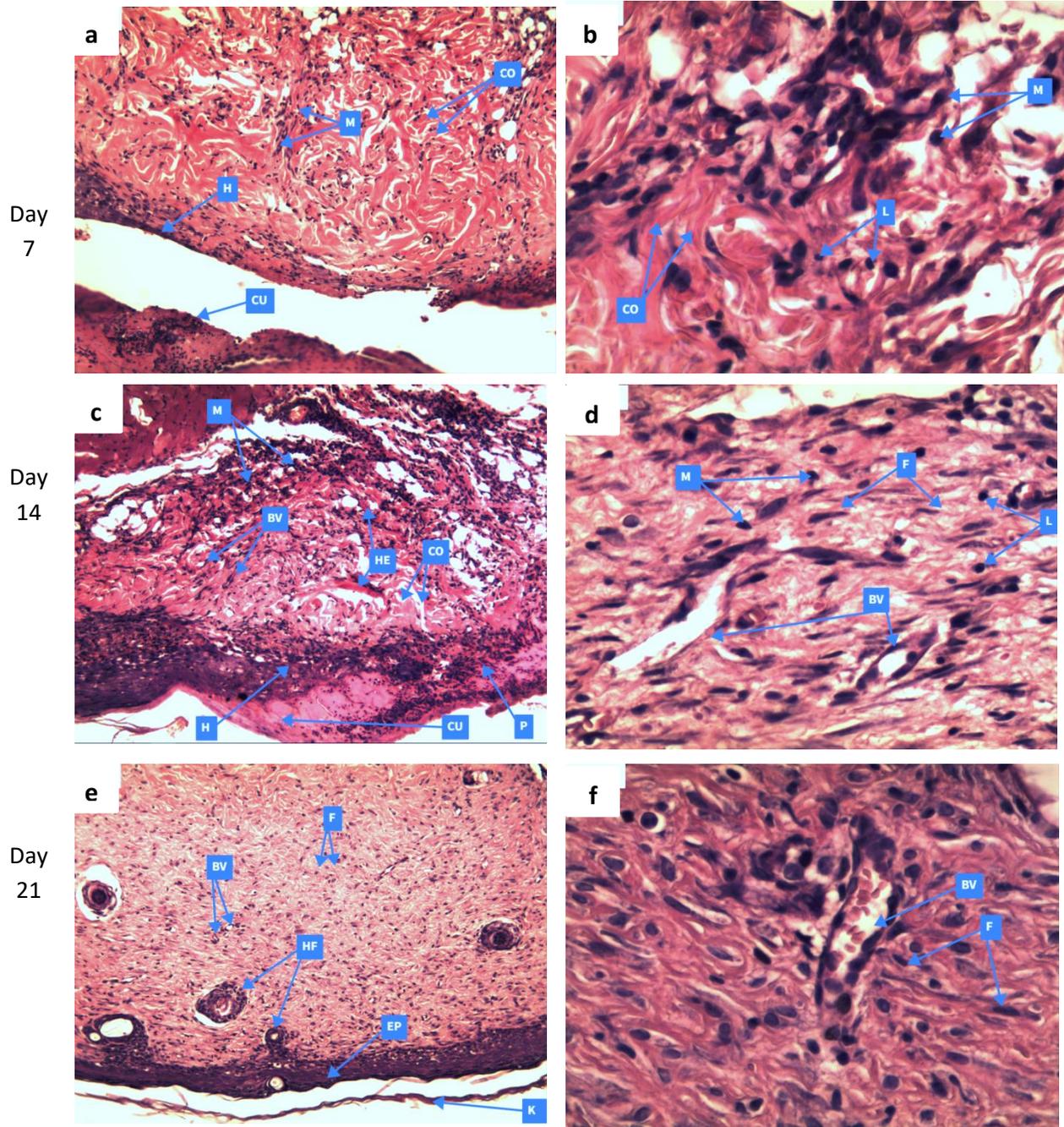


Figure.4: Epithelial tissue of rats in group 2 subjected to second-degree thermal burns, H&E staining **a**-Presence of crust (CU) above the skin surface with complete sloughing of epidermal layer with few hyperplasia of stratum basale (H), thick interlecting collagen fibers with infiltration of inflammatory cells(M) and few granulation tissue formation (50x), **b**-infiltration of inflammatory cells mainly macrophages and lymphocytes (L), thick coarse and interlecting collagen fibers (40x), **c**-There is Thick crust and purulent exudate (P) above the skin surface



with moderate and incomplete hyperplasia of stratum basales (H) hemorrhage and presence of granulation tissue and new blood vessel formation (BV) with infiltration of inflammatory cells in the dermis with thin network of collagen (50x), **d**-higher magnification . Formation of new blood vessel and proliferation of fibroblast (F) with infiltration of macrophages and lymphocytes(200x), **e**-There is complete epithelization (EP) of epidermal layer with the presence of keratinized layer (K) , also there is regular and profuse fibrosis (F) with formation of new blood vessel with the formation of developed hair follicles (HF) (50x), **f** - Higher magnification. There is well developed blood vessel which contain RBCs with proliferation of fibroblasts (200x).

Discussion

The purpose of this research was to assess the effectiveness of application of silver nanoparticles on the superficial second degree burns in rats.

The size of the burns which related to the treatment groups is cleared significant decrease with no inflammation or infection at all the time of monitoring which uncovers important effects of chitosan nanomaterials application Time-based decrements in size of burns are not observed in group 1, however the latter shows such reductions in burn size after 21 days when compared to the other treatment groups. Due to its safety, affordability, decreased toxicity, and broad-spectrum resistance against various pathogens, nanoscale silver plays an exceptional role in the healing of wounds (22,23). When dressing a wound, cotton fibers are coated with AgNPs, which act as bacterial killers and promote faster wound healing (24). This is supported by the study in G2 that found no evidence of inflammation or infection over the course of the investigation. In order to allow for the proliferation of skin cells (keratinocytes and fibroblast) and the subsequent reconstruction regarding the skin surface over time, it is shown that AgNPs block the respiratory pathway of cells, keeping them alive (25).

The surface area and wound contraction are decreased significantly in group 2 when compared with control one in seven- and fourteen-days post treatment and this accord with (26) who found that silver

nanoscales showed efficient wound healing process with clear wound contraction.

The noticeable of no signs of inflammation and infection belong group 2 are attributable to use of silver nanoparticles which plays as bactericidal agent as described by (27) who found that this nanoscale agent can inhibit visible growth of a microorganism after overnight incubation in a specified culture medication.

According to G1 in this work's microscopic analysis, inflammatory response happened shortly following injury and has been followed by tissue necrosis, while proliferative phase involved epithelialization from wound edge to resurface defect extended more than the period of the study to complete repair. Burn wound healing includes the hemostasis and coagulation, cell proliferation, inflammation, and remodeling (28). Control group exhibits in 7 days the existence of thick crust above skin surface and some hyperplasia of stratum basale with the inflammatory cells and profuse irregular thick collagen in the dermis, whereas in 14 days there is sloughing of epidermal layers and profuse collagen with infiltrations of the inflammatory cells and the existence of granulation tissue and formation of new blood vessels, while is 21 days there is incomplete hyperplasia with profuse granulation tissue and fibrosis and such results are in accordance with (29,30, 31).

Following seven days, in G2, epidermal layer had entirely shed, stratum basal underwent some stratum basal hyperplasia, and the skin's surface has developed a crusty layer. The tissue is



infiltrated by inflammatory cells, notably lymphocytes and macrophages, although little granulation tissue is produced. At 14 days, there is a thick crust and purulent discharge above the surface of the burned tissue, and the stratum basal has mildly and incompletely hyperplized. Hemorrhage, the development of new B.V., the presence of granulation tissue, the infiltration of inflammatory cells—primarily lymphocytes and macrophages—into the dermis, the development of a thin network of the collagen, and the proliferation of fibroblasts.

Epidermal layer had completely epithelized and developed a keratinized layer following 21 days. Along with growth of new blood vessels, RBCs, hair follicles, a thin network of collagen, and fibroblast proliferation, there is also frequent and extensive fibrosis. Those findings suggest a proven healing procedure. This finding is consistent with (32) who discovered epidermal regeneration at day seven, the

absence of much granular tissue, vascularization at the dermal layer, and the presence of neovascularization at the dermal tissue by day fourteen along with complete epidermal regeneration. We are of the opinion that AgNPs is biocidal and harmful to microorganisms that damage the epidermal layer and slow wound healing. This is in agreement with the findings of (33) who discovered that the impact of a burn on skin treated with AgNPs led to the appearance of a demarcation line which was visible on day 7 and was made up of polymorph nuclear cells in 7 days, while hair was visible covering the lesion by day 21.

Conclusion

The application of silver nanoparticles over the second degree burn in skin of rats revealed presence of neovascularization at dermal layer and high intensity of granulation tissue with degree excel the control group.

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