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Induced cutaneous wound healing in rabbits under the cover of silver nanoparticles.

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ABSTRACT

Wound healing is a complicated procedure involving a combination of activities of different tissues and cell lineages and has been the subject of concentrated research for a long time. The aim of this study was to evaluate the cutaneous wound healing under the cover of silver nanoparticles (Ag-NPs), in rabbits, a Ag-NPs model for surgical cutaneous wounds. This preparation was used in treatment of cutaneous skin wounds. Four healthy, adult New Zealand male rabbits, weighing 1-1.5 kg were used for the study. Anesthesia of rabbits was provided first by 10 mg /kg of xylazine hydrochloride intramuscular injection and pre- medication, then the application of 50 mg / kg of ketamine hydrochloride intramuscularly. The dorsal aspects of rabbits were clipped and prepared for aseptic surgery. On dorsal aspect of each animal, one cranially and two caudally located full- thickness skin wounds of 28.5 mm diameter were created. Wound was dressed once daily, with topical Ag-NPs, the third by normal saline for 14 days. Observations during daily wound care: each wound was evaluated for the presence of exudates and wound healing until 14 days. The wound diameters were measured by caliper both horizontally and vertically and the average was calculated postoperative on the 4^{th}, 7^{th}, 10^{th}, and 14^{th} days. Biopsy specimens, which were collected on the 4^{th}, 13^{th}. , and 18^{th} post-operative days (POD). The results of the study showed body weight and body temperature did not show significant changes. Respiratory rates none significantly increased post operation. Heart rates significantly increased($P < 0.05$) in day 9 and 15 post operation. Bleeding time none significantly increased in day 3 and 9 but it become significant increases($P < 0.05$) in day 15 post operation. Clotting time significantly decreased($P < 0.05$) in day 9 and 15 post operation. The results showed that, RBC count and Hb concentration did not show significant changes , PCV % significantly decreased($P < 0.05$) in day 3 post operation , but the decrease become none significant in day 9 and 15 post operation .The results showed that the total leucocytes count none significantly increased in day 9, but it decreased significantly($P < 0.05$) in day 15 post operation. Heterophils % none significantly increased in day 9 post operation. Lymphocytes % none significantly decreased in day 9 post operation. Esinophils% significantly deceased ($P < 0.05$)in day 9 and 15 post operation. .Basophils none significantly decreased. Monocytes none significantly increased post operation. The results showed that the better healing was achieved in preparation at concentration 20% followed by that of 10% .The results showed that the better healing was in preparation concentrated 20%.

Keywords: silver nanoparticles, Rabbits, wound healing .

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INTRODUCTION

Ultimate goal for wound healing is a speedy recovery with minimal scarring and maximal function. Wound healing proceeds through an overlapping pattern of events including coagulation, inflammation, proliferation, and matrix and tissue remodeling. For this efficient and highly controlled repair process to take place, numerous cells signaling events are required. Although cytokines are crucial in initiating, sustaining, and regulating the post – injury response, these same molecules have been implicated in impaired wound healing, abnormal scar formation, and uncontrolled inflammatory response (1, 2, 3).

Silver has been used for centuries to prevent and treat a variety of diseases including pleurodesis, cauterization, and healing of skin wounds (4, 5). Its antibacterial effect may be due to blockage of the respiratory enzyme pathways and alteration of microbial DNA and the cell wall (6). In addition to its recognized antibacterial properties, some authors have reported on the possible pro- healing properties of silver (7).

Nanotechnology has provided a way of producing pure silver nanoparticles (Ag-NPs). This system also markedly increases the rater of silver ion releases (8). Recently we demonstrated that silver nanoparticles exhibit cytoprotective activities toward HIV -1- infected cells (9).

Published studies of silver nanoparticles on wound healing are sparse, and the mechanism of action remains unknown. Herein we report that silver nanoparticles can promote wound healing and reduce scar appearance in dose – dependent manner. Furthermore, our studies show that silver nanoparticles act by decreasing inflammation through cytokine modulation. Silver nanoparticles promote healing and achieve better cosmoses (10, 11).

In our thermal injury model, the deep partial – thickness wounds normally healed after 35.4 ± 1.29 days (mean ± SE). In animals treated with silver nanoparticles (ND), these healed in 26.5 ± 0.93 days). whereas wounds treated with silver sulfadiazine (SSD need 37.4 ± 3.43 days , The rate of healing in the three groups was also compared .As with healing time , rate of healing was increased in animals treated with ND. These observations indicate that wound healing is accelerated by silver nanoparticles. We next compared the appearance of healed wounds. We found that wounds in the ND group showed the most resemblance to normal skin, with less hypertrophic scarring and nearly normal hair growth on the wound surface. The worst cosmetic appearance was observed in the SSD treatment group (10).

Under histological evaluation. Healed wounds from the ND group resembled normal skin, with a thin epidermis and nearly normal hair follicles. In contrast, histological sections from the SSD – treated group showed thickened epidermis and no evidence of hair growth (10).

Topical delivery of silver nanoparticles suppresses both local and systemic inflammation .This study examined the effects of Ag-NPs on healing used in experimentally induced , full thickness skin wounds in terms of clinical (following wound formation , scar formation until the 20th postoperative day granulation tissue development ; progress of epithelization and presence of infection were evaluated macroscopically) , biochemical (MDA ,GSH , GSH-PX , CAT) and histopathological (re-epithelization , granulation tissue , collagen accumulation , inflammatory cell , angiogenesis , ulcer) aspects (12).

The aim of this study was to evaluate the wound healing activity of the preparation as compared with isotonic saline solution, in the cutaneous wounds in rabbits, an appropriate of Ag-NPs model for surgical cutaneous wounds.

MATERIAL AND METHODS

Animals

Four healthy, adult New Zealand male rabbits , weighing 1-1.5 kg were used for the study. They were individually housed and fed in normal diet (green and concentrated) and water ad libitum in a room with natural light cycle and constant temperature (24 ±2 ^{o}C).

Instrumentation and surgical procedure

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Anesthesia of rabbits was provided first by 10 mg /kg of xylazine hydrochloride intramuscular injection and pre- medication, then the application of 50 mg / kg of ketamine hydrochloride intramuscularly. The dorsal aspects of rabbits were clipped and prepared for aseptic surgery. On dorsal aspect of each animal, one cranially and two caudally located full- thickness skin wounds of 15 mm diameter were created.

The preparation of silver nanoparticles was obtained from the Nanotechnology and Advanced Materials Research Center, Baghdad.

Treatment protocol

Wound was dressed once daily, with topical preparation, the third by normal saline for 14 days. Evaluation of wound healing by observations during daily wound care: each wound was evaluated for the presence of exudates and wound healing until 14 days. The wound diameters were measured by caliper both horizontally and vertically and the average was calculated postoperative on the 4^{th}, 7^{th}, 10^{th}, and 14^{th} days. Skin specimens for histological evaluation, which were collected on the 4th, 13th. , and 18th post-operative days (POD) according to (13).

Statistical analysis

Statistical analysis: All values are expressed as the mean ± the standard error of the mean (SEM) using t test in comparison of the means for statistical differences, the significant level of test was $P < 0.05$, according to (14).

RESULTS

The results of the study showed body weight and body temperature did not show significant changes. Respiratory rates none significantly increased post operation. Heart rates significantly increased($P < 0.05$) in day 9 and 15 post operation. Bleeding time none significantly increased in day 3 and 9 but it become significant increases($P < 0.05$) in day 15 post operation. Clotting time significantly decreased($P < 0.05$) in day 9 and 15 post operation **Figure 1.**

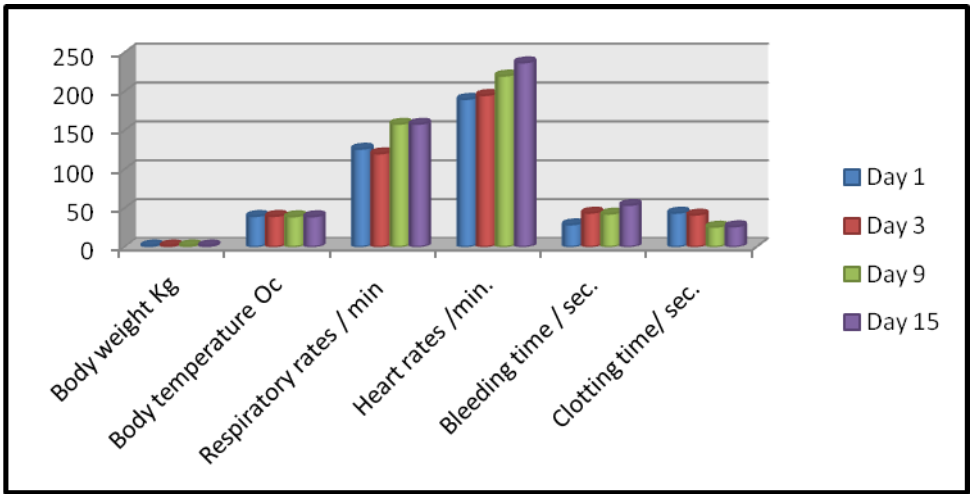

Figure -1-showing clinical parameters (Body weight, Body temperature, Respiratory rates, and Heart rates) bleeding time, and clotting time of rabbits in the study

The values are Mean ± SE. significance at $P < 0.05$, means significance in comparison 3,9,15 days with 1 day of treated group.

The results showed that, RBC count and Hb concentration did not show significant changes , PCV % significantly decreased($P < 0.05$) in day 3 post operation , but the decrease become none significant in day 9 and 15 post operation **Figure 2.**

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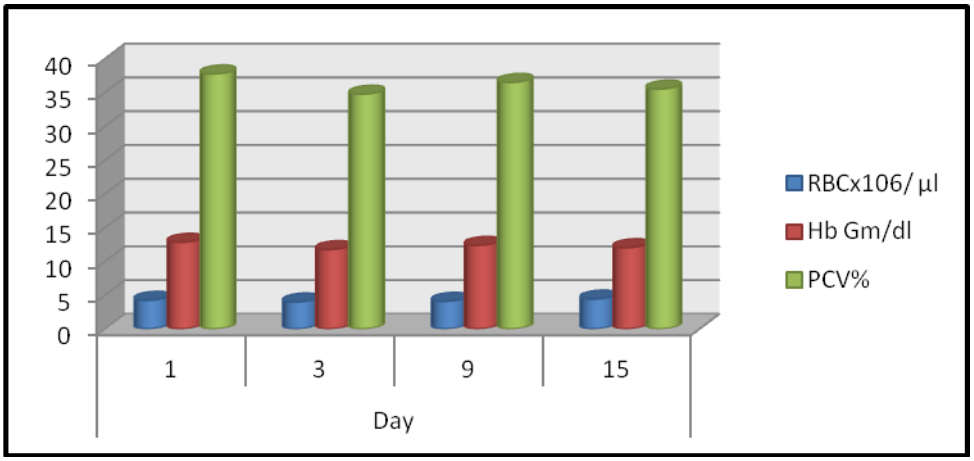

Figure -2- showing Total erythrocytes count, Hb concentration, PCV, and erythrocyte indices of rabbits in the study.

The values are Mean ± SE. significance at P < 0.05, means significance in comparison 3,9,15 days with 1 day of treated group.

The results showed that the total leucocytes count none significantly increased in day 9, but it decreased significantly($P < 0.05$) in day 15 post operation. Heterophils % none significantly increased in day 9 post operation .Lymphocytes % none significantly decreased in day 9 post operation .Eosinophil% significantly deceased($P < 0.05$) in day 9 and 15 post operation .Basophils none significantly decreased. Monocytes none significantly increased post operation **Figure 3.**

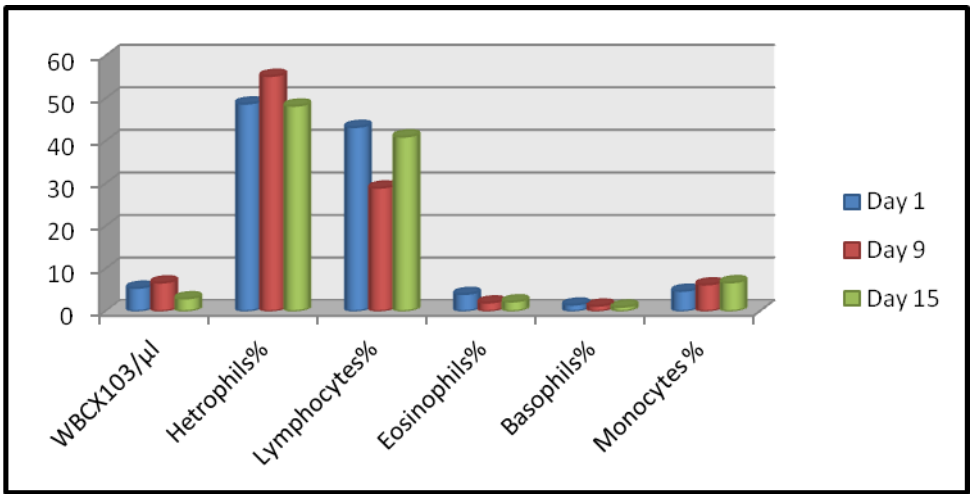

Figure -3- showing Total leucocytes counts and differential leucocytes count of rabbits in the study

The values are Mean ± SE. significance at P < 0.05, means significance in comparison 3,9,15 days with 1 day of treated group.

The results showed that the better healing was achieved in preparation at concentration 20% followed by that of 10% **Table 1.**

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Table -1- Showed diameters of wound postoperative

Parameter / Degree of healing	Days			
	1	7	9	12
Saline	21.5±3.33	26.39±0.96	24± 2.0	Scar
5%	21±1.53	27.24±2.94	23.67±2.96	Scar
10%	20.67±0.60	26.63±1.95	26.67±1.20	Scar start healing
20%	21±1	27.08±3.06	15.83±4.8	++ve better healed

The values are Mean ± SE. means significance in comparison 7,9,12 day with 1 day of treated group by comparison 5%,10%,20% concentrated .

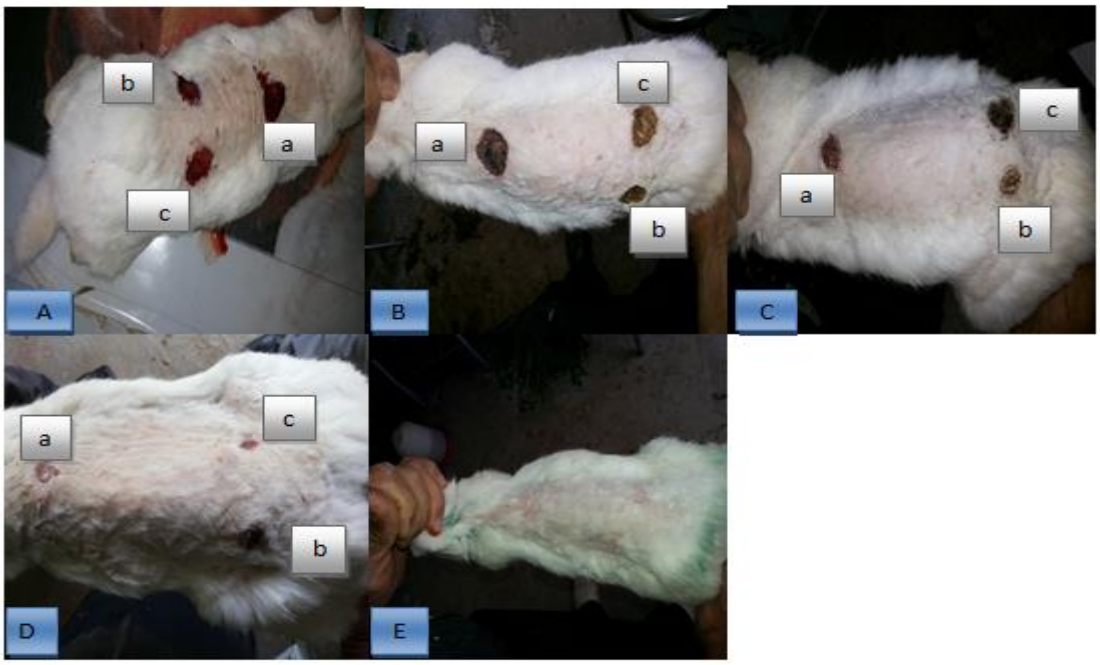
The results showed that the better healing an achieved in preparation concentrated 20%, 10%, and 5% respectively **Table 2.**

Table -2- Showing the degrees of healing of wounds

Wound No.	Concentration of preparation			
	saline	5%	10%	20%
1	Scar	Scar	Healed nearly	Healed nearly
2	Scar	Scar	Scar	Better healing ++ve
3	scar	Scar	Scar	++ve healing

Data of macroscopic appearance showed that wound healing treated with Ag-NPs at concentration 20% revealed the best wound healing. At 7th days the wound healing showed normal response to injury (inflammatory phase), and the wound itself did not ooze .

At 15th day post operation, diameters of wound were decreased ,wounds were remodeled , made up new blood vessels, and had eventually scar formation. At 30th day, our data showed complete close to the wound. We also found that wounds at 20% Ag-NPs group were somehow similar to normal skin, with less hypertrophic scarring and nearly normal hair on the skin surface as shown in **Figure 4**.



Figures- 4- showing the macroscopic appears of healing of wounds with Ag-NPs .A One day, B 4th day, C 7th day, D 15th day, E 30th day, a 5% ,b10% , c 20 %

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Histological result revealed that healed wounds at concentration 20%, 10%; and5% groups respectively resembled normal skin, with a thin epidermis and nearly hair follicles in comparison to the control group. Histological result showed that healed wounds of treated and none treated groups at 7th day showed presence of scar tissue formation, fibroblasts formation, and granulation tissue which is surrounded by inflammatory cells as shown in **Figure 5**.

At 13th day operation, our data revealed that healed wounds at concentration 20%, 10%; and5% groups showed many histological findings represented by presence of fibrosis in the epidermis of the skin, presence of granulation mass in the epidermis, perivascular inflammatory cell in the epidermis of the skin accompanied with congestion as shown in **Figure 6.**

At 18th day, histological data showed that healed wounds at concentration 20%, 10%;and 5% groups have granulation tissue in their skin, the granulation mass composed of necrotic tissue accompanied with cellular debris (inflammatory cells) , presence of fibrosis in the epidermis of the skin, re-epithelization of the dermis in the skin **Figure 7.**

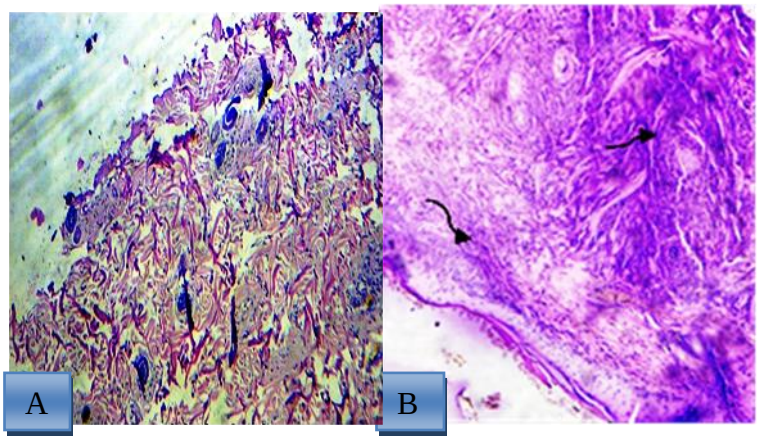

Figure (5).(A,B) Histological of skin wounds of treated and not treated groups in the 7th day showed fibroblasts → Hematoxylin and eosin staining 40 x.

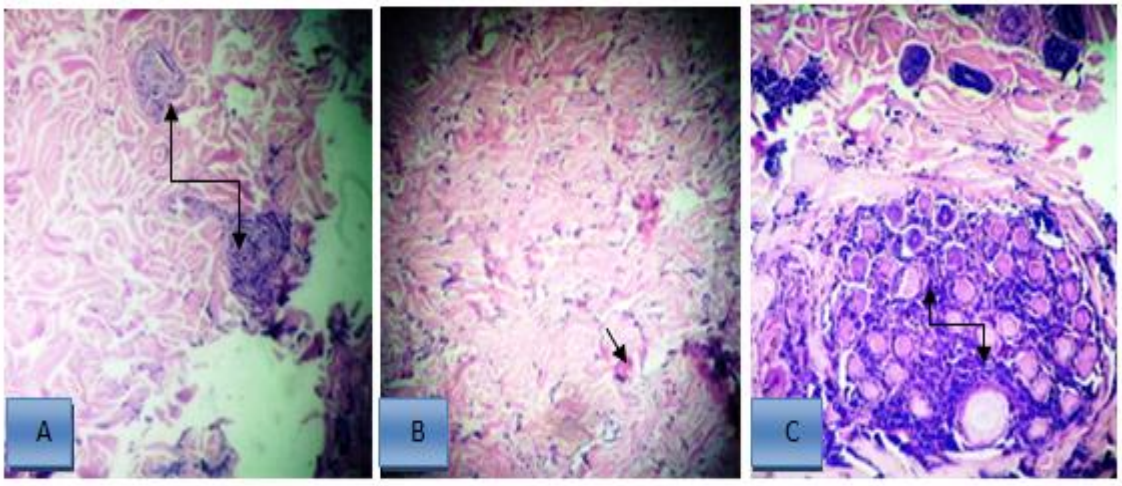

Figure -6- Histological of skin wounds of treatment by Ag-NPs on days13 after wound injury. (A,C) presence of granulation tissue ↑→ ,(B) presence of the red blood cells in the epidermis (congestion) ,congestion of the blood vessels → .Hematoxylin and eosin staining 40 x.

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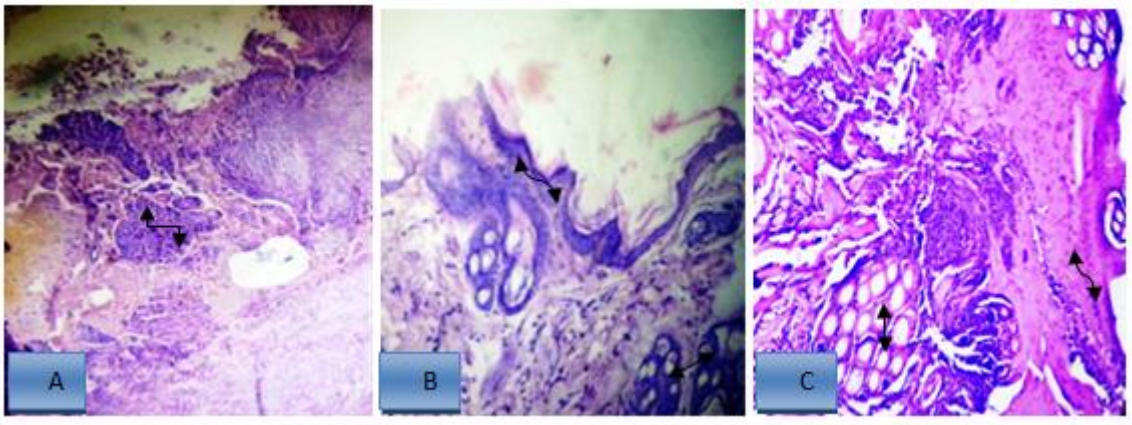

Figure -7- Histological of skin wounds of treatment by Ag-NPs on days 18 after wound injury. (A)presence of granulation tissue ,(B) re-epithelization of the dermis ,(C) normal hair follicles hematoxylin and eosin staining 40 x.

DISCUSSION

Wound healing is a complicated procedure involving a combination of activities of different tissues and cell lineages and has been the subject of concentrated research for a long time (1). The wound healing process involves many complex factors. These may be classified as local and systemic factors, organ and species variability in response to injury. In this study, we adopted an experimental design including daily observation, macroscopically and histological evaluations of the wounds healing process and statistical methods (15).

The results of body weight, body temperature and Respiratory did not show significant changes because of anti- inflammatory properties of silver nanoparticles, low systemic toxicity (16, 17).

Re-epithelization, collagen deposition and neovasculization are all among the parameters used for the analysis of healing wounds (5-7). The immature vessels are originated from mature vessels of deeper tissues. Anastomoses also occur among these vessels to provide the wound area with sufficient blood supply (8).

To better understand the action of silver nanoparticles on wound healing, we looked at histological sections of the wounds from all experimental groups. Although there was an initial influx of neutrophils into the wounds of all, experimental groups, we found significantly fewer hetrophils in the Ag-NPs group on day 7. This suggests a diminished inflammatory response at the wound site. Silver therapy, in principle, has many benefits, such as a multilevel antibacterial effect on cells, which considerably reduces the organism's chances of developing resistance; effectiveness against multi-drug-resistant organisms; and low systemic toxicity(11).

The effect of silver nanoparticles in the inflammatory response at the wound site and observed that low levels of expression of transforming growth factor b (TGF-b) coincided temporally with increased levels of interferon (IFN)-g until wound closure in animals treated with silver nanoparticles (17).

The result of macroscopic and Histological appears of wounds it treated by silver nanoparticles to this study Compatible with(18) investigated the wound-healing properties of silver nanoparticles in an animal model and found that rapid healing and improved cosmetic appearance occur in a dose dependent manner. Furthermore, through quantitative PCR, immunohistochemistry, and proteomic studies, they showed that silver nanoparticles exert positive effects through their antimicrobial properties, reduction in wound inflammation, and modulation of fibro genic cytokines. Similarly, (19)investigated the effect of silver nanoparticles in dermal contraction and epidermal re-epithelialization during wound healing and suggested that silver nanoparticles could increase the rate of wound closure, promotion of proliferation and migration of keratinocytes, silver nanoparticles could drive the differentiation of fibroblasts into myofibroblasts, there by promoting wound contraction and, silver nanoparticles play a distinct role in preventing infection and

decreasing bacterial load in the wound by their broad-spectrum antimicrobial properties, and their surface modification properties.

On the other hand compared the appearance of healed wounds wear found that wounds in the Ag-NPs group showed the most resemblance to normal skin, with less hypertrophic scarring and nearly normal hair growth on the wound surface. Under histological evaluation, healed wounds from the Ag-NPs group resembled normal skin, with a thin epidermis and nearly normal hair follicles. In contrast, histological sections from the SSD-treated group showed thickened epidermis and no evidence of hair growth (20).

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