



Comparison of Burn Treatment with Nano Silver-Aloe Vera Combination and Silver Sulfadiazine in Animal Models

Seyed Abdollah Mousavi¹, Seyed Jaber Mousavi^{2,*}, Ali Zamani³, Seyede Zahra Nourani⁴, Ali Abbasi⁵, Ebrahim Nasiri⁶ and Jason Abbas Aramideh⁷

¹Department of Pediatric Surgery, Pediatric Infectious Diseases Research Center, Mazandaran University of Medical Sciences, Sari, Iran

²Department of Community Medicine, Mazandaran University of Medical Sciences, Sari, Iran

³Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran

⁴Department of Infectious Disease, Zareh Hospital, Mazandaran University of Medical Sciences, Sari, Iran

⁵Department of Pathology, Islamic Azad University, Sari, Iran

⁶Department of Anesthesiology, Traditional and Complementary Medicine Research Centre, Mazandaran University of Medical Sciences, Sari, Iran

⁷Brain and Mind Centre, Sydney Medical School, University of Sydney, Australia

*Corresponding author: MD, Assistant of Professor, Department of Community Medicine, Mazandaran University of Medical Sciences, Farah Abad Road, Sari, Iran. Tel/Fax: +98-1133344506, Email: j_mousavi@mazums.ac.ir

Received 2018 May 20; Revised 2018 November 25; Accepted 2018 December 05.

Abstract

Background: Silver sulfadiazine cream is used extensively for burns, nonetheless, several adverse reactions and side effects are presented in multiple cases. The aim of this study was to compare the healing effect of nano silver-aloe vera composition and silver sulfadiazine on burns in experimental rat models.

Objectives: The experimental study was performed on 15 male Sprague-Dawley rats to compare the burn treatment with nano silver-aloe vera combination and silver sulfadiazine in the animal laboratory.

Methods: Two deep second degree burns on both sides of the abdomen were created. One side was covered by cream and the other by 1% silver sulfadiazine. Response to treatment was assessed by digital photography on day 28. Histological parameters, such as angiogenesis, fibrosis, epithelialization, and inflammatory reactions, were evaluated.

Results: The average burn size in the nano silver aloe vera group was significantly lower compared to the average burn size of the silver sulfadiazine group ($P < 0.05$). Upon pathologic assessment, the re-epithelialization of the epidermis on the fourteenth day in the nano silver group was significantly higher. In addition, this difference in terms of fibrosis and angiogenesis was observed on the fourteenth day and it was higher in the nano silver group ($P < 0.05$).

Conclusions: The use of nano silver compounds in combination with aloe vera compared to silver sulfadiazine cream can expedite re-epithelialization and wound healing in rats.

Keywords: Aloe Vera, Silver Sulfadiazine, Nano Silver, Burn

1. Background

Burn is considered among the major worldwide public health problems, particularly in developing countries (1). Infection is the leading cause of death in these patients (2-4). Topical antibacterial agent use and rapid removal of necrotic tissue, has significantly reduced invasive infections (2). Among these drugs 1% to 3% silver sulfadiazine (SSD) cream is used worldwide to prevent and treat infection of burn wounds. Despite their significance in treatment strategies, several adverse reactions and side effects have been reported during clinical presentations, such as renal toxicity, leukopenia, antibiotic resistance, allergic reactions, and delayed wound healing (5-7). Thus, it is nor-

mally advised that such topical creams should not be used for long periods.

In the recent times, adaptations to topical creams have been produced to accommodate for current issues arising, such as the commercial pad used for absorbent wound dressing containing nano-crystalline silver particles (Acticoat). This dressing is comprised of two layers of silver-coated high density polyethylene (HDPE) bonded on either side of an absorbent rayon/polyester core (8). Although studies have been conducted on antimicrobial effect in unburned cases of wound healing, there have been no comprehensive study conducted on the effects on burn wounds, particularly in combination with herbal medicines. This is despite previous studies addressing the

therapeutic effect of aloe vera in the treatment of burn wounds.

2. Objectives

The aim of the current study was to investigate the therapeutic effects of nano silver-aloe vera (NSA) composition in burn wounds and compare its results with conventional therapies (silver sulfadiazine) (9, 10).

3. Methods

3.1. Preparation and Formulation of Cream

All stages were undertaken in the preparation of this cream was carried out by pharmaceutical experts of Kia Health Pharmaceutical Company. The safety of this product has been approved by Pharmaceutical Sciences Research Centre of Tehran University of Medical Sciences.

The preparation trend was carried out in two phases as follows: In the first phase, the composition of acid stearic 3 g, paraffin 5 g, cetyl alcohol 1 g, isopropyl myristate 2 g, olive oil 3 g, glyceryl stearate and peg 100 stearate 3 g, Tween 80 3 g, and propylparaben 0.15 g were poured in the steel tank followed by an incremental increase in the temperature until 70°C. In the second phase, the composition of nano silver (7 cc), propylene glycol (4 g), glycerin (2 g), triethanolamine (0.2 g), aloe vera (0.2 g), methylparaben (0.02 g), and deionized water (up to 100 cc) was poured in steel tanks and the temperature was gradually increased to 75°C. The content of the first tank (first phase) was added to the second tank (second phase) and mixed. At that time, the temperature was gradually reduced and brought to a temperature of 50°C. The combination was then homogenized for 15 minutes upon the temperature reaching approximately 30°C. The contents were unloaded and kept in storage tanks. The entire process was carried out in sterile conditions and the resultant cream was filled in the tube (the source of silver was from silver bullion with 99.99% purity percentage).

3.2. Test Animals

The experimental study was performed on 15 male Sprague-Dawley rats (weighing 290 to 350 g). The protocol was approved by the Research Committee of Mazandaran University of Medical Sciences prior to commencement of this study and the study was approved by the Ethical Committee Research and Technology Deputy of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.94.1213). Sedation was established at first with intramuscular injections of 70 mg/kg of ketamine hydrochloride and 5mg/kg

of lidocaine. The skin on the dorsum was shaved with an electrical clipper. This was soon followed by the standard metal probe, originally placed in 100°C boiling water, and positioned for 30 seconds on the back of rats. Consequently, two uniform deep second degree burns on both sides of the dorsum were generated. In this experiment, approximately 10% Total Body Surface (TBSA) was burned (Figure 1).



Figure 1. How to create a standard uniform burn on the back of rats

The animals were resuscitated with an intra-peritoneal injection of 5 mL of normal saline solution. One side of the wounds were covered by corresponding cream and no dressings was applied. One percent silver sulfadiazine (Behvarzan Pharmaceutical Company, Rasht, Iran) was soaked on the other side (as the control group) without any dressing. The creams were applied on a daily basis. Additionally, animals were kept in separate cages, at standard temperature and conditions. Food, water, and enough light was available for all animals.

Pathological assessments were performed by an experienced pathologist on biopsies from wounded edges. Histological parameters, such as angiogenesis, fibrosis (collagen bundles), epithelialization, and inflammation of the tissues were evaluated. Due to the healing of burn wounds, re-epithelialization was evaluated after 25 days and the last day of the treatment period. For this purpose, skin tissue samples were taken for histological studies with a small excision containing a part of the wound area. In addition, for assessment of wound healing, digital photography was taken during periods mentioned above. These photographs were taken on days three, seven, 14, 21, and 28. The photographs were then assessed by the Image J software and the percentage of healing was determined.

In order to comply with blindness, right and left wounds were treated randomly and the pathologist reviewing the samples and statistical analyzer was not informed of the treatment group. The results of all 30 burn samples in during the mentioned time periods were collected in the form of data recording.

3.3. Statistical Analysis

The data obtained was analyzed using SPSS version 16. Statistical analysis using paired *t*-test, independent sample *t*-test, and Mann Whitney U statistical tests were used. The differences were considered significant, when *p* value was < 0.05.

4. Results

The average weight and age of the animals, were 320 g and 3 months, respectively. No infection or wound-related complication was observed. In evaluating the extent of burn wounds in both groups, the average size of the wound in the nano silver group on days 28, 21, and 14 was significantly lower compared to the silver sulfadiazine treatment group. Statistical analysis revealed that there were significant differences in mean burn surface area (cm²) between the two groups on days 14 (*P* value = 0.03), 21 (*P* value = 0.04), and 28 (*P* value = 0.04). No significant differences were recorded between the two groups on days three (*P* value = 0.21) and seven (*P* value = 0.29) (Figure 2).

A pathologic assessment of the number of inflammatory cells, such as macrophages, mononuclear cells, and Polymorphonuclear cells, found no significant differences between the two groups. However, the re-epithelialization of the epidermis on day 14 in the nano silver group was found to be significantly higher. In addition, this difference in terms of fibrosis and angiogenesis was observed on day 14, and it was higher in the nano silver group (*P* < 0.05) (Figure 3).

Although the rate of wound healing was higher in the group of nano silver compared to silver sulfadiazine, histological examination revealed that on day 28, all wounds were completely healed. The trajectory course of recovery before the second week in both groups were found to be nearly identical until nano silver was demonstrated to have an impact following the two weeks.

5. Discussion

The application of nano silver particles was observed around the world in various sciences, particularly in

medicine (11, 12). One of these applications is in treatment of skin ulcers and burns. Previous studies by Wright et al. have demonstrated the anti-fungal properties of nano crystalline silver during in vitro studies (13). Yin et al. also showed that these particles have antibacterial effects. Specifically, previous studies have identified ACTICOAT (a silver-coated wound dressing) as an antimicrobial barrier with better antimicrobial effects than either of the existing silver-based products, such as silver nitrate, silver sulfadiazine, and mafenide acetate (14). In two separate studies by Furno et al. (15) and Li et al. (16) it has also been reported that silver nanoparticles are effective in wound healing. Additionally, studies by Seyyedmir et al. have also demonstrated nano silver dressing as having a significant impact on wound healing in rats. In the study by Seyyedmir et al. the mean wound area in nano silver dressing after 12 days was significantly lower than in the control group (17). Furthermore, a study by Wright et al. has also demonstrated nano silver as effective in reducing Matrix metalloproteinase activity with more extensive apoptosis and reduction in inflammation to facilitate wound healing in the early phases. The study showed that wounds treated with nano-crystalline silver has less redness and edema and forms granulation tissue in less time compared to control group (18). Suggested reasons for explaining this were that the silver treated wounds showed a marked reduction in the level of bacterial contamination and a reduction in the extent of bacterial micro-colonies found within the wound tissue. Secondly, silver-treated wounds also appeared, histologically, to be less inflamed (18).

Nano silver is known to perform antimicrobial activity through two mechanisms. One of these methods is an ionic mechanism. Over time, nano silver particles emit Ag⁺ ions. These ions during a substitution reaction, convert -HS bands on the walls of microorganisms to -AgS bands. The results of this reaction are weed and waste of microorganisms. Another way of the effect of this drug is catalytic mechanism. With catalytic mechanism the cross-sectional destruction of contact bacteria is achievable (9). It is also worth mentioning that for many years, the effect of herbal medicine on burn wound has been extensively considered in previous studies. One of these herbal products is aloe vera. However, the actual mechanism of aloe vera in improved healing is still unclear. The most probable mechanism of action for aloe vera could constitute providing the necessary materials for healing, increasing blood flow to burn area, decreasing inflammatory response and hence the rate of infection (16).

Our study was a preliminary attempt at examining the efficacy of herbal and chemical compound creams

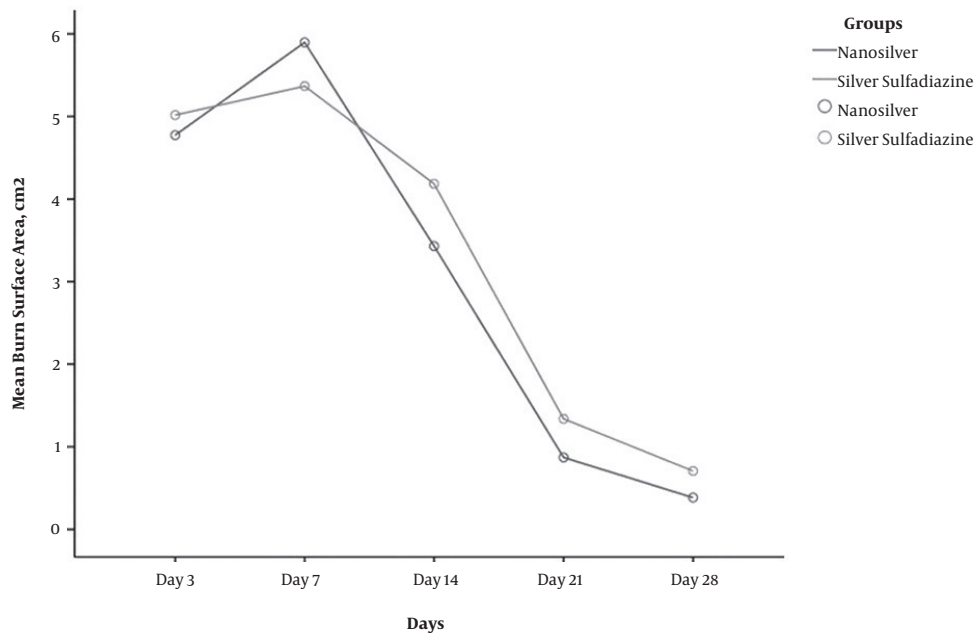


Figure 2. Comparison of the burn size in two groups of nano silver-*aloe vera* and silver sulfadiazine

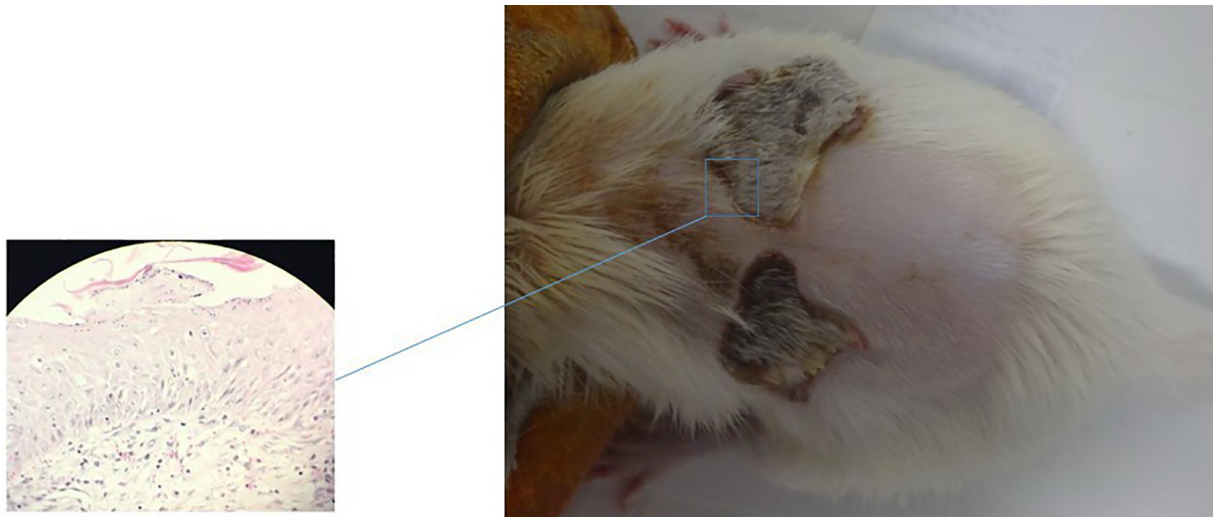


Figure 3. Macroscopic view of skin wound on the fourteen day of treated with nano silver-*aloe vera* combination. The pathologic slide is healing skin composed of regenerated epithelium and edematous tissue with congested vessels.

in the treatment of burns. This study attempted to employ a different approach toward the new chemical and herbal products in compound form. The results from this study suggest that nano silver and *aloe vera* combination speed up the healing rate compared to current treatments with SSD cream. This improvement can be demonstrated both macroscopically and histologically. Although recov-

ery speed in the first week was similar in both groups, higher amounts of angiogenesis, fibrosis, and epithelialization were observed on the fourteenth day in the nano silver group. The practical advantage of this would be that the hospital stay would be shortened. Additionally, the combination compound was found to present with lower allergic reaction in patients compared to silver sul-

fadiazine, although more studies are needed to clarify this. However, it may also be worth noting that the extent to which nano silver exhibits adverse side effects has still not been fully evaluated. There is reasonable suspicion from previous studies that suggest injected nano silver particles in mice can induce changes in white blood cells and liver enzymes (11). Further studies will also be required in order to study counter-mechanisms in alleviating treatment strategies from procuring the aforementioned adverse reactions.

The use of nano silver compounds in combination with aloe vera compared to standard methods of using silver sulfadiazine cream can speed up the re-epithelialization and wound healing in rats. The applicability of these findings through further investigation using human samples can further enhance the efficacy of these findings.

Acknowledgments

We express our thanks to the Clinical Research Development Unit of Bu-Ali Sina Hospital, Mazandaran University of Medical Sciences, for their collaboration in performing the study.

Footnotes

Authors' Contribution: Study concept and design: Seyed Abdollah Mousavi, Seyed Jaber Mousavi; analysis and interpretation of data: Seyede Zahra Nourani; contribution in management and therapeutic result of burn wounds: Ali Abbasi; assessment of pathology specimen: Ebrahim Nasiri; contribution in management and implication of animal study: Ali Zamani; contribution in pharmaceutical design of the used drugs: Jason Abbas Aramideh.

Ethical Approval: The protocol was approved by the Research Committee of Mazandaran University of Medical Sciences prior to commencement of this study and the study was approved by Ethical Committee Research and Technology Deputy of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.94.1213). (<http://syat.mazums.ac.ir/> and <http://www.mazums.ac.ir/Index.aspx?>).

Conflict of Interests: The authors declare that they have no competing interests.

Funding/Support: This project was funded by the research deputy of Mazandaran University of Medical Sciences, Sari, Iran.

References

1. Alaghebandan R, MacKay Rossignol A, Rastegar Lari A. Pediatric burn injuries in Tehran, Iran. *Burns*. 2001;27(2):115-8. doi: [10.1016/S0305-4179\(00\)00083-8](#). [PubMed: [11226645](#)]. *Trauma Mon*. 2019;24(3):e79365.

2. Howard PA, Cancio LC, McManus AT, Goodwin CW, Kim SH, Pruitt BA. What's new in burn-associated infections? *Curr Surg*. 1999;56(7-8):397-405. doi: [10.1016/S0149-7944\(99\)00166-X](#).
3. Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. *Burns*. 2004;30(4):357-61. doi: [10.1016/j.burns.2003.12.013](#). [PubMed: [15145194](#)].
4. Weber JM, Sheridan RL, Pasternack MS, Tompkins RG. Nosocomial infections in pediatric patients with burns. *Am J Infect Control*. 1997;25(3):195-201. doi: [10.1016/S0196-6553\(97\)90004-3](#). [PubMed: [9202814](#)].
5. Khorasani G, Hosseini-mehr SJ, Zamani P, Ghasemi M, Ahmadi A. The effect of saffron (*Crocus sativus*) extract for healing of second-degree burn wounds in rats. *Keio J Med*. 2008;57(4):190-5. doi: [10.2302/kjm.57.190](#). [PubMed: [19110531](#)].
6. Akhoondinasab MR, Akhoondinasab M, Saberi M. Comparison of healing effect of aloe vera extract and silver sulfadiazine in burn injuries in experimental rat model. *World J Plast Surg*. 2014;3(1):29-34. [PubMed: [25489521](#)]. [PubMed Central: [PMC4236981](#)].
7. Hang MY. Children and burn injuries. *JAMA*. 2000;283(1):154. doi: [10.1001/jama.283.1.154](#).
8. Dunn K, Edwards-Jones V. The role of Acticoat with nanocrystalline silver in the management of burns. *Burns*. 2004;30 Suppl 1:S1-9. doi: [10.1016/S0305-4179\(04\)90000-9](#). [PubMed: [15327800](#)].
9. Simonetti N, Simonetti G, Bougnol F, Scalzo M. Electrochemical Ag+ for preservative use. *Appl Environ Microbiol*. 1992;58(12):3834-6. [PubMed: [1476427](#)]. [PubMed Central: [PMC183190](#)].
10. Borkow G, Gabbay J, Dardik R, Eidelman AI, Lavie Y, Grunfeld Y, et al. Molecular mechanisms of enhanced wound healing by copper oxide-impregnated dressings. *Wound Repair Regen*. 2010;18(2):266-75. doi: [10.1111/j.1524-475X.2010.00573.x](#). [PubMed: [20409151](#)].
11. Naghsh N, Noori A, Aqababa H, Amirkhani-Dehkordi S. Effect of nanosilver particles on alanin amino transferase (ALT) activity and white blood cells (WBC) level in male wistar Rats, in vivo condition. *Zahedan J Res Med Sci*. 2012;14(7):34-7.
12. Soltani K, Zand R, Mirghasemi A. Epidemiology and mortality of burns in Tehran, Iran. *Burns*. 1998;24(4):325-8. doi: [10.1016/S0305-4179\(98\)00017-5](#). [PubMed: [9688197](#)].
13. Wright JB, Lam K, Hansen D, Burrell RE. Efficacy of topical silver against fungal burn wound pathogens. *Am J Infect Control*. 1999;27(4):344-50. doi: [10.1016/S0196-6553\(99\)70055-6](#). [PubMed: [10433674](#)].
14. Yin HQ, Langford R, Burrell RE. Comparative evaluation of the antimicrobial activity of ACTICOAT antimicrobial barrier dressing. *J Burn Care Rehabil*. 1999;20(3):195-200. doi: [10.1097/00004630-199905000-00006](#). [PubMed: [10342470](#)].
15. Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM, et al. Silver nanoparticles and polymeric medical devices: A new approach to prevention of infection? *J Antimicrob Chemother*. 2004;54(6):1019-24. doi: [10.1093/jac/dkh478](#). [PubMed: [15537697](#)].
16. Li Y, Leung P, Yao L, Song QW, Newton E. Antimicrobial effect of surgical masks coated with nanoparticles. *J Hosp Infect*. 2006;62(1):58-63. doi: [10.1016/j.jhin.2005.04.015](#). [PubMed: [16099072](#)].
17. Seyyedmir MR, Soltani HR, Akhoondi Meybodi H, Morshedi A, Fakhri E. [The effects of nanosilver dressing on wounds created in rats]. *J Isfahan Med Sch*. 2012;29(164):1-6. Persian.
18. Wright JB, Lam K, Buret AG, Olson ME, Burrell RE. Early healing events in a porcine model of contaminated wounds: Effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing. *Wound Repair Regen*. 2002;10(3):141-51. doi: [10.1046/j.1524-475X.2002.10308.x](#). [PubMed: [12100375](#)].