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Topical Applications of Silver Nanoparticles for Wound Healing Enhancement in Various Animals

J.J. Parmar[1], K.K. Hadiya[1], A.I. Shah[1], Neha Rao[1], N.R. Amin[1]

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ABSTRACT

Background: In animals due to multiple factors sometime wound management with topical anti-septic dressing along with routine medication does not give any satisfactory outcome and becomes a challenge for the veterinarians. Due to antibacterial and wound healing enhancement properties of silver nano-particles in different forms, it is widely used topically in human wound therapy. The application of silver nano-particles in wound healing in chronic complicated wounds in animals especially in clinical cases is very less documented.

Methods: Total twenty seven animals of various species, *viz*., dogs (n=20), goats (n=6), cat (n=1) and buffalo (n=1) presented with different types of chronic complicated wounds on different body parts were included in this study. The wounds after evaluation were treated according to conditions with routine antiseptic dressing with other required parenteral medications for initial 3 days. All the wounds were also subjected for topical application of silver nano-particles (100 ppm AgNOs spay) twice a day. The day of presentation was considered as a day '0' and subsequent days for granulation, wound contraction and complete healing were recorded.

Result: Amongst 20 canine wound cases, granulation of tissues with wound contraction was noted in around 5-12 days and complete healing of wound with or without scar formation was observed in 15-32 days. Among the six female goat patients, complete wound healing was observed on day 16 to day 19 according to the site and depth of wounds. In the wounds located on head, perivulvar, perineal, abdominal regions showed the marked granulation from day 3 to day 5 along with remarkable wound contraction. The wounds presented on limbs exhibited less granulation compared to wounds presented on other parts. The granulation was observed from day 5th on limb region. In all the cases healing period was found variable from day 10 to day 32.

Key words: Animals, Management, Silver nano particles, Wounds.

INTRODUCTION

Wound repair process in healthy individuals depends on several interrelated processes, including the migration of inflammatory cells to colonize the provisional matrix, proliferation of fibroblasts and vascular cells, apoptosis and synthesis of extracellular matrix proteins to reconstitute dermal architecture. Aberrant apoptosis also removes granulation tissues leading to abnormal wound healing (Walker *et al*., 1988). In animals due to various types of injuries and trauma, the deep extensive wounds are most common encountered conditions, which need emergency attention of veterinarians. The animals are more prone to various wounds on body with multiple complications and the management of such cases become very difficult and challenging. Chronic wounds need long term therapy comprised of complex managemental therapeutics with regular debridement of damaged and necrotic tissues, local and/or systemic infection control, protection of the underlying tissues and induction of cutaneous tissue regeneration (Parmar *et al*., 2022). Due to strong bactericidal effects of silver compounds and ions, these are extensively used for hygienic and wound management since centuries (Klasen, 2000). Due to the bactericidal properties various silver containing preparations were used for the treatment of chronic wounds in human patients. Apart from antimicrobial properties, having anti-inflammatory properties, it losses rubor in chronic wounds treated with colloidal silver (Demling and DeSanti, 2001). Recently, there has been an increased interest in silver, due to increased resistance of bacteria to antibiotics and improvements in polymer technology. Thus, it has resulted in a large number of silver-containing dressings being available on the market. The recent emergence of nanotechnology has provided a new therapeutic modality in silver nanoparticles for use in wounds. Nonetheless, the beneficial effects of silver nanoparticles on wound healing remain unknown. Nowadays, silver-based topical dressings have been widely used as a treatment for infections in open wounds and chronic ulcers (Mishra *et al*., 2008). The available literature for the wound healing in veterinary patients especially in

[1]Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388 001, Gujarat, India.

Corresponding Author: J.J. Parmar, Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388 001, Gujarat, India.
Email: jjparmar@kamdhenuuni.edu.in

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clinical cases is very rare. Thus, this paper presents a role of silver nanoparticles for the healing of chronic complicated wounds in different animal species.

MATERIALS AND METHODS

Total twenty seven animals of various species *viz*., dogs (n=20), goats (n=6), cat (n=1) and buffalo (n=1) presented to Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand with different types of chronic complicated wounds on different body parts. The details of breed, age, sex, location and type of wound and its duration etc in 20 dogs with would were as presented in Table 1. Amongst the six female goat patients, two years and three years old goats (n=3, each) were presented with chronic deep maggoted wounds on perivalvar region, while an eight months old cat was presented with sloughing of skin at lumber region and a fourth lactation buffalo was presented with extensive skin sloughing on right abdominal region. The evaluation of wound was done and therapeutic management was carried out according to the conditions. The day of presentation was considered as day '0'.

In all the cases, the wounds were kept open and treated with antiseptic dressing using Betadine 5% along with Inj. Cefrtiaxone and Tazobactum @ 15 mg/kg body weight in dogs and cat, 20 mg/kg body weight in goat and 6 mg/kg body weight in buffalo; and Inj. Meloxicam @ 0.2 mg/kg body weight in dogs, goats and cat and 0.5 mg/kg body weight in buffalo, intravenously, for three days. The cases presented with maggot wounds were treated initially by removing maggots manually, topical antiseptic dressing along with Inj. Ivermectin subcutaneously @ 200 µg/kg body weight in dogs and goats. All the wounds were also treated with topical applications of silver nano particles (AgNPs 100 ppm available as a topical spray) twice a day for the wound healing enhancement. The patients were kept under observations till complete healing of wounds and the duration for granulation, wound contraction and complete healing was recorded.

RESULTS AND DISCUSSION

Total twenty seven clinical cases of various species, *viz*., dogs (n=20), goats (n=6), cat (n=1) and buffalo (n=1) age ranging from two months to eleven years were presented with different types of chronic complicated wounds on different body parts. The details of 20 canine wound cases before therapy and the response of silver nano-particles therapy are presented in Table 1. In most of the canine cases, granulation of tissues at the site of wound was noted within 5-10 days with wound contraction in around next 3-5 days and complete healing of wound with or without scar formation was observed in 15-32 days depending upon the site, type, duration and depth of wound.

Amongst the six female goat patients, complete wound healing was observed on day 16 to day 19 according to site and depth of wounds. In cat and buffalo patients the granulation was observed on day 12 and 16 and complete wound healing observed on day 28 and 30, respectively. In cat, the granulation formation was slower as compared to other species.

In the wounds located on head, peri-vulvar, perineal, abdominal regions showed the marked granulation from day 3 to day 5 along with remarkable wound contraction. The wounds presented on limbs exhibited less granulation compared to wounds presented on other parts. This might be due to constant movement of animals for the locomotion, which interferes in healing process. The granulation was observed from day 5th on limb region. In all the cases healing period was found variable from day 10 to day 32. This variation might be due to depth of damage, chronicity and location of the wounds on the body of animals. The advantage of topical application of silver nano particles was that it enhanced the healing process compared to duration and chronicity of wounds before treatment and also helped in formation of granulation pad, which is necessary for the wound healing.

Wound healing is a well-orchestrated and complex series of events involving cell-cell and cell-matrix interactions. Various intrinsic and extrinsic factors like diabetes, environmental contamination, animal behaviour are intricate, which affects the wound healing and lead to delayed healing or non-healing. Sometime expansion of the wounds leads to exposure of deeper tissues and bones. The conventional treatment for the wound management like debridement, lavage and antiseptic dressing does not always act effectively. Such cases need advanced therapeutic to regenerate new tissue for enhance healing and to save the affected body parts (Parmar *et al*., 2022). The healing may be delayed due to various factors, which play a role to make wound more complicated and responsible for suffering of patients and frustration of owner. Thus, sometime the wound management may become a challenge for the veterinarian.

Silver was used as a antimicrobial over a century. In the 17th and 18th centuries, silver nitrate was already in use for ulcer treatment and in 1960 it was introduced for the management of burns. After a decrease in the use of silver salts consequent to the introduction of antibiotics in 1940, in more recent years, there has been a renewed interest in silver, due to increased resistance of bacteria to antibiotics and improvements in polymer technology. Silver is applied to burns, either in the form of impregnated bandages or as a cream containing silver sulfadiazine as the active agent, a product that was still considered the benchmark silver product (Hussain and Ferguson, 2006). Silver nitrate used to treat chronic wounds in human causes staining and irritation to contact surface. Silver sulfadiazine was introduced in the 1960s to overcome some of the shortcomings of silver nitrate, but both were limited in the clinics due to the need for a high frequency of application, inactivation of much of the silver by wound fluid and the formation of a pseudo-eschar (Mishra *et al*., 2008).

Table 1: Details of 20 canine cases of wounds before and after silver nano-particles therapy.

Breed	Age	Sex	Location of wound	Duration of wound	Post-treatment healing process (days)		
					Granulation	Wound contraction	Complete healing
German shepherd	4 yrs	M	Extensive skin sloughing at left thoracic region and medial aspect of left limb (Fig 1A)	1 month	Day 10	Day 10 (Fig 1B)	Day 30, with mild scar formation (Fig 1C)
Non-descript	7 yrs	F	Deep extensive muscles and skin tear at medial aspect of left limb since twenty days (Fig 2A)	12 days	Mild granulation on day 10 (Fig 2B), Sufficient granulation seen from deeper site on day 25	Sufficient wound contraction was seen on day 25 (Fig 2C)	Day 32
Pomeranian	5 yrs	F	Chronic ulcerated wound at right side abdominal region since one month (Fig 3A)	1 month	Sufficient granulation on day 10	Day 10 (Fig 3B)	Significant wound contraction seen on day 25 (Fig 3C)
Dalmatian	9 yrs	F	Chronic maggoted wound at left side abdominal region since twenty four days	24 days	Sufficient granulationon day 5	Day 5	Significant wound contraction seen on day 25
German shepherd	3 yrs	M	Chronic extensive maggoted wound at perineal region (Fig 4A)	2 months	Granulation from deep area on day 10	Day 10 (Fig 4B)	Day 25 (Fig 4C)
Rottweiler	2 yrs	M	Chronic deep maggoted wound at perineal region	2 weeks	Granulation from deep area on day 10	Day 10	Day 25
Labrador	2 yrs	M	Injury with hot water and sloughing of skin at head region (Fig 5A)	15 days	Marked healing on day 10 (Fig 5B)	Healing with hairs growth	Day 17 (Fig 5C)
Black pomeranian	11 yrs	F	Chronic diabetic ulcerated wound at head region since one year	1 year	Marked healing on day 5	Healing with hairs growth	Day 17
Rottweiler	2 months	M	Chronic maggoted wound on tail since ten days	10 days			Day 12
German shepherd	2 yrs	M	Chronic ulcerated wound on lower neck region	14 days			Day 13
Labrador	5 yrs	M	Maggoted wound on scrotum (Fig 6A)	5 days			Day 11 (Fig 6B)
Non-descript	2 months	M	Chronic deep wound at lateral aspect of right fore limb	10 days	Sufficient granulation on day 5th	Wound contraction on day 10th (Fig 7B)	Complete healing observed within day 17 to day 25 (Fig 7C) which was due to the varying size and depth of the wounds
Non-descript	5 months	F	Chronic infected wound at lateral aspect of left fore limb	12 days			
Non-descript	2 yrs	M	Chronic deep maggoted wound of digits of left fore limb since twenty days	12 days			
Non-descript	1 yr	M	Chronic deep wound of digits of right fore limb	15 days			
Labrador	2 yrs	F	Chronic wound of digits of right fore limb	5 days			

Table 1: Continue...

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Rottweiler	6 months	M	Chronic wound of digits of right fore limb (Fig 7A)	2 weeks
Non-descript	1 yr	M	Chronic deep maggoted wound of digits of left fore limb	15 days
Non-descript	1 yr	M	Chronic deep wound of digits of left hind limb	10 days
Non-descript	3 months	M	Chronic wound on digital region of left fore limb	2 weeks

Nanoparticles (NPs) are defined as particles having one or more dimensions in the order of 100 nm or less. Silver NPs (Ag NPs) had been shown to possess unusual physical, chemical and biological properties (Nair and Laurencin, 2007). Indeed, an active role in wound healing was attributed to silver and, along with its distinctive role in preventing infection, silver nanoparticles can also drive the differentiation of fibroblasts into myofibroblasts, which in turn promotes the wound contraction, quickens the healing rate and stimulates the proliferation and relocation of keratinocytes (Vijayakumar *et al*., 2019). An ideal Ag + dressing material promotes healing; causes minimal pain to the patient, prevents infection, results in minimal scarring and is inexpensive and easy to use. A dressing which possessed all of these qualities has yet to be developed, but currently many dressing materials met some of these criteria to varying degrees of effectiveness (Rakel *et al*., 1998). Nanotechnology has provided a way of producing pure silver nanoparticles. This system also markedly

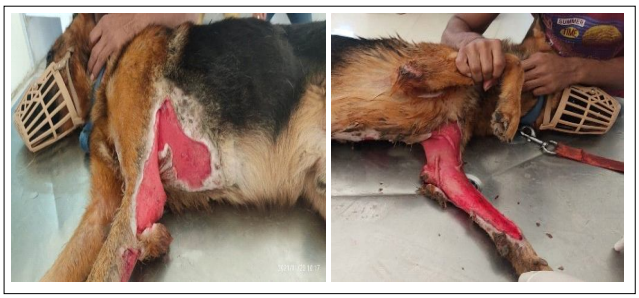

Fig 1A: Extensive skin sloughing at left thoracic region and at medial aspect of left limb region.

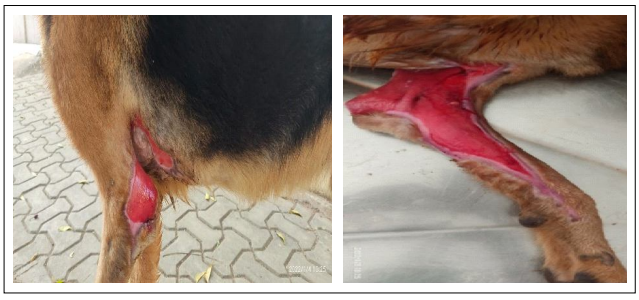

Fig 1B: Granulation and wound contraction observed on day 10.

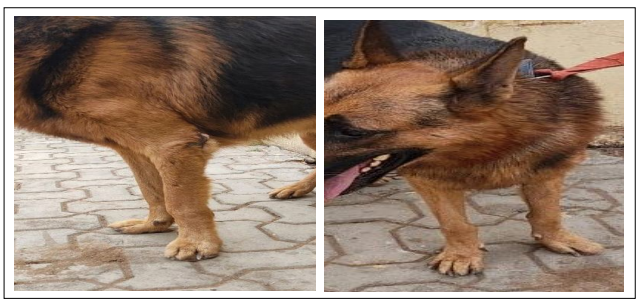

Fig 1C: Complete healing on day 30.

Fig 2A: Deep extensive muscles and skin tear at medial aspect of left limb.

Fig 2B: Mild granulation seen on day 10.

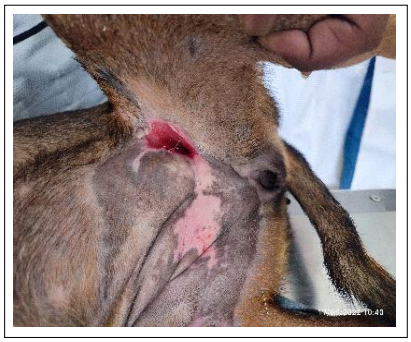

Fig 2C: Sufficient granulation and wound contraction seen on day 25.

Fig 3A: Chronic ulcerated wound at right side abdominal region.

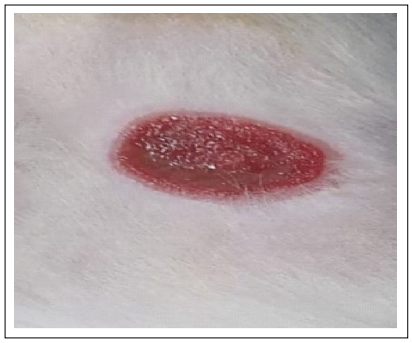

Fig 3B: Granulation and wound contraction on day 10.

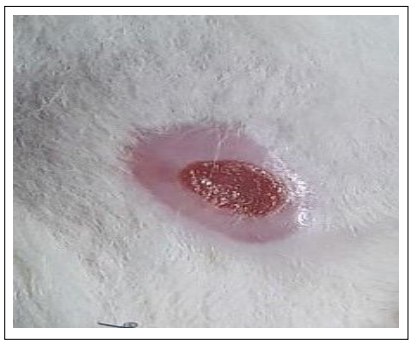

Fig 3C: Significant wound contraction seen on day 25.

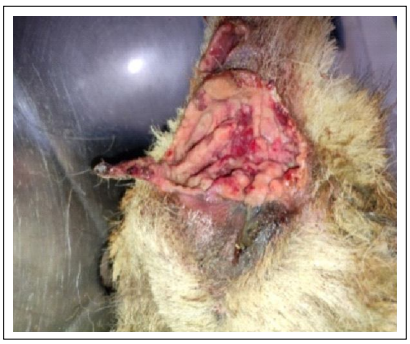

Fig 4A: Chronic extensive maggoted wound at perineal region

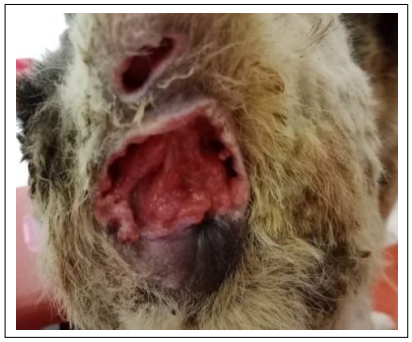

Fig 4B: Granulation from deep area and wound contraction on day 10.

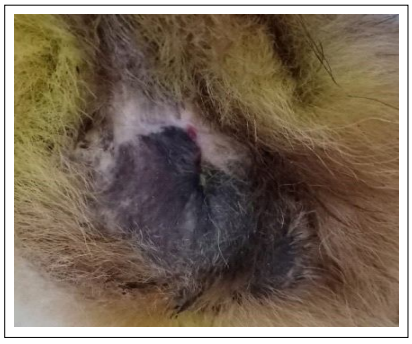

Fig 4C: Complete healing was seen on day 25.

Fig 5A: Injury with hot water and sloughing of skin at head region.

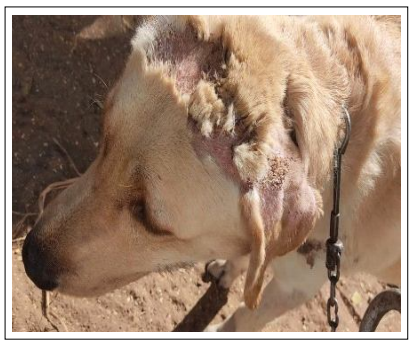

Fig 5B: Marked healing on day 10 with hairs growth.

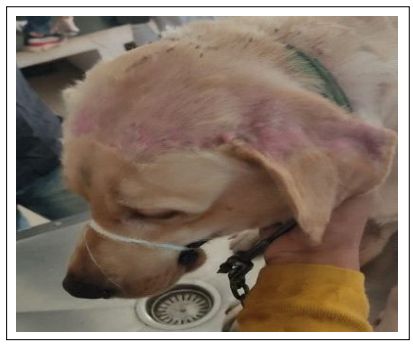

Fig 5C: Complete healing observed on day 17.

Fig 6A: Maggoted wound on scrotum.

Fig 6B: Complete healing within day 12.

Fig 7A: Chronic wound of digits of right fore limb.

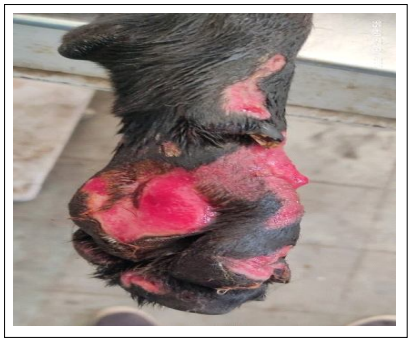

Fig 7B: Ssufficient granulation and wound contraction on day 5th.

Fig 7C: Complete healing observed on day 17.

increases the rate of silver ion release. In the present study the silver nitrate nano particles in 100 nm was used as a topical dressing material. The bio-availability of silver ion is more and rapid as compared to other silver preparations. In the present study the granulation formation and wound contraction was clearly observed at day 10 in all cases, which might be initiated before day 10.

Liu *et al*. (2010) observed that AgNPs improve the proliferation and migration of keratinocytes from the edge to the centre of the wound and trigger the differentiation and maturation of keratinocytes, thereby promoting wound contraction in rodents. In our study recorded that hair growth was seen in two years old Labrador male dog with injury by hot water and sloughing of skin at head region on day 10. Almost similar healing period was reported by Tian *et al*. (2007) in thermal wounds of mice treated with silver nanoparticles. The grossly healed wound resembled to normal skin, with less hypertrophic scarring and nearly normal hair growth on the wound surface. These observations indicate that wound healing is accelerated by silver nanoparticles. AgNPs release small silver ions, which carries positive charges, are easily attracted to negatively charged cell membrane of bacteria. It entrapped and penetrated the bacteria, disrupted respiratory processes and caused bacterial death (Jung *et al*., 2008). Therefore, AgNPs possessed antibacterial property against a wide range of pathogens (Lara *et al*., 2011). In the present study, all the cases were given antibiotics for three days to encounter the existing infection. Afterwards no antibiotics were given to any patients. The published literature of effects of silver nanoparticles on wound healing are sparse and the mechanism of action remains unknown. This article presents the effects of silver nano particles on chronic complicated wounds in different animals.

CONCLUSION

In the present study, the topical application of silver nano-particles (AgNPs 100 ppm) in spray form found to be useful to enhance the healing of different chronic complicated wounds in animals as it possessed the antibacterial property and promoted wound healing. The spray form was easy to apply over the site of affections in animals. Topical treatment of chronic complicated wounds with silver nano-particles in spray form was a safe adjunct therapy for skin wound healing in animals.

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Conflict of interest: None.

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