Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 6 June 2022

Research Article

Comparative Efficacy of Green Synthesized Silver Nano Particles of *Ehretia Laevis* and *Azadirachta Indica* on Wound Healing in Cow

Yeole SS[1], Raulkar RV[2]*, Thorat MG[3], Hatzade RI[4], Tingase SS[1] and Choudhury Ashutosh[1]

[1]*M.V.Sc. Scholar, Department of Surgery and Radiology, India*

[2]*Assistant Professor, Department of Surgery and Radiology, India*

[3] *Professor and Head, Department of Surgery and Radiology, India*

[4]*Ph.D. Scholar, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, India*

***Corresponding Author:** Raulkar RV, Assistant Professor, Department of Surgery and Radiology, India.

DOI: 10.31080/ASVS.2022.04.0401

Received: April 25, 2022

Published: May 19, 2022

Abstract

The present study was conducted on twelve cow with infected wounds, irrespective of wound type, age, sex, breed, divided into two groups, containing six cow each. Cow in Group A and Group B were treated with green synthesized silver nanoparticle ointments of *E. laevis* and *A. indica* respectively for 14 consecutive days. Hematological and biochemical changes were recorded at different intervals of time in both the groups. Cow treated with *E. laevis* ointment showed better wound healing in comparison with *A. indica* ointment treated group. Hence clinical use of *E. laevis* silver nano particles ointment can be recommended for wound dressing in cow.

Keywords: *Ehretia Laevis*; *Azadirachta Indica*; Green Synthesis; Wound; Cow

Introduction

Wound is demarcated as a discontinuity of skin, mucous membrane or tissue surface which can be caused by any physical, chemical or biological agents. Fly infestation and maggots in wide temperate climatic conditions of India aggravate the condition and add upon to the challenge of wound healing [1]. Prior to the introduction of antibiotics, silver was used for its antiseptic properties, specifically in the treatment of open wounds and burns [2]. Nano particles are defined to have their dimensions in the range of 1-100 nm [3]. Silver nano particles have mesmerized people since middle ages because of their distinctive properties and its various applications. At present, they have received high recognition due to their extraordinary biological activities. Antimicrobial properties have been discovered in various components of *E. laevis* and *A. indica* plants. The utilization of various medicinal plant materials for the biosynthesis of nano particles is considered a green technology as it does not involve hazardous chemicals.

Material and Methods

Leaves of *E. laevis* and *A. indica* were used for green synthesis silver nano particles. Five ml plant extract filtrate was treated with 95 ml aqueous 1mM silver nitrate solution in Erlenmeyer flask and

incubated at room temperature. As a result, a brown yellow solution was formed, indicating the formation of silver nano particles. The colourless to brown colouration confirms reduction of silver ions. The size of *E. laevis* and *A. indica* silver nanoparticle was determined by Transmission Electron microscopy (TEM) to be 25nm and 30nm respectively. Preparation of silver nanoparticle ointment was done by using fusion method by using 60 gm of liquid paraffin and 40 gm bees' wax in which 0.1 litre solution of *E. laevis* Silver Nano Particles (ELSNP) and *A. indica* Silver Nano Particles (AISNP) was taken respectively for preparation of 100 gm of silver nanoparticle ointment. The study was undertaken on clinical cases presented at Teaching Veterinary Clinical Complex (TVCC) Akola. Total 12 cases of cow with infected wound, irrespective of wound type, age, sex and breed were divided into two groups, containing six animals in each. Group A and Group B were treated with green synthesized silver nanoparticle ointments of *E. laevis* and *A. indica* respectively for 14 consecutive days.

Clinico-physiological parameters

Rectal temperature, heart rate, respiratory rate were recorded. Wound assessment was done on the basis of swelling at wound site, colour of wound, exudation, pain at the site and irritation. The quality of wound healing was measured on the basis of percent wound contraction [4]. All the observations were made on 0^{th}, 7^{th} and 14^{th} day of treatment.

Haematological parameters

The haematological parameters, such as haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), and fibrinogen were investigated. All of these haematological parameters were investigated using the procedure mentioned by earlier researchers [5]. All the observations were made on 0^{th}, 7^{th} and 14^{th} day of treatment.

Biochemical parameters

The biochemical parameters, such as total serum protein, alanine phosphatase (ALP) and serum calcium were investigated. All the observations were made on 0^{th}, 7^{th} and 14^{th} day of treatment.

Histochemical study

The histochemical study was carried out on 0 and after complete wound healing by using special Van Gieson stain.

Result and Discussion

Clinico-physiological parameters

Rectal temperature, heart rate and respiratory rate were found to be non significantly different. Wound assessment parameters were found to be of relevant significance for assessment of wound healing. The percent wound contraction was found to be significant within the groups. The complete wound healing from group A and group B was observed on 17 and 25 days respectively. *E. laevis* helped in removing the impurities from the wound and promotes the formation of healthy granulating tissue, adequate circulation to the wound site. It accelerated wound healing process and successfully reduced the wound size [5].

Haematological parameters

Haemoglobin

There were non-significant differences in the haemoglobin concentration between the groups or within the group. All the values were well within the normal range. In spite of this a definite decreasing trend in the mean values was observed in both the groups up to 7^{th} day and then it gradually increased till 14^{th} day.

Total erythrocyte count

There was non-significant difference in the haemoglobin concentration between the groups. All the values were well within the normal range. In spite of this there was definite increasing trend observed in the values in both the groups up to the day 14. It might be due to generalized activation of sympathoadrenal mechanism, which results into release of catecholamine's which resulted into spleen contraction, thereby causing out flux of red blood corpuscles in the blood circulation which may be responsible for causing increase in the number of TEC in the present study [6].

Total leukocyte count

There was non-significant difference in the haemoglobin concentration between the groups. The mean values of total leukocyte count gradually decreased non significantly from 0^{th} day till 14^{th} day in both the groups. It was found that on 7^{th} and 14^{th} day healing of the wound reached to stage of completion, as a result of which inflammation, exudation and infection subsided and therefore TLC also decreased up to the stage of 14^{th} day [7].

Differential leukocyte count

The levels of neutrophils, eosinophils, monocytes and lymphocytes did not differ significantly between groups. This demonstrated that the silver nano particles present in the *E. laevis* and *A. indica* ointment did not cause any allergic reaction or abnormal inflammation at the wound site.

Fibrinogen Estimation

The mean values of fibrinogen gradually increased on 7th day and then decreased on 14th day in both the groups.Present study showed that use of silver nanoparticles present in the *E. laevis* and *A. indica* ointment did not affect fibrinogen concentration markedly.

Histochemical Study

In Group A, after wound healing, histochemical study revealed formation of new blood vessels, new-epithelisation, sebaceous gland formation, mild inflammatory cell infiltration with presence of hair follicles and also well-arranged dense collagen fibres after topical application of ELSNP ointment. However, in Group B granulation tissue, mild oedema along with infiltration of mild inflammatory cells and epithelial lining projection towards dermis were observed after topical application of AISNP. The wound healing process was similar in both the groups; however, the arrangement of collagen fibres and new-epithelisation was found prominent in group A as compared to group B. As, it is already known that the major source of strength and elasticity in skin is collagen fibres [8]. This might be the prominent reason why group A wounds exhibited healing of wound at an accelerated pace on 14th day as compared to group B in which slower pace of healing of wound on 14th day was exhibited.

Biochemical estimations

Total serum proteins

The total serum protein of both the groups did not differ significantly between groups or within groups. The mean values of total serum protein gradually non significantly decreased on 7th day and then increased on 14th day in both the groups. The rate of wound healing is not correlated with plasma protein level, unless and until the protein concentration is lesser than 2 g/dl [2].

Alkaline phosphatase

The mean values of alkaline phosphatase in both the group varied significantly from each other. The mean values of alkaline phosphatase in group A gradually significantly ($p < 0.05$) decreased on 7th and 14th day. Whereas, in group B gradually significantly ($p < 0.05$) increased on 7th and 14th day respectively. The comparatively lower level of ALP in the Group A might be attributed to lower tissue damage and better wound healing properties of ointment [9]. It was observed that there was a gradual decrease of ALP activity with completion of dermal repair [10].

Serum calcium

The mean values of serum calcium in both the group varied significantly from each other. The mean values of serum calcium in group A and B gradually significantly ($p < 0.05$) increased on 7th day and decreased on 14th day. The extracellular calcium concentrations have been shown to increase upon injury, persisting through the inflammatory and proliferative phases, and then decreases during the remodelling phase [11].

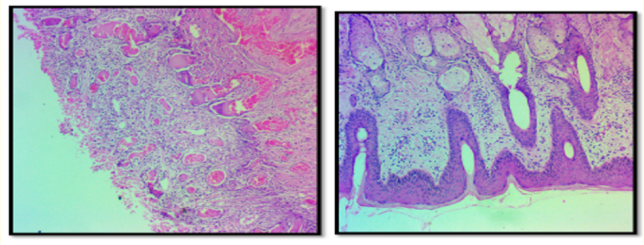

Figure 1: Histochemical observations in Group A. 0th day: After wound healing.

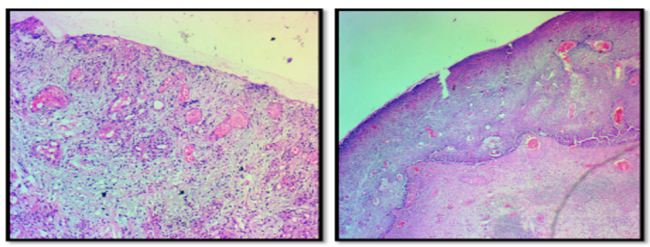

Figure 2: Histochemical observations in Group B. 0th day: After wound healing.

Parameters	Group A			Group B		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Haemoglobin (gm/dL)	11.65 ± 0.95	10.88 ± 0.91	11.28 ± 0.87	11.10 ± 1.20	10.53 ± 1.15	10.80 ± 1.12
TEC (×10^6/cu.mm)	06.66 ± 0.43	06.77 ± 0.42	07.13 ± 0.45	06.63 ± 0.46	06.71 ± 0.47	07.05 ± 0.46
TLC (×10^6/cu.mm)	11.30 ± 1.31	11.04 ± 1.29	10.77 ± 1.30	10.13 ± 1.29	09.99 ± 1.28	09.81 ± 1.29
Neutrophils (%)	30.48 ± 6.21	25.33 ± 2.6	24.30 ± 2.59	30.00 ± 4.15	29.93 ± 4.14	28.82 ± 4.14
Eosinophils (%)	01.32 ± 0.02	01.16 ± 0.01	01.31 ± 0.02	01.06 ± 0.05	01.16 ± 0.01	01.49 ± 0.05
Monocytes (%)	05.45 ± 0.43	05.58 ± 0.43	05.66 ± 0.51	05.45 ± 0.55	04.85 ± 0.54	05.22 ± 0.53
Lymphocytes (%)	55.80 ± 2.16	56.55 ± 2.16	57.20 ± 2.15	57.27 ± 1.48	58.39 ± 1.47	58.44 ± 1.47
Fibrinogen (%)	0.44 ± 0.03	0.47 ± 0.03	0.43 ± 0.03	0.43 ± 0.02	0.46 ± 0.03	0.42 ± 0.03

Parameters	Group A			Group B		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Total serum protein (gm/dL)	06.81 ± 0.4	06.73 ± 0.44	07.06 ± 0.69	06.57 ± 0.44	06.52 ± 0.38	06.69 ± 0.41
Alkalinephosphatase (IU/L)	76.45 ± 5.68	75.92 ± 6.42	69.85 ± 6.42	80.53 ± 8.96	84.96 ± 7.23	85.44 ± 7.22
Serum calcium (mg/dL)	08.87 ± 0.33	09.44 ± 0.40	09.29 ± 0.27	08.86 ±0.33	08.87 ± 0.17	08.37 ± 0.18

Table 1: Mean ± SE values of haematological parameters at different time intervals.

Conclusion

Green synthesized silver nanoparticles ointment of *E. laveis* has better wound healing property than that of *A. indica* silver nanoparticle ointment.

Bibliography

1. Basha M., *et al*. "Care and management of wound in animals". *Livestock Line* 12.12 (2019).
2. Probst CW. "Wound Healing and Specific Tissue Regeneration". In: Slatter D (ed) Textbook of Small Animal Surgery, 2nd edition, Philadelphia, W. B. Saunders (1993): 53-63.
3. Aritonang HF., *et al*. "Synthesis of Silver Nano particles Using Aqueous Extract of Medicinal Plants' (*Impatiens balsamina* and *Lantana camera*) Fresh Leaves and Analysis of Antimicrobial Activity". *International Journal of Microbiology* (2019): 1-8.
4. Parhizkar S., *et al*. "Incision choice in Laparotomy: a comparison of two incision techniques in ovariectomy of rats". *World Applied Sciences Journal* 4.4 (2008): 537-540.
5. Jain NC. "Schalm's Veterinary Haematology". 4th edition, Lea and Febiger, Philadelphia (1986): 526-527.
6. Bhat. "Comparative studies on combination of collagen particles metronidazole and mupirocin with povidone Iodine on open wound healing in dogs, M.V.Sc. and A.H. Thesis (Surgery and Radiology), submitted to NanajiDeshmukh Veterinary Science University, Jabalpur (2012).
7. Singh Y., *et al*. "Comparative evaluation of haemato-biochemical changes during infected wound healing in cow calves with the use of silver nano particle gel and povidone iodine". *Veterinary Practitioner* 18.1 (2017): 54-55.
8. Fratzl P., *et al*. "Fibrillar structure and mechanical properties of collagen". *Journal of Structural Biology* 122 (1997): 199-122.
9. Kakki D., *et al*. "Wound healing with bidenspilosa and cassia to rain Rabbits". *Indian Veterinary Journal* 3 (2016): 27-29.
10. Rai AK., *et al*. "Histochemical localization of alkaline phosphatase activity during cutaneous wound healing in a catfish under acid stress". *International Journal of Science and Research* 3 (2013): 1-9.
11. Subramaniam T., *et al*. "The role of Calcium in wound healing". *International Journal of Molecular Sciences* 22.12 (2021): 6486.