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ALTERATIONS IN THE TRACE MINERALS OF CUTANEOUS WOUNDS OF RABBITS GRAFTED WITH CALCIUM-SILVER NANOCOMPOSITE FILMS

P Ravi Kumar[1]*, RV Suresh Kumar[2], N Dhana Lakshmi[3], Ch Srilatha[4], M Vidyavathi[5], TNKVV Prasad[6] and P Veena[7]

[1]Assistant Professor, Department of Veterinary Surgery and Radiology,
NTR College of Veterinary Science, Gannavaram- 521 102(AP)
[2]Professor, Department of Veterinary Surgery and Radiology,
[3]Professor, Department of TVCC,
[4]Professor, Department of Veterinary Pathology,
[7]Professor, Department of Veterinary Surgery and Radiology,
College of Veterinary Science, Tirupati, (AP)
[5]Professor, School of Pharmaceutical Sciences
Sri Padmavathi Mahila University, Tirupati
[6]Senior Scientist, Soil Science,
Institute of frontier technology, Tirupati (AP)
E-mail: ravikumarpallitvm1018@gmail.com (**Corresponding Author*)

Abstract: The present study was conducted on eighteen rabbits divided into three groups of six animals comprising group-I with control animals, group-II with calcium-silver (35:55) nanocomposite films applied animals, group-III with calcium-silver (45:45) nanocomposite films applied animals. Cutaneous wounds were created at the loin region of all the animals and the wounds were left unsutured in control group, same size nanocomposite films were cut and applied in group-II and group-III animals. Wound healing was evaluated by the estimation of trace minerals like Iron, Copper and Zinc present in the granulation tissue. The values showed significant changes among the groups and as well as between the groups with lower value on day 7 and higher value on day 14. Based on the observations in the present study it is concluded that calcium-silver nanocomposite films could be used safely for cutaneous wound healing without any adverse effects.

Keywords: Nanoparticles, Trace minerals, Wet digestion, inflammation, antimicrobial agents.

INTRODUCTION

Successful wound care involves optimizing patient's local and systemic conditions in conjunction with an ideal wound healing environment. All the preparations aimed at providing a pathogen-free, protected, and moist area for wound healing to occur (Murphy and Evans 2012). Majority of the wound preparations contains combination of antibiotics, antifungal and corticosteroid agents, either in form of ointment or paste or gel or powders.

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However these preparations will have certain limitations like drug resistance, cost and local tissue reactions. Considering these points in mind research was diverted towards use of other materials in nano forms. Nanomaterials emerged as antimicrobial agents due to their high surface area per unit mass resulting in greater antimicrobial activity (Kemp *et al*., 2009).

Silver is one of the most powerful antiseptic materials available naturally and posses low toxicity towards the mammalian tissue (Sundaramoorthi *et al.,* 2009). The bactericidal property of silver is mainly due to its strong interaction with thiol groups present in the respiratory enzymes in the bacterial cell and also have interaction with structural proteins preferentially bind with DNA nucleic acid bases to inhibit replication (Church *et al.,* 2006).

Bishara *et al*., (2012) reported that topical silver compounds cause cytotoxicity to keratinocytes and fibroblasts in excessive doses however silver nanoparticles were preferred for external applications to other topical compounds as they offer slow, controlled release of silver ions and avoids excessive delivery, more over these are less susceptible to deactivation by the chlorides in the physiological media (Kemp *et al*., 2009). Silver decreases the activity of matrix metalloproteinase which are more in burn wounds and chronic wounds there by promoting wound healing (Fong and Wood 2006). Several scientists prepared silver nanoparticles by acticoat method (physical vapour deposition) using argon gas (Fong and Wood 2006), biosynthesized from *Aspergillus niger* (Sundaramoorthi *et al*., 2009), biosynthesized from *Streptomyces aureofaciens* (Sundaramoorthi et al., 2010), and studied the effects.

Calcium has an established role in normal homeostasis of skin and is a modulator of keratinocyte proliferation and differentiation (Kawai *et al*.,2011). Influx of free calcium into the cell causes contraction of microfilaments in the cell and alters the cell shape which helps in wound healing (Stranistreet 1982). Calcium ions released from calcium alginate into the wound bed helps in wound healing during calcium alginate dressing (Kawai *et al*., 2011). Calcium Nanoparticles show effective response over the other calcium preparations on wound healing. These calcium Nanoparticles are prepared from calcium chloride solution using beta glycerol phosphate and 0.2% acetic acid solutions (Kawai *et al*., 2011). In the present study nano calcium of phyto origin was coupled with nano silver for evaluation on the cutaneous wounds of rabbits.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

Eighteen adult rabbits weighing 1.5-2 Kgs were selected and divided into three groups of six animals each after obtaining permission from IAEC of the institution. Xylazine hydrochloride @5mg/kg was administered intramuscularly followed by Ketamine hydrochloride @35mg/kg to produce satisfactory anaesthesia in all the rabbits. Cutaneous wounds of 1cm X 1cm at the loin region were created and the wounds were left unsutured, cleaned with normal saline and covered with steripad in Group I animals. Group II animals received Calcium-silver (35:45) nanocomposite films (fig-1) with corresponding size of the wound and applied over the wounds after making pores on the material with 22G needle and added a little normal saline to have a better adherence. In Group III animals Calcium-silver (45:45) nanocomposite films (fig-2) were made into corresponding size of the wound and applied over the wounds after making pores on the material with 22G needle and added a little normal saline to have a better adherence. Steripad was applied over the nanocomposite film and covered with protective bandage. The steripad and bandage were replaced with new steripad and bandage once in two days until the wound showed healing. The accurately weighed amount of granulation tissue sample (0.2-0.5gm) was taken into a conical flask and sample was digested by wet digestion method. Then the samples were analysed for copper, iron and zinc estimation by using AAS and expressed as ppm (Parker *et al.* 1967).

RESULTS

Iron (ppm):

The changes in the iron levels in granulation tissue collected from the wounds of different groups of rabbits were shown in Table1, Fig. 1. The premean±S.E values in group I, group II and group III were 0.21±0.036, 0.2823±0.049 and 0.76±0.13 respectively. The mean values of iron at day 7 were 0.32±0.0029, 0.37±0.0033 and 1.09±0.01095 in group I, group II and group III respectively whereas the values at day 14 were 0.31±0.0039, 0.47±0.0028 and 1.18±0.0442 respectively. The values did not differ with in the group I where as group II and group III animals showed a significant ($p<0.05$) increase reaching a maximum of 0.47±0.0028 and 1.18±0.0442 respectively. The values differed significantly ($p<0.05$) among the three groups on day-7 and day-14.

Copper (ppm):

The alterations in the values of copper in granulation tissue collected from the wounds of different groups of rabbits were shown in Table 2, Fig. 2. The premean±S.E values in group I, group II and group III were 0.25±0.044, 0.29±0.051 and 0.81±0.139 respectively. The mean values of copper at day 7 were 0.33±0.0038, 0.36±0.0037 and 1.19±0.0056 respectively which were significantly ($p<0.05$) increased to 0.42±0.0039, 0.49±0.0042 and 1.23±0.0039 respectively by day-14. The values showed a significant difference ($P< 0.05$) among the groups at all periods of observation.

Zinc (ppm):

The alterations in zinc levels in the granulation tissue collected from the wounds of different groups of rabbits were shown in Table 3, Fig. 3. The premean±S.E values in group I, group II and group III were 0.04±0.01, 0.04±0.01 and 0.08±0.02 respectively. The mean values of zinc at day 7 were 0.04±0.0017, 0.05±0.0022 and 0.11±0.0056 respectively which were significantly increased to 0.07±0.0015, 0.06±0.0023 and 0.14±0.015 respectively by day-14. The values showed a significant difference ($P< 0.05$) among the groups at all periods of observation.

DISCUSSION

Majority of the wound preparations contains combination of antibiotics, antifungal and corticosteroid agents, either in form of ointment or paste or gel or powders. However these preparations will have certain limitations like drug resistance, cost and local tissue reactions. This resulted in use of collagen dressings (Swaim *et al.* 2000), full thickness skin grafts (Anjaiah *et al.* 2001) and amniotic membrane (Sreenu *et al.* 2002) for cutaneous wound healing. Considering these points in mind research was diverted towards use of other materials in nano forms. The main goals of usage of these dressings in wound care and management are prevention of infection, maintenance of a moist environment, protection of the wound and achievement of rapid and complete healing with the minimum scar formation. Nanomaterials are emerging as antimicrobial agents due to their high surface area per unit mass resulting in greater antimicrobial activity (Kemp *et al.*, 2009). In the present study calcium and silver nanocomposite films were evaluated for their efficacy on cutaneous wound healing in rabbits. Preliminary studies evaluated different combinations of calcium and silver nanocomposite films, and optimization was done to prepare calcium-silver (35:55) nanocomposite films and calcium-silver (45:45) nanocomposite films. These combinations were compared with control group rabbits. In the present study equal size of cutaneous wound was created in rabbits of all the groups. The cutaneous wounds were washed with normal saline and protected with external bandaging to prevent contamination. External bandaging helped not only in protection of wound from contamination and self mutilation but also aided in immobilisation of wound edges during healing. The nanocomposite films were closely adhered to wounds without complications. In the present study, blood clots and wound fluid was observed in control group post operatively which might be due to the haemorrhage at the wound area. None of the animals in treatment groups showed neither haemorrhage nor wound fluid. This might be attributed to haemostatic activity of the calcium (Barnett and Varley 1987). Wound fluid was absent in both the treatment groups which might be due to the rapid and uniform adherence of nanocomposite films conforming to wound bed topography preventing air or fluid pocket formation. The dressing was preferably permeable to water vapour so that a moist exudate under the dressing is maintained without pooling. Grossly none of the materials were rejected by the animals. In the present study, inflammatory reaction was absent in the animals treated with biomaterials i.e, calcium-silver (35:55) nanocomposite films and calcium-silver (45:45) nanocomposite films, which might be attributed to apoptosis of infiltrating inflammatory cells caused by silver nanoparticles (Nadworny et al. 2010). This gross finding not only explained the tolerance of the host tissue to the foreign material and also safety of the nanocomposite films. The nanocomposite films used in both the groups were found to adhere very firmly. In our study, two animals in the control group, showed purulent discharges which might be due to subsequent contamination of the wound, whereas infection was not evident in both the treatment groups. This finding was in agreement with the observations of Beam (1986), Bishara et al. (2012) who studied the antimicrobial effect of the silver and its composites on wound healing. However, in both the treatment groups silver being the common substance, which suggested that silver, as well as its derivatives and complexes were active against various microbes. The proposed antimicrobial mechanisms of silver include strong interaction with thiol groups present in the respiratory enzymes in the bacterial cell and also have interaction with structural proteins preferentially bind with DNA nucleic acid bases to inhibit replication (Church et al., 2006). In the present study, higher levels of iron were recorded in animals treated with calcium-silver (45:45) nanocomposite films followed by calcium-silver (35:55) nanocomposite films and control groups. This appears to be an indication of increased cellular metabolism and as result there was a greater requirement of iron for cellular oxidation (Ansari *et al*. 1997).

Copper is intimately assosciated with tissue oxidase as ascorbic acid oxidase, cytochrome oxidase and is an essential constituent of several protein compounds. In the present study a significant difference was observed in the copper contents of the treated and control wounds. The higher values of copper during the early phase of wound healing in treatment groups especially in group III animals might be due to role of copper in the formation of aortic elastin and the blood vessels as reported by Ansari *et al.* (1997) and Gangwar *et al.* (2006).

Zinc is the metal moiety of alkaline phosphatise. A slight increase in zinc level was recorded in the present study and was statistically significant between the groups as well as among the groups. An increase in zinc level might be due to increased activity of carbonic anhydrase and alkaline phosphatise as observed by Ansari *et al.* (1997) and Gangwar *et al* (2006).

CONCLUSION

On the basis of biochemical changes of wounds it was concluded that the treatment of wounds with calcium-silver (45:45) nanocomposite films enables the wounds to heal early in comparison with calcium-silver (35:55) nanocomposite films and control group of animals.

REFERENCES

[1] Aldo J E and King J W 1966 The management of burns with Silver Nitrate solution. Journal of the National Medical Association 58(3):165-167.

[2] Anjaiah A, Haragopal V, Raghavender K B P and Chandra Sekhar E L 2001 Effects of full thickness skin grafts and mesh skin grafts on granulating wounds in dogs : An Experimental Study . Cheiron 30(3&4) : 92-94.

[3] Ansari M A, Jadona N S, Singh S P, Amresh Kumar and Harpal Singh 1997 Effect of approaches to wound management. Clinical Microbiology Reviews 14: 244-269.

[4] Barnett S E and Varley S J 1987 The effects of calcium alginate on wound healing. Annals of Royal College of Surgeons of England. 69.

[5] Beam J W 2009 Topical siver for infected wounds. Journal of Athletic Training 44(5):531-533.

[6] Bishara S. A, Michel C, Shady N. H, and Saad A. D 2012 Effect of silver on burn wound infection control and healing. Burns 33 139-148.

[7] Church D, Elsayed S, Reid O, Winston B and Lindsay R 2006 Burn wound infections. Clinical Microbiology Reviews 19(2): 403-434.

[8] Fong J and Wood F 2006 Nanocrystalline silver dressings in wound management: A review. International journal of Nanomedicine 1(4):441-449.

[9] Gangwar A K, Sharma A K, Naveen Kumar , Kumar N, Maiti S K, Gupta O P, Goswami T K and Singh R 2006 Acellular dermal graft for repair of abdominal wall defects in rabbits. Journal of the South African Veterinary Association 77(2): 79–85.

[10] Jadon N S, Amresh Kumar and Bedi S P S 1985 Effect of amnion on wound healing in buffaloe calves, a biochemical study. Indian Veterinary Journal 62 : 514-518.

[11] Kawai K, Larson B J, Ishise H, Carre A L, Nishimoto S, Longaker M and Lorenz H P 2011 Calcium based Nanoparticles accelerate skin wound healing. PLoS One. 2011; 6(11).

[12] Kemp MM, Kumar A, Clement D, Ajayan P, Mousa S and Linhardt R J 2009 Hyaluronan and heparin-reduced silver Nanoparticles with antimicrobial properties. Nanomedicine (Lond) 4(4):421-429

[13] MacKeen P C, Person S, Warner S C, Snipes W and Stevens S E 1986 Silver-coated nylon fiber as an antibacterial agent. Antimicrobial Agents and Chemotherapy 31(1):93-99.

[14] Murphy P S and Evans G R D 2012 Advances in wound healing: A review of current wound healing products. Plastic Surgery International 2012:190436, 1-8

[15] Nadworny P L, Wang J, Tredget E E and Burell R E 2010 Anti-inflammatory activity of nanocrystalline silver-derived solutions in porcine contact dermatitis. Journal of inflammation 7:13 1-20.

[16] Sreenu M, Sastry T P, Rao T S C and Rao N V 2002 Evaluation of the efficacy of gelatin, fibrin and amniotic membrane for second intension urethral wound healing in dogs. Cheiron 31(3&4): 81-84.

[17] Stanisstreet. M 1982 Calcium and wound healing in xenopus early embryos. Journal of embryology and experimental morphology. Vol 67, 195-205.

[18] Sundaramoorthi C, Kalaivani M, Mathews D M, Palanisamy S, Kalaiselvan V and Rajasekaran A 2009 Biosynthesis of silver Nanoparticles from *Aspergillus niger* and evaluation of its wound healing activity in experimental rat model. International Journal of PharmTech Research 1(4):1523-1529.

[19] Sundaramoorthi. C, Devarasu.S and Vengadesh Prabhu.K 2010 Antimicrobial and wound healing activity of silver nanoparticles synthesised from Streptomyces Aureofaciens . International jounal of pharmaTech research. Vol.2 (12), 69-75.

[20] Swaim S F, Gillette R L, Startin E A, Hinkle S H and Coolman S L 2000 Effects of hydrolysed bovine collagen dressing on the healing of open wounds in dogs. American Journal of Veterinary Surgery 61(12): 1574-1578.

Table 1: Mean ± S.E values of Iron at different time periods in 3 groups of rabbits under study

	Day-7*	Day-14*
Group I	0.32±0.0029^{a}	0.31±0.0039^{a}
Group II*	0.37±0.0033^{bA}	0.47±0.0028^{bB}
Group III*	1.09±0.01095^{cA}	1.18±0.0442^{cB}

Means bearing different superscripts within a row (A,B..) and within a column (a,b..) differ significantly. * p<0.0

Table 2 : Mean ± S.E values of Copper at different time periods in 3 groups of rabbits under study

	Day-7*	Day-14*
Group I*	0.33±0.0038^{aA}	0.42±0.0039^{aB}
Group II*	0.36±0.0037^{bA}	0.49±0.0042^{bB}
Group III*	1.19±0.0056^{cA}	1.23±0.0039^{cB}

Means bearing different superscripts within a row (A,B..) and within a column (a,b..) differ significantly. * p<0.05

Table 3: Mean ± S.E values of Zinc at different time periods in 3 groups of rabbits under study

	Day-7*	Day-14*
Group I*	0.04±0.00165^{aA}	0.07±0.0015^{aB}
Group II*	0.05±0.00222^{aA}	0.06±0.0023^{aB}
Group II*	0.11±0.00558^{bA}	0.14±0.015^{bB}

Means bearing different superscripts within a row (A,B..) and within a column (a,b..) differ significantly. * p<0.05

Fig. 1: Graph showing the iron values at different time intervals in 3 groups of rabbits under study

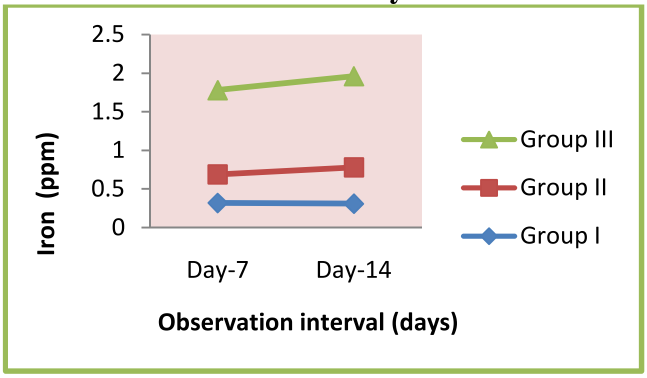

Fig. 2: Graph showing the copper values at different time intervals in 3 groups of rabbits under study

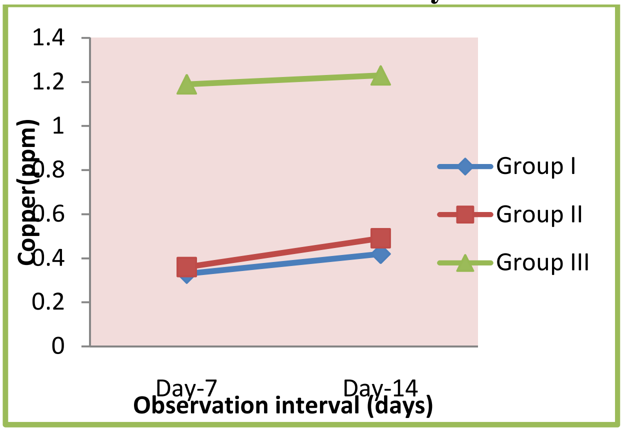

Fig. 3: Graph showing the zinc values at different time intervals in 3 groups of rabbits under study