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Study of Effectiveness of Curcumin-Chitosan-Aloe Vera Healing Agent with Mupirocin for the Treatment of Aseptic Wounds in Dogs

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ABSTRACT

Background: The study was conducted to evaluate the wound healing potential of curcumin-chitosan-aloevera based agent, for the treatment of aseptic surgical wounds in dogs presented to the Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar.

Methods: The wounds were assigned into two therapeutic regimens; -application of nano-based curcumin-chitosan-aloevera healing agent in the test group and Mupirocin in control group along with parenteral antibiotics in both the groups. Results were statistically analyzed, using analysis of variance (ANOVA), paired t-test (parametric) and Kruskal-Wallis one way ANOVA test (non parametric).

Result: Chitosan-curcumin-aloevera based healing agent resulted in early reduction of wound exudates and cast formation than control group. Time of appearance and filling of wound bed by granulation tissue was significantly better in test group than control. The percentage of wounds with early appearance of epithelium was also higher in test group than control group. Histological and histo-chemical findings on day 14 after surgery suggested less inflammatory reaction, more fibroblasts and neo-vascularisation, wider and compact granulation tissue in test group than control. The values of clinical, haematological and biochemical parameters of both the groups remained within the normal physiological limits without any significant difference. The average time taken for complete healing in the test and control groups was 9 and 14 days respectively.

Key words: Aloe vera, Chitosan, Curcumin, Dog, Mupirocin.

INTRODUCTION

The wound healing is an active, complicated and a series of closely related processes which includes haemorrhage, coagulation and starting of an acute inflammatory response to the original injury. In order to restore the disrupted anatomical continuity and disturbed functional status of the affected part, it is essential to minimize tissue damage, supply proper tissue perfusion and oxygenation, proper nutrition, moist wound healing environment (Bowler *et al*., 2001; Gosain and DiPietro, 2004; Mani *et al*., 2002 and Dinesh *et al*.,1997).

Wounds of animals treated with curcumin showed early re-epithelialization, better neo-vascularization, a higher collagen content with enhanced migration of different cells including dermal fibroblasts, myo-fibroblasts and macrophages into the site of wound bed (Sidhu *et al*., 1999). Chitosan, a cationic natural polymer, has a number of biomedical uses and is considered as an innovative substance in wound healing for its antibacterial, haemostatic and hypocholesterolemic effect (Shakeel and Saiqa, 2015). It also promotes formation of granulation tissue during proliferative stage of wound healing process. It maintains sterile wound exudates under a dry scab, restraining dehydration and wound contamination, to enhance the environment for wound healing. Similarly, aloevera has been found as a promoter of wound healing for its antibacterial and antifungal activities;

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it also increases synthesis of collagen and elastin in the wound bed (Rajeswari *et al*., 2012).

Basing on above context, a preparation was made using curcumin, chitosan and aloevera as a nano-based topical healing agent. To know its effect, a comparative study with that of Mupirocin ointment was carried out for treatment of aseptic surgical wounds in 57 clinical cases of dogs.

MATERIALS AND METHODS

The study was conducted for research purpose varying for a period of 13 months on canine surgical cases (castration and ovario-hysterectomy) presented to the Department of Veterinary Surgery and Radiology, CVSc and AH, OUAT, Bhubaneswar from different geographical locations of the state. A total of 57 numbers of dogs of different breeds, sex and various age groups were studied for this purpose. The animals were divided into two groups depending on local application of the healing agents on surgical wounds *i.e.* test group (application of Curcumin-Chitosan-Aloevera based healing agent) and control group (Mupirocin ointment application; Staphban, Savavet). Out of total 57 numbers of cases, 44 were kept under test whereas 13 under control group. The details of preparation of the test agent has been kept secret.

Fig 1: Preparation of the patient.

Fig 2: Measurement of wound.

Before initiating surgical treatment, complete history and other clinical observations like rectal temperature, respiration rate (breaths/ minute), heart rate (beats/ minute) and status of visible mucous membrane were recorded. With the consent of owner, blood was collected for haemato-biochemical examination and as per condition required, surgery were done in the presented cases and sutured (Fig 1). The characteristics of wounds like type of exudates, extent of exudates, rate of wound contraction and swelling were also recorded (Fig 2) in scale point basis (Table 1) following the method of Gopinathan *et al*. (2007) with slight modification. Tissue samples from wound margin on 0 and 14th day were collected in 10% buffered neutral formalin then processed routinely to obtain 6 µm thick tissue sections. The tissue sections were stained with Hematoxylin and Eosin (H and E) stain for studying the histo-architecture, Masson's trichrome for demonstration of collagen fibers and Verhoeff's stain for elastic fibers (Bancroft and Stevens, 1996). After operation and suturing, the curcumin-chitosan-aloevera based topical gel was applied twice daily on the animals of Test group (Fig 3). Likewise the standard therapeutic regimen *i.e*. Mupirocin ointment was applied twice daily in Control group.

Haematological observations like estimation of haemoglobin, total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume (PCV) and differential leukocyte count (DLC) were done. Biochemical parameters like blood glucose, total protein, albumin, globulin, SGPT, SGOT, phosphorous and calcium were estimated by biochemical auto analyser (TURBOCHEM-100) on day 0 and 14th day.

The mean parametric observations were compared by analysis of variance (ANOVA) as per the procedure described by Snedecor and Cochran (1989) while the non-parametric observations were compared by Kruskal-Wallis one way ANOVA.

RESULTS AND DISCUSSION

In the present study the clinical parameters like respiration (per minute), pulse rate (beats per minute), rectal temperature (°F) were recorded during post treatment which showed no significant differences between the groups during healing of wounds which corroborates with the findings of Borena *et al.* (2009). It indicates the body physiology during healing period remains normal irrespective of treatment regimen.

The findings of hematological parameters like Hb, TLC, TEC, PCV and DLC on 0 and 14th day were in good agreement with that of Gopinathan *et al.* (2007) in horses and in dogs by Borena *et al.* (2009) which showed no significant differences between test and control groups.

Table 1: Showing basis of scale point.

Type of physical evaluation	Scale point			
	0	1	2	3
Type of exudates	No exudate	Dry cast	Serous	Fibrinous
Extent of exudates	None	Slight	Moderate	Extreme
Degree of swelling at wound site	No swelling	Mild swelling	Moderate swelling	Severe swelling

The estimation of biochemical parameters like glucose, total protein and albumin were done in horses by Gopinathan *et al.* (2007) and in dogs by Borena *et al.* (2009). But in all the above cases there was no significant differences existed in their reports. Likewise in this pilot study also the parameters like glucose, SGPT, SGOT, total protein, albumin, globulin, calcium and phosphorus were estimated during treatment period which showed no significant differences and all those data remained within the normal physiological limits. This indicates that the impact of wound on the whole body system was negligible in comparison to total body area and there were normal liver and renal functioning in the body of patients.

Wound assessment was studied by parameters of gross observation of the wound *viz.* types of exudates, extent of exudates, swelling and percentage of wound contraction. The evaluation of wound was done by Bose *et al*. (2006) in bovines on 0-3 scale basis. The similar facts were also recorded by other researchers (Archibald and Blackley, 1974; Stegman and Swaim, 1990 and Curtis, 1993). The wound healing was assessed by subjective scores by Gopinathan *et al*. (2007) on 1-3, 1-5, 1-4, 1-3, 1-4 and 1-4 for color of wound, type of exudates, extent of exudates, peripheral swelling, pain and warmth respectively in horses. In the present study, the wounds in both groups were studied on 0-3 scale basis. Basing on the above observations the mean±SE values of the score for extent of exudates, type of exudates, swelling and percentage of wound contraction, significant difference ($P<0.05$) was found on 14th day of observation in both the test and control group (Table 2). It revealed that the process of wound healing was going on in enhanced manner in test group which might be due to more collagen formation by the application of curcumin based healing agent.

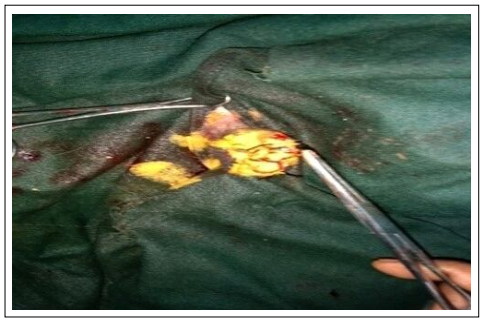


Fig 3: Application of Nano-based healing gel.

Fig 4: Healed wound.

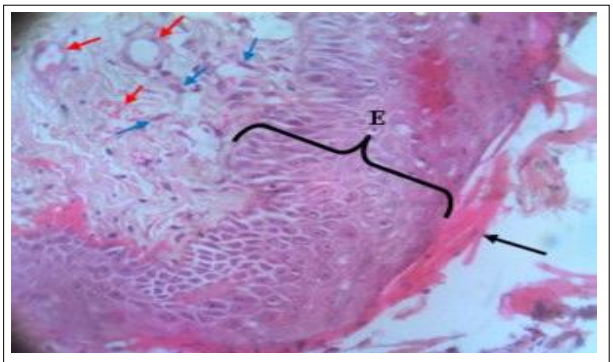
In test group, the basal mean ± SE of duration of healing of wound was 8.77±0.63 with minimum days of 7 and maximum of 14 days. On the other hand in control group, the basal mean±SE of same parameter was 13.38±2.13 with minimum of 10 and maximum of 31 days (Table 3). But, the less duration of healing period in test group might be due to early neo-vascularisation, adequate collagen formation and attracting different types of wound healing factors within short span of time. The work is being supported by Panchatcharam *et al.* (2006) while experimenting the effects of curcumin for wound healing in rats. They interpreted that curcumin based healing agent promotes wound healing process by enhancing granulation tissue, increasing biosynthesis of transforming growth factor (TGF)-β1 and proteins in extra cellular matrix.

Kirubanandan and Sehgal (2010) stated that the collagen which is one of the prominent proteins in the extracellular matrix enhances keratinocytes and fibroblast proliferation which are important factors in wound healing. In the present study, the wound samples on 14th day of topical application of test agent discerned clear evidence of the phenomena *i.e*. neo-vascularization, neo-epithelialization, fibro-collagen deposition and remodelling of epidermis were clearly evident. The epidermis had normal morphology with 10-11 numbers of cells arranged in layers starting from stratum basale to stratum corneum (Fig 5). The blood capillaries were profused in deeper and superficial part of dermis (Fig 6). Also profuse capillary bed was observed around the sebaceous and sweat glands. The collagen fibres were abundant in the dermis with large numbers of fibroblasts as well as fibrocytes. Isolated patches of skeletal muscles were reinforced in between the collagen fibre

Table 2: Effect of indigenous prepared topical gel on wound parameters (Mean±SE).

Parameters	Test		Control	
	0 (Zero) day	14th day	0 (Zero) day	14th day
Score for extent of exudates	0.31±0.17	0.08±0.04^{a}	0.38±0.18	0.15±0.03^{b}
Score for type of exudates	0.23±0.12	0.07±0.02^{a}	0.31±0.12	0.16±0.03^{b}
Score for swelling	1.23±0.32	0.02±0.01^{a}	1.38±0.42	0.08±0.02^{b}
Contraction of wound (%)	Nil	52.60±0.94^{a}	Nil	14.19±0.70^{b}

Different superscripts between columns show significant difference ($p<0.05$) within a group.



Note: The scattered fibrocytes (blue arrow) and capillaries (red arrow) in the dermis. H & E X 400.

Fig 5: Photomicrograph of wounded skin of dog from test group on 14th day showing fully formed epidermis (E) of 10-11 cell layers with distinct stratum corneum (black arrow).

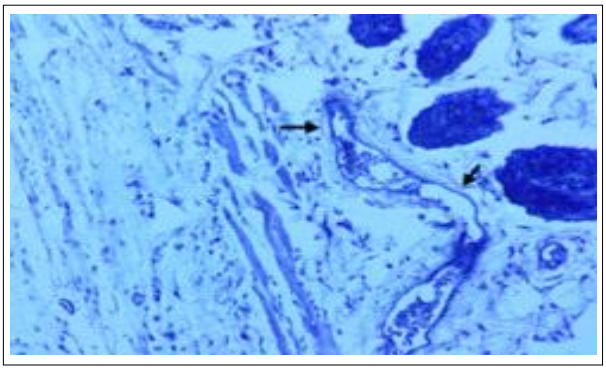
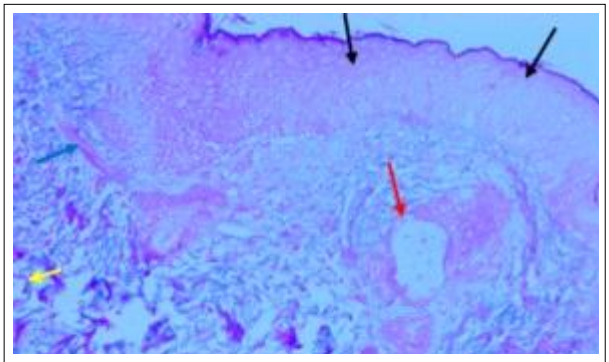
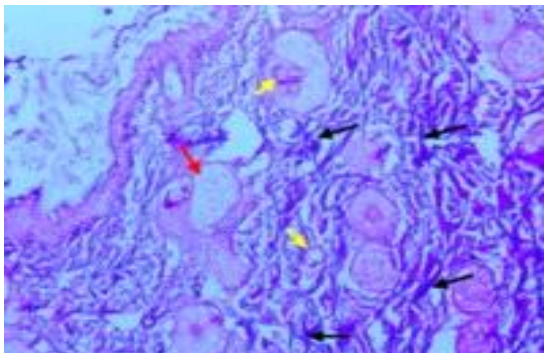


Fig 8: Photomicrograph of wounded skin of dog from test group on 14th day showing fine elastic fibers around blood vessel (arrow). Verhoeff's stain × 400.



Note: The occurrence of sebaceous glands (red arrow), isolated skeletal muscle (blue arrow) in between the collagen fiber bundles and blood capillaries (yellow arrow) in the dermis. Masson's trichrome stain X 100.

Fig 6: Photomicrograph of wounded skin of dog from test group on 14th day showing thick epidermis (black arrow).



Note: The capillaries (yellow arrow) and sebaceous gland (red arrow) in dermis. Masson's trichrome stain × 400.

Fig 9: Photomicrograph of wounded skin of dog from control group 14th day showing moderately dense, scattered arrangement of collagen fiber bundles (black arrow) in dermis.

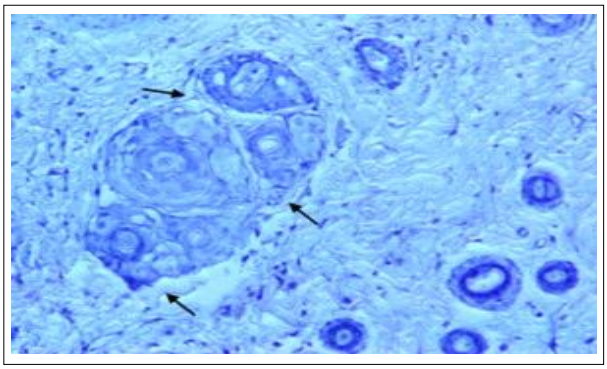
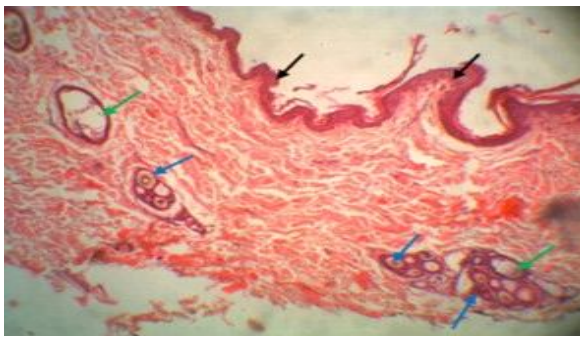


Fig 7: Photomicrograph of wounded skin of dog from test group at 14th day showing fine elastic fibers around the hair follicles (arrow). Verhoeff's stain × 400.



Note: The hair follicles (blue arrow) and sebaceous glands (green arrow). H & E X 100.

Fig 10: Photomicrograph of wounded skin of dog from control group at 14th day showing epidermis having 5-6 layers of cells (black arrow).

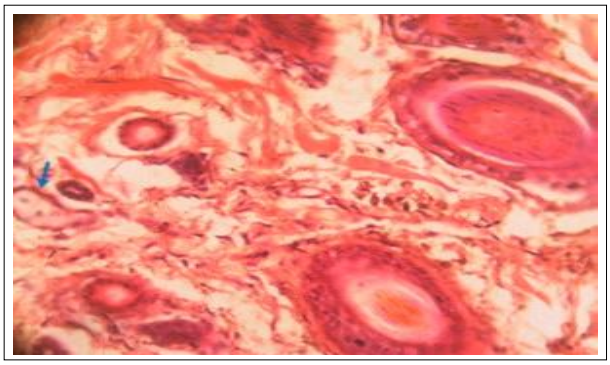


Fig 11: Photomicrograph of wounded skin of dog from control group at 14th day showing disintegrated collagen bundles and fewer blood vessels (arrow) in the dermis. H & E × 400.

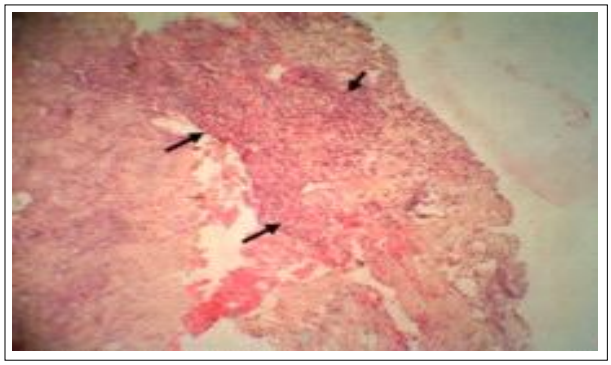
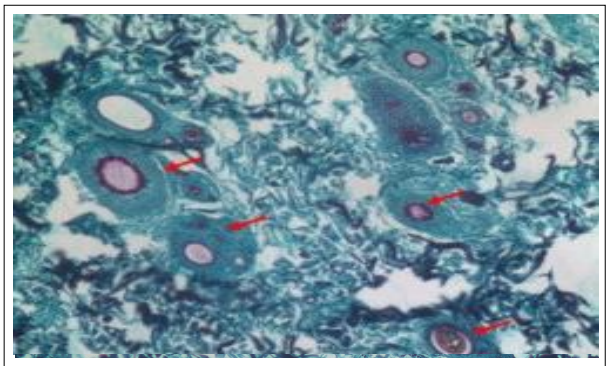


Fig 12: Photomicrograph of wounded skin of dog from control group at 14th day showing diffuse infiltration of inflammatory cells (arrow) in the dermis H & E × 100.



Note the loose packing of dermis. Masson's trichrome X 100.

Fig 13: Photomicrograph of wounded skin of dog from control group at 14th day showing less frequency of hair follicles (arrow).

Table 3: Duration in days of complete healing of incisional wounds in different groups.

Group	Minimum	Maximum	Mean±SE
Test	7	14	8.77±0.63[a]
Control	10	31	13.38±2.13[b]

Different superscripts within columns show significant difference ($p<0.05$) between the group.

bundles (Fig 6). Elastic fibers of moderate thickness were noted in the walls of some of sebaceous glands and they were continuous with those of the hair follicular wall (Fig 7). Some of the capillary walls were noted to have sparse amount of fine elastic fibers (Fig 8). There was less formation of collagen and reticular fibres on 14th day in control group than test group (Fig 9). The control group showed scanty blood vessels with moderate fibrosis, less connective tissue, less reticular fibers, less epithelialization and less rebuilding of dermal tissue due to disintegration of collagen bundles (Fig 10 and 11). There was infiltration of inflammatory cells in the dermis in the control group (Fig 12). The frequency distribution of hair follicles was comparatively less in control group than test group at 14th day of treatment (Fig 13). It was indicative of faster wound healing process (Fig 4) in test group of animals than control group as because curcumin, chitosan and aloevera have more potential wound healing capacity with different mechanism of action.

CONCLUSION

Application of Chitosan-Curcumin-Aloe vera based healing agent on the wound margins of test groups resulted in early and significantly higher percent of wound contraction and early complete healing process than mupirocin. Hitological and histo-chemical observations furthermore revealed that healing activity is enhanced by increasing cellular proliferation, formation of granulation tissue, synthesis of collagen, epithelialization, neovascularization and early histological maturation in test groups than control groups. Therefore, it is concluded that application of chitosan-curcumin based healing agent is economical, risk free; hence have great potential for use in wound healing in routine clinical cases.

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Conflict of interest: None.

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