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Evaluation of wound healing properties of neem (*Azadirachta indica*) in dogs

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Abstract
This study was conducted on 14 healthy dogs to assess the efficacy of topical neem (*Azadirachta indica*) powder on wound healing. Wound was made on right flank skin by surgical procedure and was treated with either crude neem or Neosporin antibiotics as standard control twice a day for 5 days (N=7/treatment). Degree of healing was evaluated on fifth day of surgery based on wound healing protocol of WSPA and hematology. The data were analyzed by one way ANOVA. The mean of clinical score of wound healing was not significantly different (P>0.05) between Neosporin (4.17±0.79) and neem (3.97±0.53). Fibrinogen, neutrophil and monocyte level for neem and Neosporin were not significantly different (P>0.05). Neem demonstrate a good alternative for wound management in dog and seems as effective as commercial antibiotics (Neosporin) but further investigations with histopathology of tissue is warranted for detail examination of healing activities.

Keywords: Neem, Dog, wound healing, clinical score, hematology.

1. Introduction
More than 80% of the world's population still depends upon the traditional medicine for various diseases [1]. This scenario is more evident in the developing countries like Nepal where medication is beyond the means of most people due to unaffordable cost or inaccessible facilities in the remote area [2]. Additionally, the public concern over the resistance development in pathogens against antibiotics compels urgent search for alternatives [3]. One potential alternative could be the use of plant antimicrobials which has been recognized as a potential candidate for developing safe, effective and eco-friendly drugs in future [3].
Among the plants, neem is omnipotent and has been used in Ayurvedic medicine for more than 4000 years due to its medicinal properties. Neem (*Azadirachta indica)* is naturalized in most of the tropical and sub-tropical countries. The active compounds of neem include alkaloids, flavonoids, phenolic compounds, steroids, carotenoids, ketones and azadirachtin [4]. The antibacterial activity of neem had already reported against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Esherichia coli* [1]. This antibacterial action is important to keep the wounds free from secondary infection. Besides this, neem acts as anti-inflammation as effective as cortisone acetate and helps to accelerate the wound healing [5]. Furthermore, neem oil contains fatty acids and maintains the skin's elasticity by building up collagens and by providing moist and soft texture to skin [5]. Besides the magical healing properties of neem, it is easily available, cheaper and has no adverse effect on skin. With these properties in mind, this study objectively discusses on the healing efficacy of topical neem powder. Previously, it was demonstrated that methanolic extract of neem has significant wound healing activity in laboratory rats [2], however, its activity in the field condition has yet to be documented. This is the first study, to our knowledge, conducted to manage and alleviate the wound problem with the use of neem in field conditions in surgically induced skin wound on healthy dogs.

2. Materials and Methods
2.1 Sample selection and skin wounding
A total of 14 healthy dogs were selected randomly from the dogs brought for the purpose of spaying at Veterinary Teaching Hospital, Institute of Agriculture and Animal Sciences, Nepal. The animal was anaesthetized with general anesthesia (Ketamine@10mg/kg body weight and Xylazine @ 0.5-2mg/ kg body weight). A surgical wound on the skin was made by incision (2.5 cm length x 0.5 cm breadth) on the right flank region for spaying purpose. All animal handling and care were in compliance with the guidelines set by the Veterinary Teaching Hospital, Institute of Agriculture and Animal Sciences, Nepal.

2.2 Experimental design
Dogs were divided into two treatments (T_1 and T_2), each consisting of seven dogs in a completely randomized design. T_1 and T_2 were treated twice a day for five days with neem and Neosporin® powder (standard control) respectively. The neem leaves were grinded into powder form and applied as paste for our study purpose.

2.3 Blood collection
Blood sample of approximately 5 ml was collected in a tube containing EDTA from each dog. Collection was made just before and after surgery for hematological analysis.

2.4 Measurement criteria
Degree of wound healing was evaluated based on clinical examination and hematological examination (Fibrinogen, PCV, Total plasma protein, WBC count, DLC). Clinical examination was made on fifth days after surgery according to wound healing protocol of World Society for Protection of Animal (WSPA). Fibrinogen and total protein level were determined by methods described by Schalm *et al* (1975) [6]. PCV was measured following micro-centrifugation with PCV reader. The WBC and DLC were counted under microscope.

2.5 Data analysis
The data were analyzed for One-way ANOVA by using MStat-C (Version 1.3, 1994). The means were separated by least squares means and all the results were expressed as Mean± SD. A p-value <0.05 was required for the statistical significance.

3. Results and Discussion
Table 1 shows hematological parameters and clinical scoring of dogs treated with neem and Neosporin on 5th day after surgery. There was no significant difference in hematological parameters of dogs before surgery (data not shown). Neosporin is the commonly used antibiotics on wound in humans as well as in animals. Therefore, we used Neosporin treatment as a standard control.

Total protein (TP) and packed cell volume (PCV) were not significantly different (P>0.05) between two groups (Table 1). Kirby *et al* (2000) reported a delayed wound healing due to dehydration (PCV>55%) and polycythemia by reducing the rate of epithelialization [8]. Hypo-proteinuria also adversely affect the wound healing by decreasing the tensile strength of the wound. It also reduces the number and activity of fibroblast in the granulation tissue resulting in reduced collagen production and increased frequency of the wound disruption [8, 9]. Since TP and PCV values lies within the normal range, there seems no interference of dehydration and hypo-proteinemia in healing of wound treated with neem and Neosporin.

Since fibrinogen is associated with the inflammatory conditions, the estimation of fibrinogen level is useful for the evaluation of the inflammatory response [10] and diagnosis of bacterial infection in dogs [11]. Sutton *et al* (1977) [12] reported that the fibrinogen concentration increases in inflammatory and tissue destroying disease. Fibrinogen is a plasma protein produced by the liver and plays a significant role in the body's defense mechanism. In our present study, the fibrinogen levels (gm/dl) was 0.643±0.15 and 0.657±0.1 respectively in neem and Neosporin treated dogs (P>0.05) which provides another sign indicating a healthy healing process in both treatment groups.

Table 1: Hematological value (on 5th day after surgery) and clinical scoring of wound

Treatment	Fibrinogen (gm/dl)	WBC (per mm^3)	Neutrophils (%)	Monocytes (%)	PCV (%)	TP (gm/dl)	Clinical scoring
Neem	0.64±0.15^a	12400±72^a	82.71±1.6^a	3.43±0.98^a	38±2.99^a	9.85±1.0^a	3.97±0.53^a
Neosporin	0.65±0.10^a	12380±110^a	83.71±1.2^a	3.71±0.76^a	38±2.18^a	9.67±1.2^a	4.17±0.79^a

[Means in column with same superscript is not significantly different (P>0.05)]

In differential leucocytes count, neutrophils and monocytes level were higher in Neosporin treated group (83.714±1.25% and 3.714±0.76% respectively) than neem (82.714±1.6% and 3.429±0.98%), however, a significant difference doesn't exist between these groups. The neutrophils are responsible for accelerated wound healing. Dovi *et al* (2003) [13] had reported an accelerated wound closure during neutrophilic condition in mice. Neutrophils engulf debris and also kills bacteria by releasing free radicals during respiratory burst. They clean the wound by secreting protease that break down damaged tissue. Khan and Line (2005) [14] stated that the monocytes and macrophages are major sources of cytokines that regulate inflammatory response and also functions as antigen processing cells. Monocytosis conditions may be associated with chronic inflammation, bacteremia, corticosteroid or stress response. In this study, there was no monocytosis condition and this is a sign of continual wound healing process. The white blood cells count was 12400±72 and 12380±110 per mm^3 in dogs treated with neem and neosporin respectively. There was no statistical difference (P>0.05) between these groups (Table 1) and these values lies within the normal range (5000-17000 cells/mm^3), indicating the absence of secondary infection. A low white blood count from any cause results in increased wound infection, and interfere the healing process [15]. This wound infection leads to separation of the wound edges by reducing vascular supply and also by producing certain necrotizing enzymes [8]. The separation of wound, delayed epithelial growth and necrosis are visible and incorporated in the clinical score of wound in our study.

The clinical examination of wound healing according to WSPA protocol showed mean score values of 4.171±0.79 and 3.971±0.53 respectively in Neosporin and neem treated dogs (Table 1). The highest mean score value (5) indicates the best degree of wound healing and the lowest mean scored value (1) indicates the worst degree of wound healing. This indicates that the healing efficacy of neem and Neosporin is almost similar.

4. Conclusion
In conclusion, in our study we found that neem has promising wound healing activity as effective as Neosporin. However, before recommending it for clinical use, its detail healing activities should be looked after by a well-designed detail experiment and further exploration with histopathology is warranted to examine in-depth information about wound healing efficacy of neem.

5. Conflict of Interest
The authors declare that no conflict of interest exits while

conducting this research.

6. Acknowledgments
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