www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(10): 813-817

www.thepharmajournal.com
Received: 04-08-2021
Accepted: 10-09-2021

Smriti
Assistant Professor cum Junior Scientist, Department of Veterinary Surgery, R.V.C., B.A.U., Bhagalpur, Bihar, India

Kumari N
Assistant Professor Cum Junior Scientist, Department of Animal Breeding and Genetics, R.V.C., B.A.U., Bhagalpur, Bihar, India

Sharma VK
Ex-Chairman and Head, Department of Veterinary Surgery and Radiology, Pantnagar, Uttarakhand, India

Corresponding Author
Smriti
Assistant Professor cum Junior Scientist, Department of Veterinary Surgery, R.V.C., B.A.U., Bhagalpur, Bihar, India

Efficacy of neem (*Azadirachta indica*) leaf extracts in wound healing in animals

Smriti, Kumari N and Sharma VK

Abstract
A total of 30 clinical wounds present on various body locations in animals were treated using different Neem leaf extract formulations in order to evaluate their wound healing potential. The formulations comprised of the aqueous and alcoholic extracts. The qualitative assessment of wound healing was done on the basis of clinical, histopathological and histo-chemical observations. The overall healing of wounds was better and faster in all the treatment groups compared to the control. Amongst the treatment groups, early regression of inflammatory signs and higher percent contraction were observed with the alcoholic extract in comparison to the aqueous extract treated wounds. Current research could be continued further with breed age and sex specific experimental animals to find the effect of these factors on efficacy of Neem in wound healing.

Keywords: Scabs, inflammation, histo-pathological, granulation, percent healing

Introduction
Various systemic and local reactions of the body to injury are fundamental phenomena which restore and repair the damaged tissues to their normal anatomical and physiological status. However, the rate of repair process is variable in different tissues and depends on a number of factors like state of infection, presence or absence of tissue debris, nutritional status, degree of inflammation and level of various hormones and vitamins etc. If a proper equilibrium between all the factors is maintained, then the reparative process may be optimized.

Plants and their extracts alone or in various formulations have been used for the treatment of different types of wounds. The herbal medicines used for wound healing are not only easily available but they are also economical and considered safe as hypersensitivity or any adverse reactions are rarely encountered with the use of such agents. The modus operandi of most of the herbal medicaments is not clear, however it is postulated that such agents induce healing and regeneration of lost tissues by multiple mechanisms, either by acting as antiseptics, astringents, immunomodulators or anti-inflammatory agents.

Of all the plants that have proved useful to humanity, Neem (*Azadirachta indica*) occupies topmost position. In most parts of India, it is a hardy, fast-growing evergreen, hardy tree of up to 20 metres in height. The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutics role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties (Alzohairy, 2016) [1].

The beneficial effects of Neem are attributed to its various bioactive constituents. Of these only a few have been scientifically studied and very few clinical trials have been conducted to prove their clinical efficacy as a wound healing promoter especially in animals. Keeping this in view, the present study was designed to study the wound healing property of different preparations of Azadirachta indica (Neem) leaves in animals.

Materials and Method

Table 1: Design of Experiment

Groups	No. of Wounds				Total no. of animals	Treatment protocol
	No. of infected wounds	Location of wounds on body surface	No. of contaminated wounds	Location of wounds on body surface		
I	3	Perineum, tail, neck	3	Tail, thigh	6	Aqueous extract
II	3	Bulb of heel, submandibular region, flap of nostril	3	Tail, cheek, fetlock region	6	Alcoholic extract

All animals irrespective of age, breed, sex and species suffering either with infected or contaminated wounds on anybody surface and at least of 15 days duration were included in the study (Table-1). The wounds of animals of group I and II were treated with pure form of aqueous and alcoholic extract of Neem leaves respectively, while animals of Group V served as control. Their wounds were dressed with normal saline without using any medicament.

Treatment Procedure

The wounds were gently cleaned using sterile gauze pieces and the appropriate medicament applied once daily until complete healing occurred. No local or systemic antimicrobial was used throughout the experiment.

Percent Healing

The percent healing was observed by the method of Kumar and Tyagi (1972) [8] with slight modifications.

The wound boundaries were traced on a cellophane paper with Indian ink at the time of its presentation. The subsequent wound tracings were taken on 3rd, 7th, 15th and 30th days. These tracings were placed on a graph paper and the wound area was determined by counting the number of small squares it covered. Percent healing under each treatment was calculated in all animals according to the following formula:

Percent healing = [(a- b)/A] x 100

Where, 'a' is the area of wound at the beginning of a particular period, 'b' is the area of the wound at the end of that period and 'A' is the initial area of the wound (incidentally a = A).

Aqueous and Alcoholic Extracts

For in-vitro testing of antimicrobial activity of medicaments, pus was collected from the wound surface using sterile swabs and cultured on nutrient broth as per the method described by (Singh *et al.*, 2007). A mixed growth of bacteria was obtained on the surface of nutrient broth which was used for the determination of in-vitro anti-bacterial activity of the medicaments as per the method described by (Murray *et al.*, 1995).

Histological and Histochemical Studies

For histological studies, the biopsy specimens were collected from the margins of healthy skin and healing wounds on days 3, 7, 15 and 30 and preserved in 10% buffered neutral formalin solution. The tissues were processed by routine paraffin embedding technique and longitudinal sections of 5µ thickness were cut. The sections were stained with H&E and Masson's Tri-chrome stain as described by Carleton and Drurry (1965) and Masson (1929) [10].

Result and Discussion

The assessment of wound healing was based on clinical and histopathological observations.

Fig 1: Wound at its day of presentation, note wound contamination

Fig 2: Wound after debridement, note the inflammatory signs

Fig 3: Note presence of scab covering the wound surface

Fig 4: Note presence of pale scar tissue

Fig 5: Note advancing epithelial tissue

Fig 6: Note granulation tissue formation

Fig 7: Note marked peripheral contraction of the wound margins

Fig 8: Note complete healing with healed area showing hair regrowth

Clinical Observations

Percentage reduction in wound during different durations on administration of aqueous and alcoholic extract of Neem is being shown as Table 2 (figure-9) and Table 3 (Figure-10) respectively while Table 4 shows the same for control group.

A. Group I (Aqueous Neem Extract)

On the day of presentation, three of the six wounds were having pus and emitted foul odour (Fig 1). A day following treatment, a narrow inflammatory zone with slight swelling and warmth was observed in most of the wounds (Fig 2). On day 3, signs of acute inflammation regressed. On 7th day, a marked reduction in the size of wounds dimension was observed.

By day 15, clear, pink and moist granulation tissue was observed from the depth of the wound following removal of scab (Fig. 3 and Fig. 4). Epithelialization of the wound edges was also noticed. By day 30, most of the wounds had healed completely following completion of epithelialization. Scar tissue formed was pale in colour.

Group II (Alcoholic Extract)

Clinical observations on day 0 regarding general appearance of wounds showed presence of pus and blood clots in most of the cases of this group of animals. (Fig. 5). On day 1, mild to severe inflammatory symptoms of tissue oedema, pain and localized heat were a common manifestation in almost all wounds.

By day 3, oedema and localized heat present earlier had subsided Clinical observations on the 7th day revealed a well-established granulation tissue bed in most wounds (Fig. 6). By day 15th, most wounds showed a significant reduction in size following peripheral contraction of the wound margins (Fig. 7). By the 30th day, complete healing manifested by formation of pale scars were observed in all wounds. (Fig. 8).

Current finding are supported by Maan *et al.* (2017) [9]. He observed that the animals treated with water extract of Neem exhibited significant increment in rate of wound contraction (93.39%, $P< 0.01$), hydroxyproline content (13.31 ± 6.65 mg/g of dry tissue, $P< 0.001$), DNA content (20.99 ± 0.68 µg/100 mg of tissue, $P< 0.01$), protein content (100.53 ± 7.88 mg/g of wet tissue, $P< 0.01$) and nitric oxide level (3.05 ± 0.03 mMol/g of tissue, $P< 0.001$) as well as in wound breaking strength (289.40 ± 29.45 g, $P< 0.01$) when compared with vehicle control group which was also supported by histopathological studies.

Table 2: Percent contraction in group I (Aqueous group) at different durations

Day of observation				
Animal No.	**3**	**7**	**15**	**30**
1	18.30	46.47	80.28	98.59
2	9.09	27.47	63.63	95.45
3	0.00	23.30	43.82	85.81
4	15.78	52.63	89.47	100.00
5	39.50	69.13	95.06	100.00
6	35.99	64.00	95.99	100.00
Average	19.77^{a}	47.16^{b}	78.04^{c}	96.64^{c}
SE	6.26	7.64	8.42	2.28

Means bearing different superscripts differ significantly ($P< 0.01$)

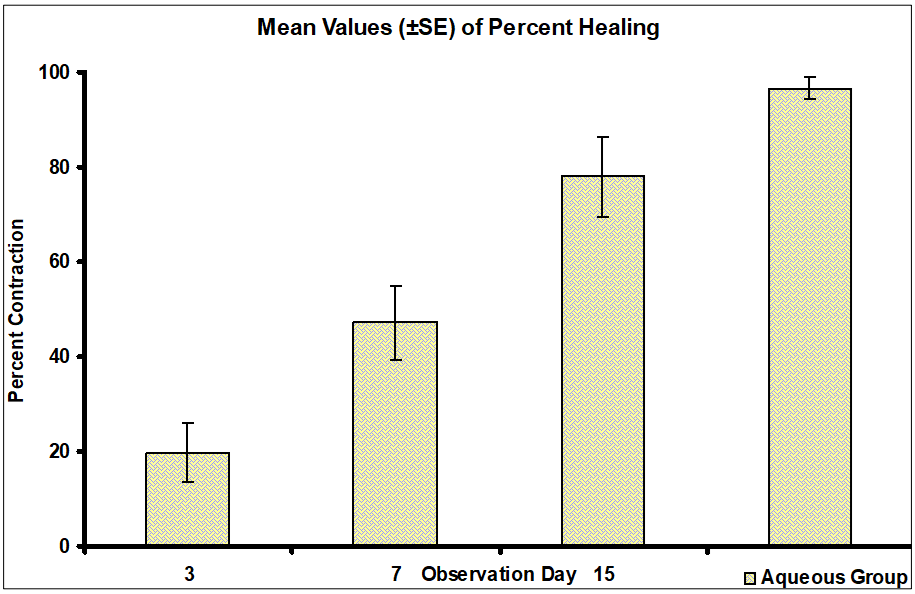

Fig 9: Mean values (±SE) of percent healing

Table 3: Percent contraction in group III (Alcoholic group) at different durations

Day of observation				
Animal No.	**3**	**7**	**15**	**30**
1	16.66	41.66	83.33	100.00
2	16.66	33.33	83.33	100.00
3	8.33	25.00	75.00	100.00
4	12.50	50.00	87.50	100.00
5	15.00	50.00	80.00	100.00
6	20.00	40.00	60.00	100.00
Average	14.85[a]	39.99[b]	78.19[c]	100.00[d]

Means bearing different superscripts differ significantly ($P< 0.01$)

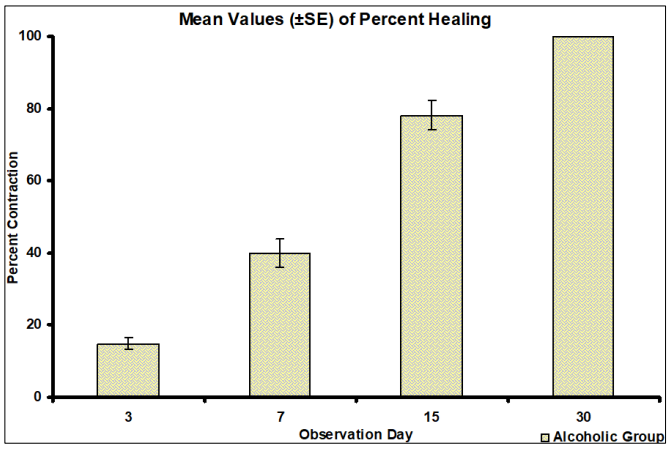

Fig 10: The extent of these changes were less

Table 4: Percent contraction in group V (Control) at different durations

Days				
Animal No.	**3**	**7**	**15**	**30**
1	9.00	27.00	54.54	81.81
2	7.60	15.38	38.46	84.61
3	8.33	25.00	41.66	83.33
4	9.09	18.18	45.45	81.81
5	7.60	38.46	53.84	84.61
6	16.66	25.00	41.66	83.33
Average	9.71 a	24.83 b	45.93 c	83.25 d
SE	1.41	3.28	2.76	0.51

Means bearing different superscripts differ significantly ($P< 0.01$)

Histopathological studies

The histological study is used to know the function of different cells at various stages of healing. The most obvious microscopic response to injury is accumulation of polymorphs in wound areas. In the initial stage of tissue repair, neutrophils and macrophages appear at the site followed by the appearance of fibroblasts which form collagenous fibres in which new capillaries grow. The aggregation of collagenous fibres along the blood capillaries constitutes granulation tissue. On 3rd day, the extent of inflammation and infiltration of macrophages were more marked in control wounds whereas in alcoholic extract treated wounds, the extent of these changes were less (figure 10 and 11).

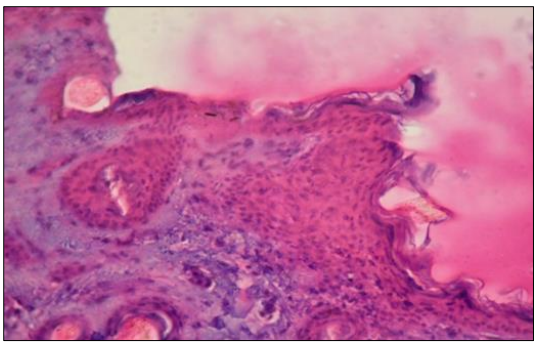

Fig 11: Photomicrograph of control group wound, day 3rd, note the extensive infiltration of neutrophils at the wound site, Stain H & E

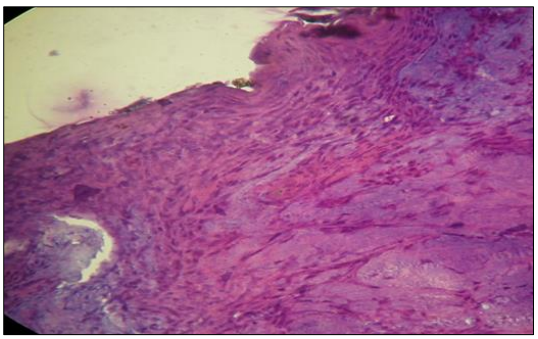

Fig 12: Photomicrograph of alcoholic extract treated wound, day 3rd, note the minimum neutrophilic infiltration at wound site, Stain H & E

Early healing in treated wounds even in the presence of microbes could be due to Neem's ability to remove harmful toxins of microbes and promoting a healthy circulation (Chattopadhyay *et al.*, 1992) [3].

The inflammatory response is an inevitable consequence of tissue injury and critical to the establishment of cutaneous homeostasis following injury (Eming *et al.*, 2007) [5]. In the present study, inflammatory signs of varying degree *viz.* swelling, warmth, pain and itchiness were observed in all wounds following their debridement.

The inflammatory signs subsided by 3rd day in group I and III and 5th day in group II and IV, whereas it persisted up to 7 days in control group. Early regression of inflammation could be due to the anti-inflammatory effects of nimbin and nimbidin found in Neem (Kraus, 1995) [7]. Further, bacterial components may also contribute to impaired repair mechanisms of the host by interference with cell-matrix interactions (Chavakis *et al.*, 2002; Athanasopoulos *et al.*, 2006) [2]. There was formation of a superficial, thin, pale yellow and serous scab over the wound surface was observed on 3rd day in group I, and III whereas, in control group, scab formation was observed on 15th day. The appearance of granulation tissue was observed in all the treated wounds at variable durations following treatment after scab detachment. In groups II, granulation tissue appeared on 7th day while in group I, it appeared on the 15th day. In control group of animals, granulation tissue appeared on 30th day which is suggestive of faster healing using medicament.

Marked granulation tissue formation up to 7th day has been observed by Patel (2002) [11] in wounds treated by different medicaments in buffalo calves.

Wound contraction results in the reduction of wound size by the inward (centripetal) movement of surrounding skin (Peacock, 1986).

In the present study, fast contraction was observed in all the groups following treatment. Up to day 7 post-treatment, highest wound contraction was observed in group I i.e., 19.77 and 47.17% on day 3rd and 7th respectively. Beyond day 10, faster rate of contraction was observed in the alcoholic extract groups compared to the aqueous ones. This clearly indicated their greater efficacy in causing wound closure. The contraction rate in all the treatment groups was much greater than that of the control group. This is suggestive of the effectiveness of the ointments in stimulating the myofibroblasts. Similar observations have been reported by different workers. Patel (2002) [11] has reported wound contraction from 8th day onwards in animals treated with different medicaments. Wound contraction was also observed in wounds treated with Adhatoda vasica (Zama *et al.*, 1991) [13] and honey (Gupta *et al.*, 1992) [6] in buffalo calves.

Summary and Conclusion

The overall healing of wounds was better and faster in all the treatment groups compared to the control. Amongst the treatment groups, early regression of inflammatory signs and higher percent contraction were observed with the alcoholic extract in comparison to the aqueous extract treated wounds. Research could be carried out further for different animals and in varying age groups to find the variation in effect of Azadirachta on wound healing w.r.t species and age of animals.

References

1. Alzohairy MA. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evidence-based complementary and alternative medicine: eCAM 2016, 7382506. https://doi.org/10.1155/2016/7382506
2. Chavakis T, Hussain M, Kanse SM, Peters G, Bretzel RG, Flock J *et al. Staphyloccocus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. Nat. Med 2002;8:687-93.
3. Chattopadhyay RR, Sarkar SK, Ganguly S, Banerjee RN, Basu TK, Mukherjee A. Hepatoprotective activity of *A. indica* leaves on paracetamol induced hepatic damage in rats. Indian Journal of Experimental Biology 1992;30(8):738-40.
4. Drurey RAB, Wallington EA. Carleton's Histological Techniques (Oxford University Press, New York) 5th ed 1980, 57-77.
5. Eming SA, Kreig T, Davidson JM. Inflammation in Wound Repair: Molecular and Cellular Mechanisms. Journal of Investigative Dermatology 2007;127:514-525.
6. Gupta KK, Rathor SS, Roy KS. Evaluation of biological tissues in wound healing in large animals. Indian. Vet. J 1992;69(11):1023-1025.
7. Kraus W. Biologically active ingredients. *In* H. Schmutterer, ed., The Neem Tree Azadirachta indica A. Juss. And Other Meliaceous Plants: Sources of Unique Natural Products for Integrated Pest sManagement, Industry and Other Purposes. VCH, Weinheim 1995, 35-88.
8. Kumar R, Tyagi YPS. A study on some factors influencing wound healing, the effect of various cleansing and debriding agents on the rate of epithelial healing in buffalo calves. Hary. Agri. Univ. J Res 1972;2:206-212.
9. Maan P, Yadav KS, Yadav NP. Wound Healing Activity of *Azadirachta indica* A. Juss Stem Bark in Mice. Pharmacogn Mag 2017;13(2):S316-S320. Doi: 10.4103/0973-1296.210163. Epub 2017 Jul 11. PMID: 28808399; PMCID: PMC5538173.
10. Masson P. J Techn. Methods 1929;12:75-90. AFIP modification.
11. Patel SM. Evaluation of hot and cold extracts of *Argyreia nervosa* as wound healing agent in buffalo calves-clinical, biochemical and histological studies, M.V. Sc., Thesis submitted to Gujarat Agricultural University, Sardarkrushinagar 2002.
12. Singh M, Govindarajan R, Nath V, Rawat AKS, Mehrotra S. Antimicrobial, wound healing and antioxidant activity of Plagiochasma appendiculatum Lehm. Et Lind. J Ethno 2006;107:67-72.
13. Zama MMS, Singh HP, Kumar A. Comparative studies on Adhatoda vasica and pancreatic tissue extract on wound healing in buffaloes. Indian Vet. J 1991;68(9):864-866.