

Evaluation of wound healing potentials of neem (*Azadirachta indica*) leaf extract on excision wounds in Wistar albino rats

¹Olorunsola, R. A., ²Oke, F., ³Bagbe, A. S. and ⁴Ayeyinbo, O. A.

¹Department of Animal production and Health, Faculty of Agriculture, Food and Natural Resources, Ondo State University of Science and Technology, Okitipupa. Ondo State.

²Department of Animal Science, Faculty of Agriculture, Federal University Dutse, Jigawa State. Nigeria.



³Department of Biological Sciences, Faculty of Science, Ondo State University of Science and Technology, Okitipupa. Ondo State.

Abstract

This study was carried out to evaluate the wound healing ability of ethanoic leaf extract of *Azadirachta indica*, incorporated into the feed of albino rats. Twenty-four (24) albino rats of both sexes weighing between 90 to 125g were randomly selected and divided into three (3) groups of 8 rats each. Animals in each group were divided into four (4) replicates of two (2) rats per replicate. Group A had penicillin, group B had neem ointment and group C had Neem incorporated in the feed. The ethanoic leaf extract of *Azadirachta indica* showed a significantly higher ($p < 0.05$) contraction rate and shortened wound closure time. The healing was 92% ($p < 0.05$) on 10th day compared to 75% and 82% of healing with ground leaf of *A. indica* incorporated into the feed (neem + feed) and procaine penicillin, respectively. The complete wound closure took place on the 12th day in the case of ethanoic leaf extract of *Azadirachta indica* while, neem + feed and procaine penicillin the wounds persisted than 12th day. The study concluded that the ethanoic leaf extract of *A. indica* had better potential wound healing activity for excision wounds; justifying its use in the traditional and orthodox medicine.

Keywords: Neem leaf, albino rats, wound contraction, Penicillin

Introduction

Wound is delineated as disruption of structural and physiological continuity of living tissue. They are inescapable events of life that may arise due to physical, chemical, or microbial agents. Wound healing is a complex cellular and biochemical cascade that lead to restitution of integrity and function, accomplished by several processes which involve different phases including inflammation, granulation, fibro genesis, neo-vascularization, wound contraction and epithelization (Clark, 1996). When healing takes place in a direction away from its normal course, it is common to have non-healing, under or over healing. Treatment is, therefore, aimed at either shortening the time required for healing or minimizing the

undesired consequences. The World Health Organization (WHO) has been promoting traditional medicine as a source of less expensive, comprehensive medical care, especially in developing countries. The WHO also recognized the importance of traditional medicine and has treated strategies, guidelines, and standard for botanical medicines (WHO, 1993). Approximately, one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3% of modern drugs (Pandey *et al.*, 2014). The tree, neem (*Azadirachta indica*) of the family Meliaceae is an evergreen tree, native to the Southeast Asia and found in most tropical countries. It has been in use since ancient times, to treat a number of human ailments and also as household pesticide (Chattopadhyay and

Evaluation of wound healing potentials of neem (Azadirachta indica) leaf extract

Bandyopadhyay, 2005). *Azadirachta indica* is a wonder plant with valuable economic and health significance attached to all its parts. In fact, it is a well known versatile medicinal plants with wide spectrum of biological activities (Siddique *et al.*, 2004). For example, its leaf, bark, roots, fruit coat, seed and flowers (Atawodi and Atawodi, 2009) have been demonstrated to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycaemic and antidiabetic, antiulcer. Extracts from the bark, leaves, fruits and roots have been used to control leprosy, intestinal helminthosis and respiratory disorders (Ketkar and Ketkar, 1995). *Azadirachta indica* (Neem) is also well-known in India for more than 2000 years, as one of the most versatile medicinal plants having a wide spectrum of biological activity. It is called "villagers' dispensary" because of its medicinal value. Every part of the tree has been used as a household remedy against various human ailments. Various studies have been carried out and it is shown to have antipyretic, immunostimulant, antiulcer, antioxidant, hypoglycemic, hepatoprotective activity (Biswas *et al.*, 2002), and because of all these properties most especially the antimicrobial qualities, it is therefore imperative to also look into its activities with respect to wound healing. Hence, this study was undertaken to evaluate the wound healing activity of **neem (*Azadirachta indica*) on body weight and excision wound model in Wistar (albino) rat.**

Materials and methods

Plant materials and plant extract preparation

Fresh *Azadirachta indica* leaves were collected and identified at Ondo State University of Science and Technology

(OSUSTECH), Okitipupa, Nigeria. The leaves were washed with tap water to remove the dust and sand, and then air dried under the shade, and the dried sample was ground into moderate powder. The 100g of the powder was soaked in one (1) litre of ethanol and stirred every 3 hours and allowed to settle for 72 hours. The mixture was filtered through Whatman filtered paper No.1, and the filtrate was then concentrated and allowed to dry. The ethanoic extract (or semi-solid mass) was used as a topical ointment for the experiment. Procaine/penicillin (CSPC Zhongnuo Pharmaceutical (Shijiazhuang) Co., Ltd, Shijiazhuang City, China) ointment was used as a standard drug for comparing the wound healing ability of the extract.

Experimental design

In this experiment, twenty-four (24) albino rats of both sexes weighing between 90 to 125g were randomly selected and divided into four (3) groups of 8 rats each. Animals in each group were sub-divided into three (4) sub-groups of two (2) rats each to have four replicates. The first group was kept as the control group whereas the remaining 3 groups served as the experimental animal group. The experiment was carried out with a complete randomized design (CRD). The animals were housed in transparent polythene cages with a stainless-steel wire line ceiling. The room temperature in which the animals were housed was about 23°C and a light/dark cycle of 12:12 hours were maintained. The animals were fed twice daily with standard commercial diet (GrowerMash) containing 28 % crude protein, 7% fat, 10% crude fibre, 1.0% calcium, 0.35% phosphorus, with 2550/Kcal/kg of metabolized energy and given tap water. The animals were kept in this condition for a week to become acclimatized to the laboratory conditions prior to any experimental manipulation.

Creating and treating of wound

The animal experiment was performed according to departmental animal right committee guidelines. The rats were depilated on the trunk using razor blade under light chloroform anaesthesia. A circular excision wound of 1cm diameter was inflicted immediately with razor blade from the central trunk marked area to get a wound measuring about 1cm diameter, by cutting away full skin thickness after ethical approval was sort. After achieving complete homeostasis, the animals were placed in two per individual cages. Animals were treated once daily till complete wound healing was achieved by applying the ointments liberally on the wounds as follows: group A; rats served as the negative control and were left untreated. While group B; rats were treated with procaine penicillin (i.e. standard drug). Group C; rats were treated with topical ointment (i.e. neem ointment). Group D; rats were treated with 100g of ground leaves of *A. indica* incorporated into the 1000g of their feed (neem + feed).

Data collection

The wound area was measured with vernier caliper and thereafter estimated on a transparent meter rule every alternate day. The time taken for complete wound closure to be achieved was determined. Wound closure was calculated as a percentage of

the original wound size using the equation as follows:

$$\% \text{ Wound Closure} = (IDW - DW) / IDW \times 100.$$

Where, IDW (Day 0) is initial diameter of wound or original wound size and DW (Day X) is diameter of wound after day 0 (Builders *et al.*, 2013).

Statistical analysis

Data from this study were analyzed using one-way analysis of variance (ANOVA) procedure and treatment means were compared using the Duncan multiple range test, SAS (2003).

Results

Effects of ethanoic leaf extract of *A. indica* on the wound and body weight are presented in Table 1 and Table 2, respectively. The effect of *A. indica* on wound healing of albino rat showed significant differences (P< 0.05) across the treatments. However, a higher effect was observed on neem ointment treated rats with total wound healing seen at 12th day of the experiment followed by procaine at 14 days while the least healing ability was observed in rat treated with addition of neem to the feed.

The effect of different treatments on body weight during wound healing period in Table 2 showed there was no significant difference (P>0.05) across the treatments from week 0 to the end of wound healing (week 2).

Table 1: Wound healing ability of neem on excision wounds in Wistar albino rats

Post wounding days	Percentage wound contraction (%)		
	Procaine penicillin Group A (as Control)	Neem ointment Group B	Neem + Feed Group C
0	1:00 (0%)	1:00 (0%)	1:00 (0%)
2	0.93±0.02 (7%)	0.92±0.03 (8%)	0.98±0.02 (2%)
4	0.80±0.03 ^{ab} (20%)	0.75±0.04 ^b (25%)	0.87±0.02 ^a (13%)
6	0.57±0.04 ^{ab} (43%)	0.47±0.06 ^b (53%)	0.65±0.06 ^a (35%)
8	0.33±0.06 ^{ab} (67%)	0.22±0.04 ^b (75%)	0.40±0.05 ^a (60%)
10	0.18±0.04 ^{ab} (82%)	0.07±0.03 ^b (93%)	0.25±0.04 ^a (75%)
12	0.07±0.03 ^{ab} (93%)	0.00±0.00 ^b (100%)	0.13±0.05 ^a (87%)
14	0.00±0.00 ^b (100%)	0.00±0.00 ^b (-)	0.07±0.03 ^a (93%)

^{ab}Means with different superscript along the column are significantly different (P <0.05).

Evaluation of wound healing potentials of neem (*Azadirachta indica*) leaf extract

Table 2: The effect of different treatments on body weight (g) of Wistar albino rats during wound healing period

Treatments	Week 0	Week 1	Week 2
Procaine penicillin	110.88±6.25	130.35±6.79	163.78±8.55
Neem ointment	108.53±6.51	132.77±7.52	156.32±9.34
Neem+Feed	109.12±4.83	125.83±5.45	154.42±7.38
Total	107.57±2.46	127.54±2.90	158.90±3.83

There was no significant different $P < 0.05$ across the treatments

Discussion

This study has showed a significant higher percentage wound closure with the wounds dressed with the ethanoic leaf extract of *Azadirachta indica* when compared to the wounds dressed with procaine penicillin and with the wounds treated with ground leaves of *A. indica* incorporated in the feed. Complete wound healing took place on 12th day in ethanoic leaf extract of *A. indica*, whereas in the standard drug and *A. indica* incorporated in the feed groups wound persisted beyond the 12th day, indicating better wound healing activity of the ethanoic leaf extract of *A. indica*. Wound healing involves a complex and superbly orchestrated interaction of cells, extracellular matrix and cytokines (Gupta *et al.*, 2008). Granulation, collagen maturation and scar formation are some of the cascade of wound healing which run concurrently, but independent of each other (Udupa *et al.*, 2006). The fibroblasts are responsible for the synthesis, deposition and remodeling of the extracellular matrix. The early re-epithelialization and faster wound closure in *A. indica* treated wounds might also be associated with the increased keratinocyte proliferation and their migration to the wound surface (Blakytyn and Jude, 2006). From the post wounding day till the end of the experiment when ethanoic leaf extract of *A. indica* was compared to the procaine penicillin group of the experiment, the wound healing potential of ethanoic leaf extract of *A. indica* was however, better than procaine penicillin as evidenced by higher

percentage of contraction of the wound, hence, promotion of neovascularization is considered as one of the factors which accelerate wound healing by ethanoic leaf extract of *A. indica*. This was in line with Falanga (2005) that neovascularization is a crucial step in wound healing process. Wounds treated with the extracts of *Azadirachta indica* was reported to possess fibrosis in the dermis and hyperkeratosis (increased thickness of the stratum carenum), which suggests that collagen formation may be necessary for wound healing and thus agreed with the postulations of Trabucchi *et al.* (1986); Shukla *et al.* (1999) who attributed enhanced wound healing activity to collagen formation and angiogenesis. Cohen and Burns (2002) reported that collagen played a central role in the healing of wounds. Baura *et al.* (2010) reported that increased angiogenesis in the *A. indica* treated group indicated better wound healing activity of the test plant. The extract of neem was reported to possess anti-inflammatory (Baura *et al.*, 2010), hence, a good promoter of wound healing.

Comparing the wound healing potential of *A. indica* incorporated in the feed group to those dressed with procaine penicillin and ethanoic leaf extract *A. indica* groups, it was observed that *A. indica* incorporated in the feed treated group showed lowest effect on wound percentage contraction and also took more time for complete wound closure. This might be associated with bacteria that invaded the wound area. The ointment used to rub wound surface area killed invaded

bacteria more than the one taken orally, this is in line with BasWa et al. (2001) who reported that bacteria present on the wound surface delay wound healing process.

Conclusion

In conclusion, the ethanoic leaf extract of *A. indica* has got potential wound healing activity for excision wound model by increasing percentage wound contraction and quick wound closure time.

This research showed that the ethanoic leaf extract of *Azadirachta indica* promoted wound healing ability through increased inflammatory response and neovascularization. The study showed that there is no relationship between the different treatments and the body weight of the experimental animals.

Recommendation

Base on the findings, it is recommended that ethanoic leaf extract of *A. indica* is good promoter of wound healing agent which could be used locally to care for wound, and it should be incorporated into the orthodox medicine for treatment of wound. Further studies with isolated constituents are required to understand the complete mechanism of wound healing ability of *Azadirachta indica*.

References

- Atawodi, S. E. and Atawodi, J. C. 2009.** *Immunopharmacology and Immunotoxicology Phytochemical Review.*; 8:601-20.
- Biswas, K. I., Chattopadhyay, R. R., Bandyopadhyay, U. and Baerjee, R. K. 2002.** Biological activities and medicinal properties of neem (*Azadirachta indica*). *Curr. Sci. J. 82(11): 1336-1345.*
- Blakytyn, R. and Jude, E. 2006.** The molecular biology of chronic wounds and delayed healing in diabetes. *Diabetic Med.*; 2:594-608.
- Builders, P. F., Toge, B. K., Builders, M., Chindo, B. A., Anwunobi, P. A. and Isimi, Y. C. 2013.** Wound healing potential of formulated extract from *Hibiscus sabdariffa* Calyx, *Indan J Pharm Sci.*, 75(1) 45-52
- Buara, C. C., Talukdar, A., Barua, A. G., Chakraborty, A., Sarma, R. K. and Bora, R. S. 2010.** Evaluation of the wound healing activity of methanolic extract of *azadirachta indica* (neem) and *tinospuracordifolia* (guduchi) in rats. *J. Pharmacology online*: 70-77.
- Chattopadhyay, R. R. and Bandyopadhyay, U. 2005.** Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. *Afr. J. Biomed. Res.* 8:101-104.
- Clark, R. A. 1996.** Cutaneous wound repairs. In: Goldsmith L.A., editor. *New York: Oxford University Press: Mediscope*, 12, 25-27.
- Cohen, S. and Burns, R. C. 2002.** Pathways of the Pulp; 8th Edition. *St. Louis: Mosby Inc.* Pg. 465.
- Falanga, V. 2005.** Wound healing and its impairment in the diabetic foot. *Lancet*; 366:1736-1743.
- Gupta, A., Upadhyay N. K., Sawhney, R. C. and Kumar, R. A. 2008.** Polyherbal formulation accelerated normal and impaired diabetic wound healing. *Wound Repair Reg.* 16:784-790.

Evaluation of wound healing potentials of neem (Azadirachta indica) leaf extract

- Ketkar, A. Y. and Ketkar, C. M. 1995.** The Neem tree: Source of unique natural products for integrated pest management, Medicine and other purpose. In (ed. Schmutterer, H.), pp. 518-525.
- Pandey, G., Verma, K. and Munna, S. 2014.** Evaluation of phytochemical, antibacterial and free radical scavenging properties of *azadirachta indica* (neem) leaves. *Int. J. Pharm Pharm. sci.* **2**: 444-447
- Shukla, C., Malinki, K., Dalla, P. and Sankar, L. 1999.** Role of nitric oxide, nitroxidative and oxidative stress in wound healing. *J. Pharmacol*, **57**, 108-119.
- Siddiqui, B. S., Rasheed, M., Ilyas, F., Gulzar, T., Tariq, R. M. and Naqvi, S. N. 2004.** Tetracyclic, triterpenoids and their derivatives from *Azadirachta indica*. *J. Nat. Prod. (Lloydia)*, **51**: 3043
- Trabucchi, E., Preis-Baruffaldi, F., Baratti, C. and Montorsi, W. 1986.** Topical treatment of experimental skin lesions in rats: macroscopic, microscopic and scanning electron microscopic evaluation of the healing process. *Int. J. Tissue. React.*, **8**: 533-544.
- Udupa, S. L., Shetty, S., Udupa, A. L. and Somayaji, S. N. 2006.** Effect of *Ocimum sanctum* Linn. on normal and dexamethasone suppressed wound healing. *Indian J Exp Biol*; **44**: 49-54.
- World Health Organization (WHO) 1993.** Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram* **28**: 13-14P.

Received: 14th July, 2018

Accepted: 19th December, 2018