



Experimental Study of Curcuma Longa Rhizomes and Azadirachta Indica Leaves Extracts on Wound Healing Activity

Shiv Kumar Purohit^{1*}, Manoj Mathur¹, Renu Solanki²

1. College of Veterinary and Animal Science, Rajasthan

2. Lachoo Memorial College of Science and technology, Pharmacy Wing, Rajasthan

ABSTRACT

Most of skin diseases are caused by pathogenic microorganisms which include *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Wounds are major responsible for physical disabilities. Wound disease spread by the invasion of bacteria inside the body through skin. Bacterial infections on skin are the common ailment for generation of other diseases in the body. Wound infections represent the invasion of tissues by number of species of microorganisms. These infections disturb the body immune system and cause inflammation, tissue damage and delayed wound healing process. Rhizomes of *Curcuma longa* (Turmeric) and leaves of *Azadirachta indica* (Neem) are used for various medicinal purposes. *Curcuma longa* possesses antibacterial, anti-inflammatory, anti-arthritic, anti-hepatotoxic and anti-allergic properties. Ayurvedic texts described it to be good for skin ailments and also as a blood purifier, wound cleanser, healer, remover of body toxins, killer of abdominal worms and a wind-repellent agent etc. While *Azadirachta indica* is useful in tiredness, cough, fever, loss of appetite, worm infestation. It heals wound, heat-rash, boils, jaundice, leprosy, skin disorders, eye disorders, stomach ulcers, chicken pox, excessive thirst, diabetes and insect poisons. It act as anti-leprotic, antimalarial, anti-hemorrhoidal and anthelmintic agent. The present study was undertaken on evaluation of combinational effect of ethanolic extract of *Curcuma longa* rhizomes and *Azadirachta indica* leaves for wound healing activity through topical route on excision wound model. The activity was compared with standard drug Povidone Iodine ointment (5% w/w). Combinational effect of both rhizomes and leaves ethanolic extract was found to have better and faster wound healing effect than standard drug Povidone Iodine ointment on excision wound model.

Keywords: *Curcuma longa*, *Azadirachta indica*, povidone iodine ointment, wound healing activity, excision wound model.

*Corresponding Author Email: solankirenu@yahoo.com

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INTRODUCTION

Wounds are major cause of physical disabilities which arises due to physical, chemical or microbial agents. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and junction¹. Many immunosuppressant, cytotoxins and non-steroidal anti-inflammatory drugs suppress the wound healing. Management of wound healing is a complicated and expensive step². Restoration of damaged tissue (wound) is an important process which plays vital role in survival of life. It is imminent for the basis of all surgical manipulations³. Many plants have proved to possess significant healing properties in different types of wounds. Using certain plants, possessing antiseptic, astringent, anti-inflammatory, antimicrobial property the rate of wound healing can be enhanced⁴. Such plant can increase the rate of tissue healing by providing different essential substances, required at various steps of wound healing. Plants being cheaper and safer than allopathic drugs, so treatment by natural ways may be useful in veterinary practice, especially in India where these are found in plenty⁵. *Curcuma longa* is a plant commonly known as Turmeric or Haldi, belongs to the family Zingiberaceae⁶⁻⁷. Its rhizomes are used for medicinal purposes. It possesses antibacterial, anti-inflammatory, anti-arthritic, anti-hepatotoxic (liver protective) and anti-allergic properties. Ayurvedic texts have additionally described it to be good for skin ailments and also as a blood purifier, wound cleanser, healer, remover of body toxins, killer of abdominal worms and a wind-repellent agent etc⁸⁻¹⁰. *Azadirachta indica* is a plant commonly known as Neem, belongs to the family Meliaceae⁶⁻⁷. Its leaves are useful in tiredness, cough, fever, loss of appetite, worm infestation. It acts as anti-leprotic, antimalarial, anti-hemorrhoidal and anthelmintic agent etc⁸⁻¹⁰. Wound healing involves a highly dynamic integrated series of cellular physiological and biochemical processes that occurs in living organisms¹¹⁻¹². The majority of world population relies on traditional medicine for their health care¹³. This is also the case in the treatment of wounds. Many research proposed that wound healing can be improved by herbal drugs having antiseptic, antibacterial, antioxidant and anti-inflammatory properties¹⁴⁻¹⁵. Based on the above source of information, the present study was aimed to evaluate combinational effect of ethanolic extract of *Curcuma longa* rhizomes and *Azadirachta indica* leaves for wound healing activity through topical route on excision wound model against standard drug Povidone Iodine ointment (5% w/w).

MATERIALS AND METHODS

Collection of plant materials

The fresh rhizomes *Curcuma longa* and leaves of *Azadirachta indica* were purchased from local

nursery garden during the month of December 2006. The plant materials were identified and authenticated at Botanical Survey of India, Jodhpur by Dr. P. J. Parmar. The fresh plant materials were dried under shade. Dried materials were powdered using mechanical grinder and passed through sieve no.60 to get the powder of desired coarseness. Powdered materials were preserved separately in an air tight container.

Extraction and drug formulation

550 g of the coarsely powdered, dried rhizomes of *Curcuma longa* and dried leaves of *Azadirachta indica* were mixed with 3500 ml of ethanol separately and were allowed to stand for 7 days. It was filtered and distilled under vacuum to get concentrated ethanolic extract. They were then filtered and distilled under vacuum to get concentrated ethanolic extracts. Ethanolic extract of both the drugs were stored under desiccators for further phytochemical and pharmacological screening.

Two 5% w/w ointment formulations were made by incorporating individual ethanolic extract with simple ointment base I.P.10 while third 5% w/w ointment formulation was made by mixing both the ethanolic extract together in a ratio of 1:1 with simple ointment base I.P10 for external application of the drug in the excision wound model.

Preliminary phytochemical analysis

A preliminary phytochemical screening was carried out for both the extract employing the standard procedure to reveal the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, glycosides, carbohydrates, phytosterols and proteins¹⁶.

Animals

Adult albino rats (wistar strain) of either sex weighing 180-200 g were taken. They were placed in polypropylene cages in a controlled room environment (25°C±2°C) at a natural day night cycle and they were provided with standard laboratory food and water *ad libitum*. Approval for the study was obtained from the institutional animal by the Institutional Animal Ethical Committee (IAEC), Reg No. 990, U.D.P.S.

Wound healing activity

Animals were assigned into 5 groups containing 6 animals in each group. All the wounds (control, standard and test groups A, B, C) were cleaned with the sterilized normal saline solution and then dried gently with sterilized gauze before every dressing. Group I was called as control group, was kept untreated, group II was called as standard group, was treated with market formulation (Povidone Iodine ointment), group III was called as test group in which it was divided into three subgroups comprising of test group III A, which was treated with ethanolic

extract ointment of *Curcuma longa* rhizomes, test group III B treated with ethanolic extract ointment of *Azadirachta indica* leaves and test group III C, was treated with ethanolic extract ointment of both *Curcuma longa* rhizomes and *Azadirachta indica* leaves in a ratio of 1:1. Excision wound model was selected for assessing wound healing activity of both the extracts on topical route of administration for 15 days. Animals were anaesthetized with ether and shaved on part to be exposed. A circular piece (500 mm² area) was impressed on the dorsal thoracic region 5cm away from ears and 1 cm away from the vertebral column. The animals were individually housed in separate cages. The test and standard preparation were topically applied once a day till epithelialisation was complete, starting from the day of the operation. The wounds were traced on 1 mm² graph paper on the day of wounding and then subsequently on the 4th, 8th, 12th and 16th post wound days and thereafter daily until healing was complete. The parameters studied were percentage of wound contraction or wound closure and period of epithelialisation¹⁷.

RESULTS AND DISCUSSION

The yield of ethanolic extract of *Curcuma longa* rhizomes and of *Azadirachta indica* leaves was found to be as 6.96%w/w and 8.56%w/w respectively. The *Curcuma longa* rhizomes extract showed the presence of phytoconstituents like curcumin, tumerone, zingerene, phellandrene, cineole, sabinene, borneol, curcumin starch, protein and high amounts of vitamin A and other vitamins etc. The *Azadirachta indica* leaves extract showed the presence of phytoconstituents like azadirachtin, nimbin, nimbidin, nimbidol, sodium nimbin, gedunin, quercetin and salannin etc. In the present study, the excision wound model is considered for evaluation of wound healing activity of *Curcuma longa* rhizomes, leaves of *Azadirachta indica* and combination of both the plant material extract as topical application against standard drug (Povidone Iodine ointment). During the initiation of study from the day 0 there was not much difference in the healing of wounds in all the groups. But after day 9, the healing process was observed faster in the groups treated with 5% w/w ointment formulation made by mixing both the plant material ethanolic extract together in a ratio of 1:1 as compared to the standard group which was treated with Povidone Iodine ointment as shown in table 1. Statistical analysis was done by ANOVA and Dunnet's multiple comparison tests. Results are expressed as mean \pm SE, n=6 in each group. Significant difference was compared to control group at p<0.01. From the results, it was observed that in the topical route, test group III C shows faster wound closure and wound contraction and the results are significant (P<0.01) when compared with standard drug Povidone Iodine ointment.

Table 1: Effect of topical administration of ethanolic extract ointment of *Curcuma longa* rhizomes and *Azadirachta indica* leaves on excision wound model

Post wounding days	Comparative mean wound area of different groups (in mm ²)				
	Group I (control)	Group II (standard)	III A	Group III (test group) III B	III C
0	200.55 ± 1.56	200.81 ± 1.27	200.55 ± 1.42	200.55 ± 1.11	201.96 ± 1.03
3	188.56 ± 1.94	148.66 ± 1.66	162.23 ± 1.33	131.76 ± 0.52	101.06 ± 1.45
6	110.04 ± 1.10	85.01 ± 1.54	72.94 ± 1.18	87.36 ± 1.02	49.01 ± 1.62
9	70.18 ± 1.00	34.28 ± 1.24	30.16 ± 0.65	36.96 ± 0.24	01.05 ± 0.03
12	34.88 ± 1.31	9.15 ± 1.21	0.00 ± 0.00	2.08 ± 0.02	0.00 ± 0.00
15	13.69 ± 1.92	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

CONCLUSION

Thus, it can be concluded that when combination of the ethanolic extracts of *Curcuma longa* rhizomes and *Azadirachta indica* leaves (ratio of 1:1) was applied externally on male albino rats by topical route through excision wound model then it showed faster as well as better wound closure and wound contraction as compared to other treatment groups. Therefore, we may interpret that the observation of such response may be due to the synergistic effect of the two plant materials possessing the presence of various phytoconstituents in them. However further studies can be performed to isolate the particular component responsible for generating the wound healing activity.

REFERENCES

1. Harshmohan. Text book of pathology. 3rd ed., New Delhi: JayPee Brothers; 2005: 121-25.
2. Gupta N, Gupta SK, Shukula VK, Singh SP. An Indian community based epidemiological study of wound. J Wound Care 2004; 13 (2): 326-28.
3. Majumdar M, Kamath JV. Herbal concept on wound healing. J Pharma Res 2005; 4 (1): 06-09.
4. Jaiswal S, Singh SV, Singh B, Singh HV. Plants used for tissue healing of animals. Natural Product Radiance 2004; 3 (4): 294-98.
5. Kirtikar KR, Basu BD. Indian medicinal plants. 5th ed., New Delhi: Sri Satguru Publications; 2001: 98-99.
6. Khandelwal KR. Practical pharmacognosy. 4th ed., New Delhi: Nirali Prakashan; 2005: 105-08.
7. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian medicinal plants. 4th ed., New Delhi: New Delhi Publication and Information Directorate; 1999: 111-13.

8. Brain KR, Turner TD. Practical evaluation of phyto-pharmaceuticals. 3rd ed., Bristol: Wright-Scientifica; 1975: 232-37.
9. Kokate CK. Practical pharmacognosy. 6th ed., New Delhi: Vallabh Prakashan; 2005: 202-04.
10. The Ayurvedic Formulary of India. Part I. Government of India, Ministry of Health and Family Welfare: New Delhi; 2003.
11. Srinivas RB, Kirankumar RR, Naidu VGM, Madhusudhana K, Sachin BA, et al. Evaluation of antimicrobial, antioxidant and wound healing potentials of *Haloptela integrifolia*. J Ethnopharmacology 2008; 11 (5): 232-37.
12. Mukherjee PK. Quality control of herbal drugs-An approach to evaluation of botanicals. . 4th ed., Bombay: Business Horizons Pharmaceuticals; 2002: 117-119.
13. Zhang X. Traditional medicine and WHO. Hamdard Medicus. 1996; 39(3): 98-100.
14. Somashekar S, Saraswati U, Laxinarayana U, Nagabushan S. Wound healing activity of *Ocimum sanctum*. Linn with supportive role of antioxidant enzymes. Int J Pharmacology. 2006; 50(2): 153-58.
15. Sunil SJ, Nitin Agrawal, Patil MB, Chimkode R, Tripathi A. Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis*. Linn. Int J Green Pharmacy 2008; 31 (1): 152-56.
16. Rangari V. Pharmacognosy and phytochemistry. Part-I. 4th ed., New Delhi: Lippincott Williams & Wilkins; 2002: 182-85.
17. Ghosh MN. Fundamentals of experimental pharmacology. 4th ed., Kolkata: Scientific Book Agency; 2005: 315-17.