Scholars Bulletin **ISSN 2412-9771 (Print)**
(A Multidisciplinary Bi-weekly Journal) **ISSN 2412-897X (Online)**
An Official Publication of "Scholars Middle East Publishers",
Dubai, United Arab Emirates
Website: http://scholarsbulletin.com/

Wound healing effect of *Azadirachta indica* and *Curcuma longa* in guinea pigs

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Abstract: *Azadirachta indica* and *Curcuma longa* is the most useful traditional plant being used in India for medicinal purposes from centuries. There is evidence that these plants have anti-inflammatory, analgesic, antipyretic, antioxidant and anticancer activities. Work has been done on the wound-healing and anti-inflammatory activities of these plants and investigation is needed to understand the mechanism of these effects for their better therapeutic utilization. This experiment was done on forty healthy ginea pigs dividing it in four groups, group A were treated with aqueous extract of *Azadirachta indica* plant leaves, group B by the fine powder of *Curcuma longa* root, group C Framycetin sulphate ointment and group D only with distilled water. Two wound of 6 cm length were made through the full thickness of the skin on the either side of the vertebral column under local anesthesia was created and treated by above mentioned drugs. Histomarphological study and time taken in wound healing was noted for different group animals. Statistical analysis of the data was done by applying unpaired t-test. Cellular infiltration, neovascularization, fibroblast proliferation, epithelialization and collageniaztion all were faster in wound healing by primary intention with aqueous extract of both *Azadirachta indica* leaves and fine powder of *Curcuma longa* rhizomes treated groups when it was compared with control. The process of healing was found faster with aqueous extract of *Azadirachta indica* leaves when it was compared with fine powder of *Curcuma longa* rhizomes.
Keywords: *Azadirachta indica*, *Curcuma longa*, Wound-healing, Anti-inflammatory activities, Ginea pigs.

INTRODUCTION

Azadirachta indica is an important medicinal plant. Its medicinal activity is known from ancient time. It has wide spectrum of biological activity. Every part of the plant is full of medicinal property and it is being used for the treatment of various diseases [1-6]. It is considered as general health promoter and has antioxidant property [7-8]. In folk medicine *Azadirachta indica* which is also known as 'neem' tree, its leaf is being used to control leprosy, intestinal helimenthiasis, chronic syphilitic sores and respiratory disorder [9]. Neem tree oil is used to control skin infection [1]. It is also consider beneficial in itching, burning sensation and skin ulcer [10]. Lotion derived from neem leaf, when locally applied, can cure these dermatological diseases within 3–4 days.

Antipyretic analgesic and anti-inflammatory property is present in various extract of *Azadirachta indica* [11]. The chloroform extract of stem bark was effective in reducing paw edema in rat. It was also found effective in reducing mouse ear inflammation [12]. Neem oil has antipyretic activity [13-14]. Methanol extract of the leaves exerts antipyretic effect in male rabbits [15].

Leaves, seeds and bark possess a wide spectrum of antibacterial, antifungal and anti viral properties. It has action against Gram-negative and Gram-positive microorganisms. It is effective against M. tuberculosis, streptomycin resistant strains [16], Streptococcus mutans, S. faecalis, [17] Vibrio cholerae, Klebsiella pneumoniae, and M. pyogenes [18]. Neem leaf, neem oil and seed extract are effective against certain human fungi like Trichophyton, Epidermophyton, Microsporum, Trichosporon, Geotricum and Candida [19].

Turmeric is an anti-inflammatory agent [20] and used in bursitis, arthritis, back pain and reducing post-surgical inflammation [21]. Turmeric lowers the production of inflammation-inducing histamine. It increases and prolongs the action of the natural anti-inflammatory action of the body. It also improves circulation. Curcumin is its main active constituent. In an experiment it was found that curcumin inhibits neutrophil aggregation associated with inflammation and platelets aggregation [22-26]. It also inhibits biosynthesis of prostaglandins from arachidonic acid [27-29], and neutrophil function during inflammatory states which is related to its mechanism of anti-inflammatory effect.

It is useful in cancer prevention, liver protection and premature aging. Its hepatoprotective effect is mainly a result of its antioxidant properties, as

well as its ability to decrease the formation of pro-inflammatory cytokines.

Curcumin is an antioxidant. Its antioxidant properties are comparable to vitamins C and E. In a study it was seen that pretreatment decreased ischemia-induced changes in the heart. It also helps in reducing blood clumps.

Turmeric extract inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. In animal study topically applied turmeric oil inhibited dermatophytes and pathogenic fungi. A study of chicks demonstrated that diets supplemented with one percent turmeric resulted in a reduction in small intestinal lesion scores.

Very little work has been done on the wound-healing and anti-inflammatory activities of these plants and investigation is needed to understand these effects to be employed in meeting human needs.

MATERIALS AND METHODS

The experiment was done in the department of pharmacology of a tertiary care center after the permission of institutional ethics committee. Forty animals were housed individually in standard laboratory environment for 7 days, fed with commercial pellet diet and water ad libitum. The experiment was done on healthy male guinea pigs dividing it into four groups, each group containing 10 animals.

Group A were treated with aqueous extract of *Azadirachta indica* plant leaves, group B by the fine powder of *Curcuma longa* root, group C Framycetin sulphate ointment and group D only with Distilled water.

Study of physical parameter, wound index and histomorphological studies were done by the method described by Rafiq et al, Biswas et al and Culling. All the animal was examined to ensure its good clinical condition. Two wound of 6 cm length were made through the full thickness of the skin on the either side of the vertebral column under local anesthesia with full aseptic precaution in opposite thigh. Above mentioned drugs were applied topically once daily on different group after washing it with sterile water. Statistical analysis of the data was done by applying unpaired t-test. Study of shrinkage of wound size and histomorphological study was done by randomly selecting ten guinea pigs on same site.

The shrinkage of length of wound was measured every 3rd day to complete healing of all the wounds. The progressive decreases in wound area were monitored periodically by tracing the wound margin on a tracing paper and the length assessed using a paper graph.

The scoring system for wound index measurement was as follows- Complete healing of wounds provided score 0, Incomplete but healthy healing:1, delayed but healthy healing:2, healing has not started:3, Formation of pus and evidence of necrosis was provided score 4.

HISTOPHARMACOLOGICAL STUDIES

Table-1: Scoring system for the measurement of histomarphological parameters for assessment of wound healing

Score	Cellular infiltration	Fibroplacia	Neovascularization	Epithelialization	Collagenization
0	absent	absent	absent	absent	absent
1	0-5 cells/HPF	Small number of fibroblast towards surface extending down to deeper areas	1-5 new blood vessels/HPF	Up to 20% epithelialization	Small amount of thin collagen
2	3-60 cells/HPF	Diffuse fibroblastic proliferation towards surface extending down to deeper areas	6-10 new blood vessels/HPF	50% epithelialization	Between 1 and 3
3	More than 30 cells/HPF	Dense fibroblast proliferation over whole area and fibroblast laying down collagen fibers	11-15 or more blood vessels/HPF	80% epithelialization	Diffuse thick collagen
4		Dense fibroblast proliferation over whole area and fibroblast laying down collagen fibers			Wavy collagen

RESULTS

Wound index at day 11 is significant of both the studied drugs in comparison to control but it is less effective than framycetin. Wound index of *Azadirachta indica* is less then Curucoma longa. Total days in wound healing were found 15th days with *Azadirachta indica* and 17th day with Curucoma longa. This period is less then control group but more then framycetin.

Azadiricta indica and *Curcuma longa* treated group cellular infiltration, neovascularization, fibroblast proliferation, epitheliazation and collagenization all were found significant when it was compared with control group although in every study group factors affecting wound healing like size, location, type of wound, mechanical factors, and nutritional status is almost the same. Both these drugs have highly significant effect in wound healing.

Table-2: Histopathological finding on 5th day of surgically incised wound.

Charactarization	*Azadirachta indica*	*Curcuma longa*	Control
Cellular infiltration	2.10±0.067**	2.05±0.007**	2.75±0.067
Neovascularization	0.97±0.048**	0.896±0.008**	0.50±0.066
Fibroblast proliferation	1.25±0.066**	1.25±0.066**	0.85±0.066
Epithelialization	0.79±0.021**	0.90±0.066**	0.50±0.066
Collagenization	2.05±0.115**	1.18± 0.007**	0.52±0.035

Values are expressed as mean ± SD (n=10) compared with control treated group*p<0.05, **p<0.01.

Table-3: Histopathological finding on 11th day of surgically incised wound.

Characterization	*Azadirachta indica*	*Curcuma longa*	Control
Cellular infiltration	1.29±0.038**	1.37±0.007	2.41± 0.037
Neovascularization	2.77±0.042**	2.40±0.010	1.0± 0.067
Fibroblast proliferation	2.54 ±0.052**	2.29±0.088	1.4 ±0.067
Epithelialization	2.75±0.067**	2.62±0.035	1.15± 0.066
Collagenization	3.15±0.211**	2.89± 0.052	1.17 ±0.042

Values are expressed as mean ± SD (n=10) compared with control treated group*p<0.05, **p<0.01.

The above data reveals that the process of healing is faster with *Azadirachta indica* at both 5th and 11th days. Wound index is significant with both the experimental drug but when these two drugs were compared together *Azadirachta indica* showed significant effect in comparison to *Curcuma longa*.

There is complete healing of the wound in 11th 15th 17th and in 22 days subsequently by Framycetin, *Azadirachta indica*, *Curcuma longa* and by control group. The process of healing was found faster with *Azadirachta indica* when it was compared with *Curcuma longa*.

DISCUSSION

The all four experimental groups showed the wound healing effect. The difference was in the time period of wound healing and in cellular reaction. The time period in complete healing of the wound was 11, 15, 17 and 22 days subsequently by Framycetin, *Azadirachta indica*, *Curcuma longa* and by control group.

We know repair begins soon after the injury. These new cells may be derived either from the parenchyma or the connective tissue stroma of the injured tissue. The healing of the primary union are finely orchestrated phenomena which takes weeks in the full process of wound healing. It is very clear why the placebo (sterile water treated group) taken the longer time in wound healing and framycetin the shortest period. The healing effect of the investigational group drugs is in between these two control groups. It indicates the wound healing potency of both the investigational group drugs. Table no 2 explains the mechanism and the comparative effect of the factors responsible for it.

Proliferation of fibroblasts and capillary buds and subsequent lying down of collagen is the usual consequence of wound healing. The process starts with infiltration by fibrin, neutrophils, epithelial cells and deposition of basal metabolites. In this study in *Azadirachta indica* treated group neovascularization, fibroblast proliferation, epithelialization and collagenization all were found significantly faster both at day 5 and 10 when it was compared with control group. Cellular infiltration is faster with control group in comparisons to both experimental groups at day 5 and 10. It explains the mechanism of its wound healing effect.

It has been found that nimbidin and polysaccharides present in neem extracts has anti-inflammatory property [30]. Nimbidin also have an antipyretic [31] and antiarthritic [32] activity. Cyclic trisulphide and tetrasulphide present in its leaf, margolone, margolonone and isomagolonone, present in bark and mahmoodin, gedunin present in its seed oil has antimicrobial [33] and antifungal [34] properties. These factors are responsible in protecting the wound from secondary infection and accelerated its healing.

A compound NB-II peptidoglycan present in neem bark is an immunomodulator [35]. This immunomodulatory effect may have a crucial role both

in humoral and cell-mediated responses. All these factors explain why healing by *Azadirachta indica* is faster than control group.

Curcuma longa is an antioxidant and have free radical scavenging property. It enhances immunity. It increases and prolongs the action of body's natural anti-inflammatory adrenal hormone and cortisol. It also improves circulation. In this study the score of *Curcuma longa* for neovascularization, fibroblast proliferation, epithelialization and collagenization are highly significantly (table: 2&3) in comparison to control group. This is probably due to above mentioned reason and its anti-inflammatory and antimicrobial (antiviral and antifungal) actions. It reduces the formation of bloods clumps. It is hepatoprotective and helps in premature aging of the cell. These properties may also help in wound healing.

CONCLUSION

Cellular infiltration, neovascularization, fibroblast proliferation, epithelialization and collagenization all are faster in wound healing by primary intention with aqueous extract of both *Azadirachta indica* leaves and fine powder of *Curcuma longa* rhizomes treated groups. Both the drugs are effective in accelerating in wound healing; however *Azadirachta indica* was found more effective than *Curcuma longa*. There is need of more specific study to determine the efficacy of these drugs.

REFERENCES

1. Chopra, R. N., Nayar, S. L., Chopra, I. C., (1956). Glossary of Indian Medical Plants, CSIR, New Delhi.
2. Chopra, R. N., Chopra, I. C., Handa, K. L., & Kapur, L. D., (1958). Indiginous drug of India. U. N .Dhur and sons, Kolkata, 51-595.
3. Kirtikar, K. R., & Basu, B. D., (1975). In: Medicinal plants, editor Blatter E, Chains JF, Mhaskar KS. Vivek Vihar, New Delhi. 536.
4. Thakur, R. S., Singh, S. B., Goswami, A., (1990). Aromatic plants. Curr. Res. Res. Med. Aromat plants. 3,135-140.
5. Koul, O., Isman, M. B., & Ketkar, C. M. (1990). Properties and uses of neem, *Azadirachta indica*. *Canadian Journal of Botany*, *68*(1), 1-11.
6. Chatterjee, A., Pakrashi, S., (1994).The treatise on Indian medical plants, editor, 3:76.
7. Arivazhagan, S., Balasenthil, S., & Nagini, S. (2000). Modulatory effects of garlic and neem leaf extracts on N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)-induced oxidative stress in Wistar rats. *Cell biochemistry and function*, *18*(1), 17-21.
8. Arivazhagan, S., Balasenthil, S., & Nagini, S. (2000). Garlic and neem leaf extracts enhance hepatic glutathione and glutathione dependent enzymes during N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)-induced gastric carcinogenesis in rats. *Phytotherapy Research*, *14*(4), 291-293.
9. Kiritikar, K. R., & Basu, B. D., (1935). In Indian Medical Plants. editor Basu LM, Allahabad.2nd edn:536.
10. Mitra, C. R., & Patel, M. S. (1963). Neem, Indian Central Oilseeds Committee, Hyderabad, 69.
11. Jacobson M. (1986). Pharmacological and toxicological effects of neem and chinaberry on warm-blooded animals. *Neem newsl*. 3:39-43.
12. Tidjani, M. A., Dupont, C., & Wepierre J., (1989). *Azadirachta indica* stem bark extract--Anti-inflammatory activity. Planta Med. Phytother. 23:259-266.
13. Murthy, SP., & Sirsi, M., (1958). Pharmacological studies on Melia azadirachta, Linn.(no Meliaceae). *Indian J. Physiol. Pharmacol*. 2:387-396.
14. Murthy, S. P., & Sirsi, M. (1958). Chemical Properties of Neem. *A. juss) Indian J Physiol Pharmacol*, *2*, 456-60.
15. Okpanyi, S. N., and G. C. Ezeukwu. Anti-Inflammatory and Antipyretic Activities of *Azadirachta indica*. *Planta medica* 41, no. 1 (1981): 34-39.
16. Chopra, I. C., Gupta, K. C., & Nazir, B. N. (1952). Preliminary study of anti-bacterial substances from Melia azidirachta. *The Indian journal of medical research*, *40*(4), 511.
17. Almas, K. (1998). The antimicrobial effects of extracts of *Azadirachta indica* (Neem) and Salvadora persica (Arak) chewing sticks. *Indian journal of dental research: official publication of Indian Society for Dental Research*, *10*(1), 23-26.
18. Satyavati, G. V., Raina, M. K., Sharma, M., (1976). eds. In: Medicinal plants of India, vol.I.
19. Khan, M., & Wissilew, S. W., (1987).In: Natural Pesticides from the Neem Tree and Other Tropical Plants, Editor Schmutterer H, Asher KRS. GTZ, Eschborn, Germany. 645-650.
20. Kohli, K., Ali, J., Ansari, M. J., & Raheman, Z., (2005). Curcumin: A natural antiinflammatory agent. *Indian J. Pharmacol.*;37(3):141-147.
21. Akram, M., Shahab-uddin., Ahmed, A., Khan, U., Hannan, A., Mohiuddin, E., & Asif, M., (2010). *Curcuma longa* and curcumin:A review article. Rom. *J. Biol. – Plant Biol*,55(2):65–70.
22. Lukita-Atmadja, W., Ito, Y., Baker, G. L., & McCuskey, R. S., (2002). Effect of curcuminoids as anti- inflammatory agents on the hepatic microvascular response to endotoxin. *Shock*,17:399-403.
23. Gukovsky, I., Reyes, C. N., Vaquero, E. C., Gukovskaya, AS., Pandol, SJ., (2003). Curcumin ameliorates ethanol and nonethanol experimental pancreatitis. *Am J Physiol Gastrointest Liver Physiol*,284:85-95.
24. Ukil, A., Maity, S., Karmakar, S., Datta, N., Vedasiromoni, J. R., Das, P. K., (2003). Curcumin, the major component of food flavour turmeric,

reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. *Br J Pharmacol*. 139:209-18.

25. Shah, B. H., Nawaz, S. A., Roomi, A., Saeed, S. A., (1999). Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor and arachidonic acid-meiated platelet aggregation through inhibition of thromboxane formation and ca^{2+} signaling. *Biochem Pharmacol*,58:1167-72.
26. Srivastava, K. C., Bordia, A., & Verma, S. K., (1995). Curcumin, a major component of food spice turmeric(*Curcuma longa*) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. *Prostaglandins Lekot Essent Fatty Acids*.52;223-7.
27. Joe, B., & Lokesh, B. R., (1997).Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages. *Lipids*, 32:1173-80.
28. Ammon, H. P., Safayhi, H., Mack, T., Sabieraj, J., (1993).Mechanism of anti-inflammatory actions of curcumin and bowsellic acids. *J Ethnopharmacol*. 38:113-9.
29. Srivastava, R., (1989). Inhibition of neutrophil response by curcumin. *Agents Actions*. 28:298-303.
30. Bhargava, K. P., Gupta, M. B., Gupta, G. P., & Mitra, C. R. (1970). Anti-inflammatory activity of saponins and ot-her natural products. *The Indian journal of medical research*, *58*(6), 724.
31. David, SN., (1969). Oil and nimbidin suppressed the secondary rise in temperature. Mediscope. 12:25-27.
32. Pillai, N. R., & Santhakumari, G. (1981). Anti-arthritic and anti-inflammatory actions of nimbidin. *Planta medica*, 43(9), 59-63.
33. Ara, I., Siddiqui, B. S., Faizi, S., & Siddiqui, SJ., (1989). Isolation and Structure Elucidation of the Triterpene Azadirinin from the Roots of *Azadirachta indica*. *Chem.Soc. Perkin Trans*.;I:343- 345.
34. Pant, N., Garg, H. S., Madhusudanan, K. P., & Bhakuni, DS., (1986). Sulfurous compounds from *Azadirachta indica* leaves. *Fitoterapia*. 57, 302-304.
35. Van der Nat, J. M., Klerx, J. P. A. M., Van Dijk, H., De Silva, K. T. D., & Labadie, R. P. (1987). Immunomodulatory activity of an aqueous extract of *Azadirachta indica* stem bark. *Journal of ethnopharmacology*, *19*(2), 125-131.