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Studies on *in-vivo* Wound Healing Activity of Leaf Extract of *Hypericum mysorense* with Different Wound Model in Rats

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Abstract – The wound healing potential of the methanol extract of *Hypericum mysorense* Wight and Arn. Leaves (Family: Hypericaceae) was evaluated on different experimental models of wounds in rats. The methanol extract of leaves of *Hypericum mysorense* (HMM), in the form of ointment in two different concentrations (5% and 10% w/w ointment of aerial part extract in simple ointment base) was evaluated for wound healing potential in excision wound model and incision wound model in rats. Both the concentrations of the methanol extract ointment showed significant responses in both the wound types tested when compared with the control group. The effect produced by the extract ointment, in terms of wound contracting ability, wound closure time, regeneration of tissues at wound site, tensile strength of the wound and histopathological characteristics were comparable to those of a standard drug Nitrofurazone ointment.

Key words – *Hypericum mysorense*, Hypericaceae, leaf, methanol extract, wound healing activity, nitrofurazone, ointment

Introduction

The genus Hypericum is well known for the therapeutic efficacy of its most potent species *Hypericum perforatum* L. (Family: Hypericaceae). Numerous compounds with documented biological activities has been reported from it (Roy Upton, 1997). The naphthodianthrones, hypericine and pseudohypericin, different flavonoids like quercetin, hyperin, etc., phloroglucinols, essential oils and xanthones have been reported to produce antidepressant, antimicrobial, antioxidant, and antiinflammatory activities (Bystrov, 1975; Gurevich *et al*, 1971; Holzl *et al*, 1989; Kitanov *et al*, 1987; Rocha *et al*, 1995; Khosa *et al*, 1982; Weyerstahl *et al*, 1995). The extracts of *Hypericum perforatum* has been shown to be more effective than placebo in the treatment of depression (Linde *et al*, 1996) and different phytochemical constituents of this plant like xanthones and flavonoid - hyperforin has been shown to be effective as antimicrobial, antiviral, antibacterial against gram positive bacteria and possess wound healing potentials (Lavie *et al*, 1995; Bombardelli, 1995).

About 20 different species of *Hypericum* are available in India in which Nilgiri district play a major role in its content (Anonymous, 1962; Fyson, 1974). Out of all the species of Hypericum available in Nilgiris, *Hypericum mysorense* Wight. and Arn., commonly known as 'Shrubby St. John's Wort' are abundantly available every where in the Nilgiris down, near to Kotagiri and Pykara (Fyson, 1974). *H. mysorense* is an erect, glabrous shrub, 1-3 m height with sessile leaves, horizontal with tips curved slightly upwards, narrow elliptic lanceolate with strong midribs (Fyson, 1974; Gamble, 1984). Flowers of this variety are golden yellow in colour, solitary or in 2-5 flowered terminal cymes, bracts foliaceous- which is a special feature of this plant (Fyson, 1974). *H. mysorense* is well known in folklore medicine for its different therapeutic potentials including spasmolytic, hypotensive and antifungal activities (Asolkar *et al*, 1992).

Presently it has come to our notice that the tribal people of Shola forest (Tamilnadu, India) use the aerial parts, most particularly the leaves of this plant for treating burns and wounds and get cure of it; it's other species *H. perforatum* has been reported to have wound healing activity (Roy Upton, 1997). In the light of these informations about the folklore use of this plant as well as reported wound healing activity of other species of *Hypericum*, the present study was undertaken to evaluate the wound healing activ-

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ity of the leaf extract of this plant, in different wound model in rats and thereby to substantiate these claims, which is being reported in the present communication.

Materials and Methods

Plant material – Fresh plant materials (aerial parts) of *Hypericum mysorense* were collected from Ootacamund, a famous hill station in southern India which belongs to the Nilgiri, Tamilnadu, India. It was identified by Botanical Survey of India, Southern circle, Coimbatore. A voucher specimen has been kept in our laboratory for future references. The aerial parts were dried under shade, leaves were separated from the stem parts, pulverized by a mechanical grinder, passed through 40 mesh sieve and stored in a closed vessel for future use.

Extracts and reference standard used – The powdered leaves of *H. mysorense* were extracted with methanol using soxhlet extraction apparatus. This methanol extract was then concentrated and dried under reduced pressure. The semi-solid mass (methanol free) thus obtained was used for the experiment. The yield was 10.75% w/w with respect to dry powdered material. Two types of formulations were prepared from the extract: (i) 5% (w/w) ointment, where 5 g of extracts of leaves were incorporated in 100 g of simple ointment base B.P. (Anonymous, 1953), (ii) 10% (w/w) ointment where, 10 g of extracts of the leaves were incorporated in 100 g of simple ointment base B.P. (Anonymous, 1953). 0.2% (w/w) Nitrofurazone ointment (Smith Kline Beecham Pharmaceuticals India Ltd., Bangalore) was used as standard drug for comparing the wound healing potential of the extract in excision and incision wound model.

Animals used – Wistar albino rats (150-180 g) of both the sexes were selected to carry out the experiment. Six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were housed in standard metal cages and provided with food and water *ad libitum.*

Excision wound model – Four groups of animals containing six in each group were anaesthetised by open mask method with anaesthetic ether. The rats were depilated on the back. One excision wound was inflicted by cutting away 500 mm^2 full thickness of skin of a predetermined area (Udupa *et al.*, 1994a; Saha *et al.*, 1997). Rats were left undressed to the open environment. Then the drug i.e. the reference standard (0.2% w/w nitrofurazone ointment), simple ointment B. P. (Anonymous, 1953); Hypericum mysorense methanol extract ointment (5% w/w and 10% w/w of the leaves) were administered till the wound was completely healed. This model was used to monitor wound contraction and epithelialisation time. Wound contraction was calculated as percentage reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on a graph paper every alternate day. To determine the changes in healing of wound, measurement of wound area on graph paper was expressed as unit (mm^2).

Histopathological examinations – From the healed wounds of the above model, a specimen sample of skin tissues of extract ointment treated, standard and simple ointment (control) treated groups were isolated from each group of rats for histopathological examinations. The tissues were stained with eosine I bluish solution and observed for the histological changes under microscope (Anderson, 1980).

Incision wound model – Four groups of animals containing six in each group were anaesthetised and two paravertebral long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from midline on each side of the depilated back of rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used through out the experiment (Udupa *et al.*, 1994b; Saha *et al.*, 1997).

All the groups were treated in the same manner as mentioned in case of excision wound model. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk thread by 0.5 cm apart. Surgical thread (No: 000) and curved needle (No: 11) were used for stitching. The continuous threads on both wound edges were tightened for good adoptation of wound. The wound was left undressed. The ointment of the leaf extract, standard drug (nitrofurazone ointment) and simple ointment B. P. (Anonymous, 1953) was applied to the wound twice daily, until complete recovery to the respective groups of animals.

Tensiometer – Tensile strength of wound represents the promotion of wound healing. Usually

wound healing agents promote the gaining of tensile strength. Tensile strength (the force required to open the healing skin) is used to measure the amount of healing. The instrument used for this purpose is called as Tensiometer, which is explained as follows.

It consists of a 6×12 inch wooden board with one arm of 4 inch long, fixed on each side of the possible longest distance of the board. The board was placed at the edge of a table. A pulley with bearing was mounted on the top of one arm. An alligator clamp with 1 cm width was tied on the tip of the another arm by a fishing line (20 lb test monofilament) in such a way that the clamp could reach the middle of the board. Another alligator clamp was tied on a longer fishing line with 1 liter polyethylene bottle on the other end. One day before performing the experiment (measurement of tensile strength) the sutures were removed from the stitched wounds of rats after recovery and tensile strength was measured as follows.

Determination of tensile strength – The sutures were removed on 9th day of wounding and the tensile strength was measured on 10th day. Extract ointments along with simple ointment (control) and nitrofurazone ointment (standard) were administered throughout the period, twice daily for 9 days. On 10th day again the rats were anaesthetised and each rat was placed on a stack of towels on the middle of the board. The amount of the towels could be adjusted in such a way so that the wound was on the same level of the tips of the arms. The clamps were then carefully clamped on the skin of the opposite sides of the wound at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally to polyethylene bottle. The position of the board was adjusted so that the bottle receive a rapid and constant rate of water from a large reservoir, until the wound began to open. The amount of water in polyethylene bag was weighed and considered as tensile strength of the wound. The mean determinations were made on both sides of the animals and were taken as the measures of the tensile strength of the wound. The tensile strength of the extract and nitrofurazone ointment treated wounds were compared with control. Tensile strength increment indicates better wound healing promotions of the applied drug.

Statistical analysis – Results obtained from both the wound model has been expressed as Mean±SE and were compared with the corresponding control (simple ointment) values. P-values were calculated by Student's t-test by comparing with control (Woodson, 1987). Percentage of wound contractions were calculated with respect to the corresponding 0 day's wound area (mm^2).

Results

The progress of the wound healing induced by *H. mysorense* leaf extract ointments (5% w/w and 10% w/w) treated groups, simple ointment (control) treated group and nitrofurazone (standard drug) treated group of animals have been shown in Table 1. It is observed that the wound contracting ability of extract ointment in different concentrations were significantly greater than that of control (i.e., simple ointment treated group). The 10% (w/w) extract ointment treated groups showed maximum contraction of wounds from sixth day onwards in both the concentrations which was comparable to that of the standard drug i.e. nitrofurazone ointment treated group of animals. The wound closer time was lesser, so much so the percentage of wound contraction was much more with the 10% w/w extract ointment treated group, where it took 16±2 days for 100% contraction. The nitrofurazone treated group as well as the 5% extract ointment treated group of animals showed almost similar wound contraction with the wound closure time of 18±2 days.

In the incision wound model, both the concentrations of the extract ointment as well as the standard drug treated groups of animals showed significant increase in tensile strength of the 10 days old wound. The measurement of the tensile strength with the effect of the extract and standard drug on the wound healing process by incision wound method has been shown in Table 2. The tensile strength of the 10% extract treated group and the nitrofurazone ointment treated group was almost same. The 5% extract ointment treated group showed lesser but significant increase in the tensile strength comparing to the control group.

The multiple sections studied in histopathological examination of the tissues of the wound area treated with the extract ointments (5% and 10% w/w), 0.2% w/w nitrofurazone ointment, and simple ointment (control) treated groups, showed that the original tis-

Table 1. Evaluation of *hypericum mysorense* leaf extract and nitrofurazone ointment on wound healing by excision wound method in rats

Post wounding days	Wound area (mm^2) (Mean±SE) and percentage of wound contraction			
	Simple ointment	Nitrofurazone ointment (0.2% w/w)	Extract ointment (5% w/w)	Extract ointment (10% w/w)
0	506±12.9	492±13.3	509±16.7	511±16.1
2	426±13.4 (15.8)	397±13.8 (19.3)	407±14.2 (20.6)	399±13.8 (21.9)
4	387±12.7[a] (23.5)	312±15.2[a] (36.6)	327±13.8[a] (35.9)	286±17.1[a] (44.0)
6	328±14.5[a] (35.2)	256±12.9[a] (48.0)	242±15.2[a] (52.5)	203±13.2[a] (60.3)
8	289±15.1[a] (42.9)	186±13.8[a] (62.2)	189±14.8[a] (62.9)	136±14.3[a] (73.4)
10	255±14.2[a] (49.6)	103±11.2[a] (79.1)	110±12.7[a] (78.4)	91±11.6[a] (82.2)
12	211±12.6[a] (58.3)	66±12.6[a] (86.6)	58±12.2[a] (88.6)	53±12.4[a] (89.6)
14	193±13.0[a] (61.8)	34±12.0[a] (93.1)	36±11.1[a] (92.9)	20±11.8[a] (96.1)
16	164±15.2[a] (67.6)	11±8.3[a] (97.8)	10±9.8[a] (98.0)	00[a] (100.0)
18	151±11.3[a] (70.0)	00[a] (100.0)	00[a] (100.0)	00[a] (100.0)

Results were compared with the corresponding control values (simple ointment) and p-values were calculated by Student's t-test.
[a]$p < 0.001$. (Figures in the parenthesis indicate % of wound contraction).

Table 2. Evaluation of *H. mysorense* leaf extract and standard drug on incision wound model in rats.

Number of Animals	Treatment	Tensile Strength (g) (Mean±SE)
6	Simple ointment	416±11.6
6	Extract ointment (5% w/w)	555±12.6[a]
6	Extract ointment (10% w/w)	567±11.2[a]
6	Nitrofurazone ointment (0.2% w/w)	569±13.2[a]

Results were compared with control and p-value was calculated by Student's t-test.
[a]$p < 0.001$

sue regeneration was much greater in the skin wound treated with extract ointments and nitrofurazone ointment without any oedema, congestion or inflammatory changes. More relative fibrosis were observed in the nitrofurazone treated wound with flattened rete ridges in the epidermis comparing to the skin wound treated with either 5% or 10% of the extract ointment.

Discussion

In addition to the mechanical breakdown of normal tissues and cellular compartments which leads to the local structural cellular damages, there is a biological response to regulate the bodys own cellular defense mechanisms which contributes a lot to the wounds and to its repairments. These repairments of healing of wound involve different phases such as contraction, epithelialisation, granulation, collagenation etc. (Charles *et al*, 1995). The leaf extracts of *H. mysorense* has been shown to possess flavonoids and glycosides on preliminary screening. The wound healing property of hyperforin, the flavonoid isolated from *H. perforatum* has already been reported (Roy Upton, 1997). It has been reported that the mixture of glycosides present in *Centella asiatica* can enhance the repairment in incised wounds and also stimulate the formation of collagen in human skin

fibroblast cell (Rosen *et al.*, 1967; Vogel *et al*, 1980). The wound healing potential of the *H. mysorense* extract may probably be due to the presence of a mixture of phytoconstituents including flavonoids, glycosides etc., the isolation of which is under way in our laboratory. Thus from the investigational work represented herein it is conclusively proved that the *H. mysorense* leaf extract has a reproducible wound healing potential and there by substantiate its use in folklore medicine in India.

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References

Anderson, J. E. *Muirs Text Book of Pathology,* 11th. ed., ELBS, 1980, pp.77-85.

Anonymous, *British Pharmacopoeia,* General Medical Council, The Pharmaceutical Press, 17 Bloomsbury Square London, WCI, 1953, p. 396.

Anonymous, *The Wealth of India, Raw Materials,* Vol. 5, Publication and Information Directorate, CSIR, New Delhi, 1962, pp. 154-156.

Asolkar, L.V., Kakkar, K.K. and Chakre, O.J., *Second Supplement to Glossary of Indian Medicinal Plant with Active Principles*, Vol. 1, Publication and Information Directorate, CSIR, New Delhi, 1992, pp. 138-139.

Bombardelli E., Morazzoni, P., *Hypericum perforatum. Fitoterapia*, **66**, 43-68 (1995).

Bystrov, N. S., Chernov, B. K., and Dobrynin, V. N., The structure of hyperforin. *Tetrahedron Letters*, 32, 2791-2794 (1975).

Charles, V. M., Rusell, R. C. G. and Williams, N. S., *Short Practice of Surgery.* 20th ed., Champan and Hall, London, 1995, pp. 9-11.

Fyson, P. F., *The Flora of the Nilgiris and Pulney Hill Tops,* Vol-1, Bishen Singh & Mahendra Pal Singh, Dehradun,1974, pp. 37-39.

Gamble, J. S., *Flora of the Presidency of Madras*, Vol-1, Bishen Singh & Mahendra Pal Singh, Dehradun, 1984, pp. 69-71.

Gurevich, A. I., Dobrynin, V. N, Kolsov, M. N., Hyperforin, an antibiotic from *Hypericum perforatum. Antibiotiki (Moscow)*, **16**, 510-513 (1971).

Holzl, J., Demisch, L., Golllnik, B., Investigation about antidepressive and mood changing effects of *Hypericum perforratum. Planta Medica*, **55**, 643 (1989).

Khosa, R. L., Bhatia, N., Antifungal effects of *Hypericum perforratum* Linn. *J. Sci. Res. Plants. Med.* **3**, 49-50 (1982).

Kitanov, G. M., Blinova, K. F., Modern state of the chemical study of species of the genus *Hypericum. Khimiya Prirodnykh Soedinenni*, **2**, 185-203 (1987).

Lavie, J. Mazur, Y., Lavie, D. and Meruelo, D., The chemical and biological properties of hypericin- a compound with a broad spectrum of biological activities. *Medicinal Research Review*, **15**, 111-119 (1995).

Linde, K., Gilbert, R., Mulrow, C. D., Pauls, A., Weidenhammer, W., Melchart, M., St. Johns Wort for depression an overview and meta-analysis of randomised clinical trials. *British Medical Journal*, **313**, 253-257 (1996).

Rocha, L., Marston, A., Potterat, O., Kaplan, M. A. C., Evans, H. S., Antibacterial phloroglucinols and flavonoids from *Hypericum brasiliense. Phytochemistry,* **40**(5), 1447-1452 (1995).

Rosen, H., Blumenthal, A. and Callum, J. M., Effect of Asiaticoside on wound healing in rats. *Experimental Medicine Surgery*, **125**, 279-280 (1967).

Roy Upton (Ed.), *American Herbal Pharmacopoeia and Therapeutic Compendium,* St. Johns Wort Monograph, American Herbal Pharmacopoeia, Santacruz, CA, 1997, pp. 3-13.

Saha, K., Mukherjee, P. K., Das, J., Pal, M. and Saha, B. P., Wound healing activity of *Leucas lavandulaefolia* Rees. *J. Ethnopharmacology*, **56**, 139-144 (1997).

Udupa, S. L., Udupa, A. L. and Kulkarni, D. R., Anti-inflammatory and wound healing properties of *Aloe vera. Fitoterapia,* **65**(2), 141-145 (1994a).

Udupa, S. L., Udupa, A. L. and Kulkarni, D. R., Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera and Aegle marmelos. Fitoterapia*, **65**(2), 119-123 (1994b).

Vogel, H. G. and De Souza, N. J., Effect of terpenoids

isolated from *Centella asiatica* on granuloma tissues. *Acta Theriologica*, **16**, 285-298 (1980).

Weyerstahh, P., Splittegerber, U. and Marschall, H. Constituents of the leaf essential oil of *Hypericum perforatum* L. from India. *Flavours and Fragrance J.*, **10**, 365-370 (1995).

Woodson, R. F., *Statistical Methods of the Analysis of Biomedical Data*, Wiley Series in Probability and Mathematical Statistics, Chichester, 1987, pp. 315-316.

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