Wound healing activity of *Hemidesmus indicus* formulation

Sir,

The discovery of drugs from medicinal plants provides new and important leads against various pharmacological targets including chronic diseases such as Alzheimer's disease, malaria, and pain.[1] *Hemidesmus indicus* (Tamil: *nannari*) is very commonly used for the treatment of gastrointestinal and cardiovascular disorders.[2] An extract from this plant's root inhibits the growth of *E. coli*, *Streptococcus*, *Corynebacterium,* and *Pneumonia*.[3] As the extract is known to have antimicrobial activity, our present study was designed to determine the wound healing activity of a herbal formulation made from *H. indicus*.

Hemidesmus indicus R. Br. (Apocynaceae) plant parts were collected in Tiruvannamalai district of Tamil Nadu. They were taxonomically identified and certified by the Botanist, Periyar Arts College, Cuddalore. Roots of *H. indicus* were collected during October–December. A voucher specimen of the plant is maintained in the Department of Pharmacology, KPCP, Tiruvannamalai, for further reference. The root of *H. indicus* was dried under the shadow for 2 weeks, and it was made as coarsely powdered for extraction.

Healthy adult Wistar rats (180–200 g) of either sex were used for the experiment. The animals were obtained from the Animal House, KPCP, Tiruvannamalai, and allowed to adapt to the laboratory conditions. All the experimental animals were housed at a temperature of 25 ± 2 °C and at a humidity of 40–50% in a 12:12 ± 1 h light–dark cycle. The rats were fed with standard rat pellets (Hindustan Limited, Bangalore, India) and water *ad libitum*. The study was approved by the Institute Animal Ethics Committee and all the animal experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Coarsely powdered shade dried roots of *H. indicus* was compactly packed in a Soxhlet extractor with methanol as a solvent and it was heated (60–80 °C) for 24 h and the final product obtained was dried. Subsequently the marc was extracted with distilled water and dried at 45–50 °C. The yields of the methanolic and aqueous extracts were 2.1% and 18.9% (w/v), respectively. A 5% (w/w) *H. indicus* ointment was formulated using the methanolic and aqueous extracts. The ointment was prepared using a simple ointment base formulated using wool fat, hard paraffin, and yellow soft paraffin.[4]

Wistar rats of either sex were divided into four groups with four animals each for the treatment as follows.

Group I : Control group
Group II : Standard drug treatment (soframycin ointment)
Group III : Treatment with 5% (w/w) *H. indicus* (methanolic extract) ointment
Group IV : Treatment with 5% w/w *H. indicus* (aqueous extract) ointment

The rats were anesthetized with anesthetic ether using the open mask method, after which their backs were depilated.

Table 1: Wound healing activity of *H. indicus* herbal formulation

Days after infliction of wound	Wound area (mm²) (percentage of wound contraction)			
	Control	Soframycin ointment	*H. indicus* ointment (methanolic extract)	*H. indicus* ointment (aqueous extract)
1	512 ± 5.18 (0)	507 ± 15.77 (0)	509 ± 10.71 (0)	507 ± 17.26 (0)
6	473 ± 16.14 (7.61)	301 ± 63.25** (40.58)	302 ± 30.02** (40.69)	467 ± 52.18 (7.79)
14	107 ± 8.04 (79.10)	57.75 ± 13.82* (88.60)	68 ± 7.30* (86.64)	94 ± 19.75 (81.45)

All the values are mean ± SEM (n = 4). *P < 0.05, **P < 0.01, compared with control, one-way ANOVA followed by Tukey's test.

One excision wound was inflicted by cutting and removing the skin entirely from a predetermined area. The wound was left undressed for 2 days, exposed to the environment. From the third day after excision, the test and standard drugs were applied once a day. The drug administration continued till the wound healed completely. The wound contraction was determined as the percentage reduction in the wound area.[4,5] The progressive changes in the wound area were monitored planimetrically by tracing the wound margin on a butter paper and tracing it out on a graph paper every 6 days. The percentage reduction was calculated using the following formula:

$$\text{Percentage reduction} = \frac{\text{Healed area}}{\text{Initial area}} \times 100$$

The mean + SEM values were calculated for each group. Significant difference between groups was determined using one-way ANOVA followed by Tukey's multiple comparison test. A P value less than 0.05 was considered to be statistically significant.

The measurements of the wounds treated with soframycin and herbal ointments are presented in Table 1. It is observed that the wound contracting ability of the *H. indicus* herbal ointment with the methanolic extract is comparable with that of the standard drug from the sixth day onwards ($P < 0.05$). The ointment made from the methanolic extract of *H. indicus* displayed significant wound healing activity. The ointment made from the aqueous extract of *H. indicus* did not display any wound healing activity.

The wound healing process is a very complex, multifactorial sequence of events involving several cellular and biochemical processes. In this investigation the herbal formulation with the methanolic extract showed significant wound healing activity by increasing cellular proliferation, promoting the formation of granulation tissue and improving the healing index. Earlier investigations have found that an extract of *H. Indicus* had antioxidant,[6] hepatoprotective,[2] and antiulcer activities.[7] Free radicals are generated at the site of injury, which impair the healing progress.[8] The herb used in the formulation has free radical scavenging properties and anti-inflammatory effects, and hence the wound healing property of the ointment containing a methanolic extract of *H. indicus* may be due to these anti-inflammatory and antioxidant properties.

It is concluded that the ointment containing the methanolic extract and not the aqueous extract of *H. indicus* promotes wound healing activity. Further studies with purified constituents of the methanolic extract of *H. indicus* are needed to understand the complete mechanism of wound healing activity of *H. indicus*.

S. Ganesan, S. Parasuraman[1], S. Uma Maheswaran, N. Gnanasekar

Department of Pharmacology, Kamalakshi Pandurangan College of Pharmacy, Ayyampalayam, Tiruvannamalai, [1]RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, Tamil Nadu, India

Address for correspondence:
S Ganesan, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry 605 006, India. E-mail: sganesh770@gmail.com

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