Wound healing property of ethanolic extract of leaves of *Hypitis suaveolens* with supportive role of antioxidant enzymes

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Ethanolic extract of leaves of *Hypitis suaveolens* was evaluated for its wound healing activity in ether-anaesthetized Wistar rats at two different doses (400 and 800mg/kg) using incision, excision, and dead space wound model. Significant increase in skin breaking strength, granuloma breaking strength, wound contraction, hydroxyproline content and dry granuloma weight and decrease in epithelialization period was observed. A supportive study made on granuloma tissue to estimate the levels of catalase and superoxide dismutase recorded a significant increase in the level of these antioxidant enzymes. Granuloma tissue was subjected to histopathological examination to determine the pattern of lay-down for collagen using Van Gieson and Masson Trichrome stains. Enhanced wound healing activity may be due to free radical scavenging action of the plant and enhanced level of antioxidant enzymes in granuloma tissue. Better collagenation may be because of improved antioxidant studies.

*Hypitis suaveolens* (Labiatae) is a sweet smelling aromatic herb found in Deccan peninsula, India and Andaman and Nicobar islands. The plant is considered to be a stimulant, carminative, sudorific, and galactogogue. Infusion of plant is used in catarrhal conditions and parasitical cutaneous diseases. The leaf juice is taken in cases of colic and stomachache. The leaves and tops are considered to be antispasmodic and are used in antirheumatic and antisuppurative baths. The decoction of root is valued as an appetiser. The plant is said to have antiseptic properties. *H. suaveolens* has been used in folk medicine for the treatment of skin complaints including burns and wounds. A survey of literature revealed that no systematic approach has been made to study the wound healing activity of this plant. Thus, the present study was undertaken to assess the effect of this indigenous herb on different parameters related to wound healing in rats and to study the influence of antioxidant enzymes on this property.

**Materials and Methods**

Plant material—Leaves of *Hypitis suaveolens* were collected during the flowering stage from the local areas of Udupi district, Karnataka, India during September 2000 and were authenticated by Professor Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi. A voucher specimen (No. pp56) has been deposited at the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, India.

**Phytochemical screening**—Preliminary phytochemical screening was done to study the presence of steroids, triterpenoids, essential oil, flavonoids, tannins, carbohydrates, and amino acids in leaves of the plant.

**Preparation of ethanol extract**—The shade dried powdered leaves (600g) were exhaustively extracted with 7.5 l of ethanol (95%) using a soxhlet apparatus and concentrated in vacuo (yield 200g).

**Animals**—Healthy Wistar albino rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. They were fed with standard chow diet (Pramav Agro Industries Ltd., Sangli, Maharashtra) and water *ad libitum*. They were housed in polypropylene cages maintained under standard condition (12/12 hr light/dark cycle; 25±3°C; 35-60% RH). The experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for experimental clearance (No. IAEK/KMC/08/2001).

**Acute toxicity studies**—Healthy adult albino rats of either sex, starved overnight, were divided into 6 groups (n= 6) and were orally fed with increasing doses (1, 2, 4, 8, 16 and 32 g/kg body wt) of ethanol.
extract. Total ethanol extract administered orally in doses of up to 8g/kg did not produce any sign of toxicity and mortality in rats when observed for 14 days after administration.

Wound models—The studies were carried out using ether-anesthesia rats in three different wound models, at two different dose levels (400 and 800 mg/kg body wt).

Incision wound—Two paravertebral incisions (6 cm long) were made through the full thickness of the skin on either side of the vertebral column of the rat. Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on the 7th day. Wound breaking strength was measured on 10th post wounding day.

Excision wounds—A circular piece of full thickness (approximately 500 mm²) was cut off from a predetermined area on the back of rat. Wounds were traced on 1 mm² graph paper on the day of wounding and subsequently on alternate days until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. Number of days required for falling of the eschar without any residual raw wound gave the period of epithelization.

Dead space wounds—These wounds were created by implanting two polypropylene tubes (2.5 cm), one on either side, in the lumbar region on the dorsal surface in each rat. On the 10th post wounding day, granuloma tissue formed on implanted tubes was dissected out carefully. Granuloma tissue from one tube was kept (at -64°C) for estimation of antioxidant enzymes. The other tube was used for determination of tensile strength after which it was dried in an oven at 60°C for 24 hr and noted dry weight. Acid hydrolysate of dry tissue was used for estimation of hydroxyproline content in the tissue.

Biochemical attributes—Granuloma tissue from dead space model was homogenized in phosphate buffer saline (pH 7.0) and centrifuged under cold condition. The clear supernatant was spectrophotometrically estimated to determine the levels of antioxidant enzymes, viz. superoxide dismutase and catalase.

Histopathology—A section of granuloma tissue was subjected to histopathological examination so as to determine the pattern of lay-down for collagen using two special stains i.e., Van Gieson and Masson Trichrome.

Statistical analysis—Results were subjected to one way ANOVA with post hoc Scheffe’s test.

Results and Discussion

Acute toxicity studies showed that drug was safe up to a maximum dose of 8g/kg body weight of the animal. In incision wound model, significant increase was observed in the skin tensile strength of ethanol extract treated group on 10th post wounding day at both the doses (Table 1). The drug treated animals of dead space wound model showed significant increase in dry granuloma weight, granuloma breaking strength and in the level of hydroxyproline content (Table 1) at both the dose levels. Histopathological study revealed increase in collagen deposition in the drug treated group (Figs 1, 2) compared to control (Figs 3, 4).

Studies on antioxidant enzymes revealed that the extract treated animals showed significant increase in the levels of superoxide dismutase and catalase, the two powerful antioxidant enzymes of the body that are known to quench superoxide radicals (Table 2).

In studies using excision wound model, animals treated with ethanol extract of Hypis suaveolens showed a significant decrease in epithelization period.

<table>
<thead>
<tr>
<th>Table 1 — Effects of ethanolic extract of Hypis suaveolens on wound healing in incision and dead space wound models</th>
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<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Treated</td>
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<tr>
<td>400mg/kg</td>
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<td>800mg/kg</td>
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* Significant at $P < 0.05$ vs control
as evidenced by shorter period for fall of eschar as compared to control. The drug extract also facilitated the rate of wound contraction significantly at both the dose levels (Table 3).

Granulation, collagenation, collagen maturation and scar maturation are some of many phases of wound healing which run concurrently, but independent of each other. Use of single model is inadequate and there is no reference standard which can collectively represent the various components of wound healing as drugs which influence one phase may not necessarily influence another. Hence in our study we have used three models to assess the effect of leaf extract on various phases of wound healing.

The results of present study showed that ethanolic extract of leaves of *H. suaveolens* possesses a definite prohealing action. This is demonstrated by a significant increase in the rate of wound contraction and by enhanced epithelization. Significant increase was also observed in skin breaking strength and hydroxyproline content which was a reflection of increased collagen levels, that was further supported by histopathological

<table>
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<th>Enzymes</th>
<th>Superoxide dismutase (IU/mg)</th>
<th>Catalase (k/see/mg)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.1175 ± 0.0111</td>
<td>2.4 × 10⁻³ ± 2.6 ± 10⁻³</td>
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<tr>
<td>Treated 400mg/kg</td>
<td>0.2443 ± 0.038</td>
<td>4.91 × 10⁻³ ± 2.52 ± 10⁻³</td>
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<tr>
<td>Treated 800mg/kg</td>
<td>0.161 ± 0.018</td>
<td>6.7 × 10⁻³ ± 6.04 ± 10⁻³</td>
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* Significant at *P* < 0.05 vs control

Figs 1-4 — (1) — Area showing granulation tissues with interspersed collagen, Van Gieson × 200 (red collagen). Treated with ethanolic extract of *H. suaveolens* leaves; (2) — Area showing granulation tissues with interspersed collagen, Masson Trichrome × 200 (green collagen). Treated with ethanolic extract of *H. suaveolens* leaves; (3) — Area showing granulation tissues with interspersed collagen, Van Gieson × 200 (Red collagen): (Control); and (4) — Area showing granulation tissues with interspersed collagen, Masson Trichrome × 200 (Green collagen: (Control).
evidence and gain in granuloma breaking strength. This indicated improved collagen maturation by increased cross-linking while an increase in dry granuloma weight indicated higher protein content. An increase in the levels of antioxidant enzymes (superoxide dismutase and catalase) was observed in granuloma tissue of dead space wound model. These enzymes are known to quench the superoxide radical and thus prevent the damage of cells caused by free radicals.12,13

Phytochemical screening revealed the presence of tannins and flavonoids. Flavonoids have been documented14 to possess potent antioxidant and free radical scavenging effect which is believed to be one of the most important components of wound healing. Thus, the enhanced wound healing may be due to free radical scavenging action of the plant, and enhanced level of antioxidant enzymes in granuloma tissues. Better collagenation seen under the influence of this plant extract may be because of improved antioxidant status.

References
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3 Chopra R N, Nayar S L & Chopra I C, Glossary of Indian medicinal plants (Council of Scientiﬁc and Industrial Research, New Delhi) 1986, 44.