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REVERSAL OF DEXAMETHASONE SUPPRESSED WOUND HEALING WITH ETHANOLIC EXTRACT OF LEAVES OF *HYPTIS* SUAVEOLENS (L) POIT.

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ABSTRACT: The dexamethasone suppressed and normal wound healing effect of ethanolic extract of leaves of *Hyptis suaveolens* (L) poit. (EEHS) at 400mg/kg and 800mg/kg was studied in Wistar rats. Two wound models viz. incision and excision wounds were used in this study. The parameters studied were breaking strength in case of incision wounds, period of epithelization and wound contraction in case of excision wound. The dexamethasone treated group showed a significant ($P<0.05$) reduction in the wound breaking strength in incision type of wound model and significant increase in epithelization period and reduction in percentage of wound contraction in excision type of wound model as compared to control group. Extract treated groups showed significant ($P<0.05$) improvement in all the wound healing parameters of incision and excision wound models as compared to control. In incision wound model, Co administration of EEHS at both the dosage levels with dexamethasone had significantly ($P<0.05$) increased the wound breaking strength as compared to dexamethasone only treated group and was seen more at dosage level of 400mg/kg. Similarly, in excision wound model, there was significant improvement ($P<0.05$) in both the parameters when EEHS was co administered with dexamethasone as compared to dexamethasone only treated group but improvement was more at dosage level of 800mg/kg.
Keywords: *Hyptis suaveolens*, wound healing, dexametasone, epithelization period, wound contraction, wound breaking strength, Incision wound, Excision wound.

INTRODUCTION

Hyptis suaveolens (Labiatae) is a medium sized aromatic annual shrub distributed in the tropical and subtropical regions, of roadsides and waste places and uncultivated land across the Deccan peninsula (South India). Its common names are American Mint, Bush mint, Vilaiti tulsi, Bhustrena etc. The leaves of *H. suaveolens* have been utilized as a carminative, sudorific and galactogogue (The Wealth of India, 1964). *Hyptis* is known to be used in traditional medicine for the treatment of various illnesses and has been found to possess anti-cancer properties (Mudgal, et al., 1997) and tumorigenic (Peerzada, et al., 1997) properties. In addition, it also has mycotoxic activity against fungus Candida *albicans*, antimicrobial activity against both gram positive *Staphylococcus aureus* and *Bacillus cereus* and negative strains of E. *coli, Pseudomonas spp.* (Olayinka, et al., 1999). Besides all these properties it also has insecticidal properties and is said to be a mosquito repellant (Mudgal, et al., 1997).

The proliferative phase of wound healing is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelization and wound contraction. In angiogenesis, new blood vessels grow from endothelial cells. In fibroplasia and granulation tissue formation, Fibroblasts secrete the precursors of all the components of the extracellular matrix, primarily the collagen and a variety of fibers.

Collagen, the major component of connective tissue which strengthens and supports extracellular tissue, contains substantial amounts of hydroxyproline, which has been used as a biochemical marker for tissue collagen (Kumar R, et al., 2006). In epithelialization, epithelial cells proliferate and spread across the wound surface. Wound contraction occurs as the myofibroblasts contract (Lawrence, et al., 1994). Chronic wounds are wounds that fail to heal despite adequate and appropriate care. Such wounds are difficult and frustrating to manage (Falanga, et al., 2004). Current methods used to treat chronic wounds include wound debridement, irrigation, antibiotics, skin grafts and proteolytic enzymes (viz. trypsin, chymotrypsin), which possess major drawbacks and unwanted side effects like bleeding, infection or pain.

Glucocorticosteroids are known to suppress wound healing (Ehrlich, et al., 1968). One such synthetic glucocorticoid, dexamethasone is known to interfere with, both the synthesis and degradation of type I and type III collagen (Oishi Y, et al., 2002). It also interferes with type VII collagen promoter activity in dermal fibroblasts, which leads to decreased anchoring of collagen and fibril formation [Kuhnt, et al., 1995).There are very few existing agents that are known to overcome the above mentioned activity of corticosteroids. In our study we have employed dexamethasone to suppress wound healing in incision and excision wound models.
Wound healing and antioxidant property of ethanolic extract of leaves of *Hyptis suaveolens* has been well established in previous studies (Shenoy R, et al., 2002; Shirwaikar, et al., 2003). In our present study, we intent to look for an answer to the research question , whether EEHS can overcome the wound healing suppression activity of dexamethasone. Hence we intent to investigate the effects of ethanolic extract of leaves of *Hyptis suaveolens* (EEHS) on incision and excision models of wound healing alone and in the presence of dexamethasone induced suppression of wound healing, with their supporting antioxidant role, in albino Wistar rats.

MATERIAL AND METHODS

Plant material- Leaves of *Hyptis suaveolens* were collected from the areas of Manipal, Udupi district, south India in the month of September and were authenticated at Department of Botany, Poorna Prajna College, Udupi.

Preparation of ethanolic extract- The shade dried powdered leaves (1.2kg) were used to extract with ethanol (95%) using Soxhlet apparatus and the net yield was 400gms.

Animals- Healthy Wistar 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 (Pranav agro industries Ltd., Saangli, Maharashtra) and water ad libitum. They were housed in polypropelene cages in a well maintained and clean environment. The experimental protocol was subjected to scrutiny of institutional animal ethics committee for experimental claearance (No. IAEC/KMC/07/2007-2008)

Acute toxicity studies- Healthy Wistar rats of either sex (n=6 in each group) were fed orally with increasing doses (1, 2, 4g/kg body wt) of ethanolic extract. The doses up to 4 g/kg body weight did not produce any sign of toxicity and mortality in rats when observed for 14 days, after administration.
Experimental procedure-
The wounding procedures were carried out on ketamine (strength 75 mg/kg body wt., i.m.) anaesthetized rats in incision and excision wound models.

Normal wound healing: Three groups comprising of control (were fed once daily with 0.8 ml of 2% gum acacia in distilled water), EEHS1 (fed orally with EEHS at a dose level of 400 mg/kg body wt.) and EEHS2 (fed once daily with EEHS at a dose level of 800 mg/kg body wt)
Dexamethasone treated groups: Dexamethasone was administered at the dose of 0.34 mg/kg body weight, i.m. on first day, there after regular dose of 0.17mg/kg, alternative days till the end of study period for control, EEHS1 and EEHS2 group.

Incision wound:

On the depilated back of the animals, two paravertebral incisions, 6 cm in length, one on either side were made, cutting through the full thickness of the skin. Interrupted sutures at 1 cm apart, were placed to approximate the cut edges of the skin[6]. The sutures were removed on the 7th post wounding day and breaking strength was measured on the 10th day by continuous water flow technique of Lee (Lee KH, et al., 1968).

Determination of wound breaking strength**:** - Rats were secured to the operation table and a line was drawn on either side of the wound 3mm.away from the wound. Two Allice forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated bottle through a string run over the pulley. Water was allowed to flow from the reservoir slowly and steadily into the bottle. As the water level rose in the graduated bottle ,the increasing weight of the bottle was transmitted to the wound site ,pulling apart the wound edges .Water flow was arrested when the wound was just opened and the volume of water collected in the bottle (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound and the procedure was repeated on the incision wound on the contralateral side. The average of six readings in one animal was taken as an individual value of breaking strength in that animal. Mean value of breaking strength of six animals gives the breaking strength of a given group.

Excision wound:

An excision wound was inflicted by cutting away a circular piece of 500 mm^2 full thickness of skin on a pre-determined area on the depilated back of the rat. Epithelization period was noted as the number of days after wounding, required for the eschar to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of the wound area on alternate days. This was achieved by tracing the wound on a 1mm^2 graph paper on alternate days. Reduction in the wound was expressed as percentage of the original wound size [5].

Statistical analysis

All the results were analyzed by one-way analysis of variance (ANOVA) and post hoc analysis was done using Scheffe's test. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Acute toxicity study shows the safety of the EEHS up to the maximum dose of 8g/kg. In incision wound model 400mg/kg and 800mg/kg EEHS (group II and III) showed the significant improvement in incision wound breaking strength as compared to control, done on 10th post wounding day at both sides of a rat (Table-1). There was significant reduction in incision wound breaking strength in dexamethasone treated control group as compared to control. Both the groups i.e. EEHS at two dose levels with dexamethasone (group V and VI) showed a significant improvement in incision wound breaking strength as compared to dexamethasone treated control group (group IV**)** **(**Table-1).

Table-1 – Effect of ethanol extract of *Hyptis* suaveolens in absence and presence of dexamethasone in incision and excision wound model.
[Values are mean± SD of 6 replications]

Treatment	Incision wound breaking strength (g)	Excision wound Epithelization period (days)
Control	211.6±7.25	23.0±1.2
Hyptis suaveolens- 400mg/kg ($EEHS_1$)	402.12±18.22*	12.3±3.7*
Hyptis suaveolens- 800mg/kg ($EEHS_2$)	381.34±13.42*	11.1±4.3*
Dexamethasone	97.43 ± 67.11	35.2±2.6
Dexamethasone + $EEHS_1$	273.65 ± 11.37£	18.4±0.4£
Dexamethasone + $EEHS_2$	237.33 ± 75.21£	15.8±1.33£

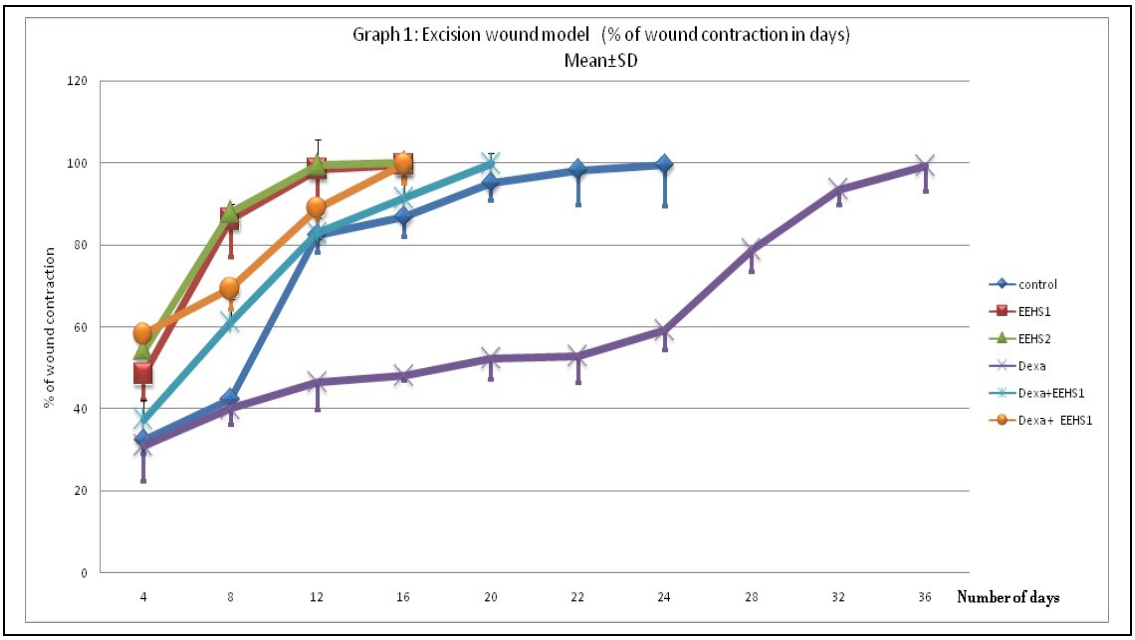
*P-value- significant if < 0.05 * significant difference as compared to control; £ significant difference as compared to Dexamethasone*

In excision wound model, the extract treated animal (group II and III) showed significant reduction in the epithelization period (Table-1) and increased percent of wound contraction (Table-2) as compared to controls. In dexamethasone treated control group significant increase in epithelization period and decreased percentage of wound contraction were observed when compared to controls. There was significant and effective reversal of dexamethasone mediated effects in above mentioned parameters with the extract treated groups (Table-1).

Table 2- Effect of ethanolic extract of *Hyptis* suaveolens on excision wound model
[Values are mean±SD of 6 replications]

Treatment	Excision wound model (% of wound contraction in days)								
	4	8	12	16	20	22	24	28	32
control	32.36±3.32	42.36±1.23	82.36±4.22	86.79±4.88	95.11±4.44	98.27±8.65	99.52±10.1	_	_
$EEHS_1$	48.34±6.3	86.11±9.12*	98.42±7.66*	99.45±3.44 *	_	_	_	_	_
$EEHS_2$	54.23±3.8*	87.78±2.25*	99.33±6.33*	_	_	_	_	_	_
Dexamethasone	30.93±8.56	39.98±3.98	46.49±6.76	48.01±1.11	52.17±5.11	52.75±6.5	59.05±4.65	78.67±5.19	93.44±3.84
Dexamethasone +$EEHS_1$	37.43±4.65£	61.11±5.54£	82.74±3.78£	91.31±7.43£	99.67±2.57£	_	_	_	_
Dexamethasone + $EEHS_2$	58.23±2.34£	69.23±4.87£	88.92±5.45£	99.78±4.76£	_	_	_	_	_

*P-value- significant if < 0.05 * significant difference as compared to control; £ significant difference as compared to Dexamethasone*
$EEHS_1$- ethanolic extract of Hyptis suaveolens at 400 mg/kg; $EEHS_2$ethanolic extract of Hyptis suaveolens at 800 mg/kg



The results of the present study clearly demonstrate that ethanolic extract of leaves of *Hyptis suaveolens* (L) poit**.** has a definitive healing activity on normal healing as well as steroid depressed wound healing. An increase in wound breaking strength points towards increase in collagen concentration and stabilization of fibres (Udupa A L, et al., 1995).

Incision and excision models evaluates the collagenation and its strength, scar maturation and wound contraction which has guided us to show the effectiveness of *Hyptis suaveolens* extract on clean cut wounds and debrided wounds. The results confirm the findings of the previous studies done by Shirwaikar Annie et al, 2003. In addition to the previous study, we intend to rule out its dexamethasone induced wound healing suppressant activity, our study showed significant reversal of effect of dexamethasone on collagen synthesis, maturation and organization. Thus it has potential for antagonizing the anti-healing effect of steroids in patients receiving steroid therapy; further studies are needed to confirm its beneficial effects in humans.

CONCLUSION

The present results provide strong evidence that the ethanolic extract of leaves of *Hyptis suaveolens* (L) poit. has a definitive wound healing activity; which confirms the findings of previous study and as well as reverses the steroid induced delay in wound healing.

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