

TISSUE - REGENERATIVE RESPONSES ON TUBER EXTRACTS OF *EULOPHIA EPIDENDRAEA* (RETZ.) FISCHER IN WISTAR RAT

M. Maridass¹, G.Raju² and S.Ghanthikumar³

¹Animal Health Research Unit, St.Xavier's College (Autonomous), Palayamkottai-627002,
²Dept. of Advanced Zoology and Biotechnology, PioneerKumaraswamy College, Nagercoil,
629 002, ³Centre for Biodiversity and Biotechnology, St. Xavier's College
(Autonomous), Palayamkottai-2, Tamil Nadu, South India.

E-mail: orchideyadass@yahoo.com

Summary

The wound healing activity of acetone tuber extract of *Eulophia epidendraea* in wistar rats demonstrated significant anti-infective and wound healing properties ($P < 0.05\%$). The epithelization period was shortest in crude extract treated wounded rats. However the reference drug exhibited higher potency against the crude extract.

Introduction

The skin is the largest organ of the body and its function is protective. Wound healing is complex series of interrelated events which combine to return the damaged tissue to as near normal condition as possible (3). The most common microorganism that cause wound infection is *Staphylococcus aureus* (9). Infected wounds heal more slowly and have an increased incidence of scarring (14). On a larger scale, infected wounds could severely compromise the overall health of individual. Therefore, the mechanisms of antibacterial protection are compromised when infection and tissue damage increase (14). Durodola and Abo *et al* demonstrated the effectiveness of crude extracts of some plants in inhibiting the growth of *Staphylococcus aureus* a major wound causing pathogen in *in-vitro* culture of the organisms (5, 1).

Orchidaceae is a highly evolved and widely distributed monocotyledonous family with a large number of terrestrial, saprophytic and epiphytic species. Orchids form 9% of our flora and about 1300 species are reported from India. More than 200 species are found in Peninsular India and Western Ghats possess most of the species. Some rare orchids occur with medicinal properties. Many orchids are used in traditional system of medicine as a remedy for a number of ailments. The orchid *Eulophia epidendraea* has been traditionally used by the local people of yadav community for the treatment of tumour, abscess and healing of wound. In the present study, wound - healing activity of tuber extract of *Eulophia epidendraea* (Retz.) Fischer was investigated in non-wounded inoculated and inoculated with *Staphylococcus aureus* on Inbred Wistar rats.

Materials and Methods

Solvent extraction

The tuber of *Eulophia epidendrea* (Retz.) Fischer was air-dried, and powdered. About 250g of the powder was extracted with acetone (56 °C) in a Soxhlet apparatus for 8h. The solvent was evaporated under reduced pressure. After determining the yield, extract was stored at 4°C until further use.

Test animal model

Adult male of inbred Wistar rats (250 ± 300g) were used. They were housed individually in cages, fed with standard rat pellets twice a day and water was allowed *ad libitum*.

Experimental wounding

The inbred Wistar rats were anesthetized with a 0.5ml intraperitoneal injection (i.p) of combination of diazepam and chloroform. After cleansing the dorsal area, sterile 8 mm punch was made to create a uniform sized (2cm x 2cm), full - thickness wound on the dorsal side of the rats. The wound was traced on a transparent plastic on the day of wounding and subsequently on alternate days until healing was complete. The size of the healed wound was expressed as the percentage of the wound area compared to the initial area of the wound (formation of regenerative of tissue).

Infected wounds

In order to obtain infected wounds, 10µl of a suspension containing approximately 5×10^8 colony forming unit (CFU) of *Staphylococcus aureus* as applied to the wound surface. The wound was traced on a transparent plastic on the day of wounding and subsequently on alternate days until healing was complete. Healing of wound size was expressed as the percentage of the wound area compared to the initial area of the wound (formation of regeneration of tissue).

Bacterial clearance rate

The quantification of *Staphylococcus aureus* inoculated rats beginning on the day of wounding healing day1, day7, day14 and day21, representative rats were euthanized. Their wounds were removed and homogenized in 1ml PBS for bacterial quantification. Wound homogenates were serially diluted (1:10), and plated on Muller- Hinton agar (MHA). Culture plates were incubated at 37±1 °C for 15 h. The bacterial colonies were detected and counted in log10 colony forming unit (CFU).

Topical treatment

Twelve animals were randomly divided into three sub-groups of four each. Crude acetone extract of *E. Epidendrea* (100mg / ml) was applied topically twice daily until complete epithelization had taken place. Gentamicin (10mg/ml) and normal saline were similarly applied as positive and negative control respectively.

Tissue regenerative studies

Animals were sacrificed on 28th day during long - term treatment. The skin was removed, washed with cold saline and preserved in 10% buffered formalin. The tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxylin and eosin (H&E) for histomorphology evaluation.

Data analysis

The data were represented as mean ± SE. and unpaired “*t*” test conducted by Stat view (Version 4. 5). *P* < 0.05 was regarded as significant.

Results

Wound contraction response in non - inoculated animals

A complete contraction of the wound was observed in the tuber-extract treated and inoculated wounded animals on day 21, which was statistically significant ($P < 0.01, 0.05, t$ test) than the control and reference (Gentamicin) groups. A similar response was observed in the reference group animals treated with Gentamicin (see Table-1)

Table 1: Effect of crude acetone extract of *Eulophia epidendrea* (Retz.) Fischer (Orchidaceae) on uninoculated wounds of wistar rats

Treatment	Wound area (cm)				Wound contraction (%)
	day 1	day 7	day 14	day 21	
Control	5.5 ± 0.334	4.63 ± 0.26	3.28 ± 0.33	2.25 ± 0.09	59.09
Extract	4.63 ± 0.18	2.55 ± 0.19	1.38 ± 0.42	0.00 ± 0.00	100
Gentamicin	5.2 ± 0.371	3.12 ± 0.29	2.06 ± 0.20	0.00 ± 0.00	100

Values are mean ± SE.

Wound contraction response in wound infected animals

The percentage of wound contraction response in the crude tuber extract treated and Gentamicin treated animals were observed for 21 days (Fig.13). Tuber extract had strong healing influence on the infected wounds when compared to control ($P < 0.05$). The percentage of wound contraction increased as a function of time of treatment. Extract treated and infected wounds showed maximum wound contraction.

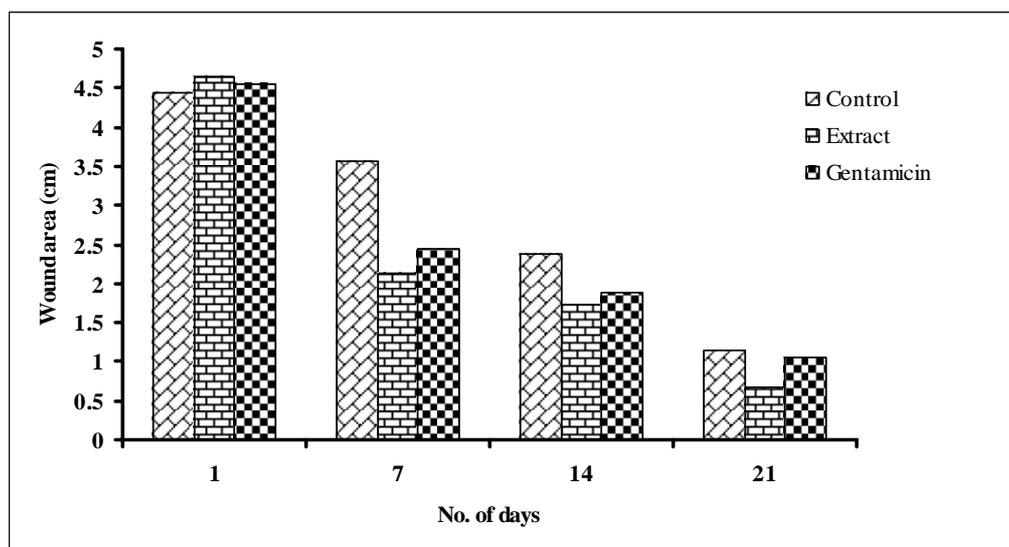


Fig.1: Wound contraction response in infected wound models of wistar rats

Bacterial clearance rate in Wistar rats

In order to assess the influence of tuber extract on the bacterial clearance response, bacteria in the wound were quantified during above experiments and represented in Fig.2. A maximum bacterial clearance response was observed in the inoculated and *E. epidendraea* extract - treated wounds in relation to the time of treatments. The total number of bacterial colonies were significantly reduced in both Gentamicin - treated ($P < 0.05$, *t*-test) and extract - treated wounds ($P < 0.05$, *t*-test).

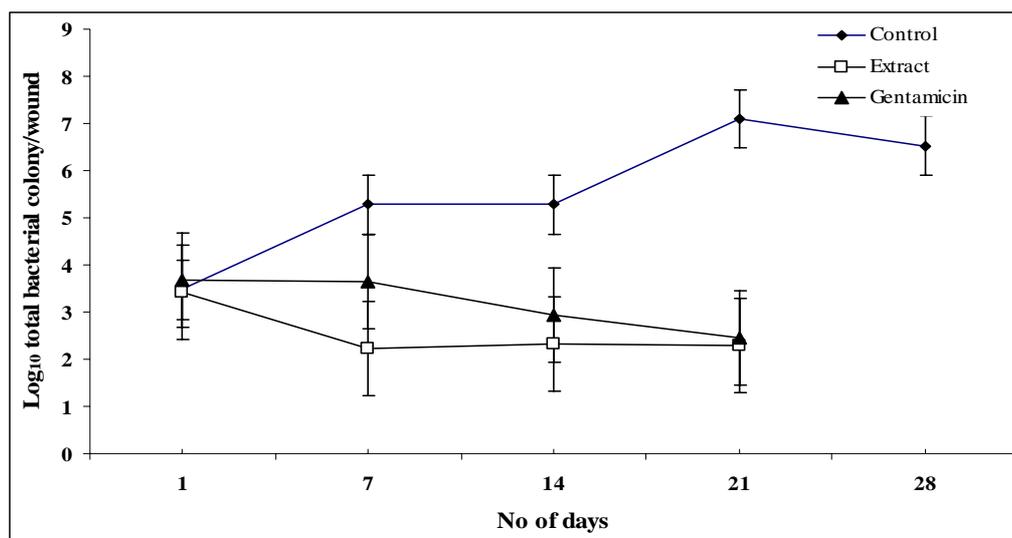


Fig. 2: Effect of *Eulophia epidendraea* tuber extract on the kinetics of bacterial clearance in the wounded Inbred Wistar rats

Tissue regenerative responses

Microscopical observation revealed that fibroblasts were generated on the 12th day along with the generative response of collagen fibrils, revascularization, and epithelial growth in the tuber - extract treated animals (Plate-1 A-F). However, in untreated groups these features were very poor and delayed.

Discussion

The present study revealed the wound-healing potential of *Eulophia epidendraea*, a plant medicine traditionally used in the treatment of wound in South India. A significant wound healing response was observed in the tuber extract treated rats. An optimum dosage (100mg/ml) was standardized and confirmed by the previous study with a significant wound – healing (1). The need for the study arose from the fact that we are faced with various wounds (12), but have few drugs that act directly on the healing process. *P. santalinus* is reputed to have wound-healing and other properties (4). Although its use to add flavor is permitted in the United States, adverse effects associated with therapeutic dosage are unknown (2). The present animal study showed no deaths or effects on the CNS after 72 hours. This permits the conclusion that at this dosage, *Eulophia epidendraea* preparation is safe.

Earlier studies with *P. santalinus* treatment promoted better wound contraction, and these wounds showed higher tensile strength. Tensile strength depended on the Van der Waals interaction among the hydrogen ion bonds of the triple helix collagen leading to twisting of the collagen fibers (6). The more twisting resulted in greater tensile strength, which caused better the healing. Also, an agent known to stimulate collagen could be expected to promote wound healing since collagen is principal component of any repaired tissue (8). There were reports (11) that crude betel nut (*Areca catechu*) extract as well as betel nut polyphenols stimulate collagen synthesis by mucous fibroblasts. *P. santalinus*-treated wounds had a mean tensile strength (421g) that reflects better collagen synthesis.

The wound healing effect of aqueous extract of latex of *Euphorbia neriifolia* has been studied in quinea pigs. Surgically produced cutaneous wounds were treated with topical application of 0.5 and 1.0% sterile aqueous solution of extract and it significantly facilitated the healing process as evidenced by increase in tensile strength, DNA content, epithelization and angiogenesis (13, 7). The multi-drug resistant *S.aureus* infected boils and wounds have been implicated to be susceptible to antibacterial agents containing amino-glycosides such as gentamicin, streptomycin and neomycin (15). In the present study, Gentamicin was used as a reference drug. Amino-glycosides act on the ribosome by interfering with mRNA attachment and thus inhibiting protein synthesis (15, 10). Therefore, it could be presumed that there are components containing amino-glycosides in the crude extract which may be responsible for the anti-infective action on the infected wound.

Conclusion

Therefore, it may be concluded that the tuber extract of *Eulophia epidendraea* had a stimulating effect in collagen synthesis thus justifying its traditional use. Further studies are in progress to ascertain the possible mechanism of the tuber extract on selected growth factors and various parameters of wound healing.

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