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Research article

EFFECT OF ETHANOLIC EXTRACT OF *PLECTRANTHUS AMBOINICUS* LEAF ON HEALING OF BURN WOUND IN WISTAR RATS

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ABSTRACT : Objectives: To study the effect of ethanolic extract of the leaf of *Plectranthus amboinicus* on the healing of burn wounds in wistar rats and compare it with silver sulfadiazine treated group.**Materials and Methods:** Five groups of rats were used in the study**.** Partial thickness burn wounds were made on each rat under ketamine anesthesia.The wounds in the five groups of rats were treated topically with petroleum base, silver sulfadiazine,1%, 2% and 3% ointment of ethanolic extract of *Plectranthus amboinicus*, respectively, once daily for 21 days or till complete healing whichever was earlier. The wound contraction rate and period of epithelization were monitored. **Results:** The rate of wound contraction was significantly more in *Plectranthusamboinicus*treated groups in comparison to the control. The mean period of epithelization was significantly decreased in *Plectranthus amboinicus* treated group when compared to control ($P < 0.01$) and silver sulfadiazine ($P = 0.02$) treated group. **Conclusion:** The ethanolic extract of *Plectranthusamboinicus*promoted healing of burn wound in wistar rats.
Keywords: *Plectranthusamboinicus*, burn wound, epithelization, wound contraction, healing.

INTRODUCTION

Burns are an important cause of emergency admissions (Turtayet al., 2010). Treatment of burn wounds is aimed at decreasing inflammation, preventing infection and scarring (Turtayet al., 2010). Various plants are used traditionally to promote burn wound healing. *Plectranthusamboinicus,* belonging to the genus *Plectranthus,* is used traditionally to treat a wide range of diseases. It is used in Brazil for the treatment of skin ulcerations (Lukhoba et al., 2006).The juice of the leaves is used in India for the treatment of skin allergies(Lukhoba et al.,2006). Studies have demonstrated its antioxidant (Patel et al., 2010), analgesic (Patel et al.,2010) and anti-inflammatory (Gurgel et al.,2009)effects in rats. It also possesses antimicrobial activity (Deena et al.,2002). The plant has been used in Malay to treat burns ((Lukhoba et al.,2006). A search of literature revealed that no scientific study has been done to evaluate the effect of *Plectranthus amboinicus* on healing of burn wound. Hence, this study was undertaken to assess the effect of ethanolic extract of the leaf of *Plectranthus amboinicus*on the healing of burn wound in wistar rats.

MATERIALS AND METHODS

Animals

Albino wistar rats of either sex weighing 180-200 g were selected. The experimental protocol was approved by Institutional Animal Ethics Committee.Each rat was housed singly in a polypropylene cage (U.N.Shah manufacturers, Mumbai) and provided with standard rat feed (Amrut lab animal feed, Pranav Agro industries Ltd., Sangli, Maharashtra) and water.The animals were maintained under standard conditions of temperature (23 ± 2)ºC , humidity of 50 ± 5% and 10-14 hour light and dark cycles.

Chemicals

Gum acacia was procured from Nice chemicals Pvt. Ltd (Mumbai, India), ketamine from Neon laboratories (Mumbai), silver sulfadiazine and petroleum base from Kasturba hospital pharmacy (Manipal).The leaves of *Plectranthus amboinicus* were obtained from a local shop and authenticated by Department of Pharmacognosy,MCOPS,Manipal.

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Preparation of ethanolic extract of *Plectranthus amboinicus* (Shanbhag et al.,2006). The dried leaves of*Plectranthus amboinicus,*weighing 500g, were soaked in absolute alcohol measuring 3 litres approximately, in a 5 litre round flask for about 24 hours. The process of extraction was done by reflux condensation at 60^0-80^0C for three hours. After cooling, the alcohol was drained through a muslin cloth into a conical flask following which 3 litres of fresh absolute alcohol was put into the flask containing the plant materials. The procedure of reflux condensation was repeated twice. The extract recovered from these three batches was concentrated under pressure by distillation till a syrupy consistency was obtained. Finally, the extract from three batches was mixed in a china dish and evaporated to dryness on a water bath. For topical application, an ointment of the extract was prepared using petroleum as a base.

Study design

Five groups, each with six rats, were used for the study.

Group I (control) – petroleum base, topical Group II (standard) – 1% silver sulfadiazine cream, topicalGroup III (test) - ethanolic extract of leaf of *Plectranthus amboinicus* 1% ointment, topical Group IV (test) - ethanolic extract of leaf of *Plectranthus amboinicus* 2% ointment, topical Group V (test) - ethanolic extract of leaf of *Plectranthusamboinicus* 3% ointment, topical. The drugs were administered topically daily from the day wound was made till 21st day or complete epithelization, whichever was earlier.

Burn wound

After overnight fasting, partial thickness burn wounds were made upon rats under ketamine anaesthesia (50mg/kg intramuscularly), by pouring hot molten wax (2g) at 80ºC through a metal cylinder with 300 mm^2 circular opening placed on the shaven dorsum of the rat. The wax was allowed to remain on the skin for 8 min for solidification. The metal cylinder with wax adhered to the skin was then removed (Bairyet al, 1997).The following parameters were observed in the study.

1. Period of epithelialization: It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind.

2. Wound contraction rate: It was noted by progressive changes in wound area excluding the day of the wounding. The wound was traced on a transparent paper every alternate day, throughout the monitoring period. The tracing was then transferred to 1 mm^2 graph sheet, from which wound surface area was evaluated. The calculated surface area was then used to calculate the percentage of wound contraction, taking the initial size of the wound, 300mm^2, as 100% by using the following formula

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Statistical analysis: The result was analysed using one way ANOVA followed by posthocTukey'stest.

RESULTS

Topical administration of *Plectranthus amboinicus*1, 2 and 3% significantly increased wound contraction rate on 4th, 8th, 12th and 16th day as compared to control (Table 1).

Table 1: Effect of ethanolic extract of *Plectranthus amboinicus* on contractionrate (%) of burn wound in rats

Group/drug	Wound contraction rate (%)
	Day 4 Day 8 Day 12 Day 16
I/petroleum base11.42±5.7420.81±8.3430.60±8. 1946.03±27.17	
II/silversulfadiazine23.59±14.8043.79±14.90*73.27±10.68*90.22±5.81*	
III/ 1% ointment35.26±9.47*46.59±3.52*80.92±20.85*95.95±3.92* Pamboinicus	
IV/2% ointment36.65±4.54*51.40±5.08*86.32±6.43**99.61±0.93**Pamboinicus	
V/ 3% ointment 38.51±5.38**56.12±11.16**87.19±2.40**99.38±0.74** Pamboinicus	
Values are expressed as Mean ± SEM ; n = 6 animals in each group; *P = 0.01 vs. control; **P < 0.03 vs. control; ANOVA followed by Tukey's test	

Values are expressed as Mean ±SEM ; n = 6 animals in each group *P = 0.01 vs. control; **p < 0.03 vs. control ANOVA followed by Tukey's test

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There was no significant difference between Plectranthus amboinicus and silver sulfadiazine treated groups on wound contraction rate

There was a significant (P<0.01) decrease in the period of epithelization in *P. amboinicus* treated groups as compared to control. The decreasein the time required for epithelizationin *P amboinicus* treated groups was also significant (P =0.02) as compared to silver sulfadiazine treated group (Table 2).

Table 2: Effect of ethanolic extract of *Plectranthus amboinicus*on period of epithelization of burn wound in rats.

Group/drug	Period of epithelization(days)
I/petroleum base	21.67±0.82
II/Silver sulfadiazine	20.00±0.89
III /Pamboinicus 1% ointment	17.67±1.63*
IV/P amboinicus 2% ointment	17.00±0.89*
V/ P amboinicus 3% ointment	15.67±1.63*,**

Values expressed as Mean ± SEM; n = 6 animals in each group ^{*p} < 0.01 vs. control, **P = 0.02 vs. silver sulfadiazine, ANOVA followed by Tukey's test

DISCUSSION

Healing of burn wounds involves infiltration of inflammatory cells, angiogenesis, granulation tissue formation, synthesis of extracellular matrix proteins, formation of collagen and remodeling (Kiran and Asad, 2008). Lysosomal enzymes from neutrophils, free radicals, leukotrienes and prostaglandins released during this process can cause tissue damage (Shuid et al., 2005). Lipid peroxidation plays a role in injuries like burns. *Plectranthusamboinicus* has been shown to have flavonoids and tannins (Kaliappan and Viswanathan, 2008). Flavonoids are known to have anti-inflammatory, antioxidant and wound healing properties.They are known to decrease lipid peroxidation by improvingvascularity and preventing or slowing down theprogress of cell necrosis (Nayak et al., 2006). They promote wound healing by their astringent and antimicrobial activity thus promoting wound contraction and hastening period of epithelization. Tannins promote wound healing by binding the free radicals thus promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts. This could be responsible for the promotion of healing of burn wound by *Plectranthus amboinicus*.In burn wounds, the skin is replaced by avascular tissue which can favor growth of microorganisms. Silver sulfadiazine has been the standard treatment for burns due to its antibacterial action ((Shuid et al., 2005). Studies have demonstrated the antimicrobial action of *Plectranthus amboinicus*. This property could be useful in prevention of infection of burn wound thus promoting healing.

In conclusion, the present study demonstrates that the ethanolic extract of leaf of *Plectranthus amboinicus* promoted healing of burn wound in wistar rats.

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