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WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL AND ROOT PART OF *LEPTADENIA PYROTECHNICA* (FORSK.) DECNE

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ABSTRACT

The ethanolic extracts of aerial and root part of *Leptadenia pyrotechnica* Plant(Family: Asclepiadaceae), ethnomedicinallyused for the treatment of wounds by the local people of Thar desert, were separately evaluated for wound healing activity on excision wound model, in Wistar albino rats, in the form of an ointment with a concentration (4 % w/w ointment) in simple ointment base. Framycetin sulphate cream (1% w/w) was used as standard drug. The rate of wound healing was assessed by the rate of wound closure and period of epithelialisation. Both the extracts showed significant response in wound healing when compared with the control group.

KEY WORDS- Wound healing, Epithelialisation, *and Leptadenia pyrotechnica*

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INTRODUCTION

Medicinal plants have been used since time immemorial for treatment of various diseases. Plants represent a large source of structurally novel compounds that might serve as leads for the development of novel drugs nutraceuticals and functional foods. There is an increasing interest in naturally occurring antioxidants to replace synthetic counterparts used for food preservation, flavoring, and cosmetics, as well as in health promotion. Polyphenolic substances, which are largely found in most plants, exhibit a wide range of biological effects including anti-inflammatory, anti-microbial and anti-cancer effects[1]. Wound healing, as a normal biological process in the human body is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling[2]. For a wound to heal successfully, all four phases must occur in the proper sequence and time frame. Many factors can interfere with one or more phases of this process, thus causing improper or impaired wound healing. Some common plants like *Aloe vera, Carica papaya, Centella asiatica, Cinnamomum zeylinicum, Cucurma longa, Plumbago zeylinica,Pterocarpus santalinus and Termanalia arjuna* have been reported in Ayurveda, Sidda and Unani systems of medicines for their wound healing potential [3]. *Leptadenia pyrotechnica* (Forssk.) Decne, commonly known as *khimp* belonging to the family Asclepiadaceae, is widespread in Indian desert, tropical Africa, Asia and the Mediterranean region and in the sandy plains in the Western Gulf countries. It is an erect, much branched, often leafless, erect shrub[4]. It is commonly used for the treatment of a variety of inflammation related disease including rheumatism, asthma, and tumors. Extracted milk of plant is used against psoriasis (skin disease). A survey of literature revealed that plant is ethnomedicinally used for the treatment of wounds by the local people of Thardesert[5]. The plant yields a fibre which is used in indigenous medicines as anantihistaminic and expectorant[6]..However, no scientific study on the wound healing activity of the plant has been reported to validate the folklore claims of this property. The objective of the present study was to evaluate the effect of ethanolic extract of aerial and root part of *Leptadenia pyrotechnica* on different parameters related to wound healing activity in Wistaralbino rats.

MATERIALS AND METHODS

Plant material

Aerial and root parts of the plant *Leptadenia pyrotechnica* were collected from the desert of Jodhpur region and identified and authenticated at Botanical Survey of India, Jodhpur by the botanist by Dr. P.J. Parmar. A voucher specimen No.847550 has been deposited in our laboratory for further reference.

Preparation of extract

Powdered aerial and root parts separately; extracted with petroleum ether (40-60^{o}C) and ethanol in the Soxhlet extractor. The ethanolic extracts were evaporated to *vacuo* and the extracts were subjected to phytochemical screening and wound healing activity.

Preparation of Ointment

The drug formulations with concentration of (4% w/w) were prepared, where 4 g of the extract was incorporated in 100 g simple ointment base I.P. Framycetin sulphate[7] cream (1% w/w) was used as standard drug, for comparing the wound healing potential of the extracts in animal model.

Animals

Healthy Wistar albino rats of either sex weighing 150-200g were used. They were housed in Polysulfone polycarbonated plastic rodent cage maintained at an ambient room temperature of (27±3ºC), 12 h light/dark cycle and fed with rodent diet of altromin pellet and distilled water ad libitum. The animals were used after an acclimatization period of seven days to the laboratory environment. All the experimental procedures and protocol used in this study were reviewed and approved by the Institutional

Animal Ethical Committee (IAEC), Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur (CPCSEA Reg.No. 541/02/C/CPCSEA)

Excision wound [8-12]

The wound healing activity was investigated in ether anaesthetized rats in excision wound models, for aerial and root extracts at concentration 4% w/w. The animals were divided into four groups of six animals each. The group I was considered as control (treated with simple ointment base IP), the group II was standard and treated with Framycetin sulphate cream (1% w/w). The group III animals were treated with 4% w/w ethanolic extract ointment of aerial part and the group IV animals were treated with 4% w/w of ethanolic extract ointment of root. Animals were anaesthetized with ether and shaved on part to be exposed. A circular piece (500 mm^2 area) was impressed on the dorsal thoracic region 5cm away from ears and 1 cm away from the vertebral column. The animals were individually housed in separate cages. The test and standard preparation were topically applied once a day till epithelialization was complete, starting from the day of the operation. The wounds were traced on 1 mm^2 graph paper on the day of wounding and then subsequently on the 4^{th}, 8^{th}, 12^{th} and 16^{th} post wounding days and thereafter on subsequent days till the healing was complete. The parameters studied were percentage of wound contraction or wound closure and period of epithelialization. Epithelialization time was noted as a number of days after wounding required for the scar to fall off leaving no raw wound behind.

STATISTICAL ANALYSIS

The results were expressed as mean ±SEM of six animals in each group. The data were evaluated by ANOVA followed by Dunnet's test and the values of $P<0.01$ were considered statistically significant.

RESULTS

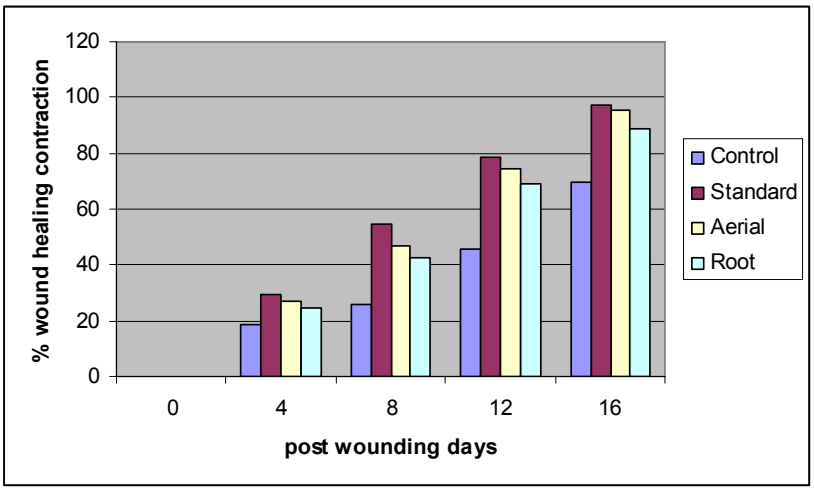
The effect of ethanolic aerial and root extract ointment on excision wound model showed wound healing activity significantly greater than that of control group. The 4 % (w/w) aerial extract ointment treated group showed significant wound healing from 8^{th} day onwards which was somewhat lesser than that of the standard drug *i.e*. Framycetin sulphate cream (1% w/w). The 4 % (w/w) root extract ointment treated group animals showed significant wound contraction from 12^{th} day onwards and achieved 100% with the wound closure time of 21± days (Table 1 and Figure 1).Aerial extract of the plant *Leptadenia pyrotechnica* showed better wound healing activity as compared to the root extract.

Table 1
Effect of ethanol extract ointment of aerial and root of Leptadenia pyrotechnica on % wound closure by excision wound method

Treatment	4^{th} Day	8^{th} Day	12^{th} Day	16^{th} Day	Period of epithelialization (days)
Control (Simple ointment base I.P.)	18.64 ±0.34	25.67±1.08	45.89±1.6	69.56±1.2	26 ±2.24
Framycetin sulphate cream (1% w/w).	29.65±0.13*	54.76±0.45*	78.56±0.5*	97.12±0.67*	18±1.36*
Aerial Ethanolic Extract (4%)	26.89±0.67	46.78±0.78*	74.39±1.8*	95.45±2.34*	20±0.58*
Root Ethanolic Extract (4%)	24.45±1.01	42.68±1.6	68.78±1.3*	88.64±0.98*	21±1.23

*Values are expressed as mean± SEM n=6 animals in each group P<0.01**

Figure 1
Graphical representation of % wound healing contraction with aerial and root extract of Leptadenia pyrotechnica on excision wound model



DISCUSSION

The present investigation describes some unique features of the aerial and root extract from the plant *Leptadenia pyrotechnica* with respect to its potential wound healing capacity in rats. Plant products are potential wound healing agents, and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations. Various activities were conducted in this study to evaluate the potential of *Leptadenia pyrotechnica* as a wound healing agent. One such activity is the phytochemical screening test. The phytochemical studies performed earlier reveals the presence of flavonoids, three terpenes; phytol, squalene and taraxerol, five sterols; cholesterol, campasterol, stigmasterol, β-sitosterol and fucosterol[13]. The constituents of the extract, such as terpenoids and flavonoids, may play a major role in the wound healing process observed in this study; however, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

CONCLUSION

It can be inferred from this study that the animals treated for wound healing activity with ethanolic extracts of the aerial and root part of the plant *Leptadenia pyrotechnica,* showed somewhat slow activity at the beginning compared to standard drug Framycetin Sulphate, but exhibited a promising wound healing activity on the subsequent days. Aerial part of the plant showed faster healing process than the root extract. The wound healing activity may be attributed to flavonoids and terpenoids which seem to be responsible for wound healing process mainly due to their astringent and antimicrobial property.

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