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WOUND HEALING ACTIVITY OF AQUEOUS EXTRACT OF *LEPTADENIA RETICULATA* IN WISTAR ALBINO RATS

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ABSTRACT: Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. *Leptadenia reticulata* belonging to the family Asclepiadaceae is an important medicinal plant which is used for various ailments. In the present study, an attempt was made to study the wound healing activity of aqueous extract of *Leptadenia reticulata* (AELR) using excision and burn wound models. In each model, animals were divided into three groups (n=6). Group I served as disease control group, received gel base, group II received AELR gel 10% and group III served as standard and received povidone-iodine 10% ointment. AELR gel 10% showed a significant ($P<0.01$) in both excision and burn wound models, which was comparable with the standard povidone iodine 10% ointment. Wound healing activity was also confirmed by histopathological studies. The present study suggested that topical application of aqueous extract of *Leptadenia reticulata* gel 10% plays an important role in wound healing activity. These finding could justify the inclusion of aqueous extract of *Leptadenia reticulata* in the management of wound healing.

INTRODUCTION: Wound is a type of injury in which the skin is torn, cut or punctured (an open wound) or where blunt force trauma causes a contusion (a closed wound) [1]. Wound healing is an intricate process where the skin (or another organ-tissue) repairs itself after injury. Wound healing is divided into three phases: the inflammatory phase, proliferative phase and remodelling phase. Inflammatory phase is characterized by increased blood flow, increased capillary permeability and increased migration of leucocytes into the affected area.

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Proliferative phase is characterized by granulation, contraction and epithelization. Remodelling phase determines the strength and appearance of the healed area [2].

Leptadenia reticulata belongs to the family Asclepiadaceae. It is commonly called as ‘jiwanti”. It is distributed in tropical and subtropical parts of Asia, Africa, Burma, Srilanka, Philippines and Madagascar. In India it is found in Gujarat, Punjab, Himalayan ranges, konkon, Nilgiris and Southern part of India [3]. Various pharmacological activities of this plant was reported which include hepatoprotective activity, anti-implantation activity, anticarcinogenic activity and anti-fungal activity [4].

The present study was designed to study the effect of aqueous extract of *Leptadenia reticulata* on wound healing activity.

MATERIALS AND METHODS:

Plant extract:

Aqueous extract of the whole plant of *L. reticulata* was procured from GR Herbals extractions (Dl. No. MP/25D/11/134) Indore, Madhya Pradesh.

Prelimnary Phytochemical studies:

The preliminary phytochemical screening of the aqueous extract of *Leptadenia reticulata* revealed the presence of sterols, triterpenoids, flavonoids, proteins and carbohydrate [5].

Experimental animals:

Wistar albino rats (150–200 g) were used for the present study. The rats were maintained on a standard pellet and water *ad libitum*. They were housed in polypropylene cages and maintained under standard conditions (12 h light-dark cycle; 23-25°C; 35-60 % relative humidity). All the experimental protocols for animal care procedures were approved by the ethical committee of Gokaraju Rangaraju College of Pharmacy. Principles of laboratory animal care guidelines were followed and prior permission was sought from the Institute Animal Ethics Committee (IAEC) for conducting the experiments. Present study was carried out in CPCSEA approved animal house (Reg. no. 1175/ac/08/CPCSEA) of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India.

Preparation of 10% gel of aqueous extract of *Leptadenia reticulata*: Simple 10% gel of aqueous extract of *Leptadenia reticulata* was prepared and applied daily once on the wound.

A. Excision wound model:

The animals were anaesthetized with ether, and their backs were shaved. An impression was made on the dorsal thoracic region 1cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. Excision wound of 500 mm^2 and 2 cm depth was created along the marked lines. The animals were then grouped and treated. Wound area was measured by tracing the wound on a millimeter scale graph paper on predetermined days *i.e* 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 days post wounding for determination of wound contraction. Falling of scar leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization. Healed wound was collected for histopathological examination [6].

B. Burn wound model:

The animals were anaesthetized with ether, and their backs were shaved. A brass bar was used to create the burn wound. The bar was heated in water bath at 100 ºC and placed on the rats back for 20 seconds to create the burn wound. The animals were then grouped and treated. Wound area was measured by tracing the wound on a millimeter scale graph paper on predetermined days *i.e* 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 days post wounding for determination of wound contraction. Falling of scar leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization. Healed wound was collected for histopathological examination [7, 8].

Evaluation of parameters for wound healing:

i. Measurement of wound contraction:

$$\% \text{ Wound contraction} = \frac{\text{Initial wound size - specific day wound}}{\text{Initial wound size}} \times 100$$

ii. Epithelization period:

The number of days required for the eschar to fall off from the wound surface exclusive of leaving a raw wound behind.

Histopathological examination:

A specimen of the skin from both the models was taken after the healing of the wound. The tissue was stored in 10% freshly prepared formalin solution and was sent to albino labs for histopathological studies.

Satistical analysis

All the results are expressed as mean ± SEM of six animals in each group. The data was evaluated using ANOVA followed by Dunnet's 't' test. A significant value of $p<0.01$ was considered satistically significant.

RESULTS:

In excision and burn wound models, a significant ($P<0.01$) increase in wound contraction and a significant decrease ($P<0.01$) in the period of

epithelization was observed in all the rats treated with AELR gel 10% when compared to the disease control group.

TABLE 1: PERCENTAGE OF WOUND CONTRACTION IN EXCISION WOUND MODEL

Compound	Percentage of wound contraction (%)							
	2nd day	6th day	10th day	12th day	14th day	16th day	18th day	22nd day
Disease control	10.0 ±0.3	27.6 ±0.5	47.4 ±0.4	58.2 ±0.4	67.4 ±0.3	76.2 ±0.2	85.5 ±0.4	96.7 ±0.1
AELR gel 10%	24.6 ±0.5*	62.3 ±0.4*	86.0 ±0.2*	95.5 ±0.2*	100 ±0.0	-	-	-
Standard povidone-iodine 10%	34.4 ±0.5*	79.3 ±0.4*	94.9 ±0.1*	100 ±0.0	-	-	-	-

Values are represented as mean ± SEM, *P<0.01 compared with the control

TABLE 2: PERIOD OF EPITHELIZATION IN EXCISION WOUND MODEL

S. no.	Compound	No of days (mean ± SEM)
1	Disease control	24.0±0.25
2	Test (AELR gel 10%)	13.8±0.40*
3	Standard (povidone-iodine 10%)	11.6±0.21*

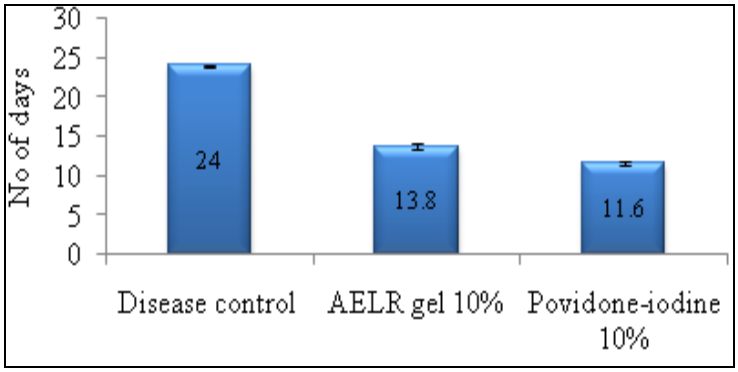


FIG.1: PERIOD OF EPITHELIZATION IN EXCISION WOUND MODEL

TABLE 3: PERCENTAGE OF WOUND CONTRACTION IN BURN WOUND MODEL

Compound	Percentage of wound contraction (%)							
	2nd day	6th day	10th day	12th day	14th day	16th day	18th day	22nd day
Disease Control	9.0 ±0.7	23.6 ±0.7	40.5 ±0.4	45.4 ±0.5	51.3 ±0.6	56.3 ±0.6	62.9 ±0.3	81.8 ±0.4
AELR gel 10%	21.3 ±0.7*	63.2 ±0.2*	86.4 ±0.1*	91.8 ±0.2*	97.7 ±0.1*	100 ±0.0	-	-
Standard povidone-iodine 10%	28.2 ±0.7*	72.2 ±0.4*	93.3 ±0.3*	100 ±0.0	-	-	-	-

Values are represented as mean ± SEM, *P<0.01 compared with the control

TABLE 4: PERIOD OF EPITHELIZATION IN BURN WOUND MODEL

S. no.	Compound	No of days (mean ± SEM)
1	Disease control	26.5±0.22
2	Test (AELR gel 10%)	15.1±0.16*
3	Standard (povidone-iodine 10%)	12.0±0.21*

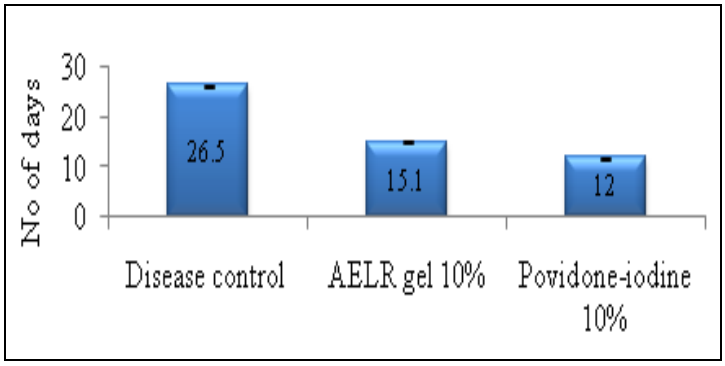


FIG.2: PERIOD OF EPITHELIZATION IN BURN WOUND MODEL

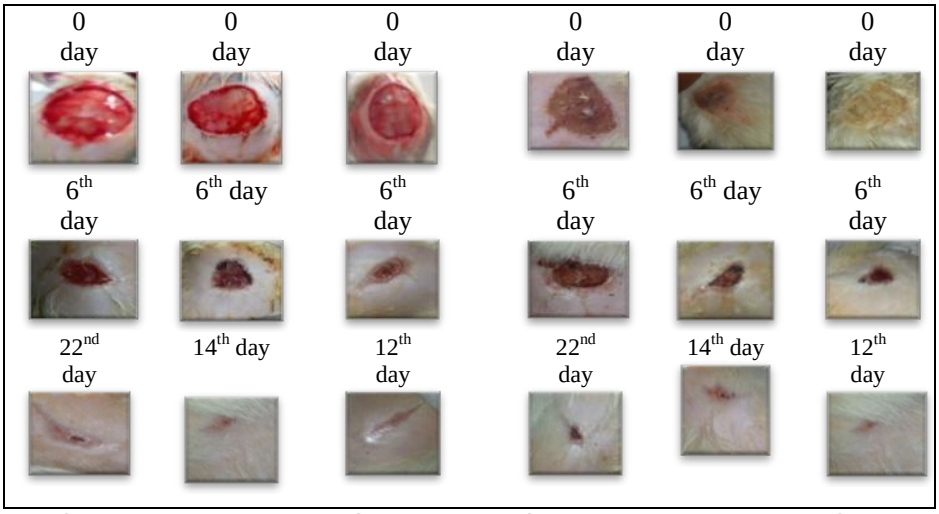


FIG.3: PHOTOGRAPHIC REPRESENTATION OF % OF WOUND CONTRACTION IN RATS.
A) Disease control B) AELR gel 10% treated rat skin C) Povidone-iodine 10%

Histopathological studies:

Histopathological studies provided further confirmation of the wound healing activity of the aqueous extract of *Leptadenia reticulata* by excision and burn wound models. Standard povidone-iodine 10% ointment showed prominent wound healing activity indicated by thicker epidermis due to hyperplasia of the epidermal cells and complete fibrosis in the dermis layer of the skin. The AELR gel 10% exhibited significant wound healing property compared to the disease control group which was evidence by moderate thickness of the epidermis layer and moderate fibrosis of the dermis layer.

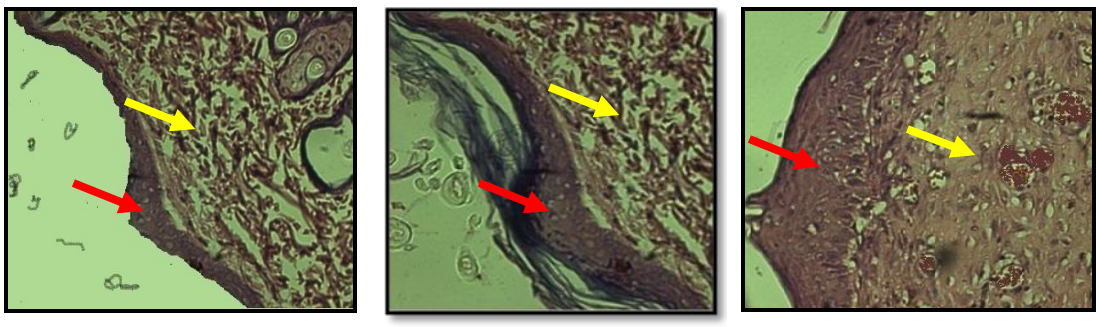
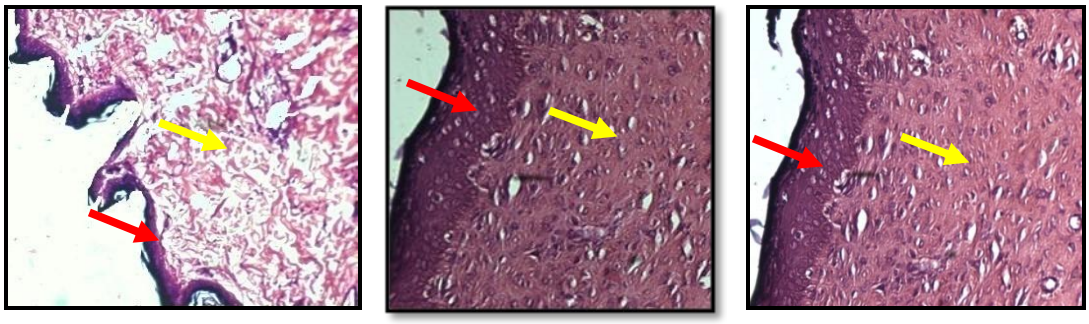


FIG.4: HISTOPATHOLOGICAL STUDIES OF THE HEALED RAT SKIN IN EXCISION WOUND MODEL
A) Disease control rat skin B) AELR gel 10% treated rat skin C) Povidone iodine 10% treated rat skin. Red arrow - epidermis layer, yellow arrow - dermis layer.



A. **B.** **C.**

FIG.5: HISTOPATHOLOGICAL STUDIES OF THE HEALED RAT SKIN IN BURN WOUND MODEL

A) Disease control rat skin B) AELR gel 10% treated rat skin C) Povidone iodine 10% treated rat skin. Red arrow - epidermis layer, yellow arrow - dermis layer

DISCUSSION: Wounds are clinical entities which are common in day to day life. The process of wound healing involves three phases *i.e*. inflammatory phase, proliferative phase and remodelling phase. These phases are concurrent but independent of each other. Any agent who accelerates this process is a promoter of wound healing. The present study describes the potential of AELR in wound healing activity using two models *i.e*. excision wound model and burn wound model.

The increased wound contraction in the AELR gel 10% treated group might be due to the enhanced activity of fibroblast which has been supported by the histopathological studies of the AELR tested rat skin. The comparative analysis revealed that AELR gel 10% and povidone-iodine 10% ointment has almost equal wound healing activity.

The earlier reports support the role of flavonoids, triterpenoids, steroids and proteins in wound healing activity. Since these chemical constituents are also present in AELR, may be responsible for wound healing activity [7].

CONCLUSION: Wound healing activity was performed using two models *i.e*. excision wound model and burn wound model. AELR gel 10% significantly stimulates the percentage of wound contraction and decrease the period of epithelization in both the models. Wound healing activity of AELR was confirmed by histopathological studies. These finding could justify the inclusion of aqueous extract of *Leptadenia reticulata* in the management of wound healing.

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