Evaluation of *Ehretia Laevis Roxb.* (Khandu Chakka/Ajan Vruksha) in the Wound Healing Adjudged by Histological Examination of the Tissue

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Abstract

Many herbal plants are mentioned in Ayurveda for wound healing. Folklore medicine for various ailments including wound healing is being practiced in India. *Ehretia Laevis Roxb*. contains many such chemical compounds useful for promotion of healing & repair. Tissue renewal goes hand in hand with inflammation influenced by multiple processes. *Ehretia Laevis Roxb*. called Khandu Chakka & Ajan Vruksha and traditionally being used for wound healing, body pain & minor fractures in the form of local application by folklore in the state of Maharashtra India. Objectives of the present study is to know the effect of *Ehretia Laevis Roxb*. in the wound healing on the histological parameters in animal model. Phenytoin ointment as a control was used. The present study has found put that leaf extract paste of *Ehretia Laevis Roxb.* has enhanced the process of wound healing ascertained on histological parameters as compared to topical phenytoin application. *Ehretia Laevis Roxb.* paste is found to be immensely useful at wound healing as compared to Phenytoin. *Ehretia Laevis Roxb.* is concluded to promote the healing significantly as observed by feature of granulation, collagenisation & re epithelisation of the wounds suggesting early healing by primary intension

***Key words**:- Ehretia Laevis Roxb., Phenytoin, Khandu Chakka, Ajan Vruksha, Wound healing , Histological study.*

Introduction

Many herbal plants are mentioned in Ayurveda for wound healing. Folklore medicine for various ailments including wound healing is being practiced in India. *Ehretia Laevis Roxb*. contains many such chemical compounds useful for promotion of healing & repair. These chemical compounds act as anti bacterial, anti fungal, anti viral, anti-inflammatory, anti parasite, analgesic, anti-oxidant, anti-apoptotic, clotting of blood plasma, improves immune responses, promotes collagen synthesis & regeneration of tissue. These properties are important for phenomenon of wound healing. Both of these processes can be mediated by external application of herbal extract of *Ehretia laevis Roxb*.[(1)]

Tissue renewal goes hand in hand with inflammation influenced by multiple processes. These processes includes the regeneration of cells by two underline processes such as wound healing by primary intension and wound healing by secondary intension.

Ehretia Laevis Roxb. called Khandu Chakka & Ajan Vruksha and traditionally being used for wound healing, body pain & minor fractures in the form of local application by folklore in the state of Maharashtra India. ([1])

Infections, old age, local tissue stress, metabolic disorder, diabetics, chemotherapy drugs, obesity, alcohol, habits such as chronic smoking, mal

nourishment influenced wound healing. Such situations require bearing of high expenditure on modern medicine drugs like expensive antibiotics.

There are not many studies in literature that has proven the role of *Ehretia Laevis Roxb*. in the wound healing, by histological evidences gathered by time graded biopsies collected from the site of wound after application of *Ehretia Laevis Roxb*. leaves paste.

Modern medicine uses Phenytoin for local application at enhancement of wound healing. [3]. Most of the studies reviewed for the present work & compared the results of Phenytoin with that of *Ehretia Laevis Roxb*.[3-9]

Study has been planned to evaluate & address their research gap involving histological evaluation.

Objectives of the present study is to know the effect of *Ehretia Laevis Roxb*. in the wound healing on the following parameters, that unable to judge the processes of wound healing secondary experimental burn into the Albino Rabbits.

· Quantification of granulation tissue after application of Ehretia Laevis Roxb. compared to Phenytoin paste application.

· Gradation of inflammatory infiltrates after application of paste of Ehretia Laevis Roxb. compared to Phenytoin.

· Quantification & pattern of collagen after application of paste of *Ehretia Laevis Roxb*. compared to Phenytoin.

Review of Literature

Drug Review:-

Ehretia laevis Roxb. known as Khandu Chakka & referred to Sant Dnyaneshwar Alandi Maharashtra, India too called as Ajan Vruksha[10], when he took Sanjivan Samadhi. Folklore believes this plant for its plenty of medicinal healing properties. [1]

Plant Description:- Following is the binomial nomenclature for the plant in the botany.

(Figure 1 & Figure 2)

Planta Tracheophyta Magnoliopsida Boraginales Boraginaceae

Ehretia Ehretia Laevis (Roxb)

Figure 1 **Figure 2**

***Ehretia Laevis Roxb*. plant shows anti microbial activity on salivary microflora , B.subtilis , S. aureus, E. Coli. Pseudomonas aeruginosa[11-14]**

Materials and Methods

Study Material:

- Healthy 10 Rabbits were selected from DMIMS animal house. Then they were divided into two groups of 5 in each group.

- Leaves paste was prepared under all aseptic precautions. Leaves paste was applied on wound in first group and considered as Group A.

- Phenytoin ointment as a control was used in second group and considered as Group B.

- Animals were anesthetized before creating wound

- The skin shaved, disinfected with 70% alcohol and injected with 1 ml of Lignocaine HCl (2%, 100 mg/5 ml).

- Approval from institutional animal ethical committee was taken.

External wound protocols

· Physically external wound was made by burn injury of 2X2cm. The edges of the wound was sampled by forcep biopsy.

· Daily leaves paste was applied in Group A and Phenytoin ointment in group B. Biopsies for assessment of healing & repair were taken on 7^{th} and on 15^{th} day.

Assessment of biopsy from wound site was done by following histological features.

- Epithelialization
- Leucocytic infiltration
- Vascularity
- Fibroblastic cell proliferation
- Appearance of granulation tissue

Parameters to calculate healing score [15]

Histological Parameter

The following histological parameters were used to calculate healing score.

1. Quantity of granulation tissue (profound -1, moderate – 2, scanty-3, absent-4)

2. Inflammatory infiltrate(plenty -1, moderate – 2, a few- 3)

3. Collagen fiber orientation (vertical -1, mixed -2, horizontal-3)

4. Pattern of collagen (reticular -1, mixed-2, fascicle-3)

5. Quantity of early collagen (profound-1, moderate -2, minimal-3, absent-4)

6. Quantity of mature collagen (profound-1, moderate -2, minimal-3)

Histological assessments of wound were Semi Quantitative and Quantitative methods as described below.

· Semi-quantitative method:- Wound reepithelialisation,Migration of keratinocytes, Bridging of cells, keratinisation, Inflammatory cells: absence/ Presence: (mild/ moderate/ marked), Fibroblasts: absence/ presence(mild/ moderate/ marked),New vessels; absence/ presence(mild/ moderate/ marked),Collagen: absence/ presence(mild/ moderate/ marked)

· Quantitative method:- Polymorpho nuclear leucocytes/ tissue macrophages ratio, Percentage of reepithelialisation, Area of the granulation tissue

Results

The following observations were made for healing score & other histological parameters in conclusion of process of healing.

Table 1 Shows the Healing score of comparison between *Ehretia Laevis Roxb*. and Phenytoin for comparison over the time grade of 0, 7^{th}, 15^{th} day

Table:-1 Histological Healing Score

Day	Date of burn injury 0 day		Biopsy done on 7th day		Biopsy done on 15th day	
Parameters	**Group A Ehretia Laevis Roxb.**	**Group B Phenytoin**	**Group A Ehretia laevis Roxb.**	**Group B Phenytoin**	**Group A Ehretia laevis Roxb.**	**Group B Phenytoin**
Quantity of granulation tissue (profound -1, moderate – 2, scanty-3, absent-4)	4	4	2	3	4	4
Inflammatory infiltrate(plenty -1, moderate – 2, a few- 3)	3	3	2	3	3	3
Collagen fibre orientation (vertical -1, mixed -2, horizontal-3)	-	-	2	2	3	2
Pattern of collagen (reticular -1, mixed-2, fascicle-3)	-	-	2	2	2	2
Quantity of early collagen (profound-1, moderate -2, minimal-3, absent-4)	-	-	1	2	1	1
Quantity of mature collagen (profound-1, moderate -2, minimal-3)	-	-	2	3	1	2

Description of Table-1

It is seen from table one, the *Ehretia Laevis Roxb*. as compared to Phenytoin application works well for the synthesis of collagen fibres & its orientation. The quantity however of mature collagen was high on the 15th day by Phenytoin as compared to the group of Albino Rabbits applied with *Ehretia Laves Roxb*. There are not many difference noted for scar on the day 7 of biopsy.

The semi quantitative & quantitative histological parameters assessed are shown in Table-2

Table-2 Assessment of other semi quantitative & quantitative histological parameters

Semi-quantitative method	Date of burn injury 0 day	Biopsy done on 7th day		Biopsy done on 15th day	
Parameters	-	**Group A Ehretia Laevis Roxb.**	**Group B Phenytoin**	**Group A Ehretia Laevis Roxb.**	**Group B Phenytoin**
Wound reepithelialisation;	-	Maximum	Minimum	Maximum	Maximum
Migration of keratinocytes,	-	Present	Present	Absent	Absent
Bridging of cells, keratin	-	Present	Present but feeble	Present	Present
Inflammatory cells: absence/ Presence: (mild/ moderate/ marked)	-	Mild	Moderate	Absent	Absent
Fibroblasts: absence/ presence	-	Present	Present	Rare	Absent
New vessels; absence/ presence	-	Present	Present	Absent	Absent
(mild/ moderate/ marked)	-	Moderate	Mild		
Collagen: absence/ presence (mild/ moderate/ marked)	-	Present Marked	Present Moderate	Present Moderate	Present Mild
Quantitative method					
Polymorpho nuclear leucocytes/ tissue macrophages ratio	-	Maintained Normal	Normal	-	-
Percentage of reepithelialisation	-	85%	80%	98%	92%
Area of the granulation tissue	-	Present	Occasional y present	Absent	Absent

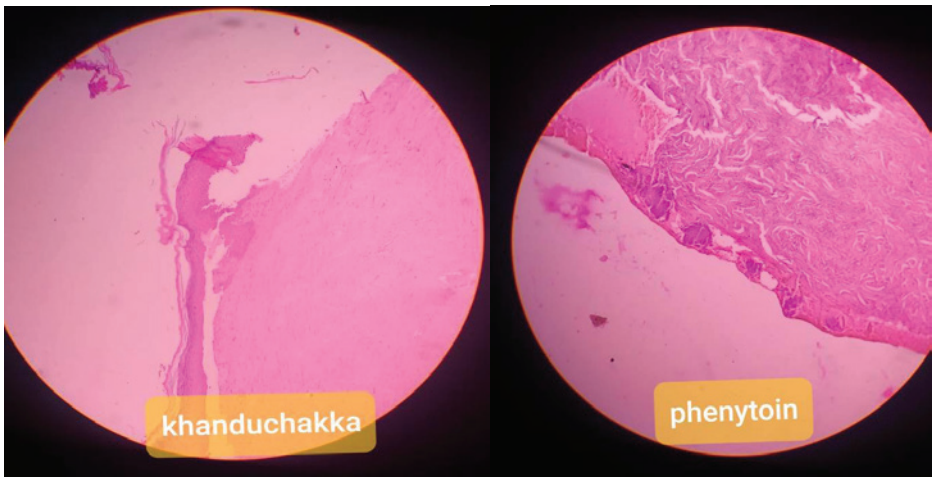
The overall epithelisation on the 15th day is much better in group of *Ehretia Laevis Roxb*.(98%) as compared to application of Phenytoin (92%). The presence of collagen was moderate on 15th day in the group of rabbits receiving *Ehretia Laevis Roxb*. as compared to group B receiving Phenytoin.

Histological changes for comparison between *Ehretia Laevis Roxb.*& Phenytoin on 7th day & 15th day have been shown in Figure- 3 A & B and Figure- 4 A & B. Figure- 4 A & B- Comparative Photographs of histology (15th day)

Figure 3:- Comparative photomicrographs of histology (7th day)

Group A:- Ehretia Laevis Roxb. Group B:- Phenytoin

Figure 3-A (Ehretia Laevis Roxb). Figure 3-B (Topical Phenytoin)



(H & E 10X)

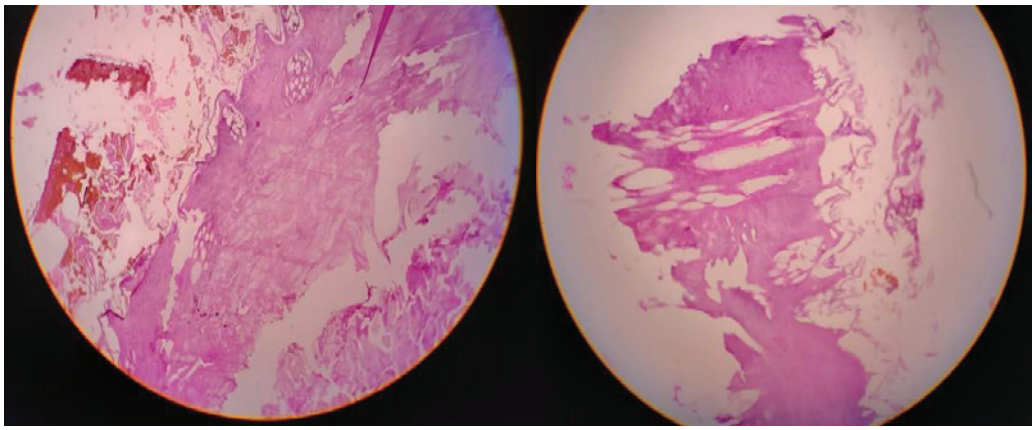
Figure 3-A Shows early epithelisation & appearance of sub epithelial fibroblast

Figure 3-B- Shows epithelisation & persistent granulation tissue.

Figure 4:- Comparative photomicrographs of histology (15th day)

Group A :- Ehretia Laevis Roxb. Group B:- Phenytoin

Figure 4-A(Ehretia Laevis Roxb) Figure 4-B(Topical Phenytoin)



(H & E 10X)

Figure 4-A- Complete epithelisation & proper organisation of mature collagen

Figure 4-B- Epithelisation marred incomplete dermal collagenosis.

Discussion

Part -I

The wound healing property has been documented. The similar observation were made in the studies but for the other drugs.

In another group Phenytoin ointment was taken, which promotes wound healing. Histological assessment was done on 7th and 15th day.

Granulation tissues were absent on date of injury and at fifteenth day from date of injury in both cases i.e. in group A and group B. Granulation tissues were

moderate in group A and scanty in group B on seventh day. Hence granulation enhances by *Ehretia Laevis Roxb.*plant compared to Phenytoin.

Inflammatory infiltrates were few at the date of injury and on fifteenth day from date of injury in both groups. Inflammatory infiltrate was few in group B and moderate in group A. Inflammatory infiltrate is more by *Ehretia Laevis Roxb.*plant as compared to Phenytoin.

Collagen fibre orientation was nil at date of injury in both groups, mixed on seventh day in both groups and on fifteenth day, horizontal in group A and mixed in group B.

Pattern of collagen was nil at the day of injury and mixed on the seventh and fifteenth day in both groups.

Quantity of early collagen was nil at the day of injury, profound in group A and moderate in group B on seventh day. Quantity of early collagen was profound in both groups on fifteenth day. Quantity of early collagen is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Quantity of mature collagen was nil at the day of injury, moderate in group A and minimum in group B on seventh day. Quantity of mature collagen is profound in group A and moderate in group B on fifteenth day. Quantity of mature collagen is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Part II: - As per semi-quantitative method and quantitative assessment:-

Wound reepithelialisation nil at the day of injury, maximum in group A and minimum in group B on seventh day and maximum in both groups on fifteenth day. Wound reepithelialisation is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Migration of keratinocytes nil at the day of injury, present in both groups on seventh day and absent in both groups on fifteenth day.

Bridging of cells keratin nil at the day of injury, present in group A and present but feeble in group B on seventh day and present in both groups on fifteenth day. Bridging of cells keratin is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Presence of inflammatory cells is nil at the day of injury, mild in group A and moderate in group B on seventh day and absent in both groups on fifteenth day. Presence of inflammatory cells is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Fibroblasts is nil at the day of injury, present in both groups on seventh day and rare in group A and absent in group B on fifteenth day.

New vessels is nil at the day of injury, moderate in group A and mild in group B on seventh day and absent in both groups on fifteenth day. Formation of new vessels is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Collagen is nil at the day of injury, present marked in group A and present moderate in group B and present moderate in group A and present mild in group B on fifteenth day. Formation of Collagen is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Part III- Quantitative assessment:-

Polymorpho nuclear leucocytes/ tissue macrophages ratio is nil at the day of injury, maintained normal in group A and normal in group B on seventh day and nil in both groups on fifteenth day.

Percentage of reepithelialisation is 85% in group A and 80% in group B on seventh day and is 98 % in group A and 92% in group B on fifteenth day. Percentage of reepithelialisation is significant by *Ehretia Laevis Roxb.* as compared to Phenytoin.

Area of the granulation tissue is present in group A and occasionally present in group B. And it is absent in both groups on fifteenth day. Area of the granulation is significant by *Ehretia Laevis Roxb.* as compared to Phenytoin.

Hence wound healed more significantly by *Ehretia Laevis Roxb.*Plant leaves paste as compared to application of Phenytoin ointment.

Anti microbial activity of plant useful in wound healing and promote healing. Anti oxidant activity plan promote the growth of tissue in wound healing. Hence both these activities help in wound healing.

The study of Sushma et al. has observed the role of natural medicine in wound healing on the basis of bio mechanical histological, biochemical, and molecular studies. However their studies included natural medicine of honey, ghee, Glycyrrhiza glabra, Nerium indicum. Histological analysis with all these application were found to have increase the rate of wound healing, rapid epithelisation, the normal collagenisation in dermis. The limited inflammatory cell response adjudged by ***Interleukin***-1β & enhances activity of myo-fibroblast & small blood vessels. The study has concluded that these natural traditional medicine brings enhances wound healing activity as observed in histological material. However the study of Sushma et al did not have group of sample, those were treated by *Ehretia Laevis Roxb.* [4]

Al-Henhena N et al evaluated topical application of Strobilanthes crispus ethanolic extract on the rat of wound closer & histology of healed wound. It was observed that group 3 & group 4 were. Strobilanthes crispus was used in 100 mg/ml & 200 mg/ml of ethanolic extract were found to have mean healing time 14.80 ± 0.37 days and 13.00 ± 0.37 days respectively. Histological evaluation when compared to control group had average healing time of 20.6 ± 0.37a days. The study concluded that Strobilanthes crispus leaf extract enhances wound healing as ascertained by histological study. [5]

The present study has found put that leaf extract paste of *Ehretia Laevis Roxb.* has enhanced the process of wound healing ascertained on histological parameters as compared to topical phenytoin application. There are not many studies that have experimental with *Ehretia Laevis Roxb.* leaves paste for the objective assessment for its property of enhance wound healing.

Conclusion

Ehretia Laevis Roxb. paste is found to be immensely useful at wound healing as compared to Phenytoin. *Ehretia Laevis Roxb.* is concluded to promote the healing significantly as observed by feature of granulation, collagenisation & re epithelisation of the wounds suggesting early healing by primary intension.

Ethical Clearance- Taken from institutional ethical committee of DMIMS(DU), Wardha

Source of Funding- Self

Conflict of Interest:- Nil

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