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WOUND HEALING ACTIVITY OF STEM BARK OF *TABERNAEMONTANA HEYNEANA* (WALL.)

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ABSTRACT

Introduction: Folk drugs represent a wealth of traditional knowledge passed down through generations. Many folk remedies have been used for wound healing over centuries. The study and documentation of these remedies are essential for preserving traditional knowledge, validating and potentially discovering new therapeutics, and enhancing treatment options. With the rise of antibiotic resistance and the need for more effective wound care solutions, traditional remedies can offer alternative strategies. *Tabernaemontana heyneana* (Wall.), belonging to Apocynaceae is one such potential herb mainly used by the folklore practitioners of South Canara and Udupi region of Karnataka effectively in the management of wound. The present study was undertaken to evaluate the wound healing activity of the stem bark of *Tabernaemontana heyneana* (Wall.) in Wistar albino rats. **Methods:** The wound healing activity of bark was studied in Excision wound model in rats. The excision wound was made on the dorsal aspect of the rats, measuring an area of 1 cm. The Wistar albino rats were randomly assigned to three distinct groups and administered group-specific medications for a duration of 24 consecutive days. Wound contraction, eschar fall and complete cure day were observed, histopathological and hydroxy proline parameters were carried out on 25th day to assess wound healing activity. **Results:** In experimental study, percentage of wound contraction and period of

epithelialization showed statistically significant when compared to standard and control. The histopathological study revealed the test group with good re-epithelialization. **Conclusion:** The experimental study results suggests that *Tabernaemontana heyneana* (Wall.) bark is effective in wound healing compared to Control and Standard group.

KEYWORDS: *Tabernaemontana heyneana* (Wall.), Excision wound, Wound healing.

INTRODUCTION

According to Ayurveda, there exists no herbal drug devoid of medicinal attributes,[1] indicating a strong emphasis on the pursuit of new drug discoveries, particularly as there are countless herbs yet to be explored. Extensive knowledge regarding numerous medicinal plants is documented in Ayurvedic texts and has also been validated through scientific research. Some plants, rooted in folklore, are not referenced in classical Ayurvedic literature; however, they are traditionally employed to treat various ailments and are referred to as *Anukta Dravyas* or Extrapharmacopoeial plants. The identification and recognition of these *Anukta Dravyas* has emerged from various ethnomedicinal survey studies.

The field of wound healing has a rich historical background that spans many centuries. Ancient Ayurvedic texts have consistently highlighted the importance of addressing wounds, whether they arise from imbalanced doshas or traumatic incidents. From the time of Acharya Sushruta in ancient India, the treatment of wounds became a significant concern, particularly due to the prevalence of battles and conflicts. Acharya Sushruta, a distinguished surgeon of his time, not only introduced foundational surgical principles but also laid the groundwork for modern plastic surgery, demonstrating remarkable foresight in the art of wound healing. Today, wounds are a common issue encountered in everyday medical practice, attracting the interest of numerous researchers globally. Despite significant advancements in surgical techniques, the management of wounds continues to be a topic of ongoing inquiry.

The plant *Tabernaemontana heyneana* (Wall.), commonly known as *Maddarasa* in Kannada belongs to the family Apocynaceae.[2] It is a small deciduous tree distributed in Konkan and Western ghats.[3] The stem bark of this plant is used to treat wounds by the folklore *Vaidyas* of South Canara and Udupi district of Karnataka. Furthermore, different parts of this plant have been reported to possess properties like Reno protective, antioxidant, antimicrobial, anti-cancerous and anti-cholinergic effects.[4]

This study aims to evaluate the wound healing efficacy of the folk drug *Tabernaemontana heyneana* (Wall.) in Wistar albino rats, utilizing an excision wound model. The fresh stem barks of *Tabernaemontana heyneana* (Wall.) are collected, dried and ground into powder form. This powder is then combined with adequate amount of distilled water and made into *Lepa* form. This *Lepa* is applied to the excision wounds inflicted on the test groups of Wistar albino rats. The effectiveness of the test drug was evaluated through various observational parameters, including the measurement of complete epithelialization area, percentage of wound contraction, histopathological analysis and hydroxyproline levels in the wound tissue.

MATERIALS AND METHODS

Collection of Samples

The stem bark of *Tabernaemontana heyneana* (Wall.) was collected from Udyavara and Udupi region and authenticated in the department of Pharmacognosy, S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi. The barks were shade dried and made into fine powder. The obtained powder form of the drug was stored in air tight container for further use.

Preparation of *Lepa*

The prepared fine powder of the stem bark of *Tabernaemontana heyneana* (Wall.) was taken in a Petri dish and quantity sufficient distilled water was added to make a thick paste. Each day, fresh *lepa* was made with the stem powder for the studies.

Drugs

Test drug-*Tabernaemontana heyneana* (Wall.) stem bark powder

Standard drug-Povidone iodine

Experimental animals

Wistar albino rats were selected from animal house of SDM Centre for Research in Ayurveda and Allied sciences, Udupi. The experimental protocol was approved by the Institutional Animal Ethical Committee.

Rats were fed with normal diet and water ad libitum throughout the study. They were acclimatized in the laboratory condition for two weeks prior to experimentation. The experimental rats were maintained at 12:12 light and dark cycle. Temperature of 25ºC and relative humidity of approximately 50%.

Inclusion criteria

Healthy albino rats of either sex weighing about 150g-250 g were included in the study.

Exclusion criteria

Rats weighing less than 150g and more than 250 grams.

Rats subjected to other experiments.

Diseased and pregnant rats.

Grouping of Animals

Table No. 1: Grouping of Animals for the Experimental study.

SI.No.	Group	No. of Rats	Drug	Purpose
1	Control	Six	No drug application	• To observe the changes occurring in the excised wound • To compare with the wound healing process of other groups
2	Standard	Six	Povidone iodine	• To assess the process of wound healing
3	Test	Six	Lepa of Stem bark powder of *Tabernaemontana heyneana* (Wall.)	• To assess the process of wound healing

Route of administration- External application

Study duration-24 days

Experimental procedure

The experimental study was carried out to know the efficacy of the *lepa* prepared from the stem bark powder of *Tabernaemontana heyneana* (Wall.) in excision wound model.

The Excision wound model technique comprises of three stages;

- ✓ Pre-operative stage
- ✓ Operative stage
- ✓ Post operative stage

Pre-operative stage: A total of 18 Wistar albino rats were selected and categorized into three groups, each consisting of six rats. Group 1 which did not receive any drug treatment, considered as normal control group. Group 2 which was applied with povidone iodine was considered as standard group. Group 3 applied with *lepa* of stem bark powder of

Tabernaemontana heyneana (Wall.) was considered as test group.

Operative stage: Excision wound method

The dorsal thoracic area was prepared by shaving with a trimmer one day prior to the excision procedure.[5] A stainless-steel stencil was used to mark the area designated for excision. The animals were anesthetized with Ketamine at a dosage of 50 mg/kg intraperitoneally, and Xylazine at 3 mg/kg intramuscularly. A surgical incision was made on the dorsal thoracic region, I cm away from the vertebral column and 5 cm away from the ear of the anesthetized rat. The skin of impressed area was excised to its full thickness, in order to obtain a wound area of about 1 cm.

Post operative stage: The external application of freshly prepared *Lepa* was done by mixing the stem bark powder of *Tabernaemontana heyneana* (Wall.) with distilled water, ensuring an adequate quantity to cover the wounds of all six rats in the test group. The standard drug, Povidone iodine was applied to the rats of standard group. The rats of the control group were not applied with anything and observed for natural healing, by providing normal diet and water ad libitum.

In order to observe the changes in wound shapes, the margins of the wounds were traced on tracing paper from the day of injury until the wounds were completely healed. This trace was retraced on a milli meter scale graph paper. The percentage of wound closure was recorded on the 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, 18^{th}, 21^{st} and 24^{th} days following the wounding. These wounds were monitored for the signs of epithelialization also. The animals were inspected daily and their health was evaluated based on various physical parameters, including the progression of wound contraction and epithelialization.

Wound contraction

The primary factor influencing wound healing is wound contraction. This was done by tracing the wound margins on transparent OP sheets with a marker, which were then redrawn on a milli meter scale graph paper. The data was subsequently analysed to determine the percentage of the original wound size for each animal in the group, based on the duration required for complete wound contraction.

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

Period of epithelialization

Eschar fall, without leaving any raw wound behind, was considered as the sign of complete epithelialization; and the duration required for this process was defined as the epithelialization period.

On the 24th day, three rats from each group were randomly selected, and the skin tissue was carefully excised for histopathological analysis.[6]

The samples were preserved in 10% formalin for examination.

For hydroxyproline content estimation, the samples were stored in normal saline at -20ºC.[7]

The results obtained from the test groups were compared with those from the standard and control groups.

Statistical analysis of the hydroxyproline data was done using one-way ANOVA, followed by Dunnett's multiple comparison "t" test as a post hoc analysis, with significance set at $p<0.05$, utilizing Graph pad prism version 3.0 software.

RESULTS AND DISCUSSION

Effect on Wound contraction

On 3rd post wounding day, the control group exhibited a wound contraction rate of 25.4%. There was decrease in wound contraction in standard and test group when compared to control group. The observed increase in wound contraction of test group when compared to control was found to be statistically significant.

On 6th post wounding day, the control group exhibited greater wound contraction than that of the test and standard group.

On 9th post wounding day, the percentage of wound contraction increased in test group than that of standard and control. But, the increase in test group was statistically non-significant when compared to control.

On 12th post wounding day, the control group exhibited more wound contraction and the test group was found to be a non-significant increase. The wound contraction of the standard was statistically very significant when compared to control.

On 15th post wounding day, in the test group 94.82% reduction in wound area was observed. When compared to control, the observed increase was found to be statistically significant.

On 18th post wounding day, the test group was found to have increased wound contraction when compared to other Group. The reduction in wound area was 95.21%, which was statistically non-significant.

On 21st post wounding day, in control group there was 96% reduction in wound area and in test group there was 98.53% reduction. The wound contraction of the test group when compared to control was statistically non-significant.

On 24th post wounding day, there was an increase in wound contraction in standard, 99%. The wound contraction in test group was 99.88%, which was statistically significant when compared to control group.

Effect on Complete epithelialization

In case of the duration required for complete epithelialization or the days taken for the complete healing of the wound, was shorter in both the standard and test groups when compared to the control group. The differences observed were statistically non-significant between standard and control group and test and control groups.

Effect on Eschar fall

Eschar refers to a layer of dead tissue that is shed from the skin's surface following an injury. It serves as a natural protective barrier against infection. Typically, eschar detaches from healthy skin autonomously. In this study, the detachment of eschar occurred earlier in the test group, specifically on the ninth day, in contrast to the control and standard groups, which experienced detachment in thirteenth and eleventh day respectively.

Histopathological changes

In the histopathological examination conducted on the 25th day, the skin tissue samples were collected from the wound beds of rats across various groups and analysed under a microscope at different magnifications. In the control group, scab area with neutrophilic aggregates, haemorrhage and cell debris were observed. Early stage granulation tissue formation was also observed. In the Standard group, compared with Control group, increased epidermal regeneration, reduced inflammatory cells, increased angiogenesis and increased fibroblast proliferation was observed. In the group treated with test drug, there was notable increase in

epithelial growth, granulation tissue, and angiogenesis when compared to the normal control group and also the amount of scab tissue was also reduced compared to that of control.

Effect on Hydroxyproline concentration

The estimation of hydroxyproline is important as it reflects the collagen deposition at the injury site and plays a role in the normal epithelialization process. In this study, the test drug showed a significant increase in hydroxyproline concentration when compared to normal control group and the results were statistically significant. Thus, the study indicates that the test drug positively affects the cellular proliferation and collagen synthesis.

Different stages of Wound healing

DAY	CONTROL	STANDARD	TEST
DAY 0			
DAY 6			
DAY 12			
DAY 18			

DAY 24			

Figure 1: Stages of Wound healing in Control, Standard & Test group.

Histopathological section of Skin

CONTROL

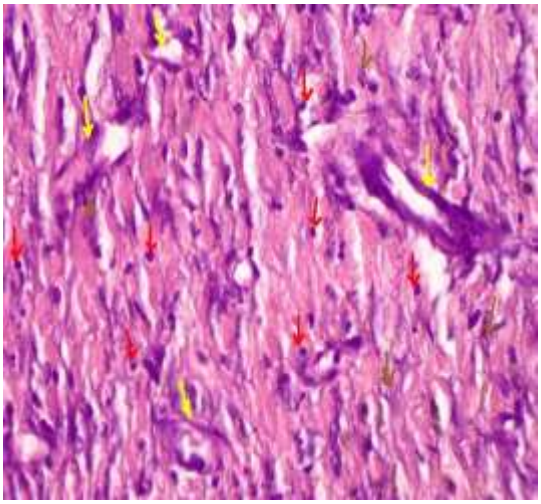

Fig.2a

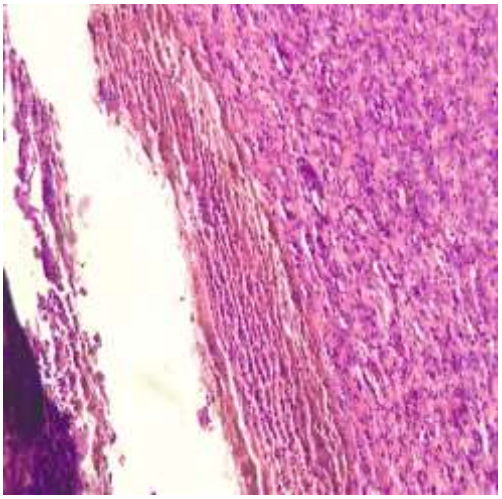

Fig.2b

STANDARD

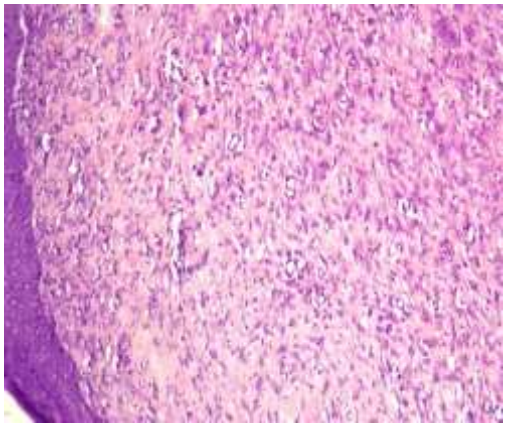

Fig.2c

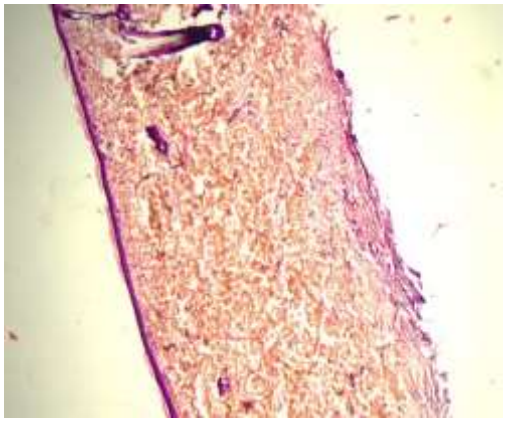

Fig.2d

TEST

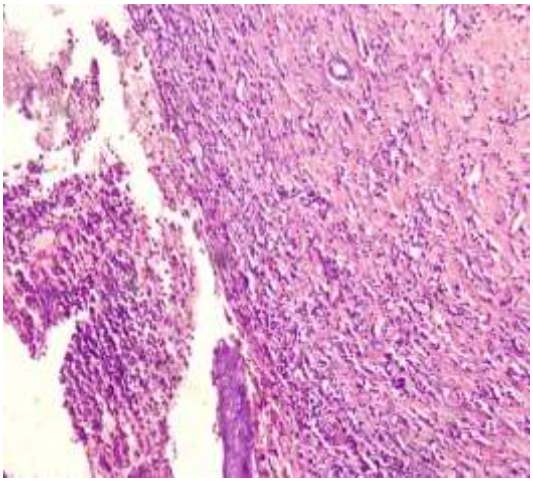

Fig.2e

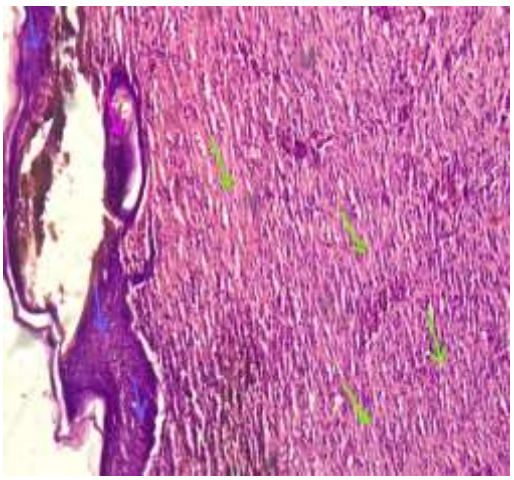

Fig.2f

Figure 2a-2f Histopathological section of Skin

Probable mode of action

According to Ayurveda, the action of a drug depends upon its *Rasapanchakas*. In *Samanyapratyarabdha Dravya*, the *Guna, Virya* and *Vipaka* are determined by its *Rasa*. In the present study, it was found that the test drug exhibits *Kashaya* as *Pradhana Rasa* and *Tikta* as *Anurasa*. According to Acharya Sushruta, *Kashaya rasa* helps in *Vrana Sandhana*; implies the *Kashaya rasa* possesses the ability to induce contraction of the wound. *Kashaya rasa* is having *Sthambhana* and *Sangrahi* properties and thus it helps in controlling the bleeding. *Kashaya rasa* is having predominance of *Prithvi* and *Vayu Mahabhuta*, therefore it helps in the viscosity of the blood and helps in the *Shoshana* of the excess *Dravamsha* in the *Vrana* respectively. The *Shoshana* property of *Kashaya rasa* helps to maintain a dry environment in the wound area, thereby preventing the invasion of *Krimi*. The *Ropana* property of the facilitates the formation of healthy granulation tissue, thus promoting the process of wound healing. The *Anurasa* of the drug was found to be *Tikta* and it is also helps in the *Shoshana* of *Kapha* and *Kleda* thereby facilitating wound healing. By its *Lekhana* property, it prevents the invasion of *Krimi*, and enhances the process of Wound contraction by the *Vishoshakari Guna*. The *Laghu Guna* is having *Kaphahara* property and it helps in *Srotoshodhana* thus facilitate wound contractions. The *Ruksha Guna* helps in the *Shoshana* which also aids in wound healing.

The test drug T.heyneana Wall. contains Phyto-constituents such as coumarins, tannins, flavonoids etc. Numerous pharmacological studies have indicated that coumarins possesses immunostimulatory, bacteriostatic, anti-inflammatory properties, which suggests its

efficiency in chronic infections. The antioxidant, free radical scavenging and anti-inflammatory properties of flavonoids also enhances the wound healing action. Tannins helps in the proliferation of the fibroblasts thus aids in the effective healing of the wound.

CONCLUSION

From the present study, it can be concluded that the test drug T.heyneana Wall. can be effectively used in the management of wound. Further research studies like Clinical trials can be conducted to assess the therapeutic efficacy of *Tabernaemontana heyneana* (Wall.) stem bark in Wound healing.

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