ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

AN EXPERIMENTAL EVALUATION OF *HUMBOLDTIA VAHLIANA WIGHT.*, A FOLK MEDICINAL PLANT ON WOUND HEALING ACTION IN WISTAR ALBINO RATS-AN IN-VIVO STUDY

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Article info

Article History:

Received: 26-08-2022

Revised: 19-09-2022

Accepted: 30-09-2022

KEYWORDS:

*Humboldtia vahliana Wight,*wound healing, excision wound contraction, breaking strength.

ABSTRACT

A wound is a break in the integrity of the skin or tissues often which may be associated with disruption of the structure and function. Globally, the magnitude of acute wounds as a healthcare problem is sharply rising mainly due to industrialization. There is still a need to revisit the ancient healing methods by using traditional medicine aiming to discover a wound healing drug with better protection, rapid granulation changes, least side effects, good bio-availability and low cost. A folk medicinal plant *Humboldtia vahliana* Wight. belongs to the family Fabaceae, is a medium sized tree with rough, dark brown bark mottled with white. Its stem bark is used for wound healing among the tribes. **Aims and objectives:** The study aims to evaluate the in-vivo wound healing action of *Humboldtia vahliana* Wight. through excision and incision animal wound models. **Methodology**: 36 male Wistar albino rats weighing 150-250gm. were selected and made into 3 groups each for both incision and excision wounds, i.e., control, standard and trial group -each containing 6 rats. The trial drug *Humboldtia vahliana* Wight. was applied over the wound area in the form of '*Kalka'*. In excision wound model, percentage of wound contraction was the parameter for the study and was achieved by using Planimetry. In incision wound model, tensile strength was the parameter and was achieved by Local Tensiometer. **Results**: In case of both Incision as well as Excision wound contraction study, the data shows a statistically non-significant increase in percentage wound contraction of Trial group when compared to Control and Standard groups. **Interpretation and Conclusion:** The stem bark of *Humboldtia vahliana* Wight. showed better wound healing action in Wistar albino rats when percentage of efficacy or mean values were compared.

INTRODUCTION

A wound is a break in the integrity of the skin or tissues often which may be associated with disruption of the structure and function[1]. Wound management and wound healing has consistently been an important tool for survival since ancient times and still it continues as a challenging clinical problem despite scientific developments in the field. Wound healing is a natural phenomenon, but the natural way of healing may lack quality, promptness and aesthetics.

Access this article online	
Quick Response Code	
	https://doi.org/10.47070/ijapr.v10iSuppl2.2513

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The main cause of modern day wounds include cuts by sharp instruments, chemical agents etc. As the skin functions as the protective covering of the body, it is the most affected organ with both major and minor wounds.

In Ayurveda, '*Vrana shodhana'* and '*Ropana'* methods have been mentioned as the line of treatment. Under the term *Vranaropaka*, several drugs of plant, mineral and animal origin has been described in Ayurveda. Acharya Sushrutha has explained in much detail about accidental wounds under '*Sadyo vrana'*and he classified *Vrana* into *Nija (Sharirika)* and *Agantuja vrana*. Wound healing is defined as a collection of complex process which comprises of different compounds including soluble mediators, blood cells, extracellular matrix and parenchymal cells[2]. With the exception of bone, all tissues heal with some scarring. The objective of proper care is to minimize the

possibility of infection and scarring. The role of wound healing drug is to prevent infection and fasten the healing process.

Different treatments have locally and systemically been used during recent era in order to help wound healing by using antibiotics, antiseptics, de-sloughing agents and extracts. Modern synthetic allopathy based medicines have their share of limitations of allergy and cost. Due to the emergence of multi-resistant organisms, decrease in newer antibiotics, wound care professionals have revisited the ancient healing methods by using traditional medicine in wound management and are fast attempting to explore the ancestral data and test its effectiveness using current experimental methods. The demand of herbal drugs is increasing day by day in the developed as well as developing countries because they are safer and are well tolerated comparatively.

A folk medicinal plant *Humboldtia vahliana* Wight. belongs to the family Fabaceae, is a medium sized tree with rough, dark brown bark mottled with white[3]. Powder or decoction of the bark is given to cure biliousness, impure blood, leprosy, ulcers and epilepsy[4]. The folklore practitioners belonging to 'Muthuvan' tribes of Kerala use its bark paste in wound management and leprosy. Till now no work has been carried out to evaluate its wound healing activity.

Considering the above facts, pharmacognostic, phytochemical and experimental study of the drug was carried out with an aim to discover a wound healing drug with better protection, good bio availability and low cost. The experimental study was conducted to evaluate the wound healing action of the drug in Wistar Albino rats by using excision and incision wound methods.

AIMS AND OBJECTIVES

- To evaluate wound healing action of *Humboldtia vahliana* Wight. on wistar albino rats through excision wound model using the parameter-Percentage wound contraction.
- To evaluate the effect of *Humboldtia vahliana* Wight. on Tensile strength of the granulation tissue on the incision wound model.

MATERIALS AND METHODS

Selection of the Animal

The experimental study was conducted at SDM Ayurveda College, Kuthpady, Udupi-Karnataka, India. Male Wistar albino rats (150-250gm) were selected for the experiment. Six rats were taken for each group. The rats were used after acclimatisation to the laboratory environment for a 7 day period. They were kept in the departmental animal house at 26±2°C and light dark cycles of 10 and 14h, respectively. Animals were provided with rodent diet and water. All the experimental procedures were approved by Institutional animal ethical committee of SDM Ayurveda College.

Inclusion Criteria

Healthy Wistar albino rats weighing about 150-250gm.

Exclusion Criteria

- Albino rats which were infected and pregnant were excluded from the study.
- Showing signs of infection during the course of study.
- Undergoing other experiments
- Weighing less than 150gm and more than 250 gm.

Table 1: Grouping of Animals

Groups	Drug	Form	Dose	Method	No. of rats
VR1-Control	Natural recovery	-	-	Excision Incision	6 6
VR2-Trial	Bark of *Humboldtia vahliana* Wight.	*Kalka*	External application	Excision Incision	6 6
VR3-Reference Standard	Betadine (Povidone-iodine)	ointment	External application	Excision Incision	6 6

The rats were divided into 3 groups (n=36): Group 1 was treated with control, Group-2 was treated with Povidone –Iodine (Standard drug), Group-3 was treated with *Humboldtia vahliana* Wight. (trial drug). Each group was again subdivided for excision (6) and incision (6) wound models. The rats were housed in individual cages and kept in a well-ventilated room under hygienic condition.

Route of drug Administration

Route of drug administration plays an important role in Ayurveda. Topical application is one of the routes for administration of drug. This method produces very constant blood levels of the substance, avoiding the need for repeated animal restraint, painful injections, sharp hazards, and lower risk of side effects. So, topical application has given utmost importance in this animal experimentation.

Methods of Analysis

By performing macroscopic and microscopic study, the stem bark of *Humboldtia vahliana* Wight. was found to be genuine. The *Rasapanchaka* study revealed about its *Kashaya rasa, Sheetha veerya, Guru guna* and *Madhura Vipaka*[5].

Experimental Study

The wound healing property of the trial drug *Humboldtia vahliana* Wight. was analysed experimentally in albino rats by two methods.

- Excision wound model (technique developed by Morton and Malone)[6]
- Incision wound model (technique developed by Hunts et al) [7]

These techniques consists of the following stages,

1. Pre-operative stage
2. Operative stage
3. Post-operative stage

1. Pre-operative Stage

The selected albino rats numbering 36 were primarily divided into 3 groups of 12 rats each, one group each for Control, Standard and Trial. They were further divided into 2 sub group for Incised and excised wound model.

2. Operative Stage

Excision Wound Model

This was conducted according to the technique developed by Morton and Malone. The animals were anaesthetized by administering pentabarbitone intra-peritoneally. After the animals were sufficiently anaesthetized, they were secured to the dissection plate in prone position. The hairs were removed using shaving blade from the part to be operated and subsequently the area was cleaned.

A round seal of 2.5cm in diameter was impressed on the dorsal thoracic central region 5cm away from the ears of the anaesthetized rats. Full skin thickness from the marked area was excised in circular fashion with the help of forceps, surgical blade and scissors. The approximate area thus formed was 500mm[2]. After achieving full haemostasis, the animals were placed in individual cages.

Incision Wound Model

The animals in all groups were anaesthetized by administering pentabarbitone intra-peritoneally, and 2cm long incision was made through the skin and cutaneous muscles at a distance of about 3 cm from the midline on the depilated back of the rat. After the incision was made, the parted skin was kept together and stitched with Surgical Linen (No.20) at 0.5cm intervals using a curved needle (No.10) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound and the wound was left undressed. After achieving full haemostasis, the animals were placed in individual cages.

3. Post-operative Stage

External application of *Humboldtia vahliana* Wight. was started from the next day of surgery (Day 1). Control groups left without applying drug to observe the natural healing process and betadine was applied for the rats in the standard group. All the rats were given normal food and water.

OBSERVATION

Excision wound model

To monitor the changes in the wound shapes, the wound margins were traced on OHP sheet from the day of wounding (0 day) and continued till the complete healing of the wound. This was again retraced on a millimetre scale graph paper. The observations of percentage of wound closure were made on the 1st, 3rd, 6th, 9th 12th, 15th, 18th, and 21st and 23rd post wounding days. On 24th day, animals were sacrificed.

Incision wound model

On 8th day, breaking strength was checked by using locally made tensiometer.

ASSESSMENT CRITERIA

Wound contraction was the parameter employed to study in excision wound model. The estimation of Breaking or Tensile strength was employed to study the incision wound model and this was achieved through local Tensiometer.

(a) Wound contraction

The main factors, which contribute wound healing, is contraction. This was done by tracing the wound margins on a OHP sheet and subsequently retracing them on a millimetre scale graph paper. This was later calculated as percentage of original wound size for each animal in the group depending on the days taken for wound contraction.

(b) Breaking strength and Tensiometer

In the study experiment, custom made Tensiometer was used, which consists of a wooden board to which four nails was fixed to restrict the movement of the rat. Apart from that, one more nail was fixed, to one end the nail thread tied which is fixed, where as to another end, easy movement of thread was allowed with the help of pulley, to the edge of thread attached to the specially made plastic container.

Group	3rd day	9th day	15th day	21st day	23rd day
Control	13.71±3.16	66.96±3.42	93.14±1.78	99.03±0.35	99.30±0.28
Standard (Betadine)	15.36±3.84	79.32±2.73 *	94.01±.70	99.34±0.21	99.68±0.10
Test (*Humboldtia vahliana* Wight.)	22.26±3.84 ↑	79.92±1.61 **↑	96.83±0.38 ↑	99.74±0.15 ↑	99.86±0.06 ↑

The rats were anaesthetized individually and were placed in wooden board between nails. The threads were then carefully tied and attached to the skin on the opposite sides of the wound at a distance of 1cm away from the wound. Specially made plastic container was used to collect the inflow normal saline which helps in increasing the weight until the healed wound opens. Once the wound opens, the flow of normal saline is stopped with the help of a clamp,and the volume of normal saline is measured and the time taken for breaking the wound is also noted. The liquid used for the flow is normal saline water. Thus the breaking strength of the wound was measured and compared.

Statistical Calculations

Values of each parameter of all the groups were expressed in MEAN±SEM. The data were analysed by one-way ANOVA followed by Dunnett's multiple comparison t-test as post hoc test. Graph Pad InStat 3 was used for calculations. A level of p 0.05 was considered as statistically significant. Levels of significance were noted and interpreted accordingly.

Group	Time taken for wound breaking (Tensile Strength) -[Sec]	Volume of Normal Saline solution [ml]
Control	30.33 ± 6.741	808.33 ± 205.11
Standard (Betadine)	87 ± 30.97	2233.33 ± 699.67
Test *(Humboldtia vahliana* Wight.)	104.83 ± 29.79 ↑	2260 ± 793.05 ↑

RESULTS

Excision Wound Contraction

Table no.2: Effect of *Humboldtia vahliana* Wight. on Percentage wound contraction measured on 3rd, 9th, 15th, 21st and 23rd post wounding days.

Data: MEAN ±SEM, * P<0.05, ** P<0.01 when compared with control group.

↑: Increase when percentages of efficacy or mean values were compared with control and standard group

Incision wound contraction

Table no.3: Time taken for Wound Breaking (Tensile Strength)- On 8th day and Volume of Normal Saline solution obtained during tensile strength determination

Data: MEAN ±SEM

DISCUSSION

The objective of present study "An experimental evaluation of *Humboldtia vahliana* Wight., a folk medicinal plant on wound healing action in Wistar Albino rats-an in-vivo study" was to evaluate in both excision and incision wound models. The efficacy of wound healing was assessed by number of days taken for the normal epithelization and wound contraction .The trial group was compared with control and standard group. The statistical inference was made using suitable statistical analysis.

In case of period of epithelization or days taken for the complete wound healing, less number of days was taken in trial and standard group when compared to control group. The observed difference was found to be statistically non-significant. Overall better wound contraction was observed in trial group when compared to standard and control group. Qualitative analysis of *Humboldtia vahliana* Wight. showed the presence of alkaloids, steroids, carbohydrates, tannins, flavonoids, terpenoids and resins. Whereas saponins, phenols, carboxylic acid, quinones, and amino acids were found to be absent.

Tannins are potentially useful in promoting the healing of wounds as these could promote cicatrisation of the wound through several cellular mechanisms like chelation of free radicals and reactive species of oxygen, promoting contraction of the wound and increasing formation of capillary vessels and fibroblasts[8]. Phytochemical screening revealed the presence of tannins and this may be one component that contributed to the plant's wound healing effect. Furthermore, most flavanoids and terpenoids exert antibacterial and astringent activities that help in infection control, wound contraction and increased rate of epithelialization[9].

These increase the migration and proliferation of fibroblasts and collagen synthesis and also possess antioxidant and anti-inflammatory activity, thus

exerting beneficial effects in wound healing process. Steroids also cause increased epithelialization, increased wound contraction and increased collagenisation. All these phytochemicals also may have contributed to its wound healing activity.

The *samanya vidhi* for *Sadhyo vrana chikitsa* include *Sheetala chikitsas* invoving *Kashaya, Madhura* and *Sheeta dravyas* and all these can be adopted for the treatment of *Sadhyovrana* till the 7th day.[10] Since the trial drug was found to have *Kashayarasa* and *Sheeta veerya*, it might have a positive influence on *Vrana ropana*.

Kashaya rasa' being *Sangrahi, Sandhanakara, Ropana, Sthambhana, Twak savarnakruth* and *Shodhaka* has a very beneficial action on *Vrana ropana.* The *Pitta shamaka* action of the trial drug might have helped to reduce the burning sensation on the *Vrana.* The *Lekhana, Kledahara, Chedana,* and *Raktashodhaka* properties of *Kashaya rasa* might also had facilitated the development of granulation tissue to build up the floor of *Vrana.* The property to the drug to bring about an adhesion (contraction) of the wound, and its cooling measure which made to thicken the local blood might be because of its *Kashaya Rasa* & *Sheeta Virya.*

CONCLUSION

The wound healing property of *Humboldtia vahliana* Wight. appears to be due to the presence of its active principles which accelerates the healing process. There was increase in the percentage excision wound contraction on 23rd post wounding day of test group when compared to the standard and control group.

The tensile/breaking strength of the incision wound was also more in test group, when compared to the standard and control group. Statistically, when percentage of efficacy or mean values were compared, *Humboldtia vahliana* Wight. appears to be slightly superior in wound healing action. Since the trial drug is found to have '*Kashaya rasa'* and '*Sheeta veerya'*, it might have a beneficial action on *Vrana ropana*. Phytochemicals such as tannins, flavanoids and terpenoids also promotes its wound healing action.

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Cite this article as:
Athira Soman, Chandrakanth Bhat, Hariprasad Shetty, Sudhakar Bhat. An Experimental Evaluation of humboldtia vahliana wight., a Folk Medicinal Plant on Wound Healing Action in Wistar Albino Rats-an in-vivo Study. International Journal of Ayurveda and Pharma Research. 2022;10(9):105-111.
https://doi.org/10.47070/ijapr.v10iSuppl2.2513
Source of support: Nil, Conflict of interest: None Declared

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Fig 1: 3rd Day-Excision Wound Contraction

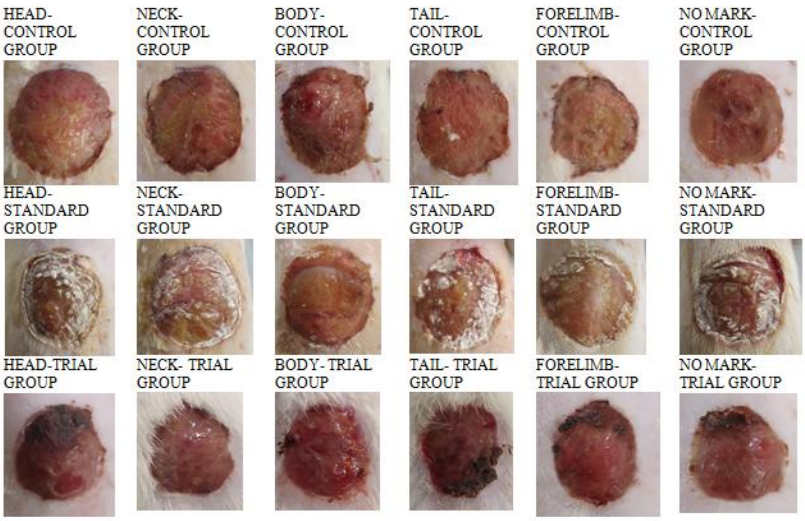

Fig 2: 15th Day-Excision Wound Contraction

Fig 3: 23rd Day-Excision Wound Contraction

Fig 4: Excision Fig 5: Excision wound

Fig 6: Application of *Lepa*-excision wound

Fig 7: Tensile strength assessment -Incision wound

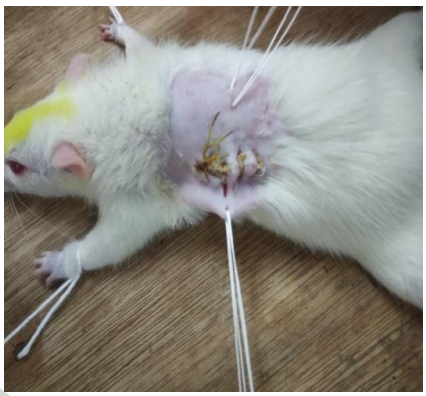

Fig 8: Local Tensiometer

Fig 9: *Humboldtia vahliana* Wight.

Fig 10: Bark cuttings

Fig 11: Kalka Preparation