



ISSN 2456-3110

Vol 6 · Issue 5

Sept-Oct 2021

Journal of
**Ayurveda and Integrated
Medical Sciences**

www.jaims.in

JAIMS

An International Journal for Researches in Ayurveda and Allied Sciences



Maharshi Charaka
Ayurveda

Indexed

A comprehensive study on *Twak Shareera* and an experimental study to evaluate histological changes observed by application of *Jathyadi Taila*

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ABSTRACT

Background: *Twak* is the outermost protective layer as well as largest sensory organ of body. Acharya Susrutha and Acharya Charaka described its layers according to their functions and diseases, which are related to those layers. It is the location of sweat channels, hairs and hair pits. It is the largest organ constituting 15 -20% of total body mass. **Objectives:** To do the literary study of *Twak Shareera* as explained in *Samhitha*, Comparative study of *Twak Shareera* with contemporary science and to study the wound healing process on the effect of *Jathyadi Taila* in experimental Wistar albino rats with control, standard and test group with the help of histological observation. **Methodology:** Literary study was done by collecting references from different *Samhitas*, contemporary science, journals and web sites. Experimental study was carried out on albino rats, wound is made under aseptic conditions and the skin is taken for histopathological study. *Jathyadi Taila* is applied at the site of wound in test group, Povidone- iodine is applied in Standard group and control group is left for natural healing. The skin is taken for histopathological studies on the 24th day in all the three groups. **Results:** The event in the wound healing like inflammatory phase, the granulation tissue, fibroblast etc are seen in histopathological studies. Application of *Jathyadi Taila* has shown significant results in wound healing and scar remodelling. The data shows there was Increase in percentage wound contraction of healing in standard and test group, when compared to the normal control group. The observed increase was found to be statistically significant. **Conclusion:** *Jathyadi Taila* does *Shodhana* and *Ropana* of *Vrana* by the virtue of its ingredients and its properties. *Jathyadi Taila* has shown significant results in wound healing and scar remodelling.

Key words: *Twak Shareera*, *Vrana*, *Vrana Ropana*, *Jathyadi Taila*, Wound Contraction, Histopathological study.

INTRODUCTION

Twak is described as the outermost protective layer as well as largest sensory organ of body. It develops at the time of conception just like cream formed while

boiling the milk. Acharya Susrutha and Acharya Charaka very minutely described its layers according to their functions and diseases. *Twak* contains *Swedvahi Strotas*, *Loma*, and *Lomakupas*.^[1,2]

Skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. The skin is continuous, with the mucous membranes lining the body's surface.^[3]

Structurally integumentary system is most complex structure and highly specialized, hence it is grouped in the sense organ. The most extensive organ system has the skin and accessory structures, including hair, nails,

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Submission Date: 17/09/2021 Accepted Date: 19/10/2021

Access this article online

Quick Response Code



Website: www.jaims.in

DOI: [10.21760/jaims.6.5.15](https://doi.org/10.21760/jaims.6.5.15)

gland and specialized nerve receptors for stimuli such as touch, cold, heat, pain and pressure.^[3]

Its functions are protection of internal structure, prevention of disease-causing microorganisms, temperature regulation, excretion through perspiration, primary protection against ultraviolet sunrays, and production of vitamin D. The body store about half its fat in the underlying hypodermis.

Vrana as pathological condition, which broadly classified into two categories as *Nija vrana* and *Agantuja vrana*. *Nija Vrana* occurs due to the intrinsic factors while *Agantuja Vrana* may arise due to external factors such as; burn, accidents, trauma & animal bites. Initially *Vrana* manifested on the skin, later on it extended to the deeper sites like; *Meda*, *Mamsa* etc.

Wound is a discontinuity or break of the surface by an injury especially in which skin or external surface is torn, pierced, cut, torn or broken. In the simple wound, only the skin is involved. When it is a complex wound, nerves, vessels, tendons are involved.

Wound healing mostly means healing of the skin. Healing is a complex process involving regulated cascade of biochemical and cellular events designed to attain restoration of tissue integrity. The healing process in human tissue combines both aspects of tissue repair and regeneration. In the repair of tissue, scar formation will occur at the site of wound.^[4]

It is important to understand the histology of the skin for the process of wound healing and to plan for a better treatment. Hence present study "A comprehensive study on *Twak Shareera* and an experimental study to evaluate histological changes observed by application of *Jathyadi Taila*" is taken.^[5]

OBJECTIVES

1. To do the literary study of *Twak shareera* as explained in *Samhitha*.
2. Comparative study of *Twak Shareera* with contemporary science.
3. To study the wound healing process on the effect of *Jathyadi Taila* in experimental wistar albino rats

with control, standard and test group with the help of histological observation.

METHODOLOGY

All of the experiments were conducted after prior permission from institutional animal ethics committee (IAEC), SDM centre for research in Ayurveda and allied sciences, Udupi. (Approval number- IAEC No. SDMCRA/IAEC/SS-03).

Study Design

Experimental study carried out on Albino rats 90-120 days old, 150 to 250 gm bred in S.D.M Animal house. The rats were maintained in the cycle of 12 hrs light and 12 hrs darkness with temperature and humidity controlled environment. Food supply of rats with standard food pellets and water is given.

Source of Data

Experimental Source

Wistar albino rats were obtained from the animal house attached to department of pharmacology, SDM centre for research in Ayurveda and allied sciences, Udupi.

Drug Source

- Test drug - Market sample obtained from SDM pharmacy, Udupi
- Standard drug
- Chemical used for anaesthesia
- Other chemicals

Dose Fixation and Schedule

The dose for experimental animals calculated based on body weight.

Duration of Study

24 days

Method of Data Collection

- Wistar albino rats of either sex, weighing between 200-250g were obtained from the animal house attached to Department of Pharmacology, S.D.M

Centre for Research in Ayurveda and Allied Sciences, Udupi.

- They were housed in standard transparent polypropylene cages with wheat husk bedding, renewed every 24 hours. They were kept under controlled room temperature at around $25\pm 3^{\circ}\text{C}$ with relative humidity of 40-60%, on a 12-hour natural day and night cycle. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. They were fed with diet Amrut brand rat pellet feed and tap water ad libitum.
- Institutional Animal Ethics Committee had approved the experimental protocol and the care of animals was undertaken as per the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

Inclusion Criteria

- Healthy Wistar albino rats of either sex were selected.
- Weighing between 200-250g.

Exclusion Criteria

- Wistar strain albino rats weighing less than 200g and more than 250g.
- Pregnant and diseased rats.
- Rats used for and under trial of other experiments.

Animal Grouping

Selected Wistar strain albino rats of either sex weighing around 200-250gm were placed randomly under 3 groups, each group containing minimum of 6 rats.

The rats are bred in the animal house of SDM Ayurveda College Kuthpady, Udupi Karnataka.

The rats were divided into three groups (n=18); group 1 was treated with control, group 2 treated with 5% povidone – iodine ointment USP (Standard drug) and group 3 was treated with *Jathyadi Taila* (test Drug).

The rats are housed in individual cages and kept in a well-ventilated room under hygienic condition.

- **Pre-operative stage**
- **Operative stage**
- **Post-operative stage**

Pre-operative Stage

- The selected albino rats numbering 18 are primarily divided in to 3 groups of 6 rats each (Control, Standard and Test).

Operative Stage

Excision Wound Model

- This was conducted according to the technique developed by Morton and Malone.
- The animals were anesthetized using Pentobarbitone (45 mg/IP) intra peritoneally.
- After the animals anesthetized they are secured to the dissection plate in prone position.
- The hairs removed using shaving blade from the part to be operated and subsequently the area is cleaned.
- A round seal of 2.5 in diameter was impressed on the dorsal thoracic central region 5cm away from the ears of the anesthetized rats.
- Full skin thickness from the marked area was excised in circular fashion with the help of the forceps, surgical blade and scissors.
- The approximate area thus formed is 500mm^2 . After achieving full haemostasis, the animals are placed in individual cage.

Post-operative stage

- The external application of *Jathyadi Taila* was started from day 1st of post wounding.
- Control group left without applying drug to observe the natural healing process and 5% povidone ointment is applied for the rats in the standard group.
- All the rats are given normal food and water.

Excision wound model

- To monitor the changes in the wound shapes, the wound margins are traced on OHP sheet from the day of wounding (0 day) and continued until the complete healing of the wound.
- This is again retraced on a millimetre scale graph paper.
- The observations of percentage of wound closure are made on the 1st, 3rd, 6th, 9th, 12th, 15th, 18th, 21st and 24th post wounding days.
- These wounds are also observed for period of epithelization and contraction.
- On the 24th day, the animal was sacrificed and skin over the wound which was healed was cut and sent for histopathological study.

Histopathological Examination of Part of Skin**Procedure Followed to Prepare Histopathological Slides**

Fixation: the tissue was excised out immediately after sacrificing the animals, cleaned of extraneous tissue, cut into pieces of appropriate thickness and were transferred to 10% formalin solution. The tissue was allowed to remain in it till they are taken up for processing.

Tissue processing: the tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. The tissue were thoroughly washed by placing them under running tap water and then passed through a series of following solvents as per schedule for dehydration clearing and paraffin infiltration.

- Alcohol 70% - 20 minutes
- Alcohol 80% - 20 minutes
- Alcohol 90% - 20 minutes
- Alcohol 95% - 20 minutes
- Isopropyl alcohol - 20 minutes
- Acetone (2 changes) - 20 minutes
- Chloroform (3 changes) - 20 minutes

- Melted paraffin wax (60° C) (2 changes) - 30 minutes each
- Next, the tissues were embedded in paraffin wax to prepare tissue blocks. The tissue blocks were fixed to a metal object holder after trimming them to suitable size.

Section cutting: the tissue section of 5-6 µm thickness were cut with the help of spencer type rotating microtome and floated in a water bath between 50-55°C for 30 minutes and then they were mounted on clear glass slide with a drop of Mayer's egg albumin dried on hot plate at 50°C for 30 minutes.

Staining: after fixing the section on the slide, the sections were stained by serially placing them in the following reagents.

- Xylol (2 changes) - 3 minutes
- Acetone - 3 minutes
- Alcohol - 3 minutes
- Running water - 3 minutes
- Haematoxylin - 20 minutes
- Running water - 20 minutes
- Eosin working solution - 2 minutes
- Alcohol 95% (3 changes) - 3 minutes
- Acetone (2 changes) - 3 minutes
- Xylol (2 changes) - 3 minutes

After passing through all the above reagents and stains, the slide were covered with D.P.X (Diphenyl pthalinen Xylene) and cover slip were placed. Care was taken to avoid the air bubble formation during mounting the slides.

The slides were viewed under binocular research Carl-Zeiss's microscope (Germany)

At various magnification to note down the changes in the microscopic features of the tissue studied.

Assessment Criteria

Wound contraction and epithelisation were the parameters employed to study in excision wound

model and this was achieved by using Histopathology and Hydroxyproline estimation.

A) Wound Contraction

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

Initial wound size - Specific day wound size

B) Period Epithelization

- Falling of scar leaving no raw wound behind is taken as end of complete epithelialization and the days required for this is taken as period of epithelialization.
- In all the three groups, on the 24th day, 3 rats were randomly selected from each group and after the sacrifice, the skin tissue carefully excised and sent for histopathological examination.
- Histopathological studies – skin tissue preserved in 10% formalin for histopathological examination.
- The obtained results of the test groups are compared with that of the standard and control groups.

OBSERVATION AND RESULTS

Effect of Jathyadi Taila on percentage wound contraction measured on 3rd post wounding day.

Excision Wound Contraction

Group	% wound contraction measured on 3 rd day	Percentage Change
Control	14.16 ± 2.49	
Standard (Betadine)	12.33 ± 5.17	12.92 ↓
Test (Jathyadi Taila)	0.33 ± 9.06	97.66 ↓

Data: MEAN ±SEM

- Data related to the effect Jathyadi Taila on percentage wound contraction measured on 3rd post wounding day presented in table.
- The data shows there was decrease in percentage wound contraction on post 3rd day of healing in standard and test group, when compared to the normal control group. The observed decrease was found to be statistically non-significant.

Effect of Jathyadi Taila on percentage wound contraction measured on 6th post wounding day

Excision Wound Contraction

Group	% wound contraction measured on 6 th day	Percentage Change
Control	33.16 ± 2.24	
Standard (Betadine)	43.66 ± 5.01	31.66 ↑
Test (Jathyadi Taila)	25.5 ± 9.66	23.10 ↓

Data: MEAN ±SEM

- Data related to the effect Jathyadi Taila on percentage wound contraction measured on 6th post wounding day presented in table.
- The data shows there was Increase in percentage wound contraction on post 6th day of healing in standard group and test group, when compared to the normal control group. The observed increase was found to be statistically non-significant.

Effect of Jathyadi Taila on percentage wound contraction measured on 9th post wounding day.

Excision Wound Contraction

Group	% wound contraction measured on 9 th day	Percentage Change
Control	53.66 ± 7.34	
Standard (Betadine)	74.5 ± 2.81*	38.83 ↑
Test (Jathyadi Taila)	63.86 ± 4.01	19.008 ↑

Data: MEAN ±SEM, *P<0.05

- Data related to the effect *Jathyadi Taila* on percentage wound contraction measured on 9th post wounding day presented in table.
- The data shows there was Increase in percentage wound contraction on post 9th day of healing in standard group, when compared to the normal control group. The observed increase was found to be statistically significant.
- The data shows there was Increase in percentage wound contraction on post 9th day of healing in test group, when compared to the normal control group. The observed increase was found to be statistically non-significant.

Effect of *Jathyadi Taila* on percentage wound contraction measured on 12th post wounding day.

Excision Wound Contraction

Group	% wound contraction measured on 12 th day	Percentage Change
Control	65.83 ± 10.72	
Standard (Betadine)	82 ± 3.29	24.56 ↑
Test (<i>Jathyadi Taila</i>)	85 ± 1.11	29.87 ↑

Data: MEAN ± SEM

- Data related to the effect *Jathyadi Taila* on percentage wound contraction measured on 12th post wounding day presented in table.
- The data shows there was Increase in percentage wound contraction on post 12th day of healing in standard and test group, when compared to the normal control group. The observed increase was found to be statistically non-significant.

Effect of *Jathyadi Taila* on percentage wound contraction measured on 15th Post wounding day.

Excision Wound Contraction

Group	% wound contraction measured on 15 th day	Percentage Change
Control	75 ± 6.81	
Standard (Betadine)	86.83 ± 1.83	15.77 ↑
Test (<i>Jathyadi</i>)	90.33 ± 1.58*	20.44 ↑

<i>Taila</i>)		

Data: MEAN ± SEM *P < 0.05

- Data related to the effect *Jathyadi Taila* on percentage wound contraction measured on 15th post wounding day presented in table.
- The data shows there was Increase in percentage wound contraction on post 15th day of healing in standard group, when compared to the normal control group. The observed increase was found to be statistically non-significant.
- The data shows there was Increase in percentage wound contraction on post 15th day of healing in test group, when compared to the normal control group. The observed increase was found to be statistically significant.

Effect of *Jathyadi Taila* on percentage wound contraction measured on 18th post wounding day.

Excision Wound Contraction

Group	% wound contraction measured on 18 th day	Percentage Change
Control	81.33 ± 2.75	
Standard (Betadine)	90.66 ± 0.95 **	11.47 ↑
Test (<i>Jathyadi Taila</i>)	95.33 ± 0.71 **	17.21 ↑

Data: MEAN ± SEM ***P < 0.01

- Data related to the effect *Jathyadi Taila* on percentage wound contraction measured on 18th post wounding day presented in table.
- The data shows there was Increase in percentage wound contraction on post 18th day of healing in standard and test group, when compared to the normal control group. The observed increase was found to be statistically very significant.

Effect of *Jathyadi Taila* on percentage wound contraction measured on 21st post wounding day.

Excision Wound Contraction

Group	% wound contraction measured on 21 st day	Percentage Change
Control	87.83 ± 2.45	
Standard	95.66 ± 0.61**	8.91 ↑

(Betadine)		
Test (Jathyadi Taila)	97.33 ± 0.55**	10.81 ↑

Data: MEAN ±SEM **P< 0.001

- Data related to the effect Jathyadi Taila on percentage wound contraction measured on 21st post wounding day presented in table.
- The data shows there was Increase in percentage wound contraction on post 21st day of healing in standard and test group, when compared to the normal control group. The observed increase was found to be statistically very significant.

Effect of Jathyadi Taila on percentage wound contraction measured on 24th post wounding day.

Excision Wound Contraction

Group	% wound contraction measured on 24 th day	Percentage Change
Control	91 ± 1.78	
Standard (Betadine)	98.83 ± 0.65**	8.60 ↑
Test (Jathyadi Taila)	99.5 ± 0.34**	9.34 ↑

Data: MEAN ±SEM **P< 0.001

- Data related to the effect Jathyadi Taila on percentage wound contraction measured on 24th post wounding day presented in table.
- The data shows there was Increase in percentage wound contraction on post 24th day of healing in standard and test group, when compared to the normal control group. The observed increase was found to be statistically very significant.

Consolidated Statement of Results

Day	Standard	Test
3	NSD	NSD
6	NSI	NSD
9	SI	NSI
12	NSI	NSI
15	NSI	SI
18	VSI	VSI

21	VSI	VSI
24	VSI	VSI

Histopathology Results

Grading done for various features as follows

- + : Slight
- ++ : Moderate
- +++ : Extensive
- : Absence

Slide No	Epidermal regeneration	Granulation tissue	Inflammatory cell infiltration	Angiogenesis	Proliferation of fibroblast cells	Collagen deposit
C1	-	++	+++	-	+	+
C2	+	++	+	++	++	++
C3	++	++	+++	+	++	++
S1	++	++	++	++	++	++
S2	++	++	+	++	++	++
S3	Wound area not seen					
T1	+	++	+	+++	++	++
T2	+	++	+	+++	++	++
T3	+	+	+++	++	++	+

DISCUSSION

Twak is one, which covers the body. Various aspect of Twak is explained in the Brihatthrayis and Laghuthrayis.

Embryology of Twak

The Twak is formed from the essence of Sukra and Sonitha like the formation of cream from boiling milk. But modern embryology opines the development of skin in the third week of the fetal life. Different cells of the skin and appendages have different origin and from the different area, they migrate to the skin.

Pramana of Twak

Acharya Susrutha has explained the Pramana of each layers of skin as having thickness of 18th, 16th, 12th, 8th, 5th, 1 and 2 Vreehi or Yava. Hence, totally it becomes 5.95 Vreehi or Angushtodhara Pramana.

Bhavaprakasha and Dalhana clarifies the thickness of each layer as 18/20, 16/20, 12/20, 5/20, 1 and 2 Vreehi, and the total thickness of Twak is 5.95 Yavas. In the context of Udara Patana, it is explained, as the incision on skin should be of Angushtodara Pramana. Angushtodara is 6-1/20th Yavas = 5.95 Yavas. Some recent authors have considered the Pramana of Twak as 1/18th, 1/16th, 1/12th, 1/8th, 1/5th 1 and 2 Vreehi. Then total thickness becomes 3.5 Yavas. As it is not equal to Angushtodara Pramana, considering this opinion is not possible

According to modern aspect, the thickness of the skin is 1-2 mm, in face it is 0.02 mm, while on the sole of the feet between 1 and 5 mm.

Acharya susruta has mention seven layers of Twak. Though the exact correlation is not possible with modern science. The subsequent comparison can be made with the modern science based on colour, appearance and structural involvement of the layers in various diseases.

Avabhasini and Lohitha as Stratum Corneum

Avabhasini is the first layer of Twak. This layer illuminates the Varna of the skin and the five Chaya. Lohitha is the second layer of Twak. Stratum Corneum can be compared with Avabhasini and Lohitha as this layer is pigmented and the disease occurring in this layer are pigmentation disorders

Sweta as Stratum Lucidum

Sweta is the third layer of Twak. Sweta word implies that clear layer. The stratum lucidum is also clear and highly refractive layer. This layer contains droplets of intermediate substance eledin that is eventually transformed to keratin. The diseases, which occur in this layer, are due to the localized over growth of melanocytes and melanin.

Tamra as Stratum Spinousm and Stratum Granulosum

Tamra is the fourth layer. Kilasa and Kushta are disease that are likely to occur in this layer.

Stratum spinousm contains keratinocytes with bundles of tonofilaments. Melanocytes and Langerhans cell

are present in this layer. At the site of allergic dermatitis Langerhans cells believed to take up antigen and present it lymphocytes in a form to which they can react by generation of antibodies. Stratum granulosum contains darkly-stained protein granules keratohya line, which is converted tonofilaments to keratin.

Tamra Varna of the Twak may be due to keratohyaline pigment.

Vedini as Stratum Basale and Papillary Dermis

Vedini is the fifth layer of the Twak. It is the Adhishtana of Visarpa and Kushta.

Stratum Basale and papillary dermis contains merkel disc, which serve as mechanoreceptors. The papillary dermis contains tactile receptors, messner corpuscles and free nerve ending it gives sensation of pain, warm, tickling etc. stratum Basale also contains cells of Langerhans and keratinocytes. Vedana is seen in the diseases of this layer. Hence, it is named as Vedini.

Rohini as Reticular Dermis

Sixth layer of the Twak is Rohini, the word meaning of Rohini which growing or ascending. In this layer abnormal growth, Granthi, Apache, Sleepada, Galaganda are seen. Reticular dermis contains fibroblast, collagen, reticular fibres and few adipose cells. This region possesses rich lymphatic and vascular supply. Due to rich vascular & lymphatic supply various growths, Granthi etc are seen in this layer.

Mamsadhara as Superficial Fascia

Mamsadhara is the 7th layer. Bhagandra, Vidrathi, Arshas are occurring in this layer.

Arshas, Bhagandra, Vidrathi is developed here. The Samprapti of all these disorder will not specify the role of Twak. But the Vyaktha Sthana of these is clearly observed through the Twak.

Twak as Sense Organ

Twak is the Gnanendria concerned with sensation of touch according to our classics. Acharya charaka has given prime importance to sparshanendriya by quoting those tactual contacts and mental contacts are the two types of contacts, which bring happiness

and miseries. In *Triyagatha Dhamani*, also we get the reference of skin responsible for *Sukha or Dukha Sparsha*.

According to modern science, also the skin is considered as an organ of sensation. The cutaneous receptors carry information concerning various stimuli. The branched myelinated and non-myelinated terminal ends in the dermis and lower layer of epidermis. It is a sensory component; they may be mechanic, chemo and thermos in skin. Hirsute skin such fibres lie in close association with hair follicles. Here merkels disc and Meissner's corpuscles found in the dermal papillae are responsible for rapid adapting mechanoreception. Dermis and hypodermis also contain Pacinian corpuscles, which are sensitive to pressure.

Varna of Twak

Varna Prasadana is because of *Raktha Dhatus*. The *Bhrajakapitta* also helps for *Twak Varna*. According to modern science, the skin colour depends on the pigments. That is melanin and blood capillary loops.

Bhavamishra explained the *Bhrajaka Pitta* in the layer of *Avabhasini*, which is responsible for *Twak Varna*.

The amount of melanin determines the skin colour. This variation is leads to yellow to red colour skin and red to brown colour. The number of melanocytes is almost same is all human being. The difference in colour is mainly depends on the amount of melanin they produce and transfer to keratinocytes. The amount of melanin is more in the dark skinned people and less in light-skinned people. Melanin pigments can be understood as *Bhrajaka Pitta*.

In the absence of large amount of melanin, the main determinant of skin colour is the oxygenated haemoglobin of the dermal vascular beds, particularly the superficial papillary plexus. These when viewed through the overlying epidermis, whose surface layer is somewhat opalescent, giving well-oxygenated skin a pink colour. These indicates the role of *Rakta Dhatu* in *Twak Varna*

Twak in Temperature Regulation

Regulation of the body heat and variations in the colour of the *Twak* are the functions of *Bhrajakapitta*

as well as *Dhamanis*. The skin plays the major role in the regulation of the body temperature.

Sensory receptor in the skin provides information about the external environment, helping the skin regulate the body temperature. The large surface area of the skin makes it ideal for temperature regulation. The rate of heat loss can be regulated by the amount of the blood flowing through the blood vessels in the dermis close to surface of the skin.

Besides capillary, there are other vascular connections between the arterial and venous network in the skin. Plexus in the subcutaneous are connected with adjustable arteriovenous anastomosis (AVA) with feeding arteries. When they open large amount of blood can pass. The AVA plays major role in thermoregulation of the body. The activation of the sympathetic nerves leads to active vasoconstriction. The decrease of sympathetic activity leads to passive vasodilatation. In the warm environment the AVA are open and cold environment AVA are almost closed. These indicates the role of *dhamanis* in maintaining body temperature

Discussion on Wound Healing

After the injury immediately, wound surface is filled with blood, which then clots. Platelet degranulation along with hypoxia results in the production of numerous cytokine such as epidermal growth factor and keratinocyte growth factor from platelets and macrophages. These will produce neovascularisation. Combination of proteolytic enzyme liberated from neutrophils, autolytic enzymes from dead tissue and phagocytic activity of macrophages clear off necrotic tissue and blood cells. Here the *Vrana* is considered as *Shuddha Vrana* because it having soft moist texture of tongue with the edges slightly swollen.

The new fibroblast originates from fibrocytes as well as mitotic division of fibroblasts. These fibroblasts secrete collagen and proteoglycans of connective tissue matrix that units wound edge together by assuming polymeric form. The collagen is the major components of the normal skin. Wound contraction is the next stage represented by centripetal movement of wound edges towards the centre of the wound. The

epithelial and granulation tissue is fills the wound space. This phase is considered as *Vrana Roohyamana* phase.

Scar remodelling is the final phase of wound healing process. The process of remodelling dramatically increases the wound bursting strength. This process is continues for some month and is visible as change colour. This can be considered as *Roodha Vrana* as wound site remains gradually to normal skin.

Discussion on Drug Action

Jathyadi Taila is indicated in *Nadivrana, Sphotaka, Kacchu, Visarpa, Dagdha Vrana, Sadhyo Vrana, kushta* etc and other type of *Dushtavrana*. *Taila* does *Shodhana* and *Ropana* on applying over *Vrana*.

Most of the ingredients of *Jathyadi Taila* are having *Tikta Kashaya Rasa* and *Laghu Ruksha Gunas*.

Kashaya Rasa: it dose *Shoshana* there by it might help in *Vrana Ropana*.

Tikta Rasa: it dose *Twak Mamsa Sthereekarana* and *Lekhana*. It might help in increasing tensile strength of wound.

Katu Rasa: it has *Vrana Shodhana* and *Avasadana* properties.

Tutha: it is one among the ingredients of *Jathyadi Taila*, having *Lekhana Karma*.

Tila Taila: it has *Ushna, Theekshna, Madhura, Vataghna, Vyavayi, Vikasi* and *Sukshma Gunas*. When it is treated with this drug, it takes the properties of those drugs. Therefore, it might help in reaching the minute channels.

As *Jathyadi Taila* includes the drug, which possess both *Shodhana* and *Ropana* qualities it, and helps in proper healing of *Vrana*.

Discussion on Experimental Study

- Experimental study was carried out using the excision wound model.
- Rats were used in this study because their biological characteristics closely resemble those of humans.
- Rats are small and cost effective making it easier to get subjects.

- Rats of either sex were selected.
- The diseased and pregnant rats were excluded as it may alter the results

Discussion on Excision Wound Model

- Morton and Malone developed this technique.
- Wistar albino rats were selected for the experiment.
- This model is standard model accepted for study of wound.
- The rats were divided into three groups (n = 18): Group I was treated with Control, Group-II was treated with 5% Povidone Iodine Ointment (Standard drug), Group-III was treated with *Jathyadi Taila*.
- After the animals are sufficiently anesthetized, a wound of 2.5 cm diameter was Excised on the dorsal thoracic central region.
- External application of *Jathyadi Taila* was started from the 1st day of post wounding. Control groups left without applying drug to observe the natural healing process and 5% Povidone Iodine Ointment is applied for the rats in the standard group.
- To monitor the changes in the wound shapes, the wound margins were traced on OHP sheet from the 1st day of post wounding and continued till the complete healing of the wound.
- This is again retraced on a millimetre scale graph paper. The observations of percentage of wound closure are made on the 0th, 3rd, 6th, 9th, 12th, 15th, 18th, 21st and 24th post wounding days.

Discussion on Wound Contraction

The *Jathyadi Taila* showed good effect on the wound. There was increase in the wound contraction time when compared with standard and control group. This shows that it has wound healing property. But reduction was statistically significant.

Discussion on Histopathology

In the histopathological study, the skin tissue collected from the different rats of control, standard and test group were examined under microscope at different magnifications. The tissue sections show

wound area. The epithelium (3-6 layers) is formed in two slides. Granulation tissue seen with acute and chronic inflammatory cells, newly formed blood vessels (Angiogenesis) and proliferating fibroblasts. Collagen deposition seen. Oedematous areas seen. Lower areas show macrophages, mast cells etc. Granulation tissue formation with mild epithelial regeneration.

In the standard group the tissue sections show wound area. The epithelium is 6 to 7 layers thickness. The granulation tissue seen beneath the epithelium consists of few chronic inflammatory cells like lymphocytes, proliferation of fibroblasts and new blood vessels. No discontinuity in epithelium. Collagen deposition seen. Abundance of pigment laden macrophages seen. Hair follicle formation seen in one slide. Granulation tissue formation with complete epithelial regeneration compared with Control, more epithelial regeneration & less inflammation.

Histopathology report in the test group shows the tissue section shows wound area. There is discontinuity in epithelium. The epithelium is 4 to 6 layers thickness. The granulation tissue seen beneath the epithelium, consists of few inflammatory cells (neutrophils and lymphocytes), more proliferation of fibroblasts and new blood vessels. Collagen deposition seen. Many pigment laden macrophages seen. Test group shows cells that are more inflammatory and less epidermal regeneration. Comparing with C, less inflammation and more angiogenesis

CONCLUSION

Twak is one of the *Indriyaadhishtana*, which completely covers the body and is more prone to diseases and trauma. The *Pramana* of the *Twak* layers, from outside to inside can be taken as 18/20, 16/20, 12/20, 8/20, 5/20, 1 and 2 *Yavas* respectively. The total thickness of the *Twak* is *Angushtodara Pramana*, which is approximately 5.95 *Yavas*. After this study, *Twak* layers can be compared with skin. *Avabhasini* and *Lohitha* as Stratum Corneum, *Sweta* as

stratum lucidum, *Tamra* as stratum spinosum and stratum granulosum, *Vedini* as stratum basale and papillary dermis, *Rohini* as reticular Dermis and *Mamsadhara Twak* as Superficial fascia. Wound healing process can be compared with the *Vrana Ropana* in our classics. The initial inflammatory phase can be considered under *Sudha Vrana*. Wound repair and regeneration phase as *Roohyamana Vrana*. Moreover, the scar and remodelling phase is *Roodha Vrana*. The events in the wound healing- like inflammatory phase, the granulation tissue, fibroblasts etc. can be seen in histopathological studies. *Jathyadi Taila* does *Shodhana* and *Ropana* of *Vrana* by the virtue of its ingredients and its properties. When compared to standard drug- Povidone iodine, the test drug – *Jathyadi Taila* has shown significant results in wound healing and scar remodelling.

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How to cite this article: Rakesh R, Nithin Kumar. A comprehensive study on Twak Shareera and an experimental study to evaluate histological changes observed by application of Jathyadi Taila. J Ayurveda Integr Med Sci 2021;5:100-110.
<http://dx.doi.org/10.21760/jaims.6.5.15>

Source of Support: Nil, **Conflict of Interest:** None declared.
