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An experimental evaluation of wound healing activity of *Paranthyadi taila* in wistar albino rats

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Abstract
Paranthyadi Taila has been mentioned in classics in the context of *mandali visha chikitsa* for the treatment of the wound caused by the bite of *mandala sarpa*. It has also mentioned that it is also a better choice of treatment for healing of any types of wounds. Wound is disease according to system of Ayurveda & *vrana shodhana* and *ropana* methods have been mentioned as a line of treatment. Three groups (control, standard and trial) had been taken for the study, each group containing 6 rats. In the trial group, P*aranthyadi taila* was applied over the wounded area. In excision wound model, percentage of wound contraction and period of epithelisation were the parameters for the study and this was achieved by using Planimetry (Technique developed by Mortone and Mallone). In *Paranthyadi taila* applied group more of mature collagen, good granulation tissue formation and profuse number of adnexia especially hair follicles in different stage of development were observed. This clearly shows that quality of wound healing process is enhanced by its application.

Keywords: *Paranthyadi taila*, wound healing, *vranaghna*

1. Introduction
Ayurveda isa science which comprise of two words viz.., '*Ayush*' and '*veda*'. *Ayush* means life and *veda* means science or knowledge. In Ashtanga Ayurveda, Agadatantra is a branch which deals with the signs & symptoms and also the treatment of various poisons[1] of both animate & inanimate origin [2]. This branch is a hidden treasure of various formulations those can be used not only in toxic conditions, but also in other diseases mentioned under other branches of Ayurveda. For e.g. *Bilwadi Gutika* indicated for *sarpa, lootha, mooshika, vrishchikavishas* is also effective in *vishuchika, ajeerna, jwara* [3] etc, *Kalyanaka Ghrita* indicated for *garavishachikitsa*, can also be used in *jwara, kasa, shosha* [4] etc.
The science of wound healing has an existing journey over the ages. From the references available in Ayurveda and History, we come across warriors being treated very effectively with then available medicines. When an individual develops an open wound nature attempts to cover the wound to prevent infection by the growth and migration of epithelial cells. This natural method is slow and explains why infections of the wound by germs or parasites are common. Poor wound healing not only cause trauma to the patient but increase the burden of financial resources.
Wounds are treated differently from another, depending upon how they happened and how serious they are. Healing is the response of the body to injury that sets into motion a sequence of events. With the exception of bone, all tissues heal with some scarring. The object of proper care is to minimize the possibility of infection and scarring. Though it is a natural phenomenon, many factors interfere with the healing of wounds. The role of wound healing medicines is to prevent infection and to fasten the healing process.
Clinically it has been seen that in case of snake bite, after the treatment for the venom, in the later stage, the wound will not be healed without a proper line of treatment. A number of formulations have been mentioned in this stage those can counteract the underlying pathology and aid in healing of the wound. *Paranthyadi Taila* has been mentioned in classics in the context of *mandali visha chikitsa*. It has been especially mentioned for the treatment of the wound caused by the bite of *mandali sarpa* [5, 6]. It has also mentioned that not only in this, it is also a better choice of treatment for healing of any types of wounds. Hence furthermore scientific evaluations are needed to determine and understand its pharmacological actions and effect in any type of wound healing in general. Hence the following study has been conducted to assess the rationality present behind the action of *Paranthyadi taila* in wound healing entitled "An Experimental Evaluation Of Wound Healing Activity Of *Paranthyadi Taila* In

Wistar Albino Rats"

Aims and Objectives

- To assess the wound healing activity of *PARANTHYADI TAILA* in wistar albino rats through excision wound model using following parameters.

1. Hydroxyproline estimation in wound tissue.
2. Histopathological changes in wound tissue.

Materials and Methods

Paranthyadi taila was procured from Vaidyaratnam Oushadhashala, Kasargod branch with batch no: 16A2684 and manufacturing date Oct 2016.

Healthy male wistar albino rats (200±50 g) were selected for the experiment. Six rats were taken for each group. The rats were used after acclimatization to the laboratory environment for a seven period. They were kept in the departmental animal house at 26±20C and light dark cycles of 10 & 14 hours, respectively. Animals were provided with rodent diet and water. All the experimental procedures were approved by Institutional Animal Ethical Committee (CPCSEA/IAEC/SDM-AT-04 dated 11/04/2016) of SDM Ayurveda College, Kuthpady, Udupi, Karnataka, India.

Grouping of animals

Wistar albino rats (200±50 g) were selected for the experiment. Six rats were taken for each group; the rats were bred in the animal house of SDM Ayurveda College, Kuthpady, Udupi, Karnataka, India. The rats were divided into three groups, Group I was treated with Control, Group II was treated with Betadine Ointment (standard drug) and Group III was treated with *PARANTHYADI TAILA.* Each group of rats were housed in individual cages and kept in a well-ventilated room under hygienic condition. The wound healing property of trial drug *PARANTHYADI TAILA* was evaluated in albino rats by excision wound model (technique developed by Morton and mallone) [7]

This technique consists of the following stages:

1. Pre-operative stage

2. Operative stage

3. Post-operative stage

I. Pre-operative stage

- The selected albino rats numbering 18 were primarily divided into three groups of 6 rats each, one group each for Control, Standard and Test.

II. Operative stage

- **Excision wound model**

This was conducted according to the technique developed by Morton and Malone97. The animals were anaesthetized using pentabarbitone intraperitoneally. 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 cm2 was impressed on dorsal plane of sub-scapular region, 5cm away from the ears on the depilated part of skin and extending to a depth of 0.2 cm from the demarcated area was excised to get a wound under mild anaesthesia with the help of forceps, surgical blade and scissors. After achieving full haemostasis, the animals were placed in their respective cages.

III. Post-operative stage

Paranthyadi taila was topically applied once daily till the complete epithelisation, starting from the day of excision. Control groups left without applying any 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 sheets 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 0th, 4th, 8th, 12th, 16^{th} and 20^{th} post wounding days. These wounds were also observed for period of epithelialisation. On 21st day animal was sacrificed and skin over the wound which was healed was cut and sent for hydroxyproline estimation.

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.

Wound contraction

The main factors which contributes to wound healing, is contraction. This was done by tracing the wound margins on an 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 size for each animal in the group depending on the days taken for wound contraction.

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

a. Period epithelisation

- Falling of scar leaving no raw wound behind was taken as end point of complete epithelisation and the days required for this was taken as period of epithelisation.
- In all groups, on 21st day, 3 rats from each group are randomly selected; the skin tissue carefully excised and sent for histopathological examination and Hydroxyproline estimation.
- Histopathological studies – preserved in 10% formalin for histopathological examination.
- Hydroxyproline content estimation – sample was stored in normal saline and stored at 20 ^{0}C.
- The obtained results of the test groups were compared with that of the standard and control groups.
- Statistical analysis of hydroxyproline data was generated by One Way ANOVA followed by Dunnet's multiple comparison't' test as post hoc test if $p<0.05$ using statistic software of Graph Pad prism 3.0 version.

Observation and Results

Table 1, 2, 3, 4, 5 shows Effect of *Paranthyadi Taila* on Percentage wound contraction measured on 4th, 8th, 12th, 16th and 20th post wounding day respectively

In Table 6 Data related to the effect of test drug on hyrdroxyproline concentration is depicted.

In Table 7, 8, 9 depict Histological Examination of skin of Control, Standard and Trial Group respectively.

Reports of the Histopathological examination

- Fig 1a and 1b - Histopathological section of skin of control group rats
- Fig 2a and 2b - Histopathological section of skin of standard group
- Fig 3a and 3b - Histopathological section of skin of trial group (*Paranthyadi taila*)

Exscision Wound Contraction

Table 1: Effect of *Paranthyadi Taila*on Percentage wound contraction measured on 4th post wounding day.

Group	% wound contraction measured on 4th day	Percentage change
Control	31.56 ± 5.92	
Standard (Betadine)	-9.79 ± 7.97 **	131.02 ↓
Test (*Paranthyadi Taila*)	19.07 ± 9.79	39.575 ↓

Data: MEAN ± SEM, **P < 0.01

Table 2: Effect of *Paranthyadi Taila*on Percentage wound contraction measured on 8th post wounding day

Group	% wound contraction measured on 8th day	Percentage change
Control	62.92 ± 10.88	
Standard (Betadine)	58.37 ± 6.05	7.23 ↓
Test (*Paranthyadi Taila*)	65.86 ± 5.62	4.67 ↑

Data: MEAN ± SEM, **P < 0.01

Table 3: Effect of *Paranthyadi Taila*on Percentage wound contraction measured on 12th post wounding day

Group	% wound contraction measured on 12th day	Percentage change
Control	90.18 ± 4.00	
Standard (Betadine)	89.66 ± 3.34	0.576 ↓
Test (*Paranthyadi Taila*)	94.69 ± 2.13	5.00 ↑

Data: MEAN ± SEM, **P < 0.01

Table 4: Effect of *Paranthyadi Taila*on Percentage wound contraction measured on 16th post wounding day

Group	% wound contraction measured on 16th day	Percentage change
Control	93.47 ± 2.44	
Standard (Betadine)	95.49 ± 0.60	2.161 ↑
Test (*Paranthyadi Taila*)	97.33 ± 1.65	4.13 ↑

Data: MEAN ± SEM, **P < 0.01

Table 5: Effect of *Paranthyadi Taila*on Percentage wound contraction measured on 20th post wounding day

Group	% wound contraction measured on 20th day	Percentage change
Control	98.13 ± 1.72	
Standard (Betadine)	99.21 ± 0.62	1.10 ↑
Test (*Paranthyadi Taila*)	99.17 ± 0.82	1.05 ↑

Data: MEAN ± SEM, **P < 0.01

Table 6: Data related to the effect of test drug on hyrdroxyproline concentration depicted.

Group	Hydroxyproline concentration (µg/g skin)	Percentage change
Control	419.20 ± 15.21	
Standard (Betadine)	360.55 ± 29.91	13.990 ↓
Test (*Paranthyadi Taila*)	375.53 ± 82.12	10.417 ↓

Data: MEAN ± SEM

Table 7: Histological Examination of skin of Control group

Rat no and section	Changes observed			Remarks
	Field 1	Field 2	Field 3	
Gp1 - R1	Epidermis continuous well formed, oedema in dermal and hypodermal regions, collagen fibres comparatively less, hair follicles numerous	Hypertrophy of epidermis, mix of young and mature collagen fibres, granulation tissue – highly vascularised in hypodermis	Epidermis well formed, collagen fibres numerous, adnexia-profuse	Oedematous changes in some places- different stages of development and regeneration
R2	Epidermis denuded, mix of young collagen fibres and granulation tissue – no adnexia	Epidermis continuous, collagen fibres present in moderate quantity, many hair follicles and sebaceous glands	Epidermis well formed, collagen fibres moderate in number, numerous hair follicles, plenty of leucocytes in hypodermis representing regeneration	
R3	Small area covered by epidermis, diffused oedema in dermis, remnants of hair follicle numerous – leucocytes in hypodermis	Small area covered by epidermis, diffused oedema in dermis, remnants of hair follicle numerous – leucocytes in hypodermis	Small area covered by epidermis, diffused oedema in dermis, remnants of hair follicle numerous – leucocytes in hypodermis	

Table 8: Histological Examination of skin of Standard group

Rat no and section	Changes observed			Remarks
	Field 1	Field 2	Field 3	
S1F1	Epidermis continuous- thin, Dermis with moderate no of mature collagen fibres; adnexia many ;	S-corneum thin; Epidermis continuous thin, collagen fibres present in moderate quantity, few hair follicles seen ; hypodermis with leuckocytes	S corneum and epidermis discontinuous, mature collagen fibres moderate in no; adnexia many	
S2F1	Thin continuous epidermis; dermis oedematous, collagen fibres mature and moderate in no; hypodermis thin with few leuckocytes	Thin continuous epidermis; dermis oedematous, collagen fibres mature and moderate in no; hypodermis thin with few leuckocytes- adnexia many	Thin continuous epidermis; dermis oedematous, collagen fibres mature and moderate in no; hypodermis thin with few leuckocytes- adnexia many	
S3F	Thin continuous epidermis; dermis slightly oedematous; collagen fibres mature and moderate in no; hypodermis thin with few leuckocytes- Hair follicles many	Thin continuous epidermis; dermis slightly oedematous; collagen fibres mature and moderate in no; hypodermis thin with few leuckocytes- hair follicles many	Continuous epidermis; dermis- collagen fibres mature and moderate in no; hypodermis thin with few leuckocytes- hair follicles many	

Table 9: Histological Examination of skin of Trial group (*Paranthyadi taila*)

Rat no and section	Changes observed			Remarks
	Field 1	Field 2	Field 3	
R1	Epidermis well formed, collagen moderate, hair follicles numerous, leukocytes in hypodermis	Mix – epidermis well formed – large; part exhibits collagen fibres with hair follicles,with underlying hypodermis showing presence of plenty of leukocytes – another part showing mix of young collagen and granulation tissue	Mix – epidermis well formed – large; part exhibits collagen fibres with hair follicles,with underlying hypodermis showing presence of plenty of leukocytes – another part showing mix of young collagen and granulation tissue Numerous young collagen fibres interspersed with leukocytes, hypertrophied epidermis	
R2	Epidermis slimmer, numerous adnexia, lower part of dermis oedematous	Epidermis slimmer, numerous adnexia, lower part of dermis oedematous, less leukocytes in the hypodermis	Epidermis slimmer, numerous adnexia, lower part of dermis oedematous, less leukocytes in the hypodermis	
R3	Very well developed epidermis- under differentiation stage, dermis full of young collagen with underlying granulation tissue-leuckocytes- no adnexia	Very well developed epidermis- under differentiation stage, dermis full of young collagen with underlying granulation tissue- leuckocytes- Highly vascularized hypodermis with plenty of leuckocytes	Very well developed epidermis- under differentiation stage, dermis full of young collagen with underlying granulation tissue, eosinphilic changes observed in upper dermis	

Histological Examination of Skin

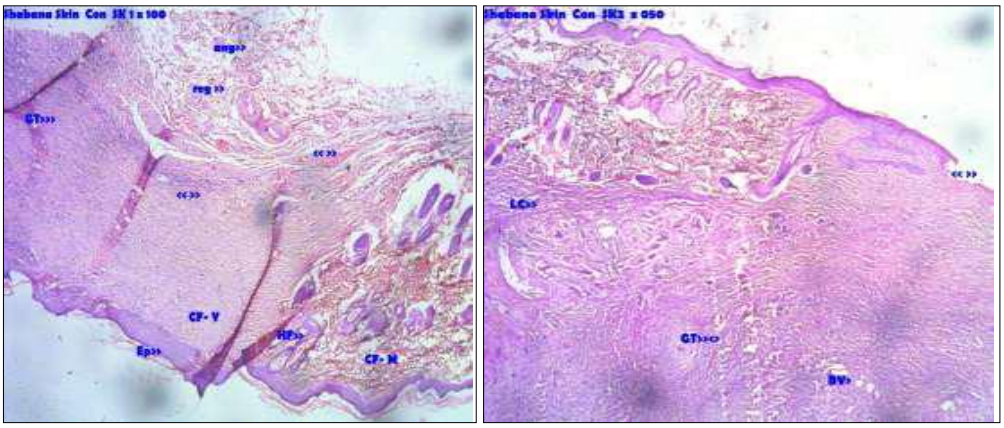

Fig 1a **Fig 1b**

Fig 1a 1b: Histopathological section of skin of control group rats

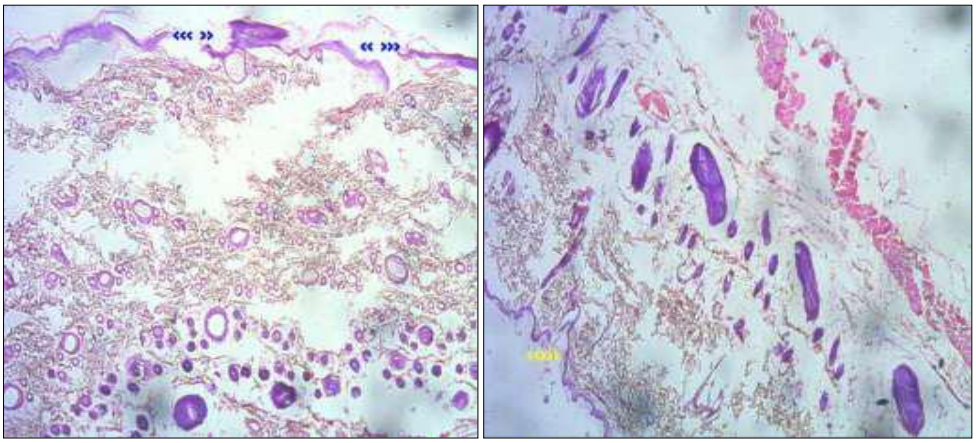

Fig 2a **Fig 2b**

Fig 2a, 2b: Histopathological section of skin of standard group

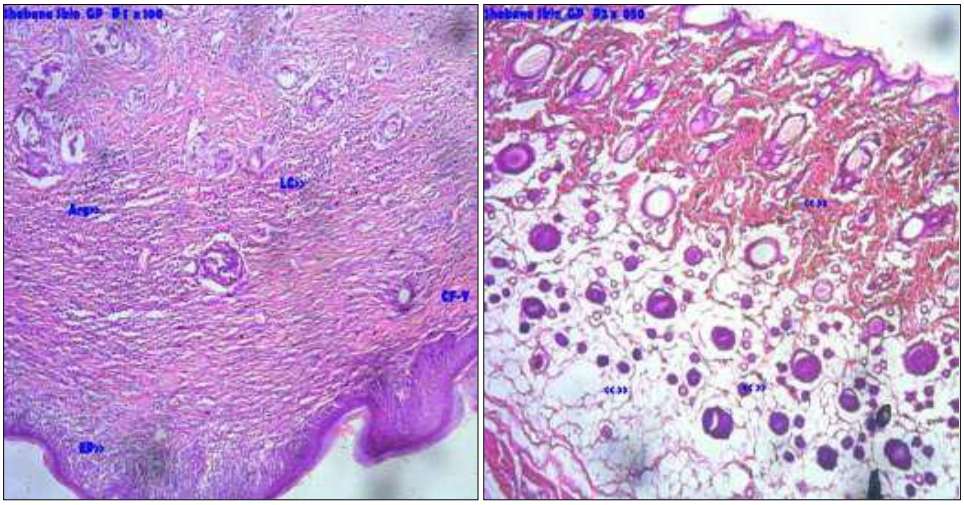

Fig 3a **Fig 3b**

Fig 3a, 3b: Histopathological section of skin of trial group (*Paranthyadi taila*)

Discussion

Histopathology: In control group, the wound area though healed almost simultaneously with test groups contained more of young collagen, mix of collagen and granulation tissue and adnexia like hair follicles, sebaceous glands were comparatively less. Sections from Standard group show developing epidermis, active regeneration in dermal components especially the adnexia, hypodermis contains only few leukocytes indicating lessened inflammation – features resemble mature skin. In *Paranthyadi taila* applied group more of mature collagen, good granulation tissue formation and profuse number of adnexia especially hair follicles in different stage of development were observed.

Experimental Study: The objective of the present study "An Experimental Evaluation of Wound Healing Activity of *Paranthyadi Taila* in Wistar Albino Rats" was to assess the wound healing enhancing effect of *Paranthyadi taila* in excision wound model. The efficacy of wound healing was assessed by number of days taking to normal epithelisation and wound contraction in excision wound by biochemical parameters such as hydroxyproline estimation and histopathological changes in wound area. The trial group is compared with control and standard group. The statistical inference was made using suitable statistical analysis.

In case of period of complete epithelisation or days taken for complete healing of the wound, less number of days was taken by wound healing in trial, standard when compared to control group. The observed difference was found to be statistically non-significant.

While comparing the data related to the hydroxyproline, there was non-significant increase in the concentration of hydroxyproline in test group rats as compared to normal control rats. The standard administered rats also had increase in the hydroxyproline concentration as compared to normal control, however the observed changes were found to be statistically non-significant. The synthesis of hydroxyproline is an important indicator of collagen deposition at the site of injury and in normal epithelisation process. In the present study there is a considerable increase in the hydroxyproline in *Paranthyadi* group as compared to control group.

The data obtained in this study were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's t-test. A Pvalue<0.05 was considered to be significant. All the values were expressed as MEAN ± SEM.

Histopathological examination revealed better wound maturation profile in comparison to the control group. In control group, the wound area though healed almost simultaneously with test groups contained more of young collagen, mix of collagen and granulation tissue and adnexia like hair follicles, sebaceous glands were comparatively less. In *Paranthyadi taila* applied group more of mature collagen, good granulation tissue formation and profuse number of

adnexia especially hair follicles in different stage of development were observed. This clearly shows that quality of wound healing process is enhanced by its application. It can be suggested that the test drug has wound contraction enhancing property in the early stages of wound healing and enhances the wound maturation process.

Conclusion

The test drug *Paranthyadi taila* in the case of period of complete epithelisation or days taken for complete healing of the wound, less number of days were taken in wound healing when compared to control group. While comparing the data related to the hydroxyproline, there was non-significant increase in the concentration of hydroxyproline in test group rats as compared to normal control rats. The histopathological examination revealed better wound maturation profile in comparison to the control group. In *Paranthyadi taila* applied group more of mature collagen, good granulation tissue formation and profuse number of adnexia especially hair follicles in different stage of development were observed. The study clearly shows that the quality of wound healing process is enhanced by its application. It can be suggested that the test drug has wound contraction enhancing property in the early stages of wound healing and enhances the wound maturation process. Hence from the present study it could be inferred that *Parnthyadi taila* has got wound healing effect. The study has helped to give a scientific validation for the textual reference.

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