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Research Article

WOUND HEALING PROPERTIES OF *VRANASHODHANAHARA TAIL* AND *DOORVADI TAIL* AS AN EXPERIMENTAL AND COMPARATIVE STUDY ON ALBINO RATS

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ABSTRACT

In Ayurveda, for oil preparations *Tila* oil is used as a base (good for wound healing), on external application attracts oil soluble toxic substances and can be washed out easily with lukewarm water. Study done on healthy albino rats of either sex were selected randomly and were divided into six groups (control group {P}, group for *Vranashodhanahara tail* {V} and group for *Doorvadi tail* {D} which subdivided into two groups), each containing four albino rats for evaluation and effect of prepared samples on wound healing. For the purpose of study two types of wounds were produced experimentally, excised or open wound (Morton and Mallone) and incised or sutured wound (Hunt's et. al.). The assessment was made by studying the regeneration tissue by different parameters viz. wound contraction and period of epithelialisation and tensile strength. The gross appearance was noted at the interval of 3 days i.e., 3rd, 6th, 9th and 12th day of applying wounds. The results show that excised wound contracted earlier in treated groups while in the trial groups complete closure was achieved around 14th day, where as in the control-P group, it was achieved around 17th day and incised wound indicating that trial V group provided better result than control-P.

KEYWORDS: *Vranashodhanahara tail, Doorvadi tail*, albino rats, plainimeter.

INTRODUCTION

In Ayurveda, the management of wounds has been divided in three phases such as *Vranashodhana* - tissue debridement; foreign body removed using instruments and irrigation of the wound by decoctions, poultice and other topical applications of certain herbal drugs. After the wound has been thus cleaned the later part of the treatment starts; where efforts are made to promote the healing (*Vrana Ropana*) by the application of different herbal preparations prepared in ghee, oil or suitable vehicles. When the wound has heals leaving the scar tissue which is sometimes hard, hypo or hyper pigmented and devoid of hairs in the hairy region. There is advocated definite medication for normalization of colour and trimming of the scar tissue. This type of treatment is known as *Vaikritapaham*.

AIM AND OBJECTIVES

- Experimental Study on *Vranashodhanahara tail* and *Doorvadi tail* W.S.R. to their wound Healing Properties.
- Conceptual study of *Vranashodhanahara tail* and *Doorvadi tail* W.S.R. to their wound Healing Properties.

MATERIAL AND METHODS

The trial drugs *Vranashodhanahara tail* contains *Tila Tail, Tila Beej* (Seasamum indicum), *Nimba Patra Swarasa* (Azadirachta indica) *Haridra* (Curcuma longa) and *Trivrit* (Operculina terpathum) where as the *Doorvadi tail* consists of *Tila Tail, Kampillaka* (Mallotus philippinensis), *Daruharidra* (Berberis aristata) and *Doorva Swarasa* (Cynodon dactylon). The formulations *Vranashodhanahara Tail* (Pharmacopiea Govt. of A.P.) and *Doorvadi Tail* (B.R. 47/79-80) were prepared in our laboratory, Department of Rasa Shastra & B.K., N.I.A., Jaipur, by adopting S.O.P. and S.M.P. as laid down in the respective pharmacopieas.

Materials: These are Albino rats 24 in numbers and other like as Ether, Scissors, Mosquito forceps, Artery forceps, Blunt forceps, Needle holder, Silk thread, Povidone – Iodine, Surgical cotton,

Surgical gloves, Scalpels, Albino rat cages, Suture needle, Feeding Bottles etc

Methods: Selection of Animals: Healthy albino rats of either sex weighing between 150 – 200 gms were randomly selected for the study. The rats were housed in the animal house of S.M.S. Medical College, Jaipur. The rats were fed with rat pellets and water ad-libidum.

Inclusion Criteria:

- Healthy albino rats of either sex were included, weighing between 150 – 200 gms.

Exclusion Criteria

- Albino rats weighing less than 150 gms. and more than 200 gms. were excluded.
- Infected and pregnant animals were excluded from the study.
- Any animals showing signs of infection during the course of the study were also excluded (eight infected animals were removed in the present study)

Grouping of animals: Twenty four albino rats of either sex were selected randomly from the animal house and were divided into six groups each containing four albino rats. The rats were housed in individual cages and kept in a well ventilated room under hygienic condition.

Table 1: Showing grouping of animals

Group	Group Model	No. of rats	Purpose
Control-P	Excision	4	To serve as prophylactic control
Control-P	Incision	4	To serve as prophylactic control
Trial – V	Excision	4	To serve as prophylactic effect
Trial – V	Incision	4	To serve as prophylactic effect
Trial – D	Excision	4	To serve as prophylactic effect
Trial – D	Incision	4	To serve as prophylactic effect

Excised wound was made on the back of Albino rats in 2.0 Sq.cm. ascetically. Control-p groups were applied *Tila tail* while treated 2 ml. of *Vranashodhanahara tail* and *Doorvadi tail* separately once daily topically was applied to drug treated groups. The gross appearance was noted at the interval of 3 days i.e., 3rd, 6th, 9th and 12th of applying wounds. The wounds were traced on transparent paper and area measured with plainimeter (millimeter scale graph paper). Period of epithelialisation is calculated as the day the scar falls off leaving no raw area behind.

Incised wound was done as paravertebral incision measuring 6 cms of full skin thickness was made. Then the incision was closed by interrupted sutures at an interval of 1 cm. The albino rats were applied trial drugs and plain *Tila* tail in control-P group from '0' wounding day on both the lips once daily. The sutures were removed on 7th post wounding day and the wounds were examined for tensile strength contents to know the quality of healing in comparison to control-P on 10th post wounding day. In incision wound model the strength of the granulation tissue was measured. Once a breach or gap has occurred in the tissue it is closed by the granulation tissue. Here the strength of the granulation tissue is assessed using a simple instrument called the Tensionometer.

Experimental study

The wound healing property of the trial drugs, *Vranashodhanahara Tail* and *Doorvadi Tail* analyzed in albino rats by two methods viz., Excision wound model (technique developed by Morton and Mallone[1]) and Incision wound model (technique developed by Hunts et. al.[2]) These techniques consists of the following stages –

- Pre – operative stage.
- Operative stage (creation of wound model)
- Post – operative stage.

Pre – operative stage: The Selected albino rats were fasted overnight and water was given ad-libidum. Each rat was hydrated with 5 ml of water and they were kept in individual cages.

Operative stage: The selected albino rats numbering 24 were primarily divided into 12 each for excision and incision wound models.

Excision wound model

This is conducted according to the technique mentioned by Morton and Mallone. The animals were anaesthetized using ether as an anesthetic agent in semi-aseptic condition. After the animals were sufficiently anaesthetized they were secured to the dissection plate in a prone position. The hairs were removed by using scissor and razor from the part to be operated and subsequently the area was cleaned with betadine solution. A round seal of 2 cm. in diameter was impressed on the dorsal region i.e. 5 cms 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 scissors and surgical blade. The approximate area

of the wound thus formed was 315 mm^2. After achieving full haemostasis the animals were placed in individual cages.

Incision Wound Model

This is conducted according to the technique mentioned by Hunts et. al. The animals were anaesthetized using ether as an anesthetic agent in semi aseptic condition. After the animals were sufficiently anaesthetized they were secured to the dissection plate in a prone position. The hairs were removed using scissors and razor from the part to be operated and subsequently the area was cleaned with betadine.

The paravertebral incision was measuring 6 cms. of full skin thickness was made. Then the incision was closed by interrupted sutures at an interval of 1 cm. After achieving full haemostasis, the animals were placed in individual cages.

Post operative stage

External application of *Vranashodhanahara Tail* and *Doorvadi Tail* was started from the day of surgery (0 day). Every post wounding day the wounds were cleaned with normal saline and the trial drugs were applied to the trial groups where as in control-P group *Tila tail* was applied for healing process. The rats were given normal food and water.

Observations: The following observations were carried out for the 2 wounds models.

Excision wound model: To monitor the changes in the wound shape, the wound margins were traced on a thin transparent polythene sheet from the day of wounding (0 day) and continued till the complete healing of the wound. This was again retraced on a millimeter scale graph paper.

The observations of percentage of wound closure were made on the 3rd, 6th, 9th and 12th post wounding day. The wound was also observed for the period of epithelialisation.

Incision Wound Model: The wound was observed for its breaking strength on the 10th post wounding day.

Assessment criteria: Wound contraction and epithelialisation were the parameters employed to study excision wound model and this was achieved using planimetry. As the role of collagen in wound healing is well studied, the estimation of tensile strength was employed to study the incision wound model and this was achieved through tensiometry.

Excision Wound Model

a. Wound contraction: The factor which attributes mainly to wound healing (closure) is contraction. This was done by tracing the wound margins on a thin transparent polythene sheet and subsequently retracing them on a millimeter 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 complete wound contraction.

b. Period of Epithelialisation: It was measured in terms of days required for the falling of the scar. Falling of scar leaving no raw area behind was taken as the end point of complete epithelialisation and the time was noted in all animals.

Incision Wound Model: Here the tensile strength or breaking strength is measured. As per the method followed, the volume of water required to open' the edges of the wound was measured and converted to the corresponding weights assuming the density to be equal to one. Using this tensionometer, the tensile strength is expressed as the minimum weight of water necessary to bring about the gaping of the wound.

As per this method the suture were removed on 7th post wounding day and the breaking strength was determined on the 10th post wounding day by method of Lee[3], as described below. All animals were anaesthetized before measuring the breaking strength using tensionometer. Each animal was secured to the operation table in its natural position and lines were drawn on either side of the wound, 3 mm away from the wound margins on adjacent normal skin, leaving about 5 mm. wound towards both ends. Two allies' forceps were firmly applied on the lines, facing each other. One side of the forceps was hooked to a metal rod and was fixed firmly to the operation table. The other side was fixed to a light polythene container through a string run over a pulley. Water was allowed to flow at constant rate into the polythene container so as to build a gradual pulling force necessary to disrupt the wound. Tensile strength corresponds to the increase in amount of collagen present.

The flow of water was regulated by means of an occlusion clamp on rubber tubing connected to a water reservoir, kept at a suitable height. As soon as the gaping of the wound was observed, the water flow was cut off. Further gaping of the wound was avoided by releasing the pulling force on the wound immediately, which was achieved by lifting up the polythene container. The volume

of water in the polythene container was measured as weight.

Results of Excision wound Model: The parameters assessed in the excision wound model are:

- Rate of wound contraction
- Epithelialisation phase

Rate of wound contraction is calculated as percentage and expressed in square millimeter. The changes were observed once in three days from the day of wounding. Thus four readings were taken i.e. 3rd post wounding day, 6th post wounding day, 9th post wounding day and 12th post wounding day. By the 14th post wounding day complete healing was achieved in the trial groups treated with *Vranashodhanahara Tail* and *Doorvadi Tail*. In the Control-P group in which the plain Tila Tail was applied, complete healing was achieved only on the 17th post wounding day. Epithelialisation phase was calculated in number of days.

The parameter assessed in the incision wound model was the breaking strength or tensile strength of the wound. On 3rd days, comparing the Control-P and trial D group the t value obtained was 2.90 which is mild significant ($p < 0.050$) i.e., trial V group is better than Control-P group, on 6th days, comparing the Control-P and trial D group t-value obtained was 2.46, which is mild significant ($p<0.050$) i.e., trial D group is better than Control-P group, on 9th days, comparing the Control-P and trial D group t – value obtained was 5.18, which is highly significant ($P < 0.005$) i.e., trial D group provided better result than Control-P group. On 12th days, comparing the Control-P and trial D group t – value obtained was 3.77, which is moderately significant ($P < 0.010$) i.e., trial D group provided better result than Control-P group.

Table 2: Showing % closure of original excision wound area (sq. mm.) on post wounding day

On Days	Group	No. Of RAT				Mean	S.D.	S.E.	t-value	P-value
		Rat 1	Rat 2	Rat 3	Rat 4					
3rd	Control P	25.85	27.48	28.82	27.13	27.46	1.65	0.83	33.22	<0.001
	Group V	37.0	38.18	32.15	35.40	35.68	6.84	3.42	10.43	<0.005
	Group D	31.33	30.08	36.21	34.1	32.95	7.68	3.84	8.59	<0.005
6th	Control P	49.40	54.58	55.25	52.88	53.03	6.84	3.42	15.50	<0.001
	Group V	67.61	69.30	63.51	64.71	66.28	7.01	3.50	18.91	<0.001
	Group D	62.60	60.44	67.04	65.59	63.92	8.79	4.40	14.54	<0.001
9th	Control P	85.03	79.36	82.00	84.45	82.71	6.71	3.36	24.64	<0.001
	Group V	90.73	92.46	87.79	89.47	90.11	1.56	0.78	113.89	<0.001
	Group D	88.65	87.44	90.23	89.79	89.03	3.90	1.95	46.22	<0.001
12th	Control P	92.73	84.05	88.14	91.03	88.99	14.43	7.21	12.34	<0.001
	Group V	98.12	100.0	95.18	97.90	97.80	1.73	0.86	112.23	<0.001
	Group D	96.21	95.50	98.32	97.76	96.95	3.94	1.97	49.66	<0.001
Showing the period of Epithelialisation (In number of days)										
	Control P	15	17	16	15	15.75	0.92	0.46	34.36	<0.001
	Group V	13	12	14	13	13.00	0.33	0.17	81.00	<0.001
	Group D	14	15	13	13	13.75	0.67	0.33	39.00	<0.001
Showing tensile strength in gms. of incision wound on 10th post wounding day										
	Control P	309.0	294.0	291	318	303	162	81	3.74	<0.050
	Group V	430.0	423	442	436	432.75	66.25	33.13	13.06	<0.001
	Group D	389	409	392	406	399	99.33	49.67	8.03	<0.001

Table 3: Showing comparative % closure of excision wound area on wounding day

On Days	Groups	t- value	P- value	Remarks
3rd	Control P & Group V	3.29	<0.01	V>C
	Control P & Group D	2.90	<0.05	D>C
6th	Control P & Group V	2.98	<0.025	V>C
	Control P & Group D	2.46	<0.05	D>C
9th	Control P & Group V	6.96	<0.001	V>C
	Control P & Group D	5.18	<0.005	D>C
12th	Control P & Group V	4.50	<0.005	V>C
	Control P & Group D	3.77	<0.01	D>C

Showing comparative epithelialisation between the groups				
Epithelialisation	Control P & Group V	8.94	<0.001	V>C
	Control P & Group D	6.14	<0.001	D>C
Showing comparative tensile strength in between the groups				
Tensile strength for incision wound	Control P & Group V	5.52	<0.001	V>C
	Control P & Group D	3.83	<0.01	D>C

(V = *Vranashodhanahara tail*, D = *Doorvadi tail*, C = Control-P)

DISCUSSION

For excised wound, the mean contraction was seen on the control-P group on 3rd day was 27.46±1.65%, where as in trial V was 35.68±6.84%, the trial-D group was 32.95±7.68% (Table No.3) on comparison trial V and D group, the mean contraction of control-P group on 6th day was 53.03±6.84%, where as trial group V and D were 66.28±7.01% and 63.92±8.79% respectively. The mean contraction of control-P group on 9th & 12th day were 82.71±6.71% and 88.99±14.43%, where as trial group V & D were 90.11±1.56%, 97.80±1.73% and 89.03±3.90%, 96.95±3.94% respectively. The overall contraction result was observed in the control-P and trial V group with a t-value was 4.50, which is highly significant (p<0.005) indicating that, trial V group provides better result than control-P group. While comparing to the Control-P group and trial D group the obtained t-value was 3.77, which is moderately significant (p<0.01) suggesting that trial D group provides better results than control-P group. The results show that the wound contracted earlier in treated groups while in the trial groups complete closure was achieved around 14th day, where as in the control-P group, it was achieved around 17th day.

The mean period of epithelialization in the trial V group was 13 days and in the trial group D was 13.75 days and that of the control group P was 15.75 days. The period of epithelialization in between control P and trial V groups t-value obtained was 8.94, which is highly significant (p<0.001) i.e. trial V group provided better result than the control-P group and on comparison with control and trial D group the t-value obtained was 6.14 which is highly significant (p<0.001) i.e. trial D group provided better results than control-P group.

For incised wound, the mean of the trial V group was 432.75 and the trial D group was 399.00 and that of the control-P group was 303.00 On comparison, tensile strength between the control-P and trial V group the t-value was 5.52, which is highly significant (p<0.001) indicating that trial V group provided better result than control-P while comparing to control P and trial D group the obtained t-value was 3.83, which is moderately significant (p<0.010) showing that trial D group provided better results than control group.

CONCLUSION

- The trial drugs were prepared according to pharmacopoeia of Govt. of A. P. after analysis of the tail shows that it confirms to the prescribed chemical and physical standard.
- *Vranashodhanahara tail* and *Doorvadi tail* showed significant result in percentage of contraction of original excision wound.
- Both *Vranashodhanahara tail* and *Doorvadi tail* showed significant result in period of epithelialization.
- *Vranashodhanahara tail* and *Doorvadi tail* showed significant result in tensile strength or breaking strength.
- Since *Vranashodhanahara tail* and *Doorvadi tail* have shown very good effect over the three parameters assessed, it can be concluded that both tails have wound healing properties.
- The studies can be done clinically on large number of patients and also in comparison with other drugs which are already proved to have wound healing properties.

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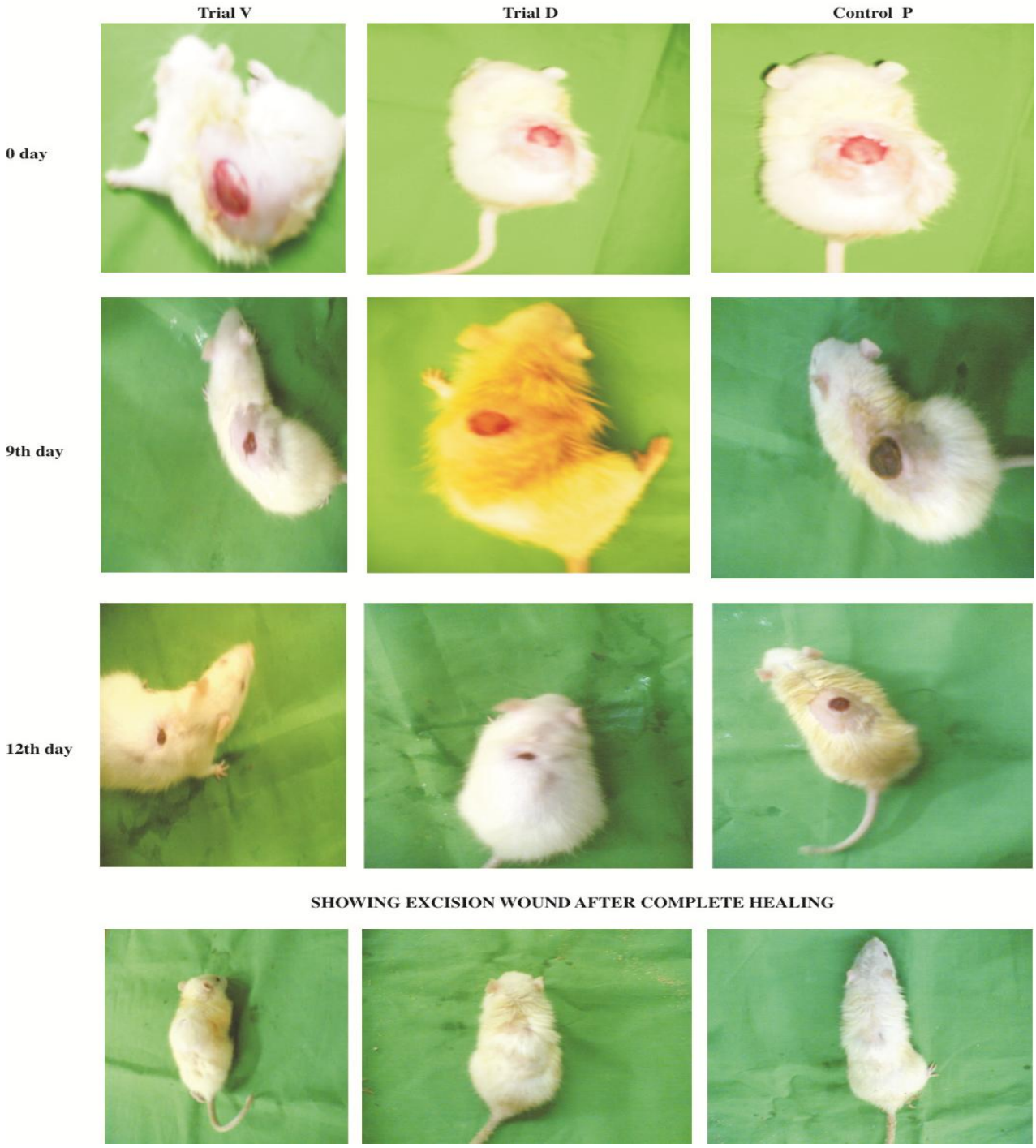
Stages of Excision Wound Healing



SHOWING EXCISION WOUND AFTER COMPLETE HEALING