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Experimental study of *Pruthvisara taila* in excised wound model in Wistar albino rats

Research Article

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Abstract

Pruthvisara taila is a formulation indicated in the *chikitsa* of *vrana* as topical medicine in classical text *Chakradatta.* It contains *Shuddha chitrakamoola*, *Shuddha vatsanabha, Shuddha karaveera, Nirgundi moola, Nadibeeja, Kanji* and *Karanja taila* as the base. Aims and Objectives: Evaluation of wound healing property of *Pruthvisara taila* through experimental study. Methodology: Wound healing property in albino rats by excision wound healing model and its histopathology study. Results: Results of the study on the parameters assessed like percentage of wound contraction and histopathology study; percentage of wound closure was observed in Control group was 94.92%, in standard it was 93.73% and in test drug the percentage of wound contraction was 95.73%.The test drug, *Pruthvisara taila* showed more angiogenesis and formation of new blood vessels than standard group and in control group there was absence of formation of new blood vessels and proliferation of fibroblast cells. The control group did not show any collagen formation and scab formation the test drug *Pruthvisara taila* showed moderate formation and deposition compared to standard drug. Compared to control and standard group, the test drug, *Pruthvisara taila* selected in this study showed remarkable wound healing property in excised wound.

Keywords: *Pruthvisara taila,* Wound healing, Histopathology, Angiogenesis.

Introduction

In Ayurveda many formulations are indicated for various kinds of wounds as topical medicine which are not been tested. In the present study, one such attempt has been made to explore the efficacy one such formulation and assess its efficacy of through wound healing model in Wistar albino rats.

Wound is the discontinuity in the skin and body tissues. Every human being irrespective of age will get affect from wound in their lifetime in one or the other. The presence of wound over the skin causes cosmetological worry for the individual. So, the wound healing is expected to be faster and in a good manner leaving minimal scar. Excised wound is a condition where deep tissues are involved. Here the medicine which is going to be used should be effective, in repairing tissue damage than any other wound healing drugs(1).Wound management includes topical agent as well as dressing. A topical agent is that which is applied to a wound. The *Shodhana* and *Ropana karma* are important factors responsible for wound healing. In classics Acharya have mentioned the *Taila* as *Twachyam* and it possesses the *Vyavayi* and *Sookshma guna* hence it enters even through minute pores and spread very quickly.

Among medicinal plant extracts, oils are mainly used for skin healing purposes, especially those rich in essential fatty acids (EFAs), because these compounds (such as linoleic acid) are necessary for maintaining epidermal integrity and the skin's water barrier(2).

The use of oils shows promising results in the treatment of skin wounds, as they have an effective impact on the phases of the wound-healing process through their antimicrobial, anti-inflammatory, and antioxidative activities and by promoting cell proliferation, increasing collagen synthesis, stimulating dermal reconstruction, and repairing the skin's lipid barrier function (3).

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Many formulations have been told in classics which are *Vranaropana* and *Vranashodhana, Pruthvisara taila* (4) is one among them. It is oil based formulation which has been mentioned as one of remedy for wounds. The study was carried out to evaluate the efficacy of *Pruthvisara taila* on the epithelial tissue repair of full thickness excision wound model in Wistar albino rats.

Materials and Methods

Drug

The *Pruthvisara taila* contains *Shuddha chitraka moola (5) (Plumbago zeylanica* Linn),*Shuddha karaveer (6)(Nerium indicum*. Mill*) Shuddha vatsanabh(7) (Aconitum ferox* Wall.) *Nirgundimoola (Vitex negundo* Linn*), Nadibeeja (Corchorus olitorius* Linn.*), Kanji(8) and Karanja taila(Pongamia pinnata* L) pierre) as a base. The preparation of formulation was carried out in *Rasashastra and Bhaishajya Kalpana* practical laboratory, Sri Dharmasthala Manjunatheshawara College of Ayurveda and Hospital, Hassan and Standard drug Povidine iodine was taken.

Experimental study and Histopathological study

The experimental protocol for this study was approved by the institutional animal ethical committee (SDMCRA /IAEC/ Ph- RS -01).Healthy Wistar albino rats of either sex were weighing 150-200gm.were selected from animal house of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. They were individually housed and maintained on normal rat diet and water at libitum throughout the study. They were acclimatised in the laboratory condition for two weeks prior to experimentation and were periodically weighed before the experiment.

Experimental protocol (9)

Equipments: Albino rat cages, Blunt forceps**,** Scissors, Surgical cotton**,** Betadine solution, surgical gauze**,** Mosquito forceps, surgical gloves**,** Artery forceps and Scalpels.

Procedure: The experimental study involves following steps

- Pre- operative stage
- Operative stage
- Postoperative stage

Pre-operative stage:

The 24 albino rats were selected for study and divided into 3 groups of 6 animals in each group.The groups were treated as follows; Group I was Control group where the animals were treated without any drug treatment. Group II was considered as the Standard group with application of Povidine iodine as drug and Group III consist of *Pruthvisara taila* as test sample which was considered as Test group.

Table No.01: Showing the grouping of animals

Sl. No.	Group	No. of rats	Drug	Purpose
1	Group I -Control group	Six	No drug application	To assess the healing process in the excised wound healing process and also to compare with the wound healing process of other groups**.**
2	Group II-Standard group	Six	Povidone iodine	To assess thewound healing process.
3	Group I III Test group	Six	*Pruthvisara taila*	To assess the wound healing process.

Phase of operation

The wound was produced at the designated wound site using the excision wound procedure. One day before the trial, the specific skin area was shaved. Ketamine [50 mg/kg, IP] and Xylazine [3 mg/kg, IM] were used to anaesthetise the rats both before and after the experimental wounds were inflicted.An impression was taken on the anaesthetised rat's dorsal thoracic area, one centimetre from the spinal column and five centimetres from the ear.

A wound measuring 2 centimetres was created by completely excising the impressed area. The cotton swab soaked in saline was used to blot the wound in order to achieve hemostasis. Diethyl ether was used in sterile settings to perform the surgical procedures. The study's postoperative phase involved separating and isolating any animals exhibiting signs of infection, as they were closely observed for infection. The administration of the drug was external and the duration of study was for twenty-four days.

Postoperative stage

As the route of drug administration was external and duration of study was carried out for 24 days. The application of standard drug povidine iodine and test drug *Pruthvisara taila* were applied to the respective groups from 1st day of wounding. The Control group rats received normal diet and water ad libitum without any drug application for natural healing.

The changes in the wound shapes during the observational stage were monitored by tracing the wound margins by trace paper/ transparent paper from the 1st day of wounding on the 4th, 8th, 12th, 16th, 20th, 24th post wounding days. The marked traced papers were again retraced on a millimeter scale graph paper. The wounds were observed for wound contraction and for the period of epithelialization. The animals were inspected daily & the health was assessed based on physical parameters.

The percentage of wound contraction

The main factors which contribute wound healing, is contraction. This was done by tracing the wound margins on a trace paper 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 complete wound contraction.

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

Epithelisation Period

The completion of epithelialisation was determined by measuring the number of days needed to achieve the end point of falling scar and leaving no open wounds.

Statistics evaluation

Utilizing Dunnet's multiple t-test as a post hoc test, the collected data was examined using an ANOVA one-way analysis. The tool used for this was Graph Pad Inst 3. For statistical significance, a p-value of less than 0.05 was used.

Histopathological study

Procedure

Fixation: The tissues were excised as soon as the animals were sacrificed. Following cleaning and suitable thickness cutting, the extraneous tissue was placed in a 10% formalin solution. Before being removed for processing, the tissues were left in solution.

Processing of tissue: The tissue was thoroughly cleaned by running tap water, and it was then put through a sequence of solvents for paraffin infiltration, clearing, and dehydration in accordance with the schedule.

Table 02: Showing the solvents used and timings of tissue processing

Sl no	Solvent	Timing
1	Alcohol 70%	20 minutes
2	Alcohol 80%	20 minutes
3	Alcohol 90%	20 minute
4	Alcohol 95%	20 minutes
5	Isopopyl Alcohol	20 minutes
6	Acetone (2 changes)	20 minutes
7	Chloroform(3 changes)	20 minutes
8	Melted paraffin wax (60^0 C) (2 changes)	30 minutes each

After the fixation of tissues they were embedded in paraffin wax to prepare tissue blocks. After trimming tissues to suitable sizes tissue blocks were fixed to a metal object holder.

Section cutting

The tissue section were cut to the 5-6 µm thickness with help of Spencer type rotating microtone of and placed in a water bath between 50-55^{0}C for 30 minutes and then they were mounted on clear glass slides with a drop of Mayer's egg albumin dried on hot plate at 50^{0}C for 30minutes.

Staining

After fixing the tissue section on the slide, the sections were stained by serially placing them in the following reagents

Table 3: Showing the utilized reagents and timings for tissue staining

Sl. No	Reagent	Timing
1	Xylol(2 changes)	3 min
2	Acetone	3 min
3	Alcohol 95%	3 min
4	Running water	3 min
5	Haematoxylin stain	20 min
6	Running water wash	20 min
7	Eosin working solution	2 min
8	Alcohol 95% (3changes)	3 min
9	Acetone (2 changes)	-3 min
10	Xylol(2 changes)	3 min

After tissue staining and passing through all the above reagents and stains, the slides were covered with D.P.X (Dibutylphthalate polystyrene xylene) and cover slip were placed. Care was taken to avoid the air bubble formation during mounting the slide. The slides were viewed under binocular research Carl-Ziess's microscope (Germany).At various magnifications to note down the changes in the microscopic features of the tissues studied.

Grading was done for following features

Regeneration of Epidermal tissue, Granulation tissue, Infiltration of inflammatory cells, Angiogenesis, Fibroblast cells proliferation, Deposit of collagen fiber and scab formation.

Results

Table 4: Showing the percentage (%) of wound contraction measured during study period

	Control		Standard-Povidine iodine		Test-*Pruthvisara taila*	
Days	% of wound contraction	% changes	% of wound contraction	% changes	% of wound contraction	% changes
3rdday	19.79±3.07	-	18.98±2.75	4.09↓	12.83±4.37**	85.69↓
6th day	47.57±3.00	-	37.36 ± 4.66	21.46↓	40.17±5.13	15.55↓
12th day	76.09±3.14	-	77.46±3.64	1.80↑	58.16±11.81	23.56↓
15th day	82.98±2.22	-	83.37±4.56	0.46↑	81.40±7.67	1.90↓
18st day	87.21±1.95	-	91.20±3.92	4.57↑	87.59±3.57	0.43↑
21th day	90.82±1.75	-	92.05±3.99	1.35↑	91.91±3.34	1.20↑
24th	94.92±1.67	-	93.73±3.70	1.25↓	95.34±1.91	0.44↑

Data: MEAN ± SEM,*P<0.05 in comparison to normal Control group

The data related to the % of change in wound area on 3rd day. Control group marginal (19.79) increase in wound area was observed. The data shows decrease in wound contraction in Standard, test group when compared to the control group, the observed decrease in wound contraction was found to be statistically non-significant. There was decrease in wound contraction in test drug when compared to the control group, the observed decrease was found to be statistically very significant. On 6th day, in control group marginal reduction (47.57) in wound area was observed, there was decrease in wound contraction in standard group and test group when compared to the control group, the observed decrease was found to be statistically non-significant.

On 12th day, in Control group marginal reduction (76.09) in wound area was observed there was increase in wound contraction in Standard when compared to the Control group, the observed increase was found to be statistically non-significant. There was decrease in wound contraction in test group when compared to the control group, which was statistically non-significant.

The change in wound area on 15th day, in control group marginal reduction (82.98) in wound area was observed. The data shows there was increase in wound contraction in standard group when compared to the Control group, the observed increase wound contraction was found to be statistically non-significant. There was decrease in wound contraction in test group when compared to the control group, the observed decrease was found to be statistically non-significant.

The change in wound area on 18th day, in control group marginal reduction (87.21) in wound area was observed. The increase in wound contraction in standard and test group when compared to the control group, the observed increase was found to be statistically non-significant. The change in wound area on 21st day, in control group marginal reduction (90.82) in wound area was observed. The data showed there was increase in wound contraction in Standard and test group when compared to the Control group, the observed increase was found to be statistically non-significant.

The data related to the change in wound area on 24th day, In control group, marginal reduction (94.92) in wound area was observed. There was decrease in wound contraction in Standard group when compared to the control group, the observed decrease was found to be statistically non-significant. There was increase in wound contraction in test group when compared to the control group, the observed increase was found to be statistically non-significant.

In the excised wound model studied, significantly improved wound healing activity has been observed with the prepared test drug *Pruthvisara taila,* compared to that of the reference standard and control group of animals.

Histopathology study Result

Skin tissues of the wound from different groups were examined under microscope at 40X, 100X and 400X magnifications. Grading was done for various features as follows

Table 5: Showing the histological changes observed in all groups

Slide No	Epidermal regeneration	Granulation tissue	Inflammatory cell infiltration	Angiogenesis	Proliferation of fibroblast cells	Collagen deposit	Scab
C-1	-	-	+++	-	-	-	
C-2	-	-	+++	-	-	-	
C-3	-	-	+++	-	-	-	
S-1	++	+	++	-	+	+	
S-2	++	+	+	+	+	+	
S-3	++	+	+	+	+	+	
T1-1	++	++	++	+	+++	++	-
T1-2	++	++	++	+	++	++	-
T1-3	+	++	+++	+	++	++	+

+ slight; ++ moderate; +++ extensive; − absence

Changes observed in tissue sections in each group

In Control group the tissue sections of wound area showed small areas of necrosis with cell debris with aggregation of neutrophils. The Hemorrhagic spots are seen. There was absence of Angiogenesis, proliferation of fibroblast cells and collagen deposits. Inflammatory cell infiltration was extensively seen. Epithelial regeneration and granulation tissue formation was absent. The tissue sections in Standard group showed granulation tissue beneath the epithelium which was 3 to 9 layers thickness. The epidermal regeneration was moderately formed. It consists of slight inflammatory cells (lymphocytes, neutrophils) and new blood vessels were seen in some slides. Necrosis was not observed. There was slight Angiogenesis; less Proliferation of fibroblast cells, and collagen deposits was seen. In test drug histopathology study, tissue section of wound area showed slight granulation tissue with few new blood vessels were seen and small area of stratified squamous epithelium of 4 to 11 layers thickness was observed. The granulation tissue seen beneath the epithelium with moderate inflammatory infiltration of cells (lymphocytes, neutrophils). Hemorrhagic areas and cell debris seen in few slides with chronic inflammatory cells. There was slight angiogenesis and reduced inflammatory infiltrate was seen. The proliferation of fibroblast cells, collagen deposit and epidermal regeneration were moderately observed which helps in healing of wound.

Discussion

Wound healing refers to the healing process that occurs after an injury to the skin or other soft tissue. The goal of wound care is to get the wound healed as quickly as possible, without causing any pain, discomfort, or scarring for the patient.

Wound closure is the resultant effect of wound contraction. The percentage of wound closure observed in this study

Table 6: Wound closure observed in each group

Group	3rd day	6th day	12th day	15th day	18th day	21st day	24th day
Control	19.79	47.57	76.09	82.98	87.21	90.82	94.92
Standard	18.98	37.36	77.46	83.37	91.20	92.05	93.73
Test-1 (NPT)	2.83	40.17	58.16	81.40	87.59	91.91	95.34
Test-2 (SPT)	12.48	16.76	60.47	69.81	78.67	79.51	83.30

In the initial days of the study the increase in wound contraction was seen in standard drug compared to control and test drug. The percentage of wound closure was observed in different groups. In Control group 94.92% of wound closure was observed in standard it was 93.73% and in test drug the percentage of wound contraction was 95.73% which was more compared to control and standard group.

The excised wound comes under *Sadhyo vrana* (fresh wound) hence the *ropana* property is required but the test drug possess the *Ushna* and *Tikshna* property which may delay in wound contraction initial days but may help in *Dushta vrana* as it requires *Ushna* and *Tikshna* property to remove the *Doshas* (slough) from wound which help in formation of granulation tissue so wound contraction was seen more in test drug compared to control in later part of the study.

In histopathology study the wound areas in the skin from different groups were examined under microscope at different magnifications. In Epidermal regeneration process, the epidermis cells, or top layers of skin, continuously replace themselves. During this journey, cells that produce keratin undergo a series of biochemical and morphological changes that result in the formation of various layers of skin (10).In this study, the control group slides did not show any epidermal regeneration the test drug *Pruthvisara taila* showed more epidermal regeneration which was similar to the standard drug Povidine iodine.

Granulation tissue plays an important role in wound healing. Wounds heal according to two main intentions. Primary intention is to approximate the wound edges easily. Secondary intention is to not approximate the wound edges. Granulation tissue matrix fills in wounds that heal according to the second intention. Granulation tissue is considered a contractile organ characterised histologically by the presence and proliferation of fibroblasts, keratinocytes, endothelial cells, new thin-walled capillaries, and inflammatory cell infiltration of the extracellular matrix (11).

The control group slides showed absence of granulation tissues which was formed well in the test drug in comparison with standard drug.

Inflammatory cell infiltration includes the neutrophils, lymphocytes and monocytes. Immigration of these cells into peripheral tissue is one of the principal purposes for inflammation, bringing to a site of injury the immune-system cells which can combat infection and clean up damaged tissue (12).

In the inflammatory stage of wound healing, immune cell activation drives the secretion of pro inflammatory cytokines which influence migration of fibroblasts, epithelial and endothelial cells. Fibroblasts contribute to collagen deposition. Simultaneously, collagen degradation releases fragments that promote fibroblast proliferation and synthesis of growth factors that lead to angiogenesis and re-epithelialisation (13). There was extensive inflammatory cell infiltration found in control group where as standard group consists slight infiltration compared to test drug where moderately cell infiltration was seen.

Angiogenesis is the formation of new blood vessels. Granulation tissue plays an important role in wound healing. Wounds heal according to two main intentions. Primary intention is to approximate the wound edges easily. Secondary intention is to not approximate the wound edges. Granulation tissue matrix fills in wounds that heal according to the second intention. When VEGF and other endothelial growth factors bind to their receptors on endothelial cells, signals within these cells are initiated that promote the growth and survival of new blood vessels (14). The control group shows the absence of formation of new blood vessels The test *Pruthvisara taila* showed more angiogenesis than standard group.

Proliferation of fibroblasts, during the formation of granulation tissue in a dermal wound, platelets, monocytes and other cellular blood constituents release various peptide growth factors to stimulate fibroblasts to migrate into the wound site and proliferate, in order to reconstitute the various connective tissue components(15). The Control group did not show any proliferation of fibroblast cells. In test drug it was moderate but standard drug showed slight/minimal proliferation.

Collagen deposit-Nitric oxide (NO) synthesis occurs during wound healing, Nitric oxide is an important regulator of wound collagen accumulation (16) which indicates the successful outcome of wound healing. The control group did not show any collagen formation, the test drug *Pruthvisara taila* showed moderate formation and deposition compared to standard drug.

Scabs are one of the most common signs of wound healing. They protect wounds from any bacteria entering wound and also to protect from any further blood loss. As the blood on the wound begins to dry it creates the crusty scab layer over the wound (17). The test drug showed scab formation when compared to Control and standard drug.

Pruthvisara taila is a formulation indicated in *Vrana* as topical agent (18). It comprises of *Shuddha Chitraka, Nirgundi, Shuddha Karaveera, Naadibeeja, Shuddha.Vatsanabha, Kanji* and *Karanja taila* as base.

Researches have proved that external application of oils is more effective than synthetic wound healing compounds. The fatty-acid components of oils are assumed to play a major role in the wound-healing process, in particular poly unsaturated fatty acids such as linoleic acid. Many natural oils possess specific compounds with antimicrobial, antioxidant, anti-inflammatory, and anti-itch properties (19). Each oil has its own unique properties that come into play when it comes to topical skin care.

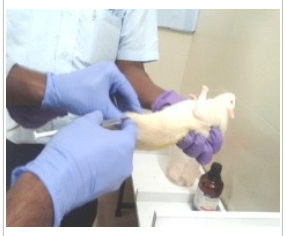
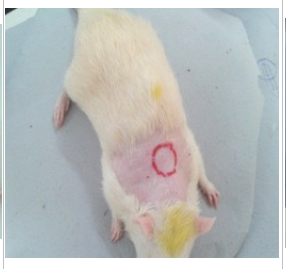
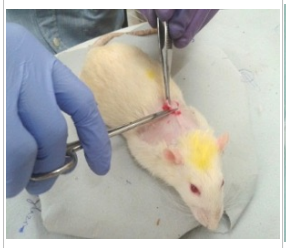
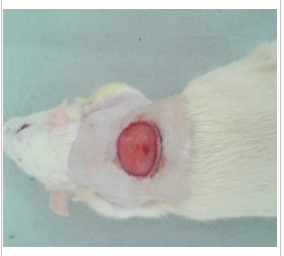
In this study the *Karanja* Seed oil is used as base in the preparation of *Pruthvisara taila*. The *Karanja taila* is indicated in skin diseases like scabies, leprosy, piles, ulcers, pain and wounds(20). The *Karanja* (*Pongamia pinnata (*L).pierre *)* oil possess the anti-inflammatory and antioxidant and analgesic properties(21) .The fatty acid composition of the *Karanja taila* possesses the wound healing property(22). Most of the drugs of *Pruthvisara taila* are having *Laghu,* and *Teekshna, Ushana, Snigdha* and *Sara guna* by this quality it helps in repairing all the blocked channels and aid in the proliferation of surrounding connective tissue elements and capillaries, which migrate in to site to be repaired. *Snigdha guna* gives moisture content which helps in wound contraction *Teekshna guna* helps the drug to act fast, spreading into deep tissues.

Even though, the wound healing is a natural restorative response to tissue injury the ingredients used in the form of *Kalka dravya* in the preparation possesses the anti-inflammatory, antimicrobial, analgesic and wound healing property (23,24,25) which may help in accelerating the wound healing process, as healing is the interaction of a complex cascade of cellular events, that are generated by the properties of above said drugs, which help in resurfacing, reconstitution and restoration of the tensile strength, of injured skin. In combination because of synergism of drugs the *Pruthvisara taila* may act as a better wound healing medicine.

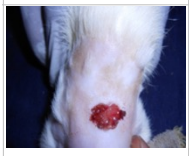
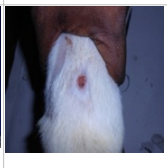
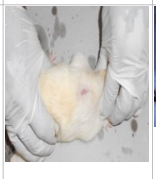
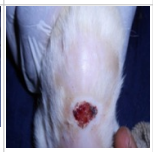
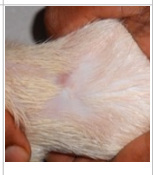
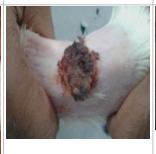
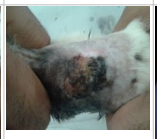
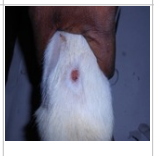
Conclusion

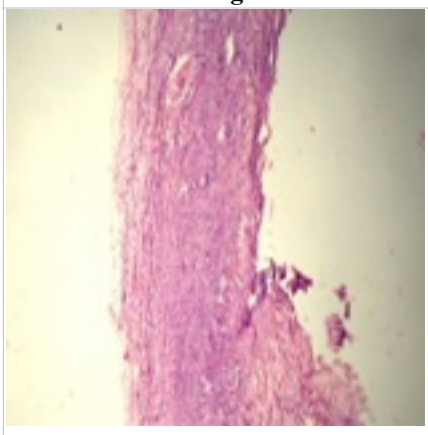
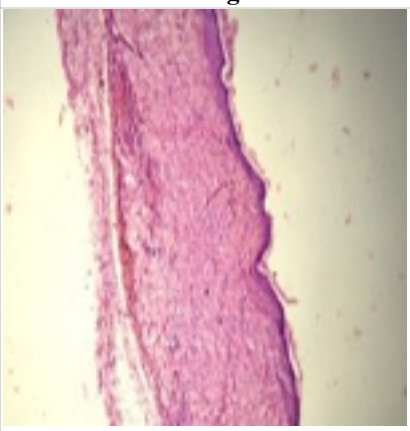
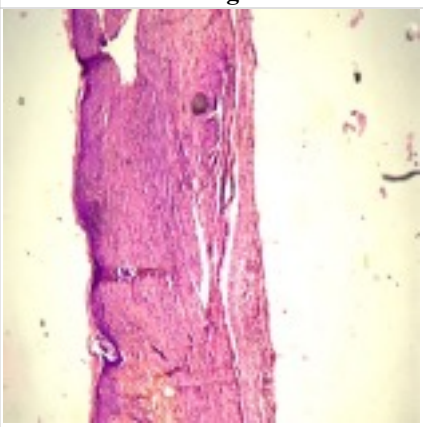
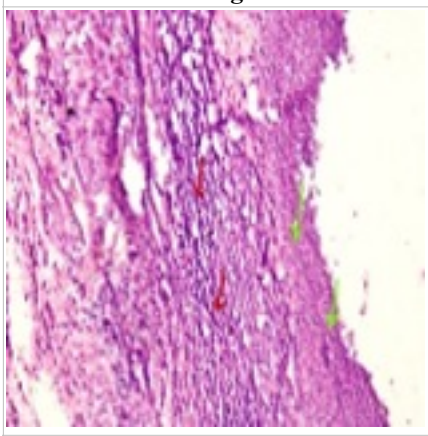
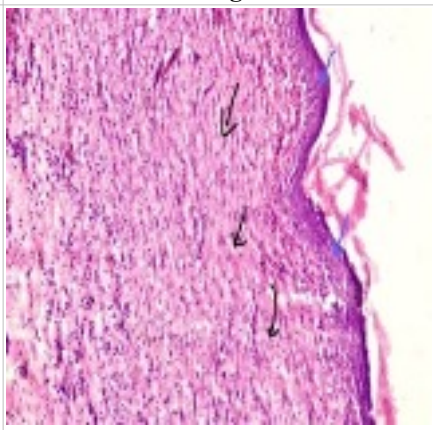
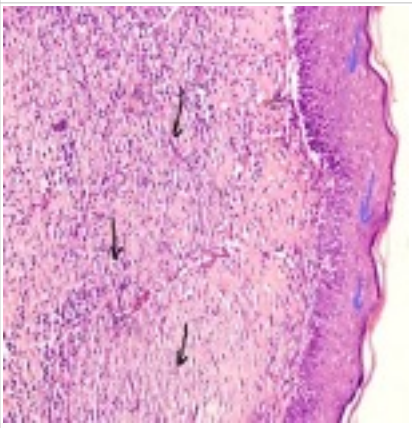
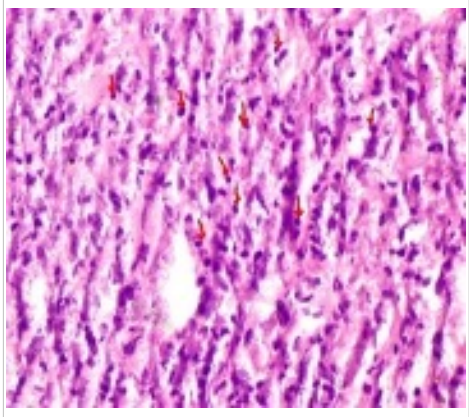
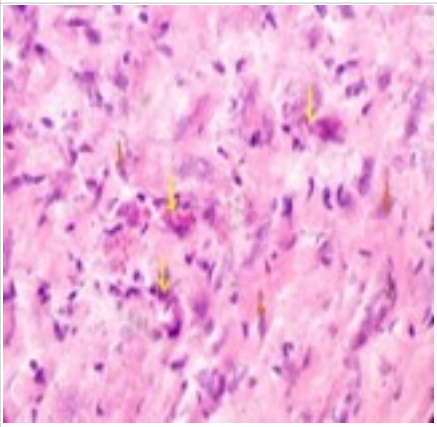
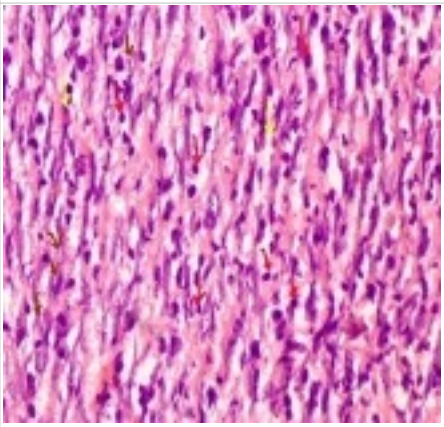
Pruthvisara taila is topical medicament indicated in all kinds of wounds it contains *Shuddha chitraka moola*, *Shuddha vatsanabha, Shuddha karaveera, Nirgundi moola, Nadibeeja, Kanji* and *Karanja taila* as the base. The test drug (*Pruthvisara taila*) showed epidermal regeneration more than control group and which was similar to the standard drug (Povidine iodine).The granulation tissues was formed well in the test drug in comparison with standard drug. The percentage of wound closure was observed in Control group was 94.92%, in standard it was 93.73% and in test drug the percentage of wound contraction was 95.73% .The histopathology study showed the extensive inflammatory cell infiltration found in control group where as standard group consists slight infiltration and in test drug it was moderate. The control group shows the absence of formation of new blood vessels and proliferation of fibroblast cells. The test drug showed more angiogenesis and formation of new blood vessels than standard group where it was minimal proliferation which might have helped in fast healing in test group. The control group did not show any collagen formation and scab formation the test drug showed moderate formation and deposition compared to standard drug. Compared to control and standard group, the test drug, *Pruthvisara taila* selected in this study showed remarkable wound healing property in excised wounds.

Photographs of experimental study

Fig-1	Fig-2	Fig-3	Fig-4	Fig-4
				
Anesthetizing Rat	**Shaved area**	**Marked area on dorsal part**	**Excision**	**Wound area**

Stages of wound healing

Control group			Standard group			Test drug group		
Fig-1	**Fig-2**	**Fig-3**	**Fig-4**	**Fig-5**	**Fig-6**	**Fig-7**	**Fig-8**	**Fig-9**
12th day	**18th day**	**24th day**	**12th day**	**18th day**	**24th day**	**12th day**	**18th day**	**24th day**
								

Photographs of Histpoathology		
Control	**Standard**	**Test drug**
Fig-1	**Fig-2**	**Fig-3**
		
40x-wound area	**40x-wound area**	**40X wound area**
Fig-4	**Fig-5**	**Fig-6**
		
100x-necrosed areas (green) with inflammatory infiltration (red	**100x-granulation tissue (black) and epithelium (blue)**	**100x-epithelium and granulation tissue**
Fig-7	**Fig-8**	**Fig-9**
		
400x- inflammatory cells	**400x-granulation tissue showing new blood vessels (yellow), fibroblasts (brown)**	**400x-granulation tissue showing inflammatory cells, new blood vessels and fibroblasts**

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