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A Clinical Study on the Efficacy of *Jatyadi* Cream in *Vrana Ropana* w.s.r to its Action on MAGS Score

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ABSTRACT

The advances in technologies and emergence of new therapeutic approaches have led to the rapid improvement in the field of wound healing. Herbal ointments have enormous role to play in wound healing and are widely used in clinical practice these days. This article reviews the wound healing effect of an *Ayurvedic* formulation in Ointment form through clinical trials with an intention to maximize its clinical effectiveness and accelerate its integration into wider clinical practice. *Ghanasatwa* (Water extract) of six individual drugs known to have good wound healing properties were prepared and the extract formulated as herbal ointment - *Jatyadi cream*. This was used to treat patients of chronic non healing wounds devoid of infection, i.e., where tissue regeneration had begun. Wound measurements were done before and during the course of treatment. Tissue biopsy to estimate the MAGS score indicating angiogenesis prior to and during the course of treatment was done. In most of the cases, there was a progressive increase in the MAGS score along with reduction in wound size, with time, indicating the efficacy of the formulation in wound healing.

KEYWORDS

Angiogenesis, Chronic non healing wounds, Jatyadi cream, MAGS Score, Vrana Ropana



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INTRODUCTION

Among the classical texts of Ayurveda, the formal perceptive of wound care have been vividly convoluted in the three great treatises (Brahatrayi) of Ayurveda viz. *Charaka Samhita*, *Sushruta Samhita* and *Astanga Sangraha*. These treatises describe the wound care under the concept of *Vrana* (various types of wounds) and present their systematic classification along with their management including various systemic and local drugs and preparations. For example, *Sushruta*, the father of Indian surgery, had compiled his surgical procedures in his famous documentation known as *Sushruta Samhita* as far back as 1000 B.C. He was perhaps, the first surgeon who had for the first time in human history elaborated the concept of *Vrana* detailing the various types of wounds, etio-pathogenesis of wounds along with their management.

The proliferative phase of healing is very much dependent on angiogenesis; in fact the term 'granulation tissue' was used by John Hunter in 1787¹ to describe the appearance of the prominent blood vessels of the initial connective tissue formed in the wound space. Without proper angiogenesis, it is very difficult for any skin wound other than the most superficial ones to enter into a stage of healing. In the

process of cutaneous wound repair, the granulation tissue which is the new stroma, begins to form approximately 4 days after injury. Abundant new capillaries bestow the neo stroma with its granular appearance. Macrophages, fibroblasts, and blood vessels enter upon the wound space as a unit which draw a parallel relation with the proposed biologic interdependence of these cells during tissue repair. Angiogenesis, hence forms an important phase of wound repair².

Acharya Sushruta describes sixty different procedures for the management of wounds. His techniques are broadly classified as *Vrana shodhana* and *Vrana ropana*³. Under these two treatment modalities, he has advocated external application of several drug combinations. Clinically also several of these drugs are found to be very effective in healing of a wound when used externally. One among such widely used drugs is *Jatyadi taila*. In the present study, 6 commonly available drugs from the combination of *Jatyai taila* have been selected, the *Ghanasatwa* (water extract) of these prepared and then formulated into a cream - *Jatyadi* cream. The effect of this cream over wound healing was estimated. Along with the improvement of the wound size, the MAGS score indicating angiogenesis was also estimated.



MATERIALS AND METHODS

The study employed a single arm before-after clinical trial design. A sample of 50 subjects attending the Wound clinic and Surgery OPD'S of the Ayurvedic and Modern wings of S.S Hospital, IMS, BHU, Varanasi with non- healing wounds of more than 6 weeks duration were enrolled into the study. There were no exclusion criteria other than malignant wounds, osteomyelitis and unwillingness of patients to attend the clinic regularly for treatment and assessment. These 50 subjects were treated with trial drug- *Jatyadi cream*. Dressings were changed daily in all the subjects. Wound size was measured and visual analogue scale-VAS for pain was recorded at weekly follow up. Initial biopsy was taken for assessment of MAGS score which was repeated after 3 weeks of treatment in 25 subjects. The approval from the Institutional Ethical Committee (IEC) was taken (Ref. No. IMS/I 43/2009/301906) and informed consent from each patient was obtained.

Details of the drug of Jatyadi Cream:

Leaves of *Jati*, *Nimba* and *Karanja*, root of *manjishta*, *yashtimadhu* and rhizome of *haridra* were collected from rural areas of Varanasi.

Method of Preparation of Dry Aqueous extract from the above mentioned drugs:

Fresh drugs were collected and cleaned with normal water and then dried for 3-5 days. They were then cut to small pieces (*Yawakuta choorna*). Then decoction (*Kwatha*) of the individual drugs were prepared as per kwatha kalpana (by adding 8 times of water and boiling till the total volume was reduced to ¼ of its initial volume), then it was filtered using a clean cloth and the liquid portion was separated from the *yawakuta* of the drug. It was refiltered to avoid presence of impurities. Hot water bath was given to this liquid portion for about 2-3 hours and when it became concentrated; it was kept in incubator at 40°C for about 4-5 days. When the material got solidified, it was powdered and stored in air tight containers. This powder was called *Ghanasatwa* or the aqueous extract of the drug. The ghanasatwa of the above said six drugs was neatly labeled separately. Finally, 60g of each drug was taken and mixed to prepare a homogenous *jatyadi* extract.

Composition of the cream

Dried extract of the drugs-As per required percentage for all the variants.

Base

Light liquid Paraffin	12%
Hard Paraffin	3%
Butylated hydroxy toluene	0.1%
Cetosteryl Alcohol	8%
Propylene Glycol	5%
Methyl Paraben	0.2%
Propyl Paraben	0.02%



Glycerine	5%
Sodium Lauryl sulphate	0.5%
Water	qs

Method of preparation of the cream

Step 1

Light liquid paraffin, glycerin, butylated hydroxy toluene, methyl paraben and propyl paraben were taken in a SS container. They were gently heated while stirring until butylated hydroxy toluene, methyl paraben and propyl paraben got fully dissolved.

Step 2

Water was taken in separate SS container & sodium lauryl sulphate was added to it. It was heated gently until SLS dissolved. The required extract was added into the water & heated gently till it got fully dissolved. The solution was filtered with 100 # filter cloth.

Step 3

Hard paraffin & cetostearyl alcohol were heated in separate container (at temp 80°C) to melt.

Step 4

The solution obtained in step-2 was poured in a homogenizer and mixed properly. The solution obtained in step-1 was then added to the homogenizer and mixed with step-2 solution. After mixing thoroughly, the solution obtained from step 3 (melted part) was mixed into solution of step 2. The solution became viscous & formation of

cream was noticed. On cooling properly, the intended cream was formed. Total mixing time was 12 minutes.

This *Jatyadi* cream thus prepared in ointment form was packed in tubes.

Method of administration of the drug:

The cream was applied on the wound area, as required, once daily with dressing until complete healing.

Study Variables:

The primary study variables were the following:

- 1) Assessment of Wound size
- 2) Assessment of pain using VAS (Visual analog scale) every week during the course of treatment.
- 3) Assessment of MAGS score based on viable wound tissue specimens.

Treatment was given to subjects of chronic non healing wounds devoid of infection, i.e., where tissue regeneration had begun, for eight weeks or until healing occurred, whichever was the earliest. Wound size (Surface Area) was assessed weekly. Biopsies were taken from 25 patients before initiating treatment. Repeat biopsies were performed after three weeks and sent for histopathological examination to assess angiogenesis through MAGS Score. This was analyzed using the microscopic angiogenesis parameters of endothelial cell regeneration: vasoproliferation, endothelial cell hyperplasia and endothelial cytology.



A numerical grade was given to each variable and a simple equation was used to calculate the overall index of endothelial regeneration - the microscopic angiogenesis grading system (MAGS) score. Statistical analysis was done using the Student's t-test. The t-test was used for changes in wound size and VAS scores.

OBSERVATIONS

As regards to the demographic data, among the 50 subjects studied, males outnumbered the females (71.4%), majority of them were found in the age group of 30-50 yrs (50%). Almost half the subjects belonged to the labour class (50%).

Wound characteristics

In 64.3% of the subjects, the mode of onset was sudden and in 35.7% the duration of wound was between 3-6 months. Percentage of subjects who complained of pain was 83.3% out of which 26.2% had mild pain, 35.7% moderate pain and 21.4% had severe pain. The shape of the wound was irregular in 47.6% and 54.8% of the wounds were in the foot. Single wound was noticed in 88.1% subjects and 11.9% had multiple wounds. Out of all wounds, 14.3% observed glossy red and edematous areas surrounding the wound, whereas 11.9%

observed eczematous and pigmented surroundings. The remaining 73.8% were either normal or in other forms. The ulcer was on the lower limb in 82% of patients with the foot being the most common site in 56%. Wound size was less than 5cm² in 10% of ulcers, 5-10cm² in 66% and more than 10 cm² in 24% of ulcers.

Based on the type of the wound 38.1% of the subjects had diabetic wound where as 35.7% had traumatic. 2.4% had tropical, 9.5% each had venous and arterial wounds. The remaining 4.8% of the subjects had leprotic wound.

RESULTS

Overall, 42 cases were available for regular follow up and assessment of healing. The remaining 8 patients did not attend follow-up assessments after a few weeks for unknown reasons. Data are given only for the 42 patients who completed the study.

1) Effect of therapy on wound size:

The surface area of the wounds in general and particularly in diabetic, traumatic, venous, and arterial wounds showed statistically significant reduction following treatment. Surface area in Leprotic wounds showed statistically insignificant reduction. The details are described in table no.1.

**Table 1** Effect of therapy on different types of wounds

Wound Types	No. of Pts.	Mean Score		% Relief	S.D	SE	t	p
		B.T	A.T					
Diabetic wound	n=15	30.83	5.65	81.67%	25.57	6.60	3.81	0.002
Traumatic wound	n=16	49.97	8.00	83.99%	55.99	13.99	2.99	0.009
Arterial wound	n=4	46.25	16.62	64.06%	12.34	9.67	2.39	0.096
venous wound	n=5	11.00	0.25	97.73%	2.87	1.43	7.48	0.005
Leprotic wound	n=2	46.00	14.00	69.57%	36.76	26.00	1.23	0.434

Number of subjects who were cured completely were 10, 21 subjects showed marked improvement, 7 subjects showed moderate improvement, 4 subjects showed mild improvements and no subjects remained unchanged to the therapy.

2) Effect of therapy on pain:

Pain in wounds showed statistically significant ($p < 0.001$) reduction following treatment. The details are described in table no.2.

Table 2 Effect of therapy on pain

No. of pts	Mean		Diff. in Mean	% Relief	Paired t test			
	B.T	A.T			S.D	SEM	t	P
n=35	1.62	0.50	1.12	69.14%	0.96	0.14	7.49	0.001

3) Effect of therapy on MAGS Score:

The microscopic angiogenesis grading system (MAGS score) is a quantitative technique for measuring the degree of angiogenesis in an area of neovascularization. It is based on 3 parameters - vasoproliferation, endothelial cell hyperplasia and endothelial cytology. This system provides a composite score ranging from 0 to 100, with biologically reasonable and quantitatively reproducible results. A formula is constructed to integrate the 3 components of endothelial cell regeneration.⁽⁴⁾

MAGS = KnN + KeE + KxX. (KnN: Vasoproliferation, KeE: Endothelial cell hyperplasia, KxX: Endothelial cytology)

Vasoproliferation (KnN)

N represents the number of vessels per high power field. A Nikon photomicroscope with 40x objective, a 1.25 objective and a 10x eyepiece i.e. a 500x total magnification was used. Field encompassed at this magnification had an area of $1 \times 10^5 \mu^2$ as measured by hemocytometer. Kn is a constant used to correct the various fields of other microscopes so that they are equal to magnified area. The maximum score for the vasoproliferative component of MAGS was set at 40. This number was chosen so that the values of vasoproliferative component corresponded the values of 2 others components in the system.

Endothelial Cell Hyperplasia (KeE)



Ke is a constant with value of 3. It is so given to allow endothelial cell hyperplasia to correspond to value of proliferation. E is the number of endothelial cell lining the cross section of capillary. Within the area of maximum proliferation the most hyperplastic capillary was chosen. Tangential section was avoided; parasites and adventitial cells were not taken. Since the number of endothelial cells varied with the size of vessels, vascular channels with a cross sectional diameter of not more than 20 were counted.

The maximum value of E was set at 10. Since the number of endothelial cells observed in a single section of capillary rarely exceeds this thus the maximal score for the parameter of endothelial cell hyperplasia (KeE) was 30.

Endothelial Cell Cytology (KxX)

Kx was a constant provided to allow the maximal cytological changes of regenerating endothelium i.e. a mitotic figure to correspond numerically to maximal proliferation and maximal

hyperplasia. X stood for histological appearance of an individual endothelial cell graded on the bases of regenerative appearance. A search was made for the most primitive group of endothelial cells within a hyperplastic zone. X was given 0-5 points based histological appearance of endothelial cells.

- 0 = Normal endothelial cell
- 1 = Plump clear nucleus
- 2 = Plump, clear nucleus + prominent nucleolus
- 3 = Large hyperchromic nucleus
- 4 = Bizzare endothelial cell
- 5 = Mitotic figure

Constant Kx was given value of 6⁵.

The average MAGS Score of the wounds was increased from 30.20 to 45.68 showing marked improvement with mean difference of 15.48± 10.73 and t value 7.20 which is statistically significant (p<0.001). The details are described in table no.3.

Table 3 Effect of therapy on MAGS Score

No. of Pts	Mean		Diff. in Mean	Paired t test		
	B.T	A.T		S.D	t	P
n=25	30.20	45.68	15.48	10.73	7.209	0.001

DISCUSSION

Chronic non-healing wounds present serious problems for both patients and clinicians. Most are associated with a small number of underlying disorders such

as diabetes, leprosy and peripheral vascular diseases. Despite treatment, the wounds are difficult to manage and therefore have significant negative



physical, economic and social effects on patients.

Action of drugs of *Jatyadi* cream

Table 4 *Rasapanchaka* of drugs of *Jatyadi* cream

Drug	Rasa	Guna	Veerya	Vipaka
<i>Jati</i>	<i>Tikta, Kashaya</i>	<i>Laghu, Snigdha, Mridu</i>	<i>Ushna</i>	<i>Katu</i>
<i>Nimba</i>	<i>Tikta, Kashaya</i>	<i>Laghu</i>	<i>Sheeta</i>	<i>Katu</i>
<i>Karanja</i>	<i>Tikta, Katu, Kashaya</i>	<i>Laghu, Teekshna</i>	<i>Ushna</i>	<i>Katu</i>
<i>Haridra</i>	<i>Tikta Katu</i>	<i>Laghu, Ruksha</i>	<i>Sheeta</i>	<i>Katu</i>
<i>Yastimadhu</i>	<i>Madhura</i>	<i>Guru, Snigdha</i>	<i>Sheeta</i>	<i>Madhura</i>
<i>Manjishtha</i>	<i>Madhura, Tikta, Kashaya</i>	<i>Guru, Ruksha</i>	<i>Ushna</i>	<i>Katu</i>

Due to its *Tikta rasa* in 5 out of 6 drugs, the drug, with its *lekhana* property⁶ inhibits excessive reactionary inflammation. In earlier phase it expels out toxins and necrotic tissue and facilitates wound healing. The *Katu* rasa and *vipaka* reduce the local discharge and irritation and act against bacteria. By the *Srotoshodhana* property of *Katu vipaka, Ushna Veerya, Laghu* and *Tikshna Guna*, the drug penetrates into deep tissues and helps in the wound healing. *Ropana karma* is induced by *Madhura rasa, madhura vipaka and Snigdha guna* which further may help in neovascularization and wound healing. All the drugs mentioned under the *Jatyadi* cream showed anti-inflammatory, analgesic and wound healing property⁷⁻¹³.

Figure 1 describes the microphotograph of a treated wound with high MAGS score showing an area having multiple capillary channels lined by plump endothelial cells and figure 2 describes the microphotograph of a treated wound with

The *rasapanchaka* of the individual drugs of *Jatyadi* cream are explained in table no.4.

high MAGS score showing granulation and multiple capillary channels lined by plump endothelial cells.

CONCLUSION

From the above clarifications it is clear that *Jatyadi cream* is effective and beneficial for wound healing. The cream can be removed easily and dressing adherence is reduced significantly with its application. Hence, the use of *Jatyadi cream* can be recommended for topical application in chronic non-healing ulcers.

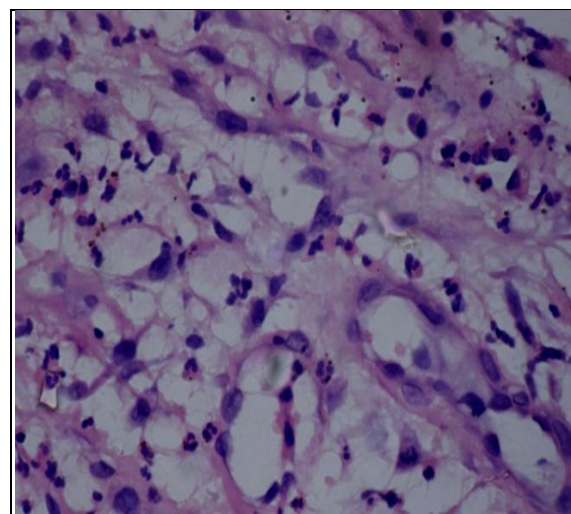


Fig 1 Microphotograph of a treated wound with high MAGS score showing an area having multiple capillary channels lined by plump endothelial cells. H & E Stain X400

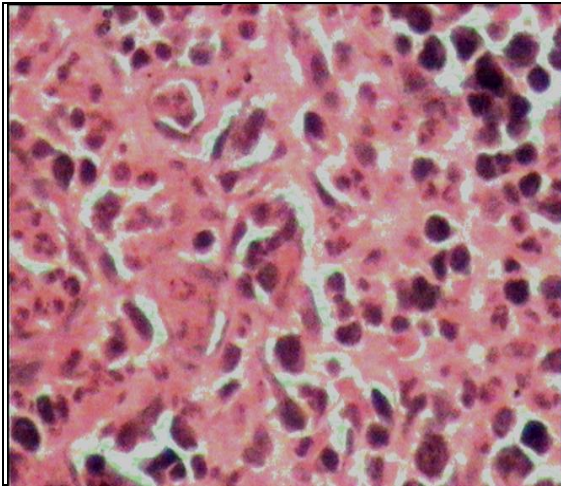


Fig 2 Microphotograph of a treated wound with high MAGS score showing granulation and multiple capillary channels lined by plump endothelial cells. H & E Stain X400



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