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Evaluation of polyherbal ointment for wound healing activity in Wistar rats

Shilpa S. Kolhe, Komal Shinde, Rajanigandha Jori, Dr. M. V. Gadhave, Dr .S. L. Jadhav, Dr. D. D. Gaikwad.

Vishal Institute of Pharmaceutical Education and Research, Ale, Taluka-Junnar, Dist-Pune, Maharashtra, India.

ABSTRACT

The aim of present study to formulate, evaluate and to check the wound healing activity of newly prepared polyherbal formulation (ointment) .Herbal ointment containing hydrochloric extracts of plants *Psorolea corylifalia, Achryanthes aspera* ,was formulated as ointment and the hydro alcoholic extract was prepared by maceration method. These Formulations were evaluated for the following parameters: pH, Spread ability, grittiness, skin irritation study, stability. The wound healing activity is assessed by the rate of wound contraction, Period of epithelisation and skin breaking strength.

Keywords: Herbal Ointment, Wound Healing Activity.

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***Address for Correspondence:**

Shilpa S. Kolhe, Vishal Institute of Pharmaceutical Education and Research, Ale, Taluka-Junnar, Dist-Pune, Maharashtra, India.

INTRODUCTION

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and aliments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore, India has after been referred to as the medicinal garden of the world.

The other main source of medicinal plants is from cultivation. The cultivated material is definitely more appropriate for use in the production of drugs. [1]

Along with other dosage forms herbal drugs are also available in the form of ointment which is semisolid preparation used topically for several purpose as protectants antiseptic, anti healing, emollient, keratolytic &astringents. [2]

Wound healing is the process of repair that follows injury to the skin and proper healing of wound is essential for the restoration of disrupted anatomical continuity and disrupted functional status of the skin. Several, medicinal plants have been used since time immemorial for treatments of cutwounds, and burns and showed promising effects. Some very common plants like *Psoroleacorylifalia, Achryanthesaspera* Aloe Vera, *AzadirachtaIndica, carica papaya, celosia argentea, centellaasiatica, cinnamomumzeylanicum, curcuma longa, Nelumbonucifera, ocimum sanctum, phyllanthusemblica, plumbagozeylanica, pterocarpussantalinus, Terminalia Arjuna*and *Terminalia chebula* have been extensively reported in Ayurveda, Siddha and Unani systems of medicines for their wound healing potential. [3]

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions .Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin .In other words wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and the function of underlying normal tissue and may also result from a contusion , haematoma, laceration or an abrasion .

Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorised into three stages ; inflammatory phase, proliferate phase and finally remodelling phase which ultimately determines the strength and appearance of the healed tissue.

Wound healing process holds several steps which involve coagulation ,inflammation, formation of granulation tissue ,matrix formation ,remodelling of connective tissue, collagenisation and acquisition of wound strength .research on wound healing agents is one of developing areas in modern biomedical sciences and many traditional practitioners across the world particularly countries like India and China have valuable information of many lesser known hitherto unknown wild plants for treating wounds and burns. Traditional forms of medicine practised for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wounds related disorders. [4]

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds ,inflammation ,skin diseases ,leprosy ,diarrhoea , scabies ,venereal disease ,ulcers ,snake bite, etc. More than 80% of world's population still depends upon traditional medicines for various skin diseases. Herbal medicines in wound management provide a moist environment to encourage the establishment of the suitable environment for natural healing process. A large number of plants are used by Folklore traditions in India for treatment of cuts, wound sand burns.[5]

Plants used:

Bavanchi is consist of leaves of *Psorolea corylifalia* family- fabaceae. Bavanchi leaves is *P. corylifalia* or Bauchi in traditional Chinese medicine is an herb used to notify the kidneys disease. It is used for helping the healing of bone fractures, for lower back &knee pain, impotence, bed wetting, hair loss & vitiligo.[6]

Aghada is consist of dried leaves of *Achryanthes aspera* Family- Amarantaceae. Aghada is has diuretic, expectorant & purgative properties. The juice of its leaves is used in fever, cough, diarrhea, dysentery, dropsy & other disease. Decoction prepared using the herb is used in stomach ache & bowel complaints, piles, boils, skin eruption, etc.[7]

Bavanchi[6]

Synonyms- Cullen Corylifolium -LotodesCorylifolia

B.S.- It Is Obtained from Dried Leaves of *Psorolea Corylifolia*

Family- Fabaceae

Figure 1: Bavanchi

Chemical Constituent

Flavonoids (Neobavaisoflavone, Isobavachalcone, Bavachalcone, Bavachinin, Bavachin, Corylin, Corylifol, Corylifolin & 6-Prenylnaringenin)

Coumarins (Psoralidin, Psoralen, Isopsoralen& Angelicin)

Meroterpenes (Bakuchiol &3-Hydroxybakuchiol)-Very High Concentration of Genistein

Aghada[7]

Figure 2: Aghada

Synonyms- Apamarg, Aghata, Onga ,ApangMadhogantha B.S.-It Is Obtained From Dried Leaves Of *Achryanthes Aspera* Family- *Amaranthaceae*

Chemical Constituent: Triterpenoids Saponins, Oleanoic Acid (Aglycone, Ecdysterone), Hormones , Long Chain Alcohol , Achryanthine , Betaine , Pentatriaontane, Hexatriacontane, Tritriacontane.[7, 8]

MATERIALS AND METHOD

Collection of Plant material: - Leaves of plants were collected from the local area of Pune.

Animal Used:

Healthy adults Albino Wistar Rats strain weighing 180-250 gram were used for the study .The animals were obtained from animal house of vishal institute of pharmaceutical education and research, Ale, Pune, India. On Arrival the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at Temp of 24±2°C and relative humidity of 30-70%. 12:12 light: Day cycle was followed .all the animals were allowed to free access to water a fed with standard commercial pelleted rat chaw(M/S Hindustan Lever Ltd. Mumbai.) All Experimental Procedures and Protocols used in this study were reviewed by the Institutional Animal Ethics committee and were in accordance with the guidelines of the CPCSEA. Approval was obtained from the **CPCSEA/ IACE** for animal studies in this project by proposal no: **VIPER /IAEC /UG/2018-01.**

Preparation of extract

Leaves of the plant were collected and washed thoroughly with distilled water and shade dried for 10 days. Dried leaves were grind into powder form. 20 gm powder was imbibed with 350ml of 90% ethanol for 3hrs. and transferred to percolator with addition of 150ml of 90% ethanol for maceration for 7 days with occasional stirring. Finally ethanol extract was collected and concentrated to get blackish green residue. The extract was stored in the airtight container at cool and dark place.

Dried leaves of Aghada were grind and the powder obtained was followed for extraction same as that for bavanchi leaves extract. The extract with blackish green colour was obtained and stored at cool and dark place in air tight container.[8]

Formulation of Ointment[9]

Table 1: Formulation of ointment base

Sr.no	Name of ingredient	Quantity to be taken
1.	Wool fat	0.5gm
2.	Cetostearyl alcohol	0.5gm
3.	Hard paraffin	0.5gm
4.	Yellow soft paraffin	8.5gm

Table 2: Formulation of Herbal ointment

Sr. no	Name of ingredient	Quantity to be taken
1.	Prepared extract sample1	0.03gm
2.	Prepared extract sample 2	0.03gm
3.	Ointment base q.s.	10gm

Procedure for preparation of herbal ointment:

a) Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.

b) Herbal ointment was prepared by mixing accurately weighed Neem and Turmeric extract to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more bases until to form homogeneous ointment, finally transferred in a suitable container.

Evaluation[10]

Colour and Odour: Physical parameters like colour and odour were examined by visual examination.

Consistency: Smooth and no greediness is observed.

PH: PH of prepared herbal ointment was measured by using digital PH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. PH was determined in triplicate for the solution and average value was calculated.

Spreadability: The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability.

Spreadability was calculated by following formulaS=M×L/T

Where,

S= Spreadability
M= Weight tide to the upper slide
L= Length of glass slide
T= Time taken to separate the slides

Extrudability: The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Diffusion study: The diffusion study was carried out by preparing agar nutrient medium. A hole board at the centre of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (After 60 minutes)

LOD: LOD was determined by placing the formulation in Petri-dish on water bath and dried for the temperature 105°C.

Solubility: Soluble in boiling water & Miscible with alcohol, ether, chloroform.

Wash ability: Formulation was applied on the skin and then ease extends of washing with water was checked.

Non-irritancy Test: Herbal ointment prepared was applied to the skin of human being and observed for the effect.

Stability study: Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2°C, 25°C and 37°C. The herbal ointment was found to be physically stable at different temperature i.e. 2°C, 25°C, 37°C within four weeks.

Pharmacological evaluation

Animals were wounded under light chloroform anaesthesia, semi aseptically. The animals were assigned into three groups (n=8). Group I was untreated group, this was taken as control. Group II Animals were received standard cipladine treatment. Group III animals were received Formulation (Test) in Excision Wound Models. NO other topical or systemic therapy was given to animals during the course of this study[11].

Control (day 1) standard (day 1) Formulation (day 1)

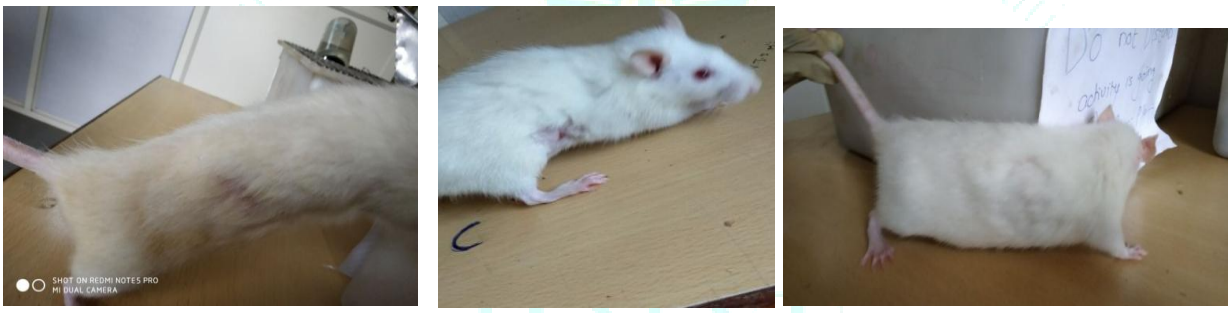
Figure 3: 1st day photograph

Control (day 5) standard (day 5) Formulation (day 5)

Figure 4: 5th day photographs

Control (day 9) standard (day 9) Formulation (day 9)

Figure 5: 9th day photographs



Standard (day 14) control (day 14) Formulation (day 14)

Figure 6: 14th day photographs

A. Excision wound model

hairs were removed from dorsal thoracic central region of anaesthetized rats .Full thickness from the demarketed area was excised to produce wound measuring around 2 cm^2.Wound was cleaned with cotton swab soaked in alcohol .The two test formulations and cipladine were applied on wound once daily for 14 days starting from the first day of wounding .Wound contraction was measured for 14 days at interval of 4 days.[11]

B. Stastical Analysis

The results were expressed as mean ±SEM. The significance of differences between the means was analyzed by student's t-Test followed by Turkey's test. A P-value <0.01 was considered significant.

RESULTS AND DISCUSSION

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using simple maceration process to obtain a good yield of extract and there was no any harm to the chemical constituents and their activity.

The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occurred which was stable during the storage.

The physicochemical properties were studied which shows satisfactory results for spreadability, extrudability, washability, solubility, loss on drying and others.

Also the formulation was placed for a stability study at different temperature conditions like 20^{0}C, 25^{o}C and 37^{o}C within four weeks. There were no changes observed in spreading ability, diffusion study as well as irritant effect.

Table 3: Results of physical characteristics

SR. no	Plant Name	Obtained value			Std. Value		
		Colour	Odour	Taste	Colour	Odour	Taste
1.	Aghada	Green	Characteristic	Pungent , bitter	Green	Characteristic	Pungent , bitter
2.	Bavanchi	Green	Characteristic	Bitter	Green	Characteristic	bitter

Table 4: Result of Extractive value Determination

Sr. No.	Plant name	Extractive value-Alcohol soluble
1.	Aghada	4%w/w
2.	Bavachi	5%w/w

Table 5: Result of Plant extracts preparation

SR. No.	Plant Species	Part Use	Percentage Yield(w/w)	Physical characteristics			
				Colour	Odour	Taste	Solubility
1.	*Achryanthes*	Leaf	4%w/w	Green	characteristic	Pungent,	Soluble in boiling
	aspera					bitter	water , miscible
							with alcohol,
							ether, chloroform
2.	*Psoralea*	Leaf	5%w/w	Green	characteristic	Bitter	Soluble in boiling
	Corylifolia						water , miscible
							with alcohol,
							ether, chloroform

Table 6: Result of preliminary phytochemical screening of extract

Sr. No.	Test	Plant extract	
		Aghada	Bavachi
1.	Carbohydrate	+	+
2.	Proteins and Amino acid	-	-
3.	Alkaloids	+	+
4.	Glycosides	+	+
5.	Steroids	+	+
6.	Triterpenoids	-	+
7.	Tannis	+	+
8.	Phenolic Compounds	+	+
9.	Flavonods	+	+
10.	Saponins	+	+
11.	Vitamins	-	-

Table 7: Physicochemical evaluation of formulated ointment

Physicochemical parameters	Observation
Colour	Greenish brown
Odour	Characteristic
Consistency	Smooth
Ph	6.5
Spreadability(seconds)	6
Extrudability	0.26gm
Diffusion study (after 60 min)	0.7 cm
Loss on drying	30%
Solubility	Soluble in boiling water, Miscible with alcohol, Ether & chloroform
Washability	Good
Non irritancy	Non irritant
Stability study(20°c,25°c,37°c)	Stable

Physicochemical parameters Observation

Figure 7: Dried extract of samples

Figure 8: pH

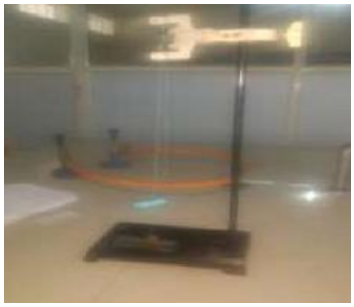


Figure 9: Spreadability test

Figure 10: Extrudability

Figure 11: Diffusion study

Pharmacological Evaluation: Photographs showing the effect of polyhedral ointment formulation on wound healing activity were shown in figure 3 to 6.

Table 8: Effect of Applied Poly Herbal Formulations on Excision Wound Models in Rats

Group	Animal Treatment	Wound Area in cm^2			
		1st day	5th day	9th day	14th day
Group 1	Control	2.0±0.0164	1.88±0.01281	1.38±0.0167	0.0687± 0.001681
Group 2	standard	2.1±0.012	1.6±0.006	1.1±0.0051	0.0±0.0
Group 3	Formulation	2.2±0.018	1.76±0.00703	0.87±0.00472	0.0±0.0

Wound contraction studies

The results of wound contraction studies were shown in photographs (figure to figure).from wound area contraction data,it was concluded at formulation group 3 produced greater wound contraction compared with the other tested formulations and wound contractions increases as the day passes. As per results shown in table. no 8, it was also concluded that the ointment formulation have significant ($p< 0.01$)wound healing activity compared to control group .

CONCLUSION

From the ancient time Bavanchi and Aghada is used for their various medicinal properties like, anti healing, ant diuretic, skin eruption, impotence, etc. Thus this ointment could become a media to use these medicinal properties effectively and easily as a simple dosage form.

Natural Remedies are more acceptable as they are safer with fewer side effects than synthetic once. So, a polyherbal wound healing formulation is non toxic, safe, effective and improves Patient Compliance as it contains herbal ingredients. From the ancient time Bavanchi and Aghada is used for their various medicinal properties like, anti healing, anti diuretic, skin eruption, impotence etc. Thus this ointment could become a media to use these medicinal properties effectively and easily as a simple dosage form. In present study the polyherbal ointment is showing better results than standard marketed formulation.[12]

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