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Research Article

WOUND HEALING ACTIVITY OF JATI KALPA GHRITA IN ALBINO RATS

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Abstract

Wound and healing are the two sides of the surgical coin, on which an expert surgeon has to play his role very sincerely. Proper healing of wounds is essential for the restoration of anatomical continuity and disturbed functional status of the skin. A group of herbs having healing property were combined and a ghee based herbal formulation called "Jati kalpa ghrita" was prepared. This formulation was evaluated for wound healing activity in excision wound model. Parameters like percentage of wound contraction and days taken for complete healing were studied. The results showed percentage of wound contraction of trial drug was highly significant when compared to control group. The days taken for epithelization was also less when compared to other groups. The days taken for complete epithelialization was 26.00 ± 01.37 days for control animals, 23.17 ± 01.38 days in vehicle control, 19.50 ± 00.67days for Jati Kalpa Ghrita treated animals and 22.33 ± 01.65 days in standard group. The results of the present study showed that Jati kalpa ghrita has potent wound healing activity.

Key words: Jati kalpa ghrita; Excision wound; Wound contraction; Epithelization.

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INTRODUCTION

The art of healing denotes to surgery is a major challenge to the surgeons, which has been described so extensively in Ayurvedic Shalya Tantra (Surgical Science of Ayurveda), that yet each measure needs the scientific touch in explanation. In this context, different types of wounds with their management have been stated in Ayurvedic texts as per clinical observations. In fact, Wound and healing are the two sides of the surgical coin, on which an expert surgeon has to play his role very genuinely.

The mission of the wound healing is to increase our basic understanding of the molecular and cellular events of the cellular repair and wound healing processes, and to use this information as the basis for developing new therapies that minimize the adverse consequences of wound. Such novel therapies could enhance cellular repair, promote rapid wound closure, minimize hypertrophic scarring, or control scar contracture.

A thorough review of research works and Ayurvedic texts showed that the different single or combined drugs have been tested for healing the wound.[1] It shows a group of drugs having ropana (healing) property, when grouped and used may give superior results. As it is specifically mentioned, prior to taking up of this study, a thorough review of research works and Ayurvedic texts were done to list out various herbs for their wound healing potential, based on this, the present formulation was framed. In this regard an innovative combination of drugs having wound healing property was formulated and assigned the name as Jati Kalpa Ghrita. (Table 1) Prior to clinical study, experimental study was planned in order to assess the wound healing activity of this formulation with scientific background.

MATERIALS AND METHODS

Test formulation

The raw materials (Table 1) of the test formulation were procured from pharmacy attached to Gujarat Ayurved University, Jamnagar. Morphological, organoleptic and microscopic studies of the powdered drugs were done as per the guidelines of Ayurvedic Pharmacopoeia of India[2] at department of Pharmacognosy. The ingredients were made into coarse powder and divided into two parts. Out of this the major part was used for decoction, whereas the other was used for making paste. Decoction was based on classical procedure[3] by mixing above said coarse powder of the drugs with water in the ratio of 1:8, which was thereafter heated at medium temperature, till it reduced to one fourth of its original quantity. The second part of the raw drug was taken and mixed with required quantity of water to convert into paste form. Thereafter, one part of paste was mixed with $1/8^{th}$ part of above made decoction and $1/4^{th}$ part of cow's ghee. The mixture was heated on medium flame till the water content evaporated. After examination of final product as per Sharangdhara text,[4] prepared Ghrita was preserved in airtight steel containers.

Standardization of test formulation

The ghee formulation was evaluated for analytical parameters like refractive index at 40°C, specific gravity, acid value, iodine value, saponification value and loss on drying as per standard analytical procedures.[5] The results obtained were compared with API standards and was found to be similar.[6][7]

Animals

Wistar strain rats of either sex weighing 180 ± 20 g were obtained from the animal house attached to the pharmacology laboratory of I.P.G.T.&R.A., Jamnagar.

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A total of 30 adult and healthy male and female rats of 12-16 weeks old age were selected. They were housed in large spacious polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum*. The animals were acclimatized for at least one week in lab conditions before the commencement of experiment in standard laboratory conditions 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60 % humidity. Before the test, the animals were fasted for at least 12 hours. Institutional animal ethics committee had approved the experimental protocol. (Approval number: IAEC 06/09-11/PhD/07)

Effect on excision wound

Prior to the operative procedure all the surgical instruments were autoclaved. The area to be excised (on the back portion of the rat – suprascapular region) was shaved carefully by scissor prior to the procedure without causing any abrasions. The rats were anaesthetized with diethyl ether and they were inflicted with excision wounds as described by Morton and Malone (1972).[8] A full thickness of the excision wound of circular area 300 mm^2 and

2 mm depth was created along the markings with a surgical blade. Homeostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The animals were randomly divided into four groups of six each. First group is served as wound control (WC) to which normal saline was applied. To the second group plain ghrita was applied and is served as vehicle control (VC) group. Third group was applied with Jati Kalpa Ghrita (JKG) whereas fourth group Reference Standard (Povidone ointment) (RS) was applied with standard drug (Povidone ointment). The test drugs (0.2 g each) were applied topically with a spatula once daily till the wound was completely healed. Wound contraction rate was monitored by planimetric measurement of the wound by tracing the wound margin on a graph paper on every 3rd day. The point at which the eschar fell off without any residual raw wound was considered epithelialization.

Statistical analysis

Students "t" test for unpaired data has been used for analyzing the data generated during the study. P value less than 0.05 is considered as statistically significant.

RESULTS

Significant wound healing activity was observed in Jati Kalpa Ghrita and RS treated groups in comparison to control group. (Table 2) Vehicle control group also have shown marked wound healing activity. The Jati Kalpa Ghrita treated group showed 100 percent wound contraction on 18th day and is better than that of RS treated group.

The days taken for complete epithelialization was 26.00 ± 01.37 days for control animals, 23.17 ± 01.38 days in vehicle control, 19.50 ± 00.67days for Jati Kalpa Ghrita treated animals and 22.33 ± 01.65 days in standard group. (Table 3)

DISCUSSION

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of anatomical continuity and disturbed functional status of the skin. Wound healing involves different phases such as contraction, epithelization, granulation and collagenation.[9]

Healing a wound though looks simple at times, yet, it is a complex phenomena. “I Dressed the wound, God heals”, doesn’t apply everywhere. With advancement of science, obstacles for healing need to be answered scientifically. Man is looking to back to the nature to get answer.

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Table 1: Formulation composition of Jati Kalpa Ghrita

Ingredients	Botanical name	Family	Part used
Jati	*Jasminum officinale* Linn.	Oleaceae	Leaves
Nimba	*Azadirachta indica* A. Juss.	Meliaceae	Leaves
Patola	*Stereospermum suaveolens* DC.	Bignoniaceae	Leaves
Sariva beeja	*Hemidesmus indicus* (Linn.) R. Br.	Asclepiadaceae	Seeds
Karanja	*Pongamia pinnata* Linn.	Fabaceae	Seeds
Usheera	*Vetiveria zizanioides* (Linn.) Nash.	Poaceae	Root
Yashtimadhu	*Glycyrrhiza glabra* Linn.	Leguminosae	Root
Manjishta	*Rubia cordifolia* Linn.	Rubiaceae	Root
Lodhra	*Symplocos racemosa* Roxb.	Symplocaceae	Stem Bark
Haridra	*Curcuma longa* Linn.	Zingiberaceae	Rhizome
Daruharidra	*Berberis aristata* DC.	Berberidaceae	Stem Bark
Padma	*Nelumbo nucifera* Gaertn.	Nymphaeaceae	Flower
Dhataki pushpa	*Woodfordia fruticosa* (Linn.) Kurz.	Lythraceae	Flower
Tuttha	Copper sulphate	---	$CuSO_4$
Madhucchishta	Bee's wax	---	Wax
Goghrita	Cow's ghee	---	Ghee

Table 2: Actual wound contraction (wound area in mm^2) recorded

Group	3rd day	6th day	9th day	12th day	15th day	18th Day	Mean % wound contraction (0-18 days)
WC	237.48 ± 18.62	189.81 ± 14.20	112.61 ± 15.66	54.85 ± 4.73	23.81 ± 01.87	12.10 ± 03.77	94.90
VC	271.53 ± 21.41	220.06 ± 19.55	91.02 ± 15.37	36.16 ± 5.93	14.24 ± 04.11*	08.09 ± 03.40	97.02
JKG	215.02 ± 8.87	158.17 ± 8.83	79.55 ± 8.13*	32.51 ± 4.92**	03.53 ± 01.58*** #	--	100.0
RS	224.78 ± 11.94	190.07 ± 15.40	115.94 ± 26.73	77.25 ± 28.41	14.73 ± 05.22*	06.09 ± 02.73	97.29

Data: Mean ± SEM; *P<0.05; ***P<0.001; **P<0.01 (Compared with control); #P<0.05 (Compared with VC)

Table 3: Days taken for complete epithelialisation

Group	Treatment given	Days taken for complete epithelialisation	% change
WC	Wound control – No application of any drug	26.00 ± 01.37	--
VC	Vehicle control – Application of plain Ghee	23.17 ± 01.38	10.88 ↓
JKG	Trial Group – *Jati Kalpa Ghrita*	19.50 ± 00.67***#	25.00 ↓
RS	Reference Standard – Povidone Ointment	22.33 ± 01.65	14.12 ↓

***P<0.001 (Compared with control), #P<0.05 (Compared with VC)

A group of herbs having healing property were combined and a ghee based herbal formulation was prepared and the wound healing activity was evaluated on excision model.

Excision wound healing model is often used for wound healing evaluation because it represents a true wound that could be reproducibly analyzed in non-subjective, highly controlled manner.

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A significant wound healing was observed in Jati Kalpa Ghrita and standard treated groups, in comparison to control group. There was a significant reduction in wound size from day 9 onward in Jati Kalpa Ghrita treated animals and also on subsequent days. Further swift wound closure rate was observed in Jati Kalpa Ghrita treated group when compared to the vehicle treated group also. The enhanced rate of wound contraction and significant reduction in healing time in Jati Kalpa Ghrita treated animals when compared to the untreated animals might be due to enhanced epithelialization. Jati kalpa ghrita contains characters which serve the objective of wound healing.[10] In this poly-herbal formulation it contains drugs like Jati,[11] Karanja,[12] Haridra[13] Manjishta,[14] Dhataki,[15] Lodhra,[16] Yashtimadhu,[17] Nimba[18] and Sariva[19] (Table 1) which are well established drugs of wound healing and anti-oxidant properties[20] that may be responsible for observed wound healing activity. Further ghee contains beta carotene and Vitamin E and both are known anti oxidants.[21]

CONCLUSION

The days taken for complete wound contraction in Jati Kalpa Ghrita was much earlier when compared with the other groups, even it had surpassed the reference standard group, which recommends it to be studied on clinical subjects. The results of the present study showed that Jati Kalpa Ghrita is having potent wound healing activity.

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