Research Article

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PHARMACO - ANALYTICAL AND EXPERIMENTAL STUDY OF DARVYAADI GHRITA WITH SPECIAL REFERENCE TO WOUND HEALING EFFECT

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ABSTRACT

Ayurveda has propagated many excellent remedies for Vrana ropan (wound healing). Darvyaadi ghrita is one among them. Gada Nigraha mentions it, as the best for Ropana (healing). Healing or closure of a wound has many associated complications like infection, scarring etc. An effort has been made for evaluation of wound healing property of Dravyadi ghrita through animal experimental study. Wound healing property of Darvyaadi ghrita was evaluated through excision wound model and incision wound model on albino rats. 24 albino rats were selected and grouped into 4 of 6 animals in each group. In excision wound, percentage contraction of the wound and period of epithelialization was studied. In incision wound, tensile strength or breaking strength of the wound was studied. Study showed that Darvyaadi ghrita possess superior wound healing properties than the control group. Statistically, Darvyaadi ghrita showed significantly results on all assessed parameters.

Key words: Vrana ropana, Wound healing, Sodhana, Darvyaadi ghrita, Epithelialization, Contraction, Tensile strength.

INTRODUCTION

In Ayurveda, many Kalpanas (preparations) are mentioned for therapeutic use. The Sneha kalpana (oily preparations) play an important role in many therapeutic procedures and as medicines. When considering this aspect ghrita and taila are good mediums of oleation externally as well as internally. In modern pharmaceutics the researchers have shown that oil is good for wound healing purpose. The incidence of wound is common in childhood as well as in adolescence, which may be considered to be unpleasant event ascertain unavoidable time and characterized by pain, bleeding and sometimes oedema.

The present study is an experimental study of Darvyaadi Ghrita on albino rats. For the preparation of Darvyaadi ghrita, 9 drugs like Daruharidra (*Berberis aristata* DC.), Yastimadhu (*Glycyrrhiza glabra* Linn.), Lodhra (*Symplocos racemosa* roxb.), Nagakesara (*Mesua ferrea* Linn.), Patola (*Tricosanthes dioica* Roxb.), Haritaki (*Terminalia chebula* Retz.), Vibhitaki (*Terminalia bellerica* Roxb.), Aamlaki (*Emblica officinalis* Gaertn.) and Go-Ghrita (cow's milk fat) were used. The Darvyaadi ghrita has been prepared according to classical procedure as per text Sarangadhara samhita.

Two wound models selected for this study were-

- Excision wound model.
- Incision wound model.

In the excision wound model the rate of contraction of the wound and period of epithelialization were studied. When a wound is created and there is disruption in the continuing of the tissues, the factor which contributes mainly towards healing is wound closure or contraction. Wounds can contract by up to 80% of original size so that the space to be filled is much reduced, resulting in faster healing with a smaller scar. Epithelial proliferation starts covering the surface from the periphery by proliferating beyond the edges and migrating under the scab. So contraction and epithelialization were the two major factors included, which are responsible for the successful and early healing of a wound.

In the incision wound model the tensile strength or the breaking strength of the wound is measured. Here actually the strength of the collagen tissue or the granulation tissue formed is tested, since the collagen tissue is the main factor which holds the cut edges together. Granulation tissue is formed by proliferation of surrounding connective tissue elements, which migrate in to the site to be repaired. This is achieved by the use of a simple technique called tensiometer or constant water flow technique. So keeping these parameters as reference, study was made to evaluate the result of Darvyaadi ghrita on wounds healing effect.

According to Acharya Sushruta the good healed wound will be – "when healing wound will be filled with proper granulation tissue, without swelling, without pain, colour of skin is similar to surrounding skin, and the granulation tissue will be in equal plane of surrounding skin"[1]. According to modern view "The word healing means the restoration of the normal mental or physical condition, but especially of an inflammation or a wound. Healing is the process or act of getting well, the restoration to normal as in the closure of an ulcer or a wound, or the union of a broken or diseased bone [2]".

So the present study was planned to evaluate effect of Darvyaadi ghrita based on classical texts through modern tools and techniques.

Therefore keeping these factors the study was designed to evaluate -

- Wound healing capacity,
- Rate of contraction in excised wound by Morton and Mallon technique,
- Tensile strength of granulation tissue in incision wound by technique of Hunts *et al* and by Tensiometer or constant water flow technique, developed by Lee *et al*.

MATERIAL AND METHODS

Pharmaceutical study

Darvyaadi Ghrita has been prepared according to sneha kalpana vidhi as per Sarangadhar samhita Madhyama Khanda after Ghrita Murchana.

"Daarvi Twak madhukam lodhram kesaram cha avachurnitam [3]
Patola patram triphalaam kuryaad Ardhapalonmitam
Pakwam Yasthaadyam kalkam ghrutam syaad Vranaropanam"

(Gada Nigraha Kayachikitsa Khanda)

Raw drugs, Daruharidra (*Berberis aristata* DC.), Yastimadhu (*Glycyrrhiza glabra* Linn.), Lodhra (*Symplocos racemosa* roxb.), Nagakesara (*Mesua ferrea* Linn.), Patola (*Tricosanthes dioica* Roxb.), Haritaki (*Terminalia chebula* Retz.), Vibhitaki (*Terminalia bellerica* Roxb.), Aamlaki (*Emblica officinalis* Gaertn.) and Go-Ghrita (cow's milk fat) were collected from the local market and was authenticated by the Department of Dravyaguna. A.L.N. Rao memorial Ayurvedic medical college, Koppa, Karnataka, India.

The raw drugs were made into coarse powder. The coarse powder was boiled with 9.6 liter water and decoction was prepared up to 2.4 lit of filtrate. The processed ghrita and paste of Yastimadhu (*Glycyrrhiza glabra* Linn.) was then added in this decoction of powdered herbs and boiled over medium flame till evaporation of water content of decoction. After cooling in triplicate darvyadi ghrita was filtered with double layer fine cotton cloth and packed. The process was carried out three times and three batches of sample were prepared. Each time the loss in the end product and the duration of process were observed.

Animal Experimental study

Selection of animal

Healthy albino rats of either sex weighing between 150 – 250 gms were selected randomly for the study. The rats were bred in the animal house of A.L.N. Rao Memorial Ayurvedic Medical College, Koppa, Karnataka, India. Regd. no: 191/CPCSEA, IAEC Approval no: AECBK 04/06

The rats were fed with (Amruth Brand) rat food and water ad-libidum.

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

Table 1: Grouping of animals

Group	Wound model	No. of Rats	Purpose
Control	Excision	6	To serve as prophylactic control
Control	Incision	6	To serve as prophylactic control
Trial	Excision	6	To serve as prophylactic effect
Trial	Incision	6	To serve as prophylactic effect.

The wound healing property of Darvyaadi ghrita was analyzed in albino rats by 2 methods-

- Excision wound model (technique developed by Morton and Mallone[4])
- Incision wound model (technique developed by Hunts *et al*[5])

These techniques consist of the following stages.

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

The selected albino rats was primarily divided into two groups of 12 in each group for excision and incision wound models.

Pre-operative stage: The selected albino rats were fasted overnight and water was given ad-libidum 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 was conducted according to the technique developed by Morton and Mallone. The animals were anesthetized using ketamine intra-peritoneal injection. After the animals were sufficiently anaesthetized, they were secured to the dissection plate in prone position. The hairs were removed from the part to be operated and subsequently the area was cleaned with antiseptic solution (savlon). A round seal of 2.5 cm. in diameter was impressed on the dorsal thoracic central region 5 cm 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 blade and scissors. The approximate area thus formed was 500 mm^2. After achieving full haemostasis, the animals were place in individual cages.

Incision wound model

This was conducted according to the technique mentioned by Hunts *et al*. The animals were anesthetized using ketamin intra-peritoneal injection; after the animals were sufficiently anaesthetized they were secured to the dissection plate in prone position. The hairs were removed from the part to be operated and subsequently the area was cleaned with antiseptic solution (savlon). Two para-vertebral incision measuring 6 cm in length 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 Darvyadi ghrita was started from the day of surgery (0 day). Every post wounding day the wounds were cleaned with normal saline and the trial drug was applied to the trial groups. Control groups were left alone to observe the natural healing process. The rats were given normal food and water ad-libidum.

Assessment criteria

Wound healing was assessed by monitoring physical attributes and mechanical attributes. But monitoring of any one attribute may not truly assess the healing. Therefore different wound models have been studied to monitor the phase of healing. Wound contraction and epithelialization 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

Wound Contraction: The factor which contributes 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.

Period of epithelialization: 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 epithelialization and the time was noted in all animals.

Incision wound model

Here the tensile strength or breaking strength was 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 tensiometer, the tensile strength is expressed as the minimum weight of water necessary to bring about the gaping of the wound. As per protocol suture were removed on 7^{th} post wounding day and the breaking strength was determined on the 10^{th} post wounding day by method of Lee [6], as described below.

All animals were anaesthetized before measuring the breaking strength using tensiometer. Each animal was secured to the operation table in its natural position and lines were drawn on either side of the wound, 3mm 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 pully. 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. At last the disrupted wound margins were re-sutured and allowed to heal without any scarification of the animals.

Observation

Pharmaceutical work

The loss during the pharmaceutical work of Darvyadi ghrita was observed as showed in table-

Table 2: Date of preparation, amount, loss, % of loss, average % of loss

Products	Started	Completed	Before preparation	After preparation	Loss	% of Loss	Average % of Loss
Murchita ghrita	4-9-06	5-9-06	2400gm	2100gm	300gm	12.5%	
Darvyaadi ghrita I	18-9-06	19-9-06	600gm	420gm	180gm	30%	
Darvyaadi ghrita II	20-9-06	21-9-06	600gm	440gm	160gm	26.6%	
Darvyaadi ghrita III	22-9-06	23-9-06	600gm	435gm	165gm	27.5%	28 %

Table 3: Analytical tests

Sample	LOD %	Sp. Gravity	Acid Value (mg)	Saponification Value(mg)	Ester value (mg)	Iodine Value (gm)
Darvyaadi Ghrita-1	0.11	0.9127	1.97	225.56	223.59	37.9
Darvyaadi Ghrita-2	0.21	0.9119	1.91	228.09	226.18	44.04
Darvyaadi Ghrita-3	0.19	0.9167	1.97	222.81	220.84	41.71
Murchita Ghrita	0.17	0.9168	1.75	221.17	219.42	40.29

Table 4: % closure of original excision wound area (sq.mm.) on 4th post wounding day

Sl. No.	Control group (Natural recovery)	Trial group (Darvyaadi ghrita)
1	36.7	35.1
2	35.1	32.9
3	30.8	35.3
4	33.8	40.3
5	32.5	39.0
6	35.7	39.2
Mean	34.1	36.966
S.D.	2.184	2.933
S.E.	0.89	1.197
t-value	38.23	30.865
p-value	<0.001	<0.001

Table 5: % closure of original excision wound area (sq.mm) on 8th post wounding day

Sl. No.	Control group (Natural recovery)	Trial group (Darvyaadi ghrita)
1	51.8	65.0
2	50.4	58.6
3	47.5	64.8
4	46.7	66.7
5	47.1	70.1
6	53.5	58.0
Mean	49.5	63.866
S.D.	2.817	4.715
S.E.	1.150	1.925
t-value	43.029	33.173
p-value	<0.001	<0.001

Table 6: % closure of original excision wound area (sq.mm) on 12th post wounding day

Sl. No.	Control group (Natural recovery)	Trial group (Darvyaadi ghrita)
1	68.4	84.5
2	69.5	84.3
3	66.7	80.9
4	66.9	84.8
5	64.3	83.3
6	68.5	78.3
Mean	67.383	82.683
S.D.	1.842	2.575
S.E.	0.752	1.051
t-value	89.597	78.635
p-value	<0.001	<0.001

Table 7: % closure of original excision wound area (sq.mm) on 16th post wounding day

Sl. No.	Control group (Natural recovery)	Trial group (Darvyaadi ghrita)
1	85.7	94.9
2	83.5	98.6
3	84.7	100.0
4	87.9	100.0
5	86.8	97.7
6	85.0	95.7
Mean	85.6	97.816
S.D.	1.569	2.151
S.E.	0.640	0.878
t-value	133.576	111.356
p-value	<0.001	<0.001

Table 8: Period of epithelialization (in no. of days)

Sl. No.	Control group / Days	Trial group / Days
1	16	13
2	18	15
3	14	12
4	16	14
5	17	14
6	20	15
Mean	16.833	13.833
S.D.	2.041	1.169
S.E.	0.833	0.477
t-value	20.2	28.984
p-value	<0.001	<0.001

Table 9: Tensile strength in gm/ml of incision wound on 10th post wounding day

Sl. No.	Control group	Trial group
1	420	515
2	390	480
3	356	508
4	382	422
5	396	475
6	410	495
Mean	392.33	482.5
S.D.	22.464	33.423
S.E.	9.171	13.644
t-value	42.778	35.361
p-value	<0.001	<0.001

Table 10: General properties of trial drug Darvyaadi Ghrita

Drug	Rasa	Guna	Virya	Vipaka	Dosha Karma
Daruharidra	Thikta, Kashaya	Laghu, Ruksha	Usna	Katu	Kaphapittahara
Yastimadhu	Madhura	Guru, Snigdha	Sita	Madhura	Tridosha-hara
Lodhra	Thikta, Kashaya	Laghu, Ruksha	Sita	Katu	Kaphapittahara
Nagakesara	Thikta, Kashaya	Laghu, Ruksha, Tikshna	Usna	Katu	Kaphapittahara
Patolapatra	Katu, Thikta,	Laghu, Ruksha	Usna	Katu	Kaphapittahara
Haritaki	Kashaya	Laghu, Ruksha	Usna	Madhura	Tridosha samaka
Bibhitaki	Kashaya	Laghu, Ruksha	Usna	Madhura	Tridosha samaka
Amalaki	Kashaya	Guru, Ruksha	Sita	Madhura	Vayasthapana
Ghrita	Madhura	Laghu, Tikshna	Sita	Madhura	Tridoshahara

Animal Experimental work

Excision wound model

To monitor the changes in the wound shape, 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. Later this was retraced on a millimeter scale graph paper. The observations of percentage of wound closure were made on the 4th, 8th, 12th, 16th and 18th post wounding days. The wound was also observed for the period of epithelialization.

Incision wound model

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

RESULT

Analytical Report

The samples of Murchita ghrita and 3 batches of Darvyaadi ghrita were analyzed for qualitative and quantitative standard parameters. (Table 3)

In all the 3 processes, there was a small amount of loss near about 150 to 200 g in each process. The apparent loss may be due to small amount of ghrita being absorbed by the kalka, small amounts of ghrita may be lost during the preparation and also due to adherence to the vessel and cloth used for the process.

The mean contraction was seen in the control group on the 4th day which was 34.1 ± 2.184% with 't' value 38.23 (p value < 0.001). Whereas the mean contraction in the trial group on the same day was 36.966 ± 2.933 with 't' value 30.865 (p value < 0.001).

So the above statistical observation indicates that both control and trial group were highly significant. But on the database trial group was more significant than the control group. (Table 4)

The mean contraction in the control group on the 8th day was 49.5±2.817% with 't' value 43.029 (p value < 0.001). Whereas the mean contraction in the trial group on the same day was 63.866 ± 4.715% with 't' value 33.173(p value < 0.001).

In this way, above statistical observation indicates that both control and trial group were highly significant. But on the database the trial group was more significant than the control group. (Table 5)

The mean contraction in the control group on the 12th day was 67.383±1.842% with 't' value 89.597 (p value < 0.001). Whereas the mean contraction in the trial group on the same day was 82.683 ± 2.575 % with 't' value 78.635 (p value < 0.001).

So the above statistical observation, it indicates that both control and trial group were highly significant. But on the database the trial group was more significant than the control group. (Table 6)

The mean contraction in the control group on the 16th day was 85.6 ±1.569 % with 't' value 133.576 (p value < 0.001). Whereas the mean contraction in the trial group on the same day was 97.816 ± 2.15 % with 't' value 111.356 (p value < 0.001).

So the above statistical observation, it indicates that both control and trial group were highly significant. But on the database the trial group was more significant than the control group. (Table 7)

The average period of epithelialization in the control group was 16.833 ± 2.041% with 't' value 20.2 (p value < 0.001). Whereas the average period of epithelialization in the trial group was 13.833 ±1.169% with 't' value 28.984 (p value < 0.001).

So the above statistical observation, it indicates that both control and trial group were highly significant. But on the database the trial group was more significant than the control group. (Table 8)

The tensile strength shown in the control group on 10th day was 392.33±22.464% with 't' value 42.778 (p value < 0.001). Where as in the trial group it was 482.5±33.423 % with 't' value 35.361 (p value < 0.001).

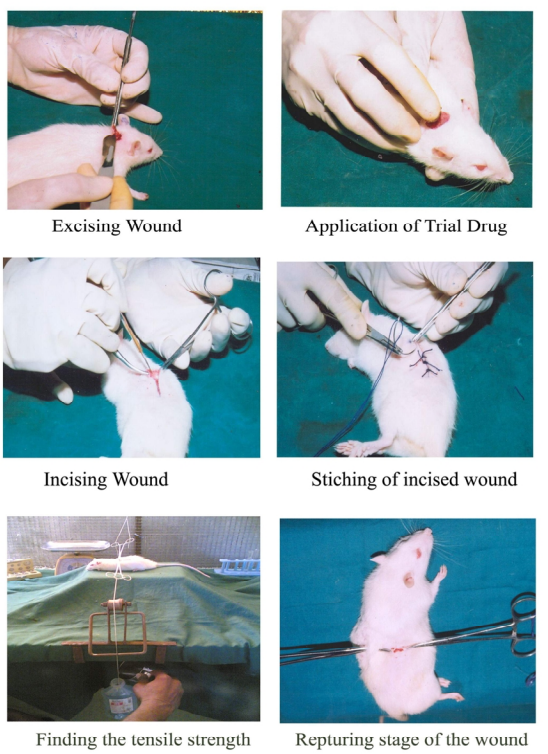
Therefore statistical observation, indicates that both control and trial group were highly significant. But on the database the trial group was more significant than the control group. (Table 9)

DISCUSSION

The present study has been undertaken to evaluate the efficacy of Darvyaadi ghrita for its wound healing property as mentioned by Acharya Sodhala in his text Gada Nigraha. kayachikitsa khanda .

Ayurveda has put forward two unique concepts in the context of wound healing sodhana and ropana. Sodhana is literally cleaning of wound and Ropana means healing process**.** Darvyaadi ghrita have been described in wound healing property.

EXPERIMENTAL STUDY



Excising Wound | Application of Trial Drug

Incising Wound | Stiching of incised wound

Finding the tensile strength | Repturing stage of the wound

Mechanism of wound healing

Wound healing is a complete phenomenon involving a number of processes, including of an acute inflammatory

process by the wounding, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of ECM proteins, remodeling of connective tissue, parenchymal components and collagenization and acquisition of wound strength. As noted, the deposition of connective tissue matrix particularly collagen, its remodeling into a scar, and the acquisition of wound strength are the ultimate effects of orderly wound repair. This mechanism can be understood by the three phases, beginning with the phase of inflammation (days 1-4), and merging into phase two, which is the phase of proliferation(granulation tissue, days 5-20) and then into phase three, the phase of differentiation (Scar tissue) from day 20 onwards. The inflammatory response occurs in the vascular connective tissue, including plasma, circulating cells, blood vessels and cellular and extra cellular constituents and connective tissue[7].

In the experimental study, 24 healthy albino rats of either sex were selected and grouped in to four. Two methods viz, excision wound model (technique developed by morton and mallone) and incision wound model (technique developed by Hunts *et al*) was followed to induce wound healing. Three parameters i.e. % contraction of original excision wound, period of epithelialization and tensile strength were calculated to find out the effectiveness of the formulation Darvyaadi ghrita. The results were analyzed statistically using students 't' test . (Table 10)

The result shows that the trial drug has wound healing properties and it has shown better significance compared to the control group.

In the excision wound two parameters were assessed,

- Percentage contraction of original wound area and
- Period of epithelialization

To prove any drug as a wound-healing agent, it should have significant effect on rate of contraction. It was measured once in four days i.e., 4^{th}, 8^{th}, 12^{th} and 16^{th} day. In the trial groups complete closure was achieved on 18^{th} day, but in the control group closure achieved on 21^{st} day. The mean period of epithelialization in the trial group was 13.833 days and that of control group was 16.833 days. This clearly shows that epithelialization was achieved early in the trial group than the control group.

In the incision wound model, the strength of the granulation tissue was measured. Once a breach or gap has occurred in the tissue, it was closed by the granulation tissue. The two ends of the breach were held together by the granulation tissue. Here the strength of the granulation tissue was assessed using a simple instrument called the tensiometer. This was calculated in grams. Here the mean of the trial group was 482.5 g and that of control group was 392.33 gm. This clearly shows that the trial group was better tensile strength than the control group.

All these result showed that trial drugs were having significant wound healing properties when used externally in experimental models.

CONCLUSION

Hence, from the experimental studies it can be speculated that, the drugs namely "Darvyaadi Ghrita" possess sufficient efficacy in "wound healing" without producing any adverse effects. The drugs also increase the rate of contraction produces healthier granulation tissue and rapid healing time in experimental studies which seems to be an optimum combination, in local, systemic or in combined form. Experimental and Statistical analysis signifies the effectiveness of Darvyaadi Ghrita upon Vrana Ropana.

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