



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article


October 2023 Vol.:28, Issue:3

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Wound Healing Potentials of Medicated Ghee - An Experimental Study



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals



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Submitted: 18 September 2023
Accepted: 23 September 2023
Published: 30 October 2023

Keywords: Ayurveda, Medicated Ghee, Excision Wound, Jatyadi ghee

ABSTRACT

Ghee-based medications are special preparations of Ayurvedic medicine. Cow's ghee is treated using specialized herbal decoctions as well as herbal pastes. When the same process is performed with the addition of herbs and medicated ghee, it is known as Avartita Ghee. Therefore, the purpose of this study was to demonstrate the superiority of jatyadi avartita ghee over jatyadi ghee in treating excision wounds in Wistar male rats. Jatyadi ghee was prepared by boiling paste of Leaves of *Jasminum officinalis* Linn, stem bark of *Azadiracta indica* A.Juss Syn Melia, Whole plant of *Triconthoes dioica*, Rhizome of *Curcuma longa* Linn, Stem of *Berberis aristata*, Root of *Picrorhiza kurrora* Royle ex Benth, Root of *Rubia cordifolia* Linn, Root of *Glycyrrhiza glabra* Linn, Root of *Hemidusmus indicus*, Seed of *Pongamia pinnata* Pierre, Root of *Veteveria zizanioidas* Linn⁹⁻²¹, Blue vitriole ($\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$) and Bee's wax with Cow's ghee and water. Avartita Jatyadi ghee was prepared by boiling decoction of the same herbs with Jatyadi ghee. The Wound model and assessment criteria were followed as described by Morton and Malone. The results shows both Jatyadi Ghrita and Jatyadi Ghrita Avartini seven significantly promotes wound healing compared to plain Ghrita. Both preparations showed significant wound-healing effect. But when compared to mean values; Jatyadi Ghrita Avartini (70.11 ± 5.86) showed increased healing results compared to Jatyadi Ghrita (68.23 ± 7.70) on the 8th day onwards. It was 99% healing on 16th day and 100% healing was seen on the 18th day in Jatyadi Ghrita Avartini group.



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INTRODUCTION

According to Ayurveda, cow's ghee is superior and has numerous medicinal uses. Cow's Ghee has a special quality known as *samskaranuvartana*, which indicates that when being processed with herbal liquids or pastes, Cow's Ghee combines the effects of herbal medicines that are applied without sacrificing its own³ therapeutic properties. Ayurvedic medicated Ghee is prepared by processing Cow ghee along with herbal paste and decoction as per classical reference¹. These medications are demonstrated to have the greatest therapeutic effects when taken orally². According to Ayurveda, Ghee has Mrudu, Somaya Guna, Madhura Rasa, Sheeta Virya, Madhura Vipaka, Balya, Medya, Rasayana etc properties. Ghee compositions are Fat, Protein, Carbohydrates, Sugar (Lactose), Cholesterol, Calcium, Vit-A,D,E, and K, Saturated, monounsaturated, polyunsaturated fatty acids like Linoleic acid, Butyric acid, Caproic acid, Caprylic acid, Capric acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Arachidic acid, Oleic acid and antioxidants like β kerotene⁴. The lipophilic action of Ghee facilitates the transportation of necessary elements to a target organ and to the internal organelles of the cell including mitochondria, microsome and nuclear membrane. This is because of the lipophilic nature of the cell membrane⁴.

Different medicated ghee is described in the context of *Sneha Kalpana* by processing Ghee and Oil (Sesame oil) along with herbal, mineral and animal origin drugs at specific temperatures, duration and in specific proportion⁵. There are references of *Dashapaka* (ten times boiling), *Shatapaka* (hundred times boiling) and *Sahastrapaka* (thousand times boiling) in Ayurvedic classics in which *Avartini*^{6,7} concept has been adopted. The concept of *Avartini* means repeated processing; which is known to enhance the potency of medicated Ghee by boiling with herbal decoctions. The purpose of potentiating is to minimize the dose, faster drug delivery & get multiple benefits from the preparations. Potentiating the preparation is the need of hour.

*Jatyadi Ghrita*⁸ is one among the medicated *Ghrita* preparation which contains Jati leaves (*Jasminum officinalis* Linn), Nimba stembark (*Azadiracta indica* A.Juss Syn *Melia*), Patol whole plant (*Triconthoes dioica*), Haridra Rhizome (*Curcuma Longa* Linn), Daruharidra stem (*Berberis aristata*), Katuka root (*Picrorhiza kurrora* Royle ex Benth), Manjistha Root (*Rubia Cordifolia* Linn), Yasimadhu root, (*Glycyrrhiza Glabra* Linn), Sariva root (*Hemidusmus indicus*), Karanj seed (*Pongamia pinnata* Pierre), Usira root (*Veteveria zizanioidas* Linn)⁹⁻²¹. Tutta (Blue vitriole $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$) and Bee's wax.

It is indicated in the treatment of fistula, fissures, different types of wound such as excision wound, open wounds, deep-rooted wounds etc. Avartini (repeated by adding decoction) was planned to Jatyadi Ghrita to observe effect of Avartita Jatyadi Ghrita on excision wound in male Wistar rats.

MATERIALS AND METHODS

Preparation of Jatyadi Ghee and Jatyadi Ghee Avartini.

The raw drugs were procured from GMP-certified KLE Ayurved pharmacy Belgaum, and authenticated at AYUSH approved drug testing laboratory of KAHER, BMK Ayurved Mahavidyalaya, Belgaum.

The Powder form (60-80 No. mesh) of each ingredient was selected. Soft paste was prepared using an appropriate quantity of water. Jatyadi Ghrita was prepared by boiling herbal paste, Ghrita, along with water in a 1:4:16 ratio respectively and boiled till confirmative signs of Ghrita appeared, and the same was confirmed with tests on paste. Tuttha (*Copper Sulphate* $CuSO_4 \cdot 7H_2O$ (blue vitriol), Sikth (Bee's wax) were added after filtration of Ghrita when it was still hot. Greenish-yellow coloured Jatyadi Ghrita was obtained. Avartita Jatyadi Ghrita was prepared by reprocessing the same Jatyadi Ghrita along with decoction of same herbal ingredients till confirmative signs of Ghrita appeared. The process was repeated seven times to make Jatyadi Ghrita Avartita seven.

Animals

Male Wistar rats weighing 150-250gms supplied by M/s Venkateshwara Enterprises Bangalore. were selected for the study. An experimental study was conducted at the Animal house of K.L.E.University's, Shri B.M.K. Ayurved Mahavidyalaya, Belgaum. (Registration No. 1017/C/ 06, CPCSEA, dated 19/12/2006). All animals were housed in colony cages at an ambient temperature ($25^{\circ}C \pm 5^{\circ}C$) and 45-55% relative humidity with 12/12 hr natural light & dark cycle. All animals were acclimatized in the laboratory about 7 days before the commencement of the study. They were fed with free access to standard pellet diet (from VRK's Scientist's Choice Laboratory Animal Feed, Baramati) and water *ad libitum*. The experimental protocol was approved by the institutional animal ethics committee resolution Number BMK/IAE/Res-04/2011.

Study design:

Eighteen animals were randomly divided into three groups (n=6 in each), Group I received plain Ghrita, while Group II received Jatyadi Ghrita & and Group III received Jatyadi Ghrita Avartini seven.

The excision wounds were induced as described by Morton and Malone.²² Under halothane anesthesia, an impression of 2.5cm was made on the dorsal interscapular region using a circular seal. Full thickness skin was excised, the wound was traced on the polythene sheet, and the animals were housed in separate cages. The external application was done with sufficient quantity of medicament using a spatula once daily till complete closure of the wound. Wound closure rate was assessed using planimetry, by tracing the wound on polythene paper on a wounding day, followed by 4th, 8th, 12th, 16th, and 18th days till the complete epithelization. The wound area was calculated by counting the number of small squares in the wound area traced on a graph paper from the polythene sheet. The degree of wound healing was calculated as percentage closure of the wound area from the original wound area using the formula-

$$\text{Percentage closure} = \frac{1 - A_d}{A_0} \times 100$$

where, A₀= wound area on day zero

A_d= wound area on corresponding days

Falling of the scab without any raw area was considered as the time for complete epithelization and was noted. Scars were traced to assess wound contraction by noting the scar size and shape.

Statistical analysis: All the values were expressed as Mean ± S.D. and data were analyzed by applying ANOVA followed by the Dunnett‘t’ test and p<0.05 was considered as significant.

RESULTS

[Table No. I-V]: There was no significant difference between the three groups in the percentage closure of the excision wound on 4th day. There was significant difference observed in the percentage closure of excision wounds between Jatyadi Ghrita and Jatyadi Ghrita Avartini as compared to control on various days.(8th , 12th, 16th,18th day). [Table

No.VIII]: Complete epithelization of group –I was seen on day 20th day, whereas for Group – II & and III it was seen on day 19th& 18th respectively. Shape of scar of group-II & III was stellate & of group-I was triangular. Scars were stellate shaped in the Jatydi Ghrita and Avartini group while they remain oval or oblong in control group. This indicated maximum contraction of the wound as compared to control. The result of the present study clearly indicates that both Jatyadi Ghrita and Jatyadi Ghrita Avartini significantly promote the wound healing process compared to the control group (P<0.01 on 8th day).

Table number 1 showing the percentage closure of excised wound on 4th day

| Group | Mean ± SD | ANOVA | Dunnett sig. p>0.05 |
|---------------------------------|------------|---------|---------------------|
| Gr I-Control | 19.07±4.36 | F= 1.68 | |
| Gr II -Jatyadi Ghrita | 21.63±6.81 | p>0.22 | 0.68 |
| Gr III -Jatyadi Ghrita Avartini | 25.28±6.26 | | 0.15 |

Table number 2 showing percentage closure of excised wound closure on the 8th day

| Group | Mean ± SD | ANOVA | Dunnet t sig. p<0.01 |
|-----------|------------|---------|----------------------|
| Group I | 53.81±7.52 | F= 9.52 | |
| Group II | 68.23±7.70 | P<0.002 | .006 |
| Group III | 70.11±5.86 | | .002 |

Table number 3 shows the differences in percentage of wound closure on 12th day

| Group | Mean ± SD | ANOVA | Dunnet t sig. p<0.001 |
|-----------|------------|----------|-----------------------|
| Group I | 81.30±7.25 | F=13.98 | |
| Group II | 93.33±4.15 | p<0.0055 | 0.001 |
| Group III | 95.21±1.89 | | 0.0001 |

Table number 4 showing the differences in the percentage of wound closure on 16th day

| Group | Mean ± SD | ANOVA | Dunnett sig. p<0.001 |
|-----------|------------|----------|----------------------|
| Group I | 88.88±3.97 | F= 27.81 | |
| Group II | 97.70±1.88 | p<0.0006 | 0.0001 |
| Group III | 99.28±0.99 | | 0.00001 |

Table number 5 shows the percentage closure of excised wound on 18th day

| Group | Mean ± SD | ANOVA | Dunnett sig. p<0.05 |
|-----------|-------------|--------|---------------------|
| Group I | 96.37±3.67 | F=5.37 | |
| Group II | 99.73±0.47 | p<0.02 | 0.03 |
| Group III | 100.00±0.00 | | 0.02 |

Complete epithelization of group –I was seen on day 20th day, whereas for Group –II & III it was seen on day 19th& 18th respectively. The shape of scar of groups II and III was stellate & of group I was triangular.

DISCUSSION

The concept of Avartini has been adopted to enhance the therapeutic potency of the Ghee preparations. The objective of this study was to evaluate the efficacy of Jatyadi Ghrita and Jatyadi Ghrita Avartini on excision wounds in male Wistar rats. The excision wound was induced on all rats and closure rate was assessed on different days. The result of the present study clearly indicates that both Jatyadi Ghrita and Jatyadi Ghrita Avartini significantly promote the wound healing process compared to the control group.

Cow's Ghee promotes wound healing^{23, 24}. The ingredients used in medicated Ghee preparation are Jati, Nimba, Patola, Haridra, Daruharidra, Manjistha, Karanja, Yastimadhu, Ushira, Kutaki, Sariva, Sikta, Tuttha. The ingredients of Jatyadi Ghrita are proved scientifically for their antimicrobial properties.

This effect on wound healing could be attributed to the properties like Tikta, Kasaya Rasa which does Vrana Shodhana and Stambhana karma, that are responsible for prevention of secondary infection and wound contraction respectively and thereby improving healing

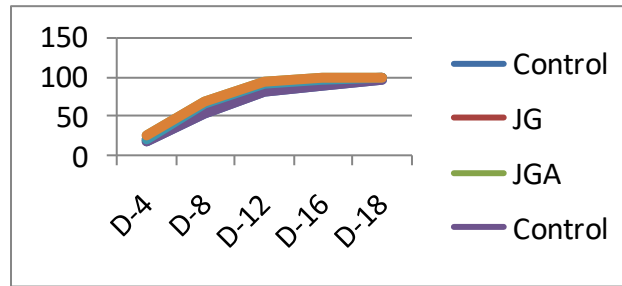
process. Properties like krimighna, kandughna have bactericidal effect. These observations can be further supported by the results of a study that proved the effectiveness of local application of Manjishthadi Ghrita which showed reduction of the wound size, promotion of healing and cosmetically effective with the least scar formation²³. In another study animals treated with Jatyadi Taila showed a significant increase in protein, hydroxyproline and hexosamine content in the granulation tissue when compared with the untreated controls. These proteins are essential for wound healing²⁵.

Sneha Kalpana¹ is a special concept & contribution of Ayurveda, in which coarse powder of herbal drugs/Medicinal plants are boiled along with Ghrita. None of medical science has such description. Sneha kalpana¹ is mass transfer of the aqueous and lipid-soluble active principles of all treated herbal drugs and material of animal and mineral origin to lipid media, in accordance of established formulae quoted in authoritative textbooks of Ayurveda, which should serve therapeutic objectives as per indications of the classical treatise of Ayurveda. The intension of boiling is not to evaporate the water, but thermo-sensitive active constitutes should be transferred into Ghee meida. These processed Ghrita are easily permeable into body tissue. The active principals adhered to Ghrita easily enters into body tissue through lipid media.

The concept of Avartini was followed from Samhita named as, Amalak Ghrita² Dashapaki, shata paka³ etc. The objective of Avartini or repeated processing concept in Sneha Kalpana is one such process by which oleaginous medicaments are boiled repeatedly along with herbal decoctions.

Avartini concept was applied to know the increase in potency at the efficacy level. So the objective of this study was to evaluate the efficacy of Jatyadi Ghrita and Jatyadi Ghrita Avartini on excision wounds in male Wistar rats. The excision wound was induced on all rats and closure rate was assessed on different days.

Graph number 4 Showing Wound Healing results between groups.



* Group 1-Control * Group 2- Jatyadi Ghrita * Group 3- Jatyadi Ghrita Avartini

The result of the present study clearly indicates that both Jatyadi Ghrita and Jatyadi Ghrita Avartini significantly promote the wound healing process compared to the control group ($p < 0.01$ on 8th day).

The difference in the mean value of the percentage of wound closure on 4th day in the inter group (ANOVA) was not- significant ($p > 0.05$). The difference in mean value of the percentage of wound closure of all treated group (Dunnett t) when compared to control was not- significant ($p > 0.05$). The difference in mean value of the percentage of wound closure on 8th day in the inter group (ANOVA) was significant ($p < 0.01$). The difference in mean value in percentage of wound closure on 8th day of all treated groups (Dunnett t) when compared to control was significant ($p < 0.01$). The difference in the mean value of percentage of wound closure on 12th day in the inter group (ANOVA) was significant ($p < 0.001$). The difference in mean value of percentage of wound closure on 12th day of all treated group (Dunnett t) when compared to control was significant ($p < 0.001$). The difference in mean value of percentage of wound closure on 16th day in the inter group (ANOVA) was significant ($p < 0.001$). The difference in mean value the of percentage of wound closure on the 16th day of all treated group (Dunnett t) when compared to control was 99.99 % significant. ($p < 0.001$) . The difference in mean value of percentage of wound closure on 18th day in the intergroup (ANOVA) was significant ($p < 0.05$). The difference in mean value the of percentage of wound closure on 18th day of all treated group (Dunnett t) when compared to control was significant ($p < 0.05$).

The percentage of wound healing was not significant ($p > 0.05$) in both groups of trial drug till 4th day (table number 1). It was significant ($p < 0.05$) in both groups of trial drug from 8th day onwards. It means both trial drugs started showing their therapeutic efficacy from 8th day onwards (table number) However, Jatyadi Ghrita Avartini showed better results in wound healing as compared to Jatyadi Ghrita. The mean value of difference in percentage of healing

of Jatyadi ghrita Avartini group was higher than Jatyadi Ghrita after 8th day onwards and it was 99% on 16th day, 100% on 18th day. (table number I-V).

The ingredients used in Ghrita preparation are Jati, Nimba, Patola, Haridra, Daruharidra, Manjistha, Karanja, Yastimadhu, Ushira, Kutaki, Sariva, Sikta, Tuttha.

All ingredients are mainly Tikta, Kasaya Rasa which does Vrana Shodhana and Stambhana karma (Table Number 1) which are responsible for prevention of secondary infection and wound contraction respectively and improve healing process. The properties like krimighna, kandughna have bactericidal effects. Local application of Manjishthadi ghrita provided good result by reduction of the wound size and promotion of healing and it proved to be cosmetically effective with least scar formation also. Pigmentation similar to that of skin was found as another updating effect which was not seen in povidone iodine group¹².

CONCLUSION

Addition of herbs and the heating process was applied in the preparation of Jatyadi Ghrita, but addition of herbs decoction and heating was done repeatedly for seven times in case of Jatyadi Ghrita Avartini. Changes in organoleptic characters as well as analysis were seen in both preparations which were due to process effect and addition of herbs. Both preparations showed significant wound healing properties. But when compared to mean values; Jatyadi Ghrita Avartini (70.11 ± 5.86) showed increased healing results compared to Jatyadi Ghrita (68.23 ± 7.70) on 8th day onwards. It was 99% healing on the 16th day and 100% healing was seen on 18th day in Jatyadi ghrita Avartini group. The addition of bio constituents was increased the therapeutic efficacy in the Jatyadi Ghrita Avartini. The result of this study supports the basic concept of literature. Though the aim of this study was not to explore the mechanism involved in the healing effect, it can be hypothesized that the increased amount of active constituents due to repeated processing in the Jatyadi Ghrita Avartini was responsible for the better results.

ACKNOWLEDGEMENT:

The authors are highly thankful to VGST SPiCE (Vision Group of Science & Technology, Bangalore) for their financial support to this research.

REFERENCES

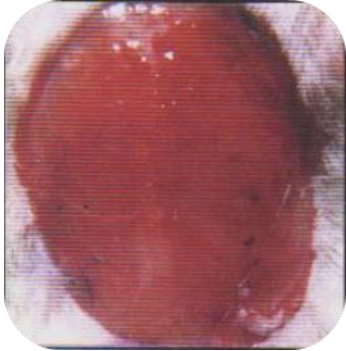
- 1) <https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-015-0568-9>
- 2) Agnivsha's Charak Samhita, Elaborated by charak & Drudhabala, Vaidyamanorama Hindi commentary by Shukla V, Tripathi R, 1st edition, Choukhamba Sanskrit Pratisthana, Varanasi, 1998, pp.199, Sutr sthana 13 verse 13.
- 3) Cakrapanidutta, Agnivesha revised by caraka & drudhabala, carakasamhita, Nirmaya sagar press, Mumbai p28, nidana sthana 1 verse 39.
- 4) Kinnari Dhruve, Anand K Chaudhary. Sneha kalpana- A probable pharmaceutical explanation, Aryavaidan Vol. XX. No. 3 Feb- Apr. 2007, pg 181-189.
- 5) Sarangdhar samhita, madhyam khand, edt by Tripathi B, Chaukhamba publications, Varanasi, reprint 2011, chap. 9/1
- 6) Agnivsha's Charak Samhita, Elaborated by charak & Drudhabala, Vaidyamanorama Hindi commentary by Shukla V, Tripathi R, 1st edition, Choukhamba Sanskrit Pratisthana, Varanasi, 1998, pp.29, Chikitsa sthana 1 / 2 verse 4
- 7) Agnivsha's Charak Samhita, Elaborated by charak & Drudhabala, Vaidyamanorama Hindi commentary by Shukla V, Tripathi R, 1st edition, Choukhamba Sanskrit Pratisthana, Varanasi, 1998, pp.744, Chikitsa sthana 29/115-20
- 8) Sarangdhar samhita, madhyam khand, edt by Tripathi B, Chaukhamba publications, Varanasi, reprint 2011, chap. 9/12-16, pp. 220-21
- 9) Pandey Gyanendra 'Textbook of Dravya guna Vignana', Krishnadas Academy Varanasi, 2002, Vol.1. 737-46, 841-47, 552-54, Vol.2, 690-710, 212-218, 500-505, 456-68, 127-36, Vol.3, 90-94, 740-45, 395-405
- 10) Hiremath S. 'text book of Bhaishajya Kalpana' I.B.H. Prakashana, Bangalore, 2000, Chapter 26, pp. 299
- 11) Acharya Yashodhar 'Rasaprakash sudhakar', Siddhananad Misra, Chaukhamba Publication, varanasi, 1998, chapter 5, pp. 102, verse 74-6
- 12) Morton JJP, MH. Evaluation of vulnary activity an open wound procedure in rats. Arch Int Pharmacodyn. 1972; 196, p. 117-26
- 13) Jyoti Baria, S. K. Gupta, C.Bhuyan, www.ayujournal.org on Thursday, March 15, 2012, IP: 223.227.133.104.
- 14) Sathish HS, Jyoti T, Ashok BK et al. Ayurpharma Int J Ayur Sci, Vol 2, No.8 (2013) Page 242-247
- 15) Singh Baljinder, Jindal Neerja, Bansu Renu et al. IJPR 2011, 2 (1) 151-156

PHOTO PLATE-3

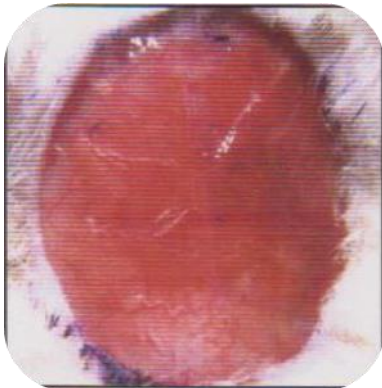
Experimental study

Day-0

Control



Jatyadi Ghrita



Avartita Ghrita



Day-16

Control



Jatyadi Ghrita



Avartita Ghrita

