Short Communication

Effect of “Murcchana samskara” on therapeutic efficacy of Bhallatakadi Ghrita

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A polyherbal formulations (herbs in combination) demonstrate better therapeutic effects with reduced untoward effects and increased palatability [1]. The ‘Ghrita’ is an Ayurvedic preparation comprising cow ghee, treated with medicinal plants either as fresh or in the form of decoctions [2]. Cow ghee possesses regenerative properties, and therefore it is generally used to treat wounds in Ayurveda. Wound healing involves an orderly advancement of the events to resume the tensile strength by re-establishing the integrity of damaged tissues. In Ayurveda, many ghrita formulations are reported for wound management [2,3].

Bhallatakadi ghrita (BG), a polyherbal Ghrita formulation, is prepared using medicinal plant extracts viz. Semecarpus anacardium Linn. (Anacardiaceae), Argemone mexicana Linn. (Papaveraceae), Cocculus hirsutus Linn. (Menispermacae), and Woodfordia fruticosa Kurz. (Lythraceae) [4].

1. Introduction

A polyherbal formulations (herbs in combination) demonstrate better therapeutic effects with reduced untoward effects and increased palatability [1]. The ‘Ghrita’ is an Ayurvedic preparation comprising cow ghee, treated with medicinal plants either as fresh or in the form of decoctions [2]. Cow ghee possesses regenerative properties, and therefore it is generally used to treat wounds in Ayurveda. Wound healing involves an orderly advancement of the events to resume the tensile strength by re-establishing the integrity of damaged tissues. In Ayurveda, many ghrita formulations are reported for wound management [2,3].

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The 'Murcchana samskara,' i.e., the process of use of 'Murcchita' ghee (prepared by incorporating antioxidant herbs) before 'ghrita-paka kalpana' ensures better shelf life, improved acceptability, and therapeutic efficacy of ghee formulation. The wound healing potential of BG [5] and the role of 'Murcchana samskara' on the shelf life and oxidative stability of ghee preparations is reported [4–7]. Hence, the present study aimed to evaluate the effect of the 'Murcchana' process on the wound healing potential of BG using excision and incision animal models.

2. Materials and methods

2.1. Preparation of BG

The plant materials viz. fruits and leaves of S. anacardium, whole plant of A. mexicana, and C. hirsutus, and leaves and flowers of W. fruticosa were collected and authenticated under the supervision of Dr. Janyathi J, Scientist ‘C’ and Dr. Benninamin A., Scientist ‘C’ at Botanical Survey of India (B.S.I.), Pune, Maharashtra, India for identification and authentication (Reference number BSI/WRC/Tech./2013/SND-1 Dated 06/12/2013; BSI/WRC/Tech./2013/JRB-01 Dated 27/11/2013; BSI/WRC/Techn./2013/GVG-01 Dated 31/12/2013; BSI/WRC/Techn./2013/GG-01 Dated 31/12/2013). The powdered materials of selected plant parts were used for BG preparation were defatted for 2 h with petroleum ether (60–80 °C) using the Soxhlet apparatus followed by extraction (Soxhlet) with alcohol to obtain the alcoholic extract [4,8]. The extracts thus obtained were used to prepare BG formulation as per the Ayurvedic procedure (Supplementary File/Fig. 1).

2.2. Preparation of M-BG

The prepared BG was processed with 'Murcchana samskara' referring to the ancient Ayurvedic procedures and denoted by M-BG (Murcchita Bhallatakadi Ghrita) (Supplementary File/Fig. 2).

2.3. Pharmacological evaluation of M-BG

M-BG formulation was evaluated for wound healing potential following the guidelines of the CPCSEA (JSPM/CCOPR/IAEC/2017–18/02 dated 12.08.2017) [9]. Wistar rats (200–250 g) of either sex were procured and were kept under standard conditions at 25 ± 2 °C with a 12/12 h light/dark cycle and fed with commercially available feed and water ad libitum regularly.

2.3.1. Acute dermal toxicity test

The animals were separated into two groups, n = 6, where Group-I was treated as control and Group-II with M-BG topically (2000 mg/kg body wt.) The acute dermal toxicity test (OECD guidelines no. 402) of M-BG was carried by monitoring changes in fur, eyes, behaviour, and toxic dermal reactions for 14 days [10].

2.3.2. Wound healing activity

Wistar rats (200–250 g) were alienated randomly into four groups, n = 6, viz. Group-I: Normal control treated with cow ghee, Group-II: reference standard group treated with 5% w/w Povidone-Iodine ointment USP (PI), Group-III: treated with BG, and Group-IV: treated with M-BG. The excision and incision wound animal models were used to assess the wound healing efficacy of M-BG [5].

The excision wounds were evaluated for wound closure (Planimetric method) and epithelization time. The cow ghee-treated (control) and PI, BG, and M-BG-treated test groups were subjected to hydroxyproline estimation. The wound breaking strength (WBS) was determined by a continuous constant water technique for the incised skin of each animal in the incision wound model.

The results are reported as mean ± S.E.M. The comparisons between groups were conducted using one-way ANOVA and Dunnett’s t-test. Multiple comparisons with control were adopted to determine the statistical significance (p < 0.05) between groups [7,11].

3. Results and discussion

Previous studies have reported the therapeutic efficacy, i.e., wound healing property of BG and the effect of the 'Murcchana' process on the stability of BG [4,12]. Furthermore, the presence of phenolic and flavonoidal phytoconstituents in BG and their antioxidant property encourage its use in wound healing [4]. In addition, reported bioactive from Murcchita herbs such as gallic acid, ellagic acid, chebulagic acid, phyllemblin, belleric acid, rutin, quercetin, isoquercetin, vitamin C, luteolin, curcuminoids [13–15] may have corroborated the promising role in augmenting the therapeutic efficacy of M-BG.

The present research attempted to measure BG’s enhanced therapeutic efficacy processed with ‘Murcchana samskara’. The ‘Murcchana’ process ensures maximum acceptability and better shelf life of BG formulation [4,12]. The use of ‘Murcchita ghee’ is generally recommended in Ayurveda to enhance the therapeutic property of ghee formulations. The herbs used in ‘Murcchana samskara’ (supplementary procedure) revealed profound antioxidant and anti-lipid peroxidation properties, thus protecting ghee from oxidative damage.

3.1. Pharmacological evaluation of M-BG

The basic tissue repair processes in animal wound healing models, viz. excision and incision, were recorded to understand, develop, and validate the clinical treatment strategies. The M-BG was safe up to the 2000 mg/kg dose in the acute dermal toxicity test and devoid of any changes in fur, eyes, skin irritation, and behaviour in treated animals.

3.1.1. Excision wound contraction

The wounded animals were assessed for about 21 days for contraction of excision wound area (mm²) and re-epithelization time (days) (Fig. 1, Supplementary file/Fig. 3), and the percentage wound contraction was calculated (Supplementary file/Table 1).

The wound contraction was observed to be profoundly more from the 3rd day (p < 0.001) in M-BG treated group compared to the control (cow ghee), and BG treated group. On 21st day post excision wounding, the control group showed 97.32 ± 0.49, 17.67 ± 1.09% wound contraction, whereas 100% wound contractions was observed in BG treated group from the 18th day and in M-BG treated group from the 15th day, respectively. The re-epithelization time for all four groups was recorded (Supplementary file/Table 1). The ghee, PI, BG and M-BG treated groups showed complete re-epithelization on 23.67 ± 0.33, 17.67 ± 0.49, 15.17 ± 0.40 and 13.50 ± 0.22 days, respectively. The significantly (p < 0.05) higher level of hydroxyproline in the M-BG treated group (Supplementary file/Table 1) proposes the ancillary role of M-BG in collagen formation in the wound healing proliferative stage.

3.1.2. Wound breaking strength (WBS)

Breaking strength is an important factor in the wound healing process which reflects the sub-dermal organization of fibers in the newly deposited collagen [5]. The M-BG treated group shows significantly (p < 0.001) higher WBS (562.33 ± 7.37 g) on the 10th day, whereas BG treated group and PI treated group showed 531.50 ± 8.9 g and 485.33 ± 8.6 g respectively as compared to control (Supplementary file/Table 1 and Supplementary file/Fig. 4).
3.1.3. Visual examination

The visual examination of the M-BG-treated group was carried out. Wounded animals were significantly clean and found to be free from swelling and redness (inflammatory reactions) compared with the cow ghee treated group.

4. Conclusion

The present study confirms the effect of the Murcchana process on therapeutic efficacy. The improved wound healing property of M-BG may be attributed to the antioxidants, flavonoids, and phenolics present in the Murcchita herbs used in the development of formulation and preparation of ‘Murcchita ghee’.

Source of funding

None.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Shailendra Gurav and Manish Wanjari: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration.

Sandesh Wayal, Sonali Barke, Pradnya Jagtap and Ganesh Nigade: Methodology, Animal Study, Software, Validation, Investigation.

Nilambari Gurav, Muniappan Ayyanar and Satyendra prasad: Writing - Original Draft, Visualization, Formal analysis, Resources.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2022.100547.

References

[1] Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: importance, challenges and future. J Tradit Compl Med 2016;7:234–44. https://doi.org/10.1016/j.jtcm.2016.05.006.

[2] Anonymous. The ayurvedic pharmacopoeia of India. 1st ed. New Delhi: Ministry of Health and Family Welfare Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy; 2007.

[3] Tommi S, Wali A. Dietary considerations of wound healing in Ayurveda. J Nutr Food Sci 2013;3:1–4. https://doi.org/10.4172/2155-9600.1000227.

[4] Wayal SR, Gurav SS. Bhallatakadi ghrita: development and evaluation with reference to Murcchana and Shata-Dhauta process. J Ayurveda Integ Med 2020;11:261–9. https://doi.org/10.1016/j.jaim.2020.05.005.

[5] Wayal SR, Gurav SS. Evaluation of wound healing potential of Bhallatakadi Ghrita—cow ghee based polyherbal formulation: in-vivo excision and incision wound model. J Ayurveda Integ Med 2021. https://doi.org/10.1515/jaim-2020-0179.

[6] Gurav SS, Gurav NS, Patil AT; Duragkar NJ. Effect of explan source, culture media, and growth regulators on callogenesis and expression of secondary metabolites of Curcuma longa. J Herbs, Spices, Med Plants 2020;26(2):172–90. https://doi.org/10.1080/10496475.2019.1688542.

[7] Gurav N, Gurav S, Wanjari M, Prasad S, Wayal S, Karokar N. Development and evaluation of aphrodisiac potential of a classical ayurvedic formulation, Kaamdev ghrita in rat model. J Ayurveda Integ Med 2021;12(2):294–301. https://doi.org/10.1016/j.jaim.2020.09.007.

[8] Amalraj S, Mariyammal V, Murugan R, Gurav SS, Krupa J, Ayyanar M. Comparative evaluation on chemical composition, in vitro antioxidant, anti-diabetic and antibacterial activities of various solvent extracts of Dregea volubilis leaves. South Afr J Bot 2021;138:115–23.

[9] CPCSEA, CPSCSA guidelines for laboratory animal facility. Indian J Pharmacol 2003;35:257–74.

[10] OECD. OECD guideline for testing of chemicals. 2015. https://www.oecd.org/env/els/testing/2-TC_402_draft_26_Oct_2015.pdf. [Accessed 30 July 2016].

[11] Murade V, Waghmare A, Pakhare D, Dihayal S, Patil R, Wanjari M, et al. A plausible involvement of GABA/benzodiazepine receptor in the anxiolytic-like effect of ethyl acetate fraction and quercetin isolated from Ricinus communis Linn. leaves in mice. Phytomedicine 2021;11(3):100041. https://doi.org/10.1016/j.phymed.2021.100041.

[12] Gurav NS, Gurav SS, Sakhawarde SN. Studies on Ashwagandha Ghrita with reference to murchan process and storage conditions. J Ayurveda Integ Med 2020;11(3):243–9. https://doi.org/10.1016/j.jaim.2019.10.004.

[13] Kumar N, Khurana SM. Phytochemistry and medicinal potential of the Terminalia bellirica Roxb.(Bahera). Indian J Nat Prod Resour 2018;9(2):97–107.

[14] Vaniya BC, Bakrania AK, Patel SS. Emblica officinalis (Amla): a review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. Pharmacol Res 2016 Sep;111:180–200. https://doi.org/10.1016/j.phrr.2016.06.013.

[15] Nigam M, Mishra AP, Adhikari-Devkota A, Dirar AI, Hassan MM, Adhikari A, et al. Fruits of Terminalia chebula Retz.: a review on traditional uses, bioactive chemical constituents and pharmacological activities. Phytother Res 2020 Oct;34(10):2518–33. https://doi.org/10.1002/ptr.6702.