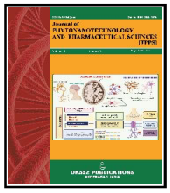
Journal of Phytonanotechnology and Pharmaceutical Sciences 4(3): 34-38, 2024

Online ISSN:2583-0376

http://jpps.ukaazpublications.com

DOI: http://dx.doi.org/10.54085/jpps.2024.4.3.5

Journal of Phytonanotechnology and Pharmaceutical Sciences



Original Article : Open Access

Wound healing and analgesic activity of herbal oil formulation in rats

Farha Khanam, Anas Islam, Badruddeen*◆, Ranjan Kumar, Juber Akhtar, Mohammad Irfan Khan, Mohammad Ahmad and Asad Ahmad

Faculty of Pharmacy, Integral University, Dasauli, Kursi Road, Lucknow-226026, Uttar Pradesh, India

Article Info

Article history

Received 2 August 2024

Revised 10 September 2024

Accepted 11 September 2024

Published Online 30 September 2024

Keywords

Herbal oil formulation

Analgesic activity

Wound healing

Natural remedies

Abstract

This study evaluated the analgesic and wound healing efficacy of herbal oil formulation (HOF) comprising menthol, eucalyptus oil, camphor, neem oil, and turpentine oil on sprague dawley rats. The analgesic activity was assessed using the hot plate method, while wound healing was evaluated through an excision wound model. Results indicated that the HOF significantly (*$p<0.05$) increased pain tolerance and accelerated wound contraction compared to the vaseline-treated (control group), while betadine (standard group) showed highly significant (**$p<0.01$) results. The study demonstrates that the HOF offers a potent alternative or complement to conventional treatments for pain relief and wound care, highlighting the potential of natural formulations in clinical settings.

1. Introduction

Wound healing is a multifaceted biological process including tissue repair and regeneration. The process involves a meticulously coordinated integration of four separate but overlapping phases: haemostasis, inflammation, proliferation, and remodelling (Chouhan *et al.,* 2019). The proliferative phase, including angiogenesis, collagen deposition, granulation tissue development, and epithelialisation, is essential for re-establishing the structural integrity of injured tissues (Häkkinen *et al.,* 2011). Pain management is an essential component of patient treatment, especially in cases of acute accidents or chronic illnesses. The main difficulty in analgesia is to efficiently mitigate pain while minimising substantial side effects. Nonsteroidal anti-inflammatory medications (NSAIDs) and opioids, fundamental components of pain therapy, often exhibit side effects that include gastrointestinal complications, addiction, and tolerance. This requires the investigation of alternative analgesic methods, especially those originating from natural sources (Bach-Rojecky *et al.,* 2019).

Herbal medications include several active constituents that may function synergistically to provide therapeutic effects. The comprehensive approach of herbal treatment is especially beneficial in addressing intricate processes such as wound healing and pain, which need the simultaneous modulation of several physiological pathways (Jahromi *et al.,* 2021). Herbal oil compositions embody a comprehensive strategy in therapeutic interventions, using the synergistic effects of many natural constituents recognised for their medicinal attributes. These oils, rich in bioactive chemicals, are meticulously chosen and combined to address several pathways associated with inflammation, pain, and tissue regeneration. These formulations not only aim to improve wound healing by facilitating cellular regeneration and minimising infection risks but also provide analgesic benefits that may relieve pain without the negative side effects often linked to synthetic pharmaceuticals (Andjiæ *et al.,* 2021). The main aim of this research is to assess the wound healing and analgesic properties of the suggested herbal oil formulation in a controlled experimental environment.The primary objective of this study was to evaluate the wound healing and analgesic activity of the proposed herbal oil formulation in a controlled experimental setting. By doing so, it aims to provide a scientific basis for the traditional use of these oils and to explore their potential as an alternative or complementary therapy for wound management and pain relief.

2. Materials and Methods

2.1 Chemicals

All chemicals used were of analytical grade and purchased from Taj Pharmaceuticals Ltd., Kemwell Biopharm Ltd., Alka Chemical Industries, G.S. Pharmbutor Pvt. Ltd., and Royal Spirits India Pvt. Ltd.

2.2 Preparation of herbal oil

The herbal oil was prepared by mixing menthol (20% v/v), neem oil (20%), eucalyptus oil (10%), camphor (10%), and turpentine oil (4%).

2.3 Animals

Rats weighing 150-200 g were obtained from CDRI Lucknow's animal house and housed at Integral University's departmental animal facility. They were acclimatized in polypropylene cages under controlled conditions of 23 ± 2°C temperature and 50-60% relative humidity with a 12 h light/dark cycle for one week before and during the experiment. The animals were fed a standard pellet diet and had

Corresponding author: Dr. Badruddeen

Professor, Faculty of Pharmacy, Integral University, Lucknow-226026, Uttar Pradesh, India

E-mail: badarmiracle@gmail.com

Tel.: +91-9918196188

ad libitum access to water throughout the study period. Ethical approval was secured from the Institutional Animal Ethical Committee (IAEC) of the Faculty of Pharmacy, Integral University, Lucknow, India.

2.4 Evaluation parameters

2.4.1 Determination of analgesic activity

Analgesic activity involves groups of five rats, regardless of sex, each weighing between 150 to 200 g. The experiment utilized a commercially available hot plate, set to a controlled temperature of 55 to 56°C, which could be either a copper plate or a heated glass surface. Animals were placed on the hot plate, and the time taken for them to lick or jump was timed with a stopwatch. Latency periods were recorded at 10 and 30 min after the topical application of either diclofenac gel (standard) or the herbal oil formulation (test compound) to all four paws. Rats treated with vaseline served as the control group (Tita *et al.,* 2001).

2.4.2 Wound healing model

Excision wounds were created by excising a full-thickness 1:2 cm² area of skin from the animal's back under ether anesthesia. The herbal oil formulation (test drug) and betadine (standard drug) were applied topically twice daily from the first day until the wound completely healed or until the 21st postoperative day. Animals treated with vaseline served as controls. Wound contraction was monitored by tracing the wound on polythene paper and then transferring these tracings to a 1mm² graph sheet to evaluate the wound surface area on days 7, 14, and 21 (Haritha *et al.,* 2012). The wound contraction percentage was calculated using the formula:

Wound contraction % = (Initial wound size-specific day wound size)/(Initial wound size) × 100

2.5 Treatment protocol

2.5.1 Treatment protocol of analgesic activity

Group I consisted of rats treated with vaseline (control), applied topically to all four paws 30 min before response measurement. Group II received the herbal oil (Test) treatment, applied in the same manner and timing as Group I. Group III was treated with diclofenac gel (standard) and also applied topically to all four paws 30 minbefore taking responses. Each Group consisted of 5 animals.

2.5.2 Treatment protocol forwound healing

Group I was treated with vaseline (control) and applied twice daily on wounds. Group II received herbal oil (test), also applied twice daily on wounds. Group III was treated with betadine (standard), and applied in the same manner and frequency as the other groups.

2.6 Statistical analysis

Statistical analysis was conducted on the experimental results, which were expressed as mean ± standard error of the mean (SEM). The data underwent one-way analysis of variance (ANOVA), and variances were further analyzed using dunnett's multiple comparison test. A p value of less than 0.05 was considered statistically significant.

3. Results

3.1 Analgesic activity

The analgesic activity of herbal oil and the standard drug was found to be 125 ± 9.57 (p<0.05) and 205.5 ± 19.60 (p<0.01), respectively, which was significantly increased when compared with the normal control group (59.25 ± 5.73).

Table 1: Analgesic activity of herbal oil formulation by hot plate method

Group (n=4)	Drug/Treatment	Analgesic activity (Sec)
I	Vaseline (control)	59.25 ± 5.73
II	Herbal oil formulation (HOF) (test)	125 ± 9.57*
III	Diclofenac gel (standard)	205.5 ± 19.60**

Values were expressed as mean ± SEM of 4 rats in each group. *p<0.05, **p<0.01, significant when compared with Group I.

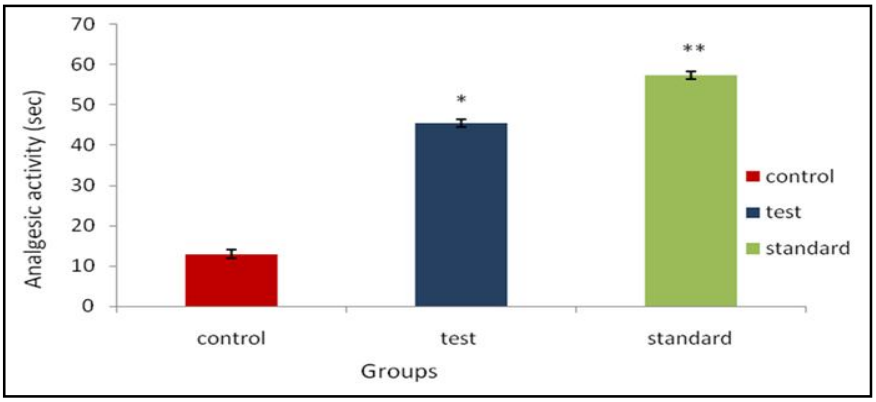


Figure 1: Analgesic activity by hot plate method.

Values were expressed as mean ± SEM of 4 rats in each group.*p<0.05, **p<0.01, significant when compared with Group I.

3.2 Determination of wound healing

The wound healing of herbal oil and standard drug on day 7th was found to be 44.37 ± 0.82 ($p<0.05$) and 54.87 ± 0.44 ($p<0.01$), respectively, which was significantly increased when compared with the normal control group (6.75 ± 0.14). On days 14th and 21st, it was found to be 58.47 ± 0.37, 76.05 ± 0.31 and 77.63 ± 0.33, 96.69 ± 0.44, respectively, which was significantly increased when compared with the normal control group (14.5 ± 0.40 and 29.57 ± 0.39, respectively).

Table 2: Percentage of wound contraction at different time intervals

Treatment (n = 4)	% Area of wound contraction		
	7th day	14th day	21th day
Vaseline	6.75 ± 0.14	14.5 ± 0.40	29.57 ± 0.39
Herbal oil	44.37 ± 0.82*	58.47 ± 0.37*	77.63 ± 0.33*
Betadine	54.87 ± 0.44**	76.05 ± 0.31**	96.69 ± 0.44**

Values were expressed as mean ± sem of 4 rats in each group. *$p<0.05$, **$p<0.01$, significant when compared with Group I.

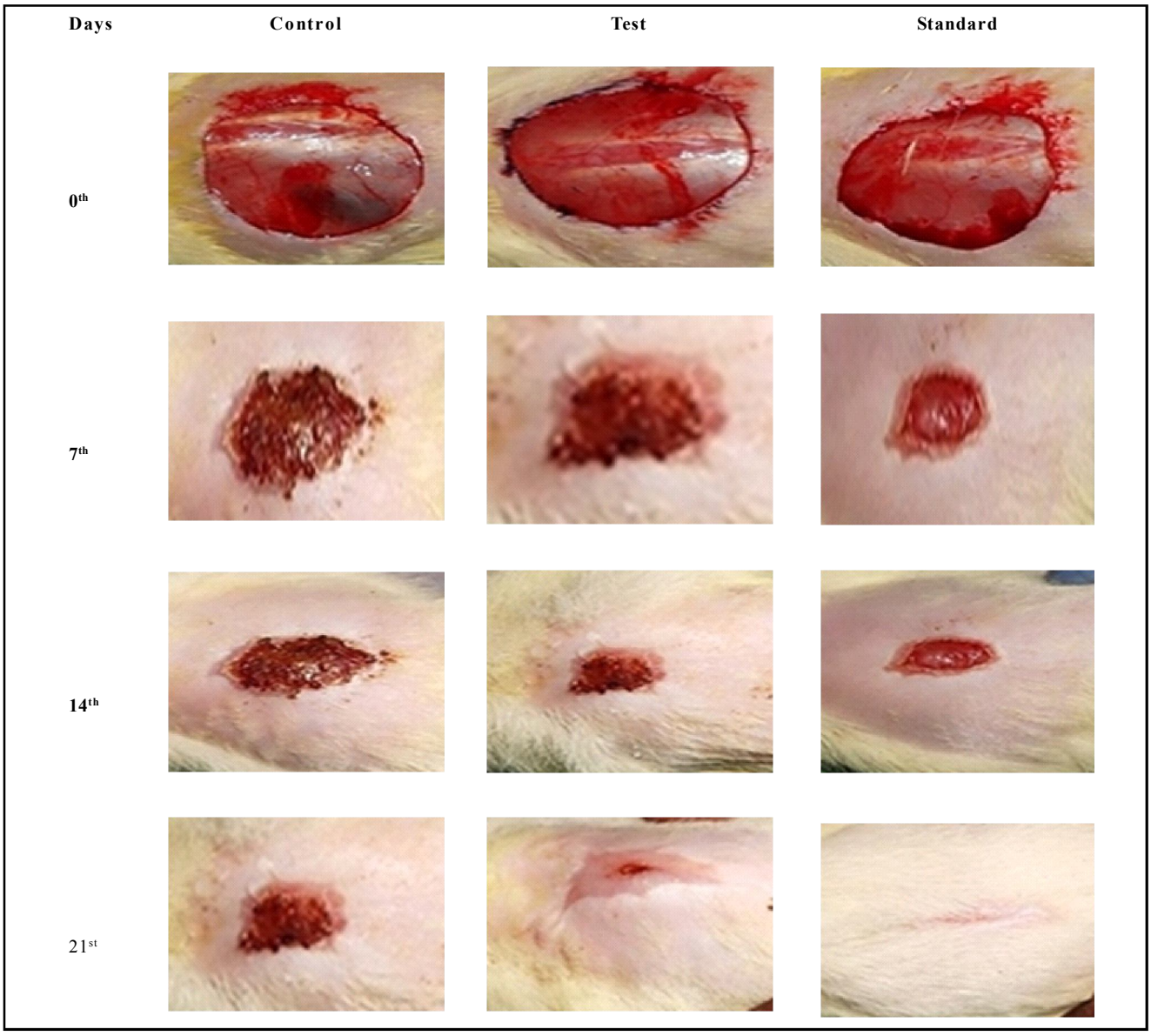


Figure 2: Wound on rat skin, Group 1= Normal control, Group 2=Treated with herbal oil formulation, Group 3= Treated with standard drug.

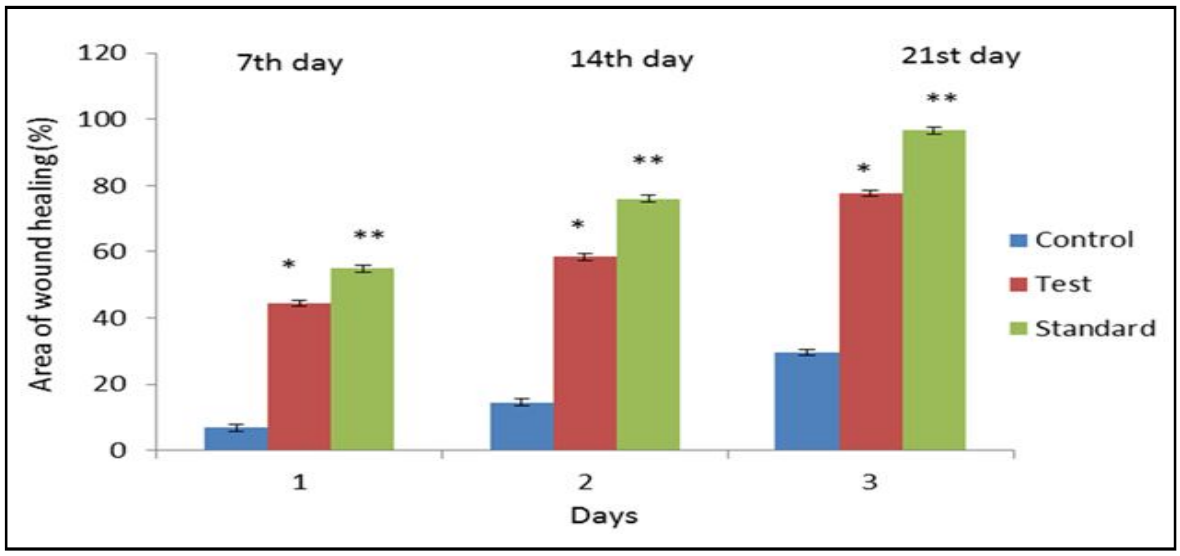


Figure 3:Percentage area of wound contraction at different time intervals.
Values were expressed as mean ± SEM of 4 rats in each group. *p<0.05, **p<0.01, significant when compared with Group I.

4. Discussion

The results of the current study reveal significant findings regarding the analgesic and wound healing efficacy of the herbal oil formulation (HOF) tested on albino rats. The HOF demonstrated a substantial improvement in pain relief and wound contraction compared to the control group, suggesting the potential utility of this formulation in clinical settings.The analgesic activity assessed by the hot plate method showed that the herbal oil formulation significantly increased pain tolerance in treated rats, as indicated by increased latency times in comparison to the control group treated with vaseline. This suggests that the herbal oil possesses potent analgesic properties, which could be attributed to the combined effects of its components like menthol, eucalyptus oil, and camphor, known for their pain-relieving properties. These findings align with previous research, which reported significant analgesic effects of eucalyptus oil, thus supporting the notion that natural products can serve as effective alternatives to traditional analgesics, which are often marred by adverse effects (Silva *et al.,* 2003).

In terms of wound healing, the HOF also showed remarkable efficacy, as demonstrated by the enhanced rate of wound contraction over 21 days. The percentage of wound contraction was significantly higher in the HOF-treated group compared to the control, with notable improvements observed as early as the 7th day post-wounding. These results may be largely due to the anti-inflammatory and antimicrobial properties of the components within the HOF, particularly neem oil, which has been documented for its effectiveness in enhancing wound healing (Banerjee *et al.,* 2021). The rapid rate of wound closure observed in this study suggests that the HOF facilitates an environment conducive to tissue repair and regeneration, likely through mechanisms involving the modulation of inflammatory cytokines and enhancement of collagen synthesis.

The comparative analysis with betadine, a standard wound care treatment, also highlighted that while betadine remains effective, the herbal oil formulation exhibits competitive wound healing capabilities, potentially offering a more natural and less irritating option for wound management. This is particularly relevant in clinical scenarios where patients may experience allergies or adverse reactions to conventional treatments.

5. Conclusion

This study demonstrated that the herbal oil formulation (HOF) possesses significant analgesic and wound healing activities, outperforming the control and showing comparable efficacy to standard treatments like betadine. The formulation, containing menthol, eucalyptus oil, camphor, turpentine oil and neem oil, effectively enhanced pain relief and accelerated wound contraction in treated rats. These findings suggest that HOF could serve as a beneficial alternative or complementary therapy in managing pain and promoting wound healing, with the potential for fewer side effects associated with synthetic drugs. Future research should focus on clinical trials to further validate the effectiveness and safety of this herbal formulation in human healthcare settings.

Acknowledgements

The authors are thankful to the Faculty of Pharmacy, Integral University for providing technical assistance to carry out the researchwork.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Farha Khanam, Anas Islam, Badruddeen, Ranjan Kumar, Juber Akhtar, Mohammad Irfan Khan, Mohammad Ahmad and Asad Ahmad (2024). Wound healing and analgesic activity of herbal oil formulation in rats. J. Phytonanotech. Pharmaceut. Sci., 4(3):34-38. http://dx.doi.org/10.54085/jpps.2024.4.3.5**