



EVALUATION OF WOUND HEALING ACTIVITY OF HEXANE AND METHANOLIC EXTRACT OF FRUIT PULP OF *MOMORDICA BALSAMINA* LINN IN ALBINO RATS

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ABSTRACT

Wound healing is an important process which is the basis of various surgical manipulations. Restoration of damaged tissue, wound or fracture is an important process which plays vital role in survival of life. *Momordica balsamina* Linn (Cucurbitaceae) is a monoecious, very slender, branched, grooved, sub glabrous tendrils. It has tremendous medicinal values such as leprosy, piles, jaundice etc., The present study was undertaken on hexane and methanolic extract of fruit pulp of *M.balsamina* on wound healing activity through oral and topical routes on excision wound model. The activity was compared with standard drug Ciprofloxacin (10 mg/kg) and Povidone iodine ointment (5% w/w) respectively. The hexane extract was found to have better wound healing effect than methanolic extract on both route of administration and the oral route of administration shows faster rate of healing when compared with topical application.

Key words: *Momordica balsamina*, Wound healing activity, Oral and topical administration.

INTRODUCTION

Wounds are inescapable events in life. Wound may arise due to physical, chemical or microbial agents. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and junction¹. Many cytotoxins, immunosuppressant and non-steroidal anti inflammatory drugs suppress wound healing. The Management of wound healing is a complicated and expensive programme². Restoration of damaged tissue, wound or fracture is an important process which plays vital role in survival of life. It is imminent for the basis of all surgical manipulations³. Many herbs have proved to possess significant pro-healing properties in different types of wounds. Using certain herbs, which possess antiseptic, astringent, anti-inflammatory, anti-microbial and bio-stimulator property can also enhance the rate of wound healing⁴. These herbs increase the rate of tissue healing by providing different essential substances, required at various steps of regeneration. These herbs being cheaper and safer than

allopathic drugs may be useful in veterinary practice, especially in India where these are found in plenty⁵.

M.balsamina is a plant commonly known as Balsam apple or Bitter melon. The whole plant powder is used for dusting over leprosy and other intractable ulcers and in healing wounds⁶⁻⁷. The leaf juice is given in bilious affections. The fruit and leaves are both administered internally in leprosy, piles, jaundice etc., Fruit is tonic, stomachic, stimulant, emetic, antibilious, laxative etc. Fruits are useful in gout, rheumatism and sub acute cases of the spleen and liver⁸⁻⁹. Based on the above light of information, the present study aimed to evaluate the wound healing activity of fruit pulp of *Momordica balsamina*.Linn belonging to the family Cucurbitaceae.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh fruits of *M.balsamina* were purchased from local market during the month of January 2009. The plant material was identified and authenticated by Prof. P. Jayaraman,

Taxonomist, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, India. A voucher specimen (PARC/2009/356) has been deposited in the herbarium for further references. The fresh plant material was dried under shade. Dried plant material was powdered using mechanical grinder and passed through sieve no.60 to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Extraction and Drug formulation

700g of the coarsely powdered, dried fruit pulp of *M.balsamina* was mixed with 4500 ml of Hexane and allowed to stand for 2 days. It was filtered and distilled under vacuum to get concentrated Hexane extract (HE). To the marc, methanol, water mixture (3:1) was added and allowed to stand for 2 days. It was filtered and distilled under vacuum to get concentrated methanolic extract (ME). The extracts were stored under desiccators for further phytochemical and pharmacological screening. A 25% w/w ointment formulation were made by incorporating the extracts HE and ME separately with simple ointment base I.P¹⁰ for external application of the drug in excision wound model.

Preliminary Phytochemical Analysis

A preliminary phytochemical screening was carried out for the extract employing the standard procedure to reveal the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, glycosides, carbohydrates, phytosterols and proteins¹¹.

Animals

Adult albino rats (wistar strain) of either sex weighing 180-200 g were

obtained from Tamil Nadu Veterinary and Animal Science University, Madhavaram, Chennai, India. They were placed in polypropylene cages with wire-net floors in a controlled room environment (25°C±2°C) at a natural day night cycle and they were provided with standard laboratory food and boiled water *ad libitum*. Approval for the study was obtained from the institutional animal ethical committee (IAEC/BPC/12)

Acute-toxicity study

The staircase method was adopted for the determination of acute toxicity. Swiss albino mice of either sex weighing 20-25 g of 70 days old were used to determine the safer dose according to Ghosh, MN et al¹².

Wound healing activity

Animals were assigned into 8 groups containing 5 animals in each group. Excision wound model was selected for assessing wound healing activity of hexane and methanolic extract of *M.balsamina* on oral and topical route of administration at 300 mg/kg body weight and 25%w/w ointment in simple ointment base respectively for 15 days. Animals were anaesthetized with ether and shaved on part to be exposed. A circular piece (500 mm² area) was impressed on the dorsal thoracic region 5cm away from ears. Except the drug under study no topical or systemic therapy was given to animals. Animals showing infection / deterioration of wounds were excluded from the study and replaced with other animals¹³. The animals were individually housed in separate cages. Wound contraction was monitored by measuring wound area with 3 days gap till 15th post operative day.

Table 1: Treatment Schedule for wound healing activity of HE and ME of *M. balsamina*

Groups	Oral route	Topical route
I (Control)	0.5%w/v Gum acacia (Vehicle)	Simple ointment base I.P
II (Standard)	Ciprofloxacin 10mg/kg for 15 days	Povidone iodine 5%w/w ointment for 15 days
III (Test-I)	Hexane extract 300 mg/kg for 15 days	Hexane extract ointment 25% w/w for 15 days
IV (Test-II)	Methanolic extract 300 mg/kg 15 days	Methanolic extract ointment 25% w/w for 15 days

Table 2: Comparative percent yield, LD₅₀ Cut off value and Presence of phyto constituents in HE & ME of *M.balsamina*

Extracts	Percentage yield (w/w)	LD ₅₀ cut off value (mg/kg of body weight)	Presence of phytoconstituents
Hexane	3.56	3000	Steroids, Glycosides and Carbohydrates
Methanol	7.89	3000	Steroids, Glycosides, Saponins, Terpenoids and Carbohydrates

RESULTS AND DISCUSSION

Preliminary Studies

The results of comparative extractive value, preliminary phytochemical screenings, and acute toxicity studies for HE and ME of *M. balsamina* were shown in the table no.2. From this data both the extracts shows that they were non-toxic upto 3000 mg/kg body weight and absence of toxicity of the extracts.

Pharmacological Screening

Wound healing involves a highly dynamic integrated series of cellular physiological and biochemical processes that occurs in living organisms¹⁴. The majority of world population relies on traditional medicine for their health care¹⁵. This is also the case in the treatment of wounds. In developing

countries, remedies prepared from herbal, plants have been widely used for the treatment, trained by traditional practioners. Many research proposed that wound healing can be improved by herbal drugs having antiseptic, anti-oxidant and anti-inflammatory properties¹⁶⁻¹⁷. In the present study, the excision wound model is considered for evaluation of wound healing activity of HE & ME of fruit pulp of *M.balsamina* on oral as well as topical application. The rate of wound contraction in mm² was taken as a measure of wound healing. During the initiation of the study from the day 0 there was not much difference in the healing of wounds in all 8 groups. But after day 9, the healing process was faster in the groups treated with test formulation.

Table 3: Effect of oral administration of HE and ME of *M.balsamina* on Excision wound model.

Post wounding days	Comparative mean wound area of different groups (in mm ²)			
	Group 1	Group 2	Group 3	Group 4
0	226.85 ± 1.86	254.34 ± 0.62	283.38 ± 2.78	283.38 ± 2.25
3	176.62 ± 1.96	176.62 ± 1.84	132.66 ± 1.63	176.60 ± 1.82
6	113.04 ± 1.20	113.04 ± 1.62	113.04 ± 5.32	153.86 ± 1.64
9	94.98 ± 1.07	63.53 ± 9.82	12.56 ± 0.88	113.04 ± 1.42
12	63.00 ± 9.46	19.62 ± 1.96	3.14 ± 8.62	50.24 ± 12.62
15	38.46 ± 7.22	0.00 ± 0.00	0.00 ± 0.00	19.62 ± 4.63

Statistical analysis was done by ANOVA and Dunnet's multiple comparison tests. Results are expressed as mean ± SE. n=5 in each group. Significant difference compared to control group at p<0.01.

From the results, it was observed that the oral route of administration of drug was more effective in healing when compared with topical route of administration. In the case of oral route when the results are compared with

Group I, III and IV shows faster wound closure and wound contraction and it was significant(P<0.01) when compared with standard drug ciprofloxacin. Similarly in the case of topical route when the results are compared with Group I, II & IV shows faster wound closure and wound contraction and the results are significant(P<0.01) when compared with standard drug Povidine iodine ointment.

Table 4: Effect of topical administration of HE and ME of *M. balsamina* on Excision wound model.

Post wounding days	Comparative mean wound area of different groups (in mm ²)			
	Group 1	Group 2	Group 3	Group 4
0	226.85 ± 1.78	226.86 ± 1.98	200.96 ± 2.03	346.18 ± 2.23
3	176.62 ± 1.96	132.66 ± 2.27	113.04 ± 1.47	226.86 ± 2.22
6	113.04 ± 1.20	63.58 ± 9.56	63.58 ± 1.24	153.86 ± 1.86
9	94.98 ± 1.07	07.06 ± 1.76	19.62 ± 0.99	78.50 ± 1.46
12	63.58 ± 9.46	0.78 ± 0.21	7.06 ± 0.64	19.62 ± 5.88
15	38.46 ± 7.22	0.00 ± 0.00	0.78 ± 0.10	3.14 ± 1.64

The present study suggests that the wound healing process can be enhanced by the use of hexane and methanolic extracts of *M.balsamina*. The experimental results indicate that the hexane extract of *M.balsamina* given through oral route of administration was found to be more effective in healing when compared to the methanolic extract.

REFERENCES

1. Harshmohan. Text Book of Pathology. 5th ed. New Delhi: JayPee Brothers Medical Publishers (P) Ltd; 2005.
2. Gupta N, Gupta SK, Shukula VK, Singh SP. An Indian community based epidemiological study of wound. Journal of Wound Care 2004; 13:323-325.
3. Majumdar M, Kamath JV. Herbal Concept on Wound Healing. Journal of Pharmaceutical Research 2005; 4 (1):01-03.
4. Jaiswal S, Singh SV, Singh B, Singh HV. Plants Used for Tissue Healing of Animals. Natural Product Radiance 2004; 3 (4):284-290.
5. Wallis TE. Text book of Pharmacognosy. 5th ed. New Delhi: CBS; 2004.
6. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 1st ed. New Delhi: National Institute of Science and Communication; 1956.
7. Nadkarni KM. Indian Materia Medica. 3rd ed. Vol. I. Popular Prakashan Book Depot; 1954.
8. Ram P, Rastogi, Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. III, Lucknow: CDRI; 1984.
9. Anonymous. The Wealth of India, Raw Materials. Vol. IV. New Delhi: Council of Scientific and Industrial Research; 1962.
10. Anonymous. Indian Pharmacopoeia. Vol. II. New Delhi: Information Directorate, Ministry of Health and Family Welfare; 1996.
11. Rangari V. Pharmacognosy and Phytochemistry. Part-I. 1st ed. Nashik: Career Publication; 2002.
12. Ghosh MN. Fundamentals of Experimental Pharmacology. 3rd ed. Kolkata: Scientific Book Agency; 2005.
13. Srinivas Reddy B, Kirankumar Reddy R, Naidu VGM, Madhusudhana K, Sachin BA, Ramakrishna S, et al. Evaluation of Antimicrobial, Antioxidant and Wound Healing Potentials of *Haloptela integrifolia*. J. Ethnopharmacol 2008; 115:249-256.
14. Mukherjee PK. Quality Control of Herbal Drugs-An approach to Evaluation of Botanicals. 1st ed. Bombay: Business Horizons Pharmaceuticals Publishers; 2002.
15. Zhang X. Traditional Medicine and WHO. Hamdard Medicus 1996; 39(3):102-103.
16. Somashekar S, Saraswati U, Laxinarayana U, Nagabushan S. Wound Healing Activity of *Ocimum sanctum*. Linn with Supportive Role of Antioxidant Enzymes. Ind. J. Physiol. Pharmacol 2006; 50(2):163-168.
17. Sunil SJ, Nitin Agrawal, Patil MB, Chimkode R, Tripathi A. Antimicrobial and Wound Healing Activities of Leaves of *Alternanthera sessilis*. Linn. International Journal of Greenpharmacy 2008; 141-144.

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