Research Article

EFFECT OF *MICHELIA CHAMPACA LINN* FLOWERS ON BURN WOUND HEALING IN WISTAR RATS

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ABSTRACT

Plants are a source of large variety of potent drugs to alleviate suffering from disease. In traditional medicine the plant *Michelia champaca (M. champaca*) is widely used in the treatment of inflammation, dysmenorrhoea, fever, ulcers, wounds and skin diseases. In this study ethanolic extract of *M. champaca* flowers were used to evaluate its burn wound healing activity in rats. Rate of wound contraction and period of epithelialisation were taken as parameters. In the burn wound model, oral and topical administration of *M. champaca* showed significant improvement in the rate of wound contraction on the 16th day as compared to control but not with standard. Oral administration and topical application of *M. champaca* significantly ($p=0.005$ and $p< 0.01$) reduced the period of epithelialisation as compared to control in the burn wound model. In the dexamethasone suppressed burn wound model, wound contraction rate was improved significantly by topical ($p<0.0001$) and oral ($p<0.006$) administration of *M.champaca*. Moreover, dexamethasone suppressed epithelialisation was significantly reversed by topical ($p<0.0001$) and oral ($p<0.001$) administration of *M. champaca*. The present study gives the evidence for burn wound healing activity of *M. champaca*.

Keywords: *Michelia champaca,* Burn wound, Dexamethasone suppressed wound healing, Wound contraction, Epithelialisation period.

INTRODUCTION

The wound healing refers to the process of replacement of destroyed tissue by living tissue and is a dynamic interactive process that begins at the time of wounding. Three different phases constitute physiologic process of wound-healing: (i) substrate phase, (ii) proliferative phase (iii) remodelling phase[1].

The plant *M. champaca* used in this study is widely used in both Ayurvedha and Siddha medicine. Root bark is used in the treatment of inflammation, constipation and dysmenorrhoea[2]. Stem bark is used in gastritis, fever and cough[2]. Flower, flower bud and fruit are useful in healing ulcers, wounds and skin diseases[2]. A survey of literature revealed that the effect of flowers of *M. champaca* on healing of burn wounds in rats has not been studied. So, it was decided to determine the activity of flowers of this plant on burn wound healing and its effect on dexamethasone suppressed healing of burn wound.

MATERIALS AND METHODS

Experimental Animals

Healthy, male albino Wistar rats weighing 250-300 g were used. Rats were housed under controlled conditions of temperature 23±2^{o}C, humidity 50±5% and 10-14 hours light and dark cycle respectively[3]. The animals were housed individually in polypropylene cages containing sterile paddy husk as bedding after making burn wound till completion of wound healing. Animals were maintained on normal diet (Amrut lab animal feed, Pranav agro industries Ltd., Sangli, Maharashtra, India) and water *ad libitum*. The study was undertaken after obtaining approval of Institutional Animal Ethics Committee, Manipal.

Preparation of ethanolic extract of flowers of *M. champaca*

The flowers were shade dried and powdered. The powder was loaded in Soxhlet extractor in 8 batches of 200 g each and was subjected to extraction for 30-40 hours with 95% ethanol[4]. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50^{o}C to a syrupy consistency. Then it was dried in a dessicator. The yield was around 4%.

Acute toxicity study

Acute toxicity study was done in rats weighing between 150-200 g. Rats were fasted overnight. They were divided into 5 groups of two animals each. The ethanolic extract of *M. champaca was* administered orally through the feeding tube to the pair of rats of each group in ascending and widely spaced doses viz. 10, 30, 100, 300, 1000 mg/kg. The animals were observed continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality was recorded. No signs of toxicity were observed even with 1000 mg/kg of *M. champaca*. So the dose of the extract chosen for the study was 100 mg/kg which is corresponding to the 1/10th of the maximum tolerated dose (1000 mg/kg)[5].

Drugs and their administration

Ketamine injection was obtained from Neon Laboratories Limited (Mumbai, India), Silver sulphadiazine was obtained from Kasturba Hospital Pharmacy (Manipal, India.), Dexamethasone was obtained from Zydus Alidac

(Ahmedabad, India) and 2% gum acacia was obtained from Nice Chemicals Ltd (Cochin, Kerala, India). The flowers of *M. champaca* were purchased from local market and authenticated by the Professor of Botany, Mahatma Gandhi Memorial College, Udupi. Voucher specimen was kept in the Department of Pharmacology, Kasturba medical college, Manipal.

For oral administration, a suspension of ethanol extract (8%) was prepared using 2% gum acacia. For topical application, ointment of ethanol extract (10%) was prepared using simple ointment base. The drugs were administered once a day from day 1 and continued till the day of falling of eschar in both models[6].

Study Design

Burn wound model and dexamethasone suppressed burn wound model were used to assess the wound healing property of *M. champaca* in rats. Four groups of animals were used for burn model and three groups of animals were used for dexamethasone suppressed burn model. There were six animals in each group. The drug treatment was as follows:

For Burn Wound Model

Group I: Control - 2% Gum acacia (2 ml/ oral).

Group II: Standard- Silver sulphadiazine (0.5 g of 1% cream topical)[7].

Group III: Test-I - *M. champaca* extract (100 mg/kg.BW/oral).

Group IV: Test-II - *M. champaca* extract (10%/ topical).

For dexamethasone suppressed burn wound model:

Group V: Control- Dexamethasone (0.17 mg/kg, i.p.)[8].

Group VI: Test-III- Dexamethasone (0.17 mg/kg, i.p.) and *M. champaca* extract (100mg/kg-BW/oral).

Group VII: Test-IV- Dexamethasone (0.17mg/kg, i.p.) and *M. champaca* extract (10%/ topical).

Wound models

Burn wound model

Partial thickness burn wounds were made on overnight-fasted animals under ketamine (50 mg/kg, i.m.) anesthesia by pouring hot molten wax (2 g) at 80°C[4]. The wax was poured on the shaven back of the animal through a cylinder of 300 mm^9 circular opening. The wax was allowed to remain on the skin for 8 minutes by that time it got solidified. This was day 0.

Dexamethasone suppressed burn model

Dexamethasone was administered from day 0 (0.17mg/kg, i.p.) and was continued on subsequent days till the day of eschar falling[10].

Evaluation of burn wound healing activity of *M. champaca*

Wound contraction rate

Wound area was measured by tracing the wound size on a transparent butter paper on every alternate day of post-wounding. The tracing was then transferred to $1mm^2$ graph sheets, from which the wound area was evaluated[11]. The evaluated surface was then employed to calculate the percentage of wound contraction, taking the initial size of wound 300 mm^2 as 100% by using the following equation:

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size - Specific day wound size}}{\text{Initial wound size.}} \times 100$$

Period of epithelialisation

Falling of the eschar leaving no raw wound behind was taken as end point of complete epithelialisation and the days required for this was taken as period of epithelialisation[12].

Statistical Analysis

Results were expressed as mean ± SEM. The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Post hoc Test viz Tukey's test and significance was set at $p<0.05$[13].

RESULTS AND DISCUSSION

Wound contraction rate

In the burn wound model, oral and topical administration of *M. champaca* showed significant ($p<0.05$) increase in wound contraction rate only on 16th day as compared to control (Table 1). But there was no significant difference in rate of wound contraction between plant extract and standard.

In the dexamethasone suppressed burn wound model, wound contraction rate was increased significantly by topical application of extract in all the days; i.e day 4 ($p < 0.05$), day 8 ($p < 0.0001$), day 12 ($p < 0.0001$) and day 16 ($p < 0.0001$) as compared to dexamethasone control group, whereas oral dosage showed significant reduction in the rate of wound contraction only on 12th day. Topical wound contraction than oral extract on 8th day (Table 2).

Period of Epithelialisation

In burn wound model, oral ($p =0.005$) and topical ($p< 0.01$) administration of *Michelia champaca* significantly reduced the period of epithelialisation as compared to control (Table 3).

In the dexamethasone suppressed burn wound model, only topical ($p < 0.05$) administration of *M. champaca* significantly reversed dexamethasone depressed epithelialisation period (Table 4).

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. The processes of wound healing consist of vascular response, inflammation, proliferation and maturation; a short period of vasoconstriction occurs owing to the release of chemical mediators such as serotonin, ATP etc. Inflammation occurs due to release of PGs[14]. During the

proliferative stage, the wound is filled with new connective tissue, which is dependent on the formation of new blood vessels in the wound. Fibroblast is responsible for synthesis of collagen and other connective tissues are required for the repair process[15]. The final phase of the maturation begins when the wound has been closed by connective tissue and epithelialization. The wound scab, essential to and part of the natural healing process sloughs off as the epidermis is restored to its natural thickness. Wounds occurring as a result of burn injury are a major cause of morbidity and mortality.

Table 1: Effect of *M. champaca* on the rate of wound contraction in the burn wound model

Group (n=6)	Drug/Dose	Wound contraction (%)(Mean ±SEM)			
		Day 4	Day 8	Day 12	Day 16
I Control	Gum acacia- (2%, oral)	38 ± 10.37	58.78 ± 6.83	77.94 ± 7.39	85.80 ± 6.43
II Test-1	Extract-(100mg/kg, oral)	31.14± 6.21	64.19 ± 15.94	89.58 ± 7.87	95.82 ± 4.93[a]
III Test-2	Extract- (10%, topical)	42.39 ± 10.23	57.25 ± 11.76	76.54 ± 10.30	97.07 ± 2.74[a]
IV Standard	Silver sulphadiazine (0.5 g of 1%, topical)	46.37 ± 12.23	77.77 ± 3.77	88.76 ± 6.22	95.75 ± 1.94[a]

[a] p <0.05 Vs Control

Table 2: Effect of *M. champaca* on the rate of wound contraction in the dexamethasone suppressed burn wound model

Group	Drug/Dose	Wound contraction (%) (Mean±SEM)			
		Day 4	Day 8	Day 12	Day 16
V Control	Dexa (0.17mg/kg, i.p.)	26.75 ± 16	45.50 ± 9.76	64.83 ± 10.17	79.29 ± 8.88
VI Test-3	Dexa (0.17mg/kg, i.p.)+ Extract(100mg/kg, oral)	32.36 ± 16.32	59.06 ± 12.61	81.77 ± 8.34[a]	88.24 ± 5.96
VII Test-4	Dexa (0.17mg/kg, i.p.)+ Extract (10%, topical)	51.15 ± 11.50[a]	80.14 ± 5.03[b,c]	91.49 ± 2.37[b]	95.40 ± 2.49[b]

Dexa=Dexamethasone; [a] p < 0.05 Vs Dexa, [b]p < 0.0001 Vs Dexa , [c]p < 0.05 Vs Dexa + oral.

Table 3: Effect of *M. champaca on* period of epithelialisation in the burn wound model

Groups (n=6)	Drug/Dose	Epithelialisation period (Mean±SEM)
I Control	Gum acacia (2%, oral)	25.33 ±3.67
II Test-1	Extract (100 mg/kg, oral)	18.00 ± 3.03[a]
III Test-2	Extract (10%, topical)	18.33 ± 2.42[b]
IV Standard	Silver sulphadiazine (0.5 g of 1%, topical)	21.67 ± 3.01

[a] p < 0.005 Vs Control, [b] p < 0.01 Vs Control.

Table 4: Effect of *M. champaca* on period of epithelialisation in the dexamethasone suppressed burn wound model

Group	Drug/Dose	Epithelialisation period (Mean±SEM)
V Control	Dexa (0.17mg/kg, i.p.)	27.00 ± 2.19
VI Test-3	Dexa(0.17mg/kg, i.p.) + Extract (100mg/kg, oral)	22.67 ± 3.88
VII Test-4	Dexa (0.17mg/kg, i.p.) + Extract (10%, topical)	21 ± 3.58[a]

Dexa= Dexamethasone; [a]p< 0.05 Vs Dexa

Only topical application of ethanolic extract of *M. champaca* has shown significant wound contraction in the late period of wound healing process. Both oral and topical administration has shown significant reduction in epithelialisation period in the burn wound model.

Dexamethasone delays burn wound healing by reducing transforming Growth Factor-β (TGF-β), collagen & fibroblast deposition, neovascularisation and epithelial migration in wounds[16]. The *M. champaca* used to treat healing ulcers, wounds and skin diseases[17, 18, 19]. Since the topical application of ethanolic extract of *M. champaca* flowers significantly reverses the steroid suppressed wound healing process, it could be made use of clinically in patients as a supportive therapy to treat chronic wounds in catabolic conditions.

Having considering the above data, it could be possible that topical application of ethanolic extract of *M. champaca* can be useful for the treatment of burn wounds in immunocompromised patients. However, further well designed clinical evaluation is required to support this suggestion.

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