Michelia Champaca: Wound Healing Activity In Immunosuppressed Rats

S Dwajani, T Shanbhag

Citation

Abstract

Objectives: To evaluate wound healing profile of alcoholic extract of Michelia champaca in different wound models in rats and to study its effect on Dexamethasone suppressed wound healing.Materials and methods: For assessment of wound healing activity, excision, incision and dead space wound models were used. Group I was assigned as control, Group II received alcoholic extract of M. champaca orally. Group III received Dexamethasone intra muscularly (i.m) and Group IV was given Dexamethasone i.m and alcoholic extract of M.champaca orally. Parameters observed were breaking strength of incision wound; epithelization and wound contraction in excision model and breaking strength, dry weight and hydroxyproline content in dead space wound model.Results: In incision model, it was noted that on co-administration of Dexamethasone and M.champaca the breaking strength was significantly increased. In dead space wound model, the hydroxyproline content was significantly increased in Dexamethasone + M.champaca treated group.Conclusion: It is concluded that M.champaca is an effective agent for healing of wounds in immunocompromised patients.

INTRODUCTION

A wound is a disruption in the normal anatomical structure and function of living tissue that can be caused by physical, chemical, microbiological or immunological injury. Wound healing is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and function. When healing takes place in a direction away from its normal course, it may result in non-healing, under-healing or over healing. Therefore attempts have been made to accelerate wound healing either when it is progressing normally[1] or when it is suppressed by corticosteroids[2], anti-neoplastic[3] and non-steroidal anti-inflammatory agents[4]. Treatment is aimed at either shortening the time required for healing or minimizing the undesired consequences.

A large section of world population relies on traditional remedies to treat a plethora of diseases. Medicinal herbs are an indispensable part of traditional medicine practiced all over the world due to low cost, easy access and ancestral experience. The greatest contribution of plant kingdom to mankind is that it has provided a large variety of potent drugs to alleviate suffering from disease. The plant Michelia champaca (Sampige), used in this study is widely used in both Ayurveda and Homeopathic medicine. It belongs to the family Magnoliaceae. Root and bark are purgative,

emmenagogue and are useful in the treatment of inflammation, constipation and dysmennorhea. The stem bark is astringent, febrifuge used in gastritis, fever and cough. Flower and flower buds, fruits are useful in ulcers, skin disease wounds[6]. A survey of literature revealed that the wound healing activity of Michelia champaca, has not been evaluated. So, it was decided to determine the wound healing activity of this plant and to study its effects on Dexamethasone suppressed wound healing in various animal models in Wistar rats.

MATERIALS AND METHODS

Ethical clearance: Institutional Animal Ethics Committee cleared all the protocol of the study.

ANIMALS

Twelve-week-old healthy Wistar rats weighing 150-200g of either sex, bred locally in the animal house of Kasturba Medical College, Manipal, were selected for the study. They were housed under controlled conditions of temperature (23 ± 2oc), humidity ($50\pm5\%$) and 10-14 hours of light and dark cycles. The animals were housed individually in polypropylene cages containing sterile paddy husk bedding and free access to food and water ad libitum .

STUDY DESIGN

The animals were randomly allocated into four groups of six animals each.

Group I received 2ml gum acacia.

Group II received alcoholic extract of M.champaca orally

Group III received Dexamethasone intramuscularly

Group IV received Dexamethasone + alcoholic extract of M.champaca

DOSING SCHEDULE

Gum acacia and alcoholic extract of M.champaca were administered orally daily from day 0 to the day of complete healing or the 10th post operative day, whichever occurred earlier, in the wound healing model. Dexamethasone was given i.m on alternative days (from day 0 to the day of complete healing or 10th postoperative day)

WOUND MODEL

For assessment of wound healing activity, excision, incision and dead space wound model were used. All wounding procedures were carried out under ketamine anaesthesia i.m. In the present study no animals showed visible signs of infection.

Incision wound model: Two long paravertebral straight incisions of 6cm each were made 1cm lateral to the vertebral column on the either side of the depilated back of the animal, cutting through the entire thickness of the skin[11]. The wounds were closed with the sutures 1cm apart with No.4 black silk thread and straight needle[12]. The sutures were removed on the 7th post wounding day and breaking strength was measured on the 10th day by continuous constant water flow technique of Lee[4].

Excision wound model: An excision wound was made by cutting away a circular area of full thickness of skin measuring 500mm2 on the depilated back of the rat, in the dorsal interscapular region, 5cm away from the ears[13]. Period of epithelization was noted as the number of days after wound healing required for the eschar to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of wound area on alternate days. This was done by tracing the wound area on a graph paper. Reduction in the wound size was expressed as percentage of original wound size[14].

Dead space wound model: Dead space wounds were created by implanting 2.5x0.5cm polypropylene tubes subcutaneously in the lumbar region, beneath the dorsal paravertebral lumbar skin, through a small transverse incision[15]. Granulation tissue formed on the tube was harvested by careful dissection on the 10th post wounding day and breaking strength of granulation tissue was measured. The granulation tissue was dried in an oven at 60oC for 24 hours and the dry weight was noted. Acid hydrolysate of the dry tissue was used for determination of hydroxyproline content by the method of Neuman and Logan[16].

STATISTICAL ANALYSIS

The results were analyzed by One way Analysis of Variance (ANOVA) followed by Scheffe's test, as applicable,using SPSS computer package version 10.

RESULTS

Incision wound model: The mean breaking strength in the Group 1 was 204.58±8.04g and it was increased to 236.66±16.10g in the Group 2. In Group 3, the breaking strength was significantly reduced to 164.58±9.16g as compared to Group 1, and significantly ($p < 0.028$) increased in Group 4 (217.08±9.1g) compared to Group1 and Group 3 [Table 1].

Excision wound model: The percentage of wound contraction in the group 1 was 25.46±5.3, 51.16±0.58, 92.20±1.24 and 98.06±0.39 as measured on the 4th, 8th, 12th 16th day respectively. The wound contraction rate was not significantly altered in groups 2 & 4 as compared to group 1. The mean period of epithelization in the group 1 was 21.00±0.68 days. The rate of wound contraction was not significantly altered in animals treated with both Dexamethasone and M. champaca [Table 2].

Dead space wound model: The mean breaking strength of the granulation tissue in the group 1 was 230.83±21.11g. An increase in the breaking strength of granulation tissue was observed in the M.champaca treated group. The breaking strength of Dexamethasone treated group was significantly reduced to 155±6.05g as compared to group 1.

There was increase in breaking strength of granulation tissue in group 4 when compared to group 3 (table-3)

The mean dry weight of granulation tissue in the group 1 was 0.060±0.012mg. This was increased to 0.065±0.061mg in the group 2. The mean dry weight of granulation tissue in group 3 was 0.070±0.015mg (statistically significant) (table

3)

The mean hydroxyproline content of granulation tissue of the group 1 was 926.6±239.6mg/g of the tissue. It was significantly altered in group 4 (p<0.000) [Table 3]

Figure 1

Table 1 :Effect of drugs on Incision wound parameters

Drugs	Dose and Route	Breaking strength(g) Mean±SEM
Gum acacia	2ml oral	204.58±8.04
Michelia champaca	250mg/kg oral	236.66±16.10
Dexamethasone	0.17mg/kg i.m	164.58±9.16
Dexamethasone + *M.champaca*	0.17mg/kg i.m + 250mg/kg oral	217.08±9.1ᵃ
One way ANOVA (df=3)	F=7.528 P<0.001	------------

Number of animals in each group = 6

a → p<0.028 compared to Dexamethasone group.

Figure 2

Table 2:Effect of drugs on Excision wound parameters

Drugs	Dose and route	Period of wound Contraction (days) Mean±SEM				Period of Epithelization (days) Mean±SEM
		4	8	12	16	
Gum acacia	2ml oral	25.46±5.39	51.16±0.58	92.20±1.24	98.06±0.39	21.00±0.68
Michelia champaca	250mg/kg oral	27.66±3.34	51.8±5.09	87.66±2.35	97.56±0.24	20.66±0.42
Dexamethasone	0.17mg/kg i.m	19.56±7.68	40.13±5.92	71.48±8.10	86.78±5.72	26.33±1.20
Dexamethasone + *M.champaca*	0.17mg/kg i.m +250mg/kg oral	17.06±4.65	33.06±3.76	76.6±5.24	89.3±3.14	23.66±1.08
One way ANOVA (df=3)	F	0.811	4.347	3.672	3.073	8.560
	p	<0.503	< 0.16	<0.30	< 0.051	<0.001

Number of animals in each group = 6

Figure 3

Table 3: Effect of Drugs on dead space wound parameters

Drugs	Dose and route	Breaking strength of granulation tissue(g) Mean±SEM	Hydroxyproline content of granulation tissue(mg/g) Mean±SEM	Dry weight of granulation tissue(mg) Mean±SEM
Gum acacia	2ml oral	230.83±21.1	926.6±239.6	0.060±0.012
Michelia champaca	250mg/kg oral	240±10.80	915.94±112.25	0.065±0.061
Dexamethasone	0.17mg/kg i.m	155±6.05	677.18±200.06	0.070±0.015
Dexamethasone + *M.champaca*	0.17mg/kg i.m +250mg/kg oral	177.5±9.89	2428.9±280.50ᵃ	0.086±0.012
One way ANOVA	F	9.717	13.659	0.842
	p	<0.000	<0.000	<0.487

Number of animals in each group = 6

a = p<0.000 compared to Dexamethasone

DISCUSSION

In the present study, the breaking strength of the incision wound was not significantly increased in the M.champaca treated group as compared to control. In the dead space wound model also, there was no marked increase in breaking strength of granulation tissue in the test group. The hydroxyproline content and dry weight of granulation tissue were however, not altered significantly. Thus it could be that M.champaca might not have increased the collagen content, but probably altered the maturation process, by affecting cross linking of collagen.

Dexamethasone inhibits wound contraction, granulation tissue and collagen formation[2]. This is the cause of suppressed wound healing in the Dexamethasone treated group in all wound models. The effect of Dexamethasone was reversed by M.champaca in incision and dead space wound models, where the breaking strength of Dexamethasone + M.champaca treated was not significantly higher than that of the group treated with Dexamethasone alone. Hydroxyproline content of Dexamethasone + M.champaca was significantly higher than group treated with Dexamethasone alone.

Oxidative stress has been implicated in a variety of degenerative processes and diseases including wound healing[17]. This could contribute to its pro-healing effects. M.champaca also reversed the suppression of wound healing by Dexamethasone.

The enhanced wound contraction effect and epithelization by M.champaca could possibly be made use clinically for healing wounds in immunocompromised patients and in patients on long term steroid therapy.

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Author Information

S. Dwajani

1. Department of Pharmacology, S.S.Institute of Medical Sciences and Research Centre, Davangere, India

Tara V. Shanbhag

1. Additional Professor, Department of Pharmacology, KMC, Manipal, India