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Original Article

Wound healing evaluation of chloroform and methanolic extracts of *Mimosa Pudica* roots in rats

Jejo Paul[a], Saifulla Khan[b], Syed Mohammed Basheeruddin Asdaq[*a]

[a]*Department of Pharmacology;* [b]*Department of Pharmacognosy, Krupanidhi College of Pharmacy, Bangalore-560 035, India.*

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ABSTRACT

The roots of *mimosa pudica* (MP) are employed as folklore remedy for various types of wounds. The present research was carried out to evaluate wound-healing activity of chloroform (CEMP) and methanolic (MEMP) extracts of *mimosa pudica* roots in experimental models of animals. The wound healing evaluation was done using excision, incision, burn and dead space rat wound models. Aloe vera was used as standard wound healing agent. A formulation of CEMP and MEMP was prepared in carbopol and simple ointment respectively at 2.5% & 5% concentrations and applied topically to the wounds. The observations from excision and burn wound models demonstrated that both CEMP and MEMP at high as well as low doses possess significant reduction in wound contraction and period of epithelization compared to control. In the incision wound model, a significant rise in breaking strength was observed in all treated group animals. Low and high doses of CEMP (100 and 500 mg/kg, p.o.) and MEMP (100 and 500 mg/kg, p.o.) produced a significant increase in the breaking strength, dry weight and hydroxyproline content of the granulation tissue in dead space wound model. Conclusion: The results suggest that both CEMP and MEMP applied topically or administered orally possess wound-healing activity.

1. Introduction

Healing of wounds has been one of thirst area of research as the present day management is not able to reverse the normal state rapidly. Herbal therapies/remedies are established as agents hastening the healing processes of variety of wounds. Large numbers of scientific reports are available confirming the efficacies of plant products for wound healing activity in various experimental models and patients; however, potency of many herbal agents remains unexplored. In some instances, active principles of herbs are isolated and recognized for wound healing properties [1]. Therefore it is necessary to promote the scientific evaluation of agents which can facilitate the healing process.

The *plant mimosa pudica* (Lajalu, sensitive plant, touch-me-not, family: fabaceae) is grown throughout the globe. The decoction of mimosa pudica leaves found to have anticonvulsant [2] and anti-bacterial activities [3]. The root extract of *mimosa pudica* reported to possess antifertility effect [4]. Further, roots of *mimosa pudica* produced an antidepressant-like profile similar to clomipramine and desipramine [5]. Furthermore, the hyaluronidase and protease activities of Indian snakes venom are dose dependently inhibited by aqueous root extract of *mimosa pudica* [6]. The extract of *mimosa pudica* is also reported to possess the antioxidant property [7]. Traditionally, roots of Lajalu (mimosa pudica) are considered as one of remedy for treatment of wounds especially piles [8]. It is believed to arrests bleeding and fastens the wound healing process. However, there is no scientific confirmation of ethnic claim of wound healing activity using modern experimental models in animals. The current research was designed to determine wound healing activity of chloroform and methanolic extracts of *mimosa pudica* (MP) roots on experimental models of wounds in rats.

2. Materials and Methods

2.1.Experimental animals

The experiments were performed in healthy Sprague dawley rats (either sex) weighing 250-300 gm. The experimental protocol was approved by institutional animal ethics committee and committee for the purpose of control and supervision of animals (CPCSEA) recognized the animal house in which experimental animals were kept. Chemicals- Ketamine injection was procured from Prem Pharmaceuticals Pvt. Ltd. (Indore, India) and xylazine was from Indian Immunological Ltd. (Guntur, India), hydroxyproline and paradimethylamino benzaldehyde were procured from SD Fine Chemicals Pvt. Ltd. (Mumbai, India),

* Corresponding Author : Syed Mohammed Basheeruddin Asdaq,
Asst. Professor, Department of Pharmacology,
Krupanidhi College of Pharmacy, Chikka Bellandur, Carmelaram post
Bangalore-560 035. INDIA. Phone: +91-80-25535751Fax: +91-80-51309161
E-mail: basheer_1@rediffmail.com ; sasdaq@gmail.com

sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and copper sulphate ($CuSO_4$) were purchased from Nice Chemicals Pvt. Ltd. (Mumbai, India), hydrochloric acid (HCl) was procured from Ranbaxy Chemicals Pvt. Ltd. (Mumbai, India). Carbopol 940 was taken from Loba Chemie (Mumbai, India).

2.2. Extraction of mimosa pudica root

The roots of *mimosa pudica* was collected from Carmelarm, Bangalore and Regional Research Institute (RRI-Ay), Bangalore authenticated the roots. A specimen (RRCBI-4958, receipt no: 355) has been kept for future reference. After washing, roots were shadow dried for 15 days. The dried roots were powdered in root cutter to get coarse powder. The powdered roots were extracted for 30 hours, using soxhlet apparatus by using methanol and chloroform as solvents to get MEMP and CEMP respectively. The two different extracts were collected and vacuum dried followed by desiccation to get the constant weight.

2.3.Phytochemical estimations of the extract

Both methanolic and chloroform extracts of *mimosa pudica* were subjected to qualitative analysis for the various phytoconstituents like phytosterols, carbohydrates, saponins, flavonoids, glycosides, tannins, amino acids, proteins and alkaloids.

2.4.Selection of dose and gel base

The oral dose of MEMP and CEMP for dead space wound was selected based on acute oral toxicity studies. The OPPTS guidelines (Office of Prevention, Pesticide and Toxic Substance) were followed for performing acute oral toxicity study with the limit test procedure (www.epa.gov/opptsfrs). To the overnight fasted mice, test dose of 2 and 5 g/kg were given orally. Both high and low doses did not caused any mortality with methanolic and chloroform extracts. Hence, 1/10th and 1/50th of the 5 g/kg corresponding to 500 and 100 mg/kg orally were taken in our study as high and low doses respectively. The tween 80 (2%) and acacia (5%) were used as suspending/emulsifying agents for preparing CEMP and MEMP suspension respectively. *Aloe vera* extract (300 mg/kg, po) [9] in the form of suspension (acacia 5%) was used as standard drug. The traditional healer's knowledge was considered while selecting the dose of CEMP and MEMP for topical application in excision, incision and burn wound models. The formulation of chloroform extract was made as 2.5 % (w/w) and 5 % (w/w) in hydrophilic base like carbopol (1%) containing methyl paraben (0.01%) and propyl paraben (0.1%). The formulation of methanolic extract was prepared as 2.5% (w/w) and 5 % (w/w) in hydrophobic base like simple ointment. The 2.5% (w/w) of MP was considered as a low dose and 5 % (w/w) of MP was taken as a high dose for topical application. Since, modern medicine uses mainly antibacterial agents for treatment of wound, an herbal drug, Aloe vera (5%) was used as standard for topical application models [10]. The methanolic extract of aloe contains all the chemical constituents responsible for wound healing activity.

2.5.Excision wound [11,12]

The ketamine (100 mg/kg, im) and xylazine (16 mg/kg, im) was used to anesthetize animals. About 1 cm away from vertebral column and 5 cm away from ear, an impression was made on the dorsal thoracic region on the anaesthetized rat. One day before the experiment, particular skin area was shaved. About 500 mm2 of wound area was obtained by excising the impressed area. Cotton swab (normal saline soaked) was blotted on the wound to achieve haemostasis. The animals were then grouped and different formulations were applied to cover the entire wounded area as follows: Group I, Control; Group II, Simple ointment; Group III, Carbopol (1%) gel; Group IV, Aloe vera (5%) gel formulation; Group V, MEMP (2.5%); Group VI, MEMP (5 %); Group VII, CEMP (2.5%) and Group VIII, CEMP (5%). The wounds were traced on millimeter scale graph paper on every alternate day upto 22 days from the day on induction of wound. The wound contraction-50% (days) was evaluated by plotting the wound area Vs days on a graph paper. Complete epithelization was achieved by falling of scab with no raw wound left behind.

2.6.Incision wound [13-15]

On both sides of the vertebral column, 6 cm length of para vertebral straight incision were made through the entire thickness of the skin. After stopping the blood flow completely, interrupted sutures were made at distance of 1 cm. Drug treatment was done daily as explained in excision model upto 9th day from the day of wounding. Breaking strength of wound was estimated on 10th day by continuous, constant water flow technique.

2.7.Burn wound [16]

Under ketamine (100 mg/kg, im) and xylazine (16 mg/kg, im) anesthesia, overnight-fasted animals were subjected to partial thickness burn wounds by pouring hot molten wax (2 g) at 80 0C. Through a cylinder of 300 mm2 circular opening, wax was added and left on the shaven back of the animal till it gets solidified. Immediately after the injury and on subsequent days, the drugs or base was applied topically as mentioned above.

2.8.*Dead space wound model* [17]

This type of wound was created by implanting subcutaneously a 2.5x0.5 cm polypropylene tube in the lumber region in anesthetized rats. Animals received one of the following treatments from 0th day to 9th post wounding day. Group I, 5% acacia solution (control); Group II, *Aloe vera* extract (300 mg/kg, po); Group III, MEMP (100 mg/kg, po); Group IV, MEMP (500 mg/kg, po); Group V, CEMP (100 mg/kg, po) and Group VI, CEMP (500 mg/kg, po). On the 10th day from wounding, the animals were sacrificed and the harvested granulation tissue on the implanted tube was dissected out along with the tube carefully. A sheet of granulation tissue was obtained by cutting lengthwise the tubular granulation tissue as explained in incision wound model and breaking strengths were measured. To obtain a constant weight, collected granulation tissue were dried at 60 $^{\circ}$C for 24 hr before subjecting for hydroxyproline estiamtion [18].

2.9.Statistical analysis

Results are given as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by Bonferroni's test did the comparisons between different groups. $P<0.05$ were considered statistically significant.

3. Results

Preliminary phytochemical investigation- The chloroform extract of *mimosa pudica* showed the presence of glycoside, tannins, saponins, flavanoids and alkaloids, whereas, methanolic extract of *mimosa pudica* possessed glycoside, flavanoids, tannins and alkaloids.

Excision and incision wound (Table 1) - All the prepared formulations; MEMP (2.5%), MEMP (5%), CEMP (2.5%) and CEMP (5%) demonstrated significant fall in period of epithelization compared to control ($P<0.001$). Aloe vera extract treatment also produced significant decrease in epithelization

period (P<0.001). Topical applications also resulted in significant reduction in wound contraction (50%) when compared to control (P<0.001). The decline in period of epithelization and wound contraction with MEMP and CEMP formulations at both high and low doses were found to be significant compared to their respective emulsifying bases. High doses of MEMP and CEMP were found to be more effective than their lower doses.

Table 1. Effect of methanolic (MEMP) and chloroform (CEMP) extracts of mimosa pudica on the period of epithelization and wound contraction 50% in excision wound model and breaking strength in incision wound model

Groups	Treatment	Excision wound		Incision wound
		Epithelization period (days)	Wound Contraction -50% (days)	Breaking strength(ml)
Group I	Control	21.00 ± 0.44	10.06±0.24	308.00± 10.30
Group II	Simple ointment	18.66±0.42*	8.03±0.30***	387.66±9.06**
Group III	carbapol	17.00±0.44***	7.33±0.30***	362.83±19.08ns
Group IV	*Aloe vera* (5%)	14.66±0.42***	5.56±0.15***	494.33±8.81***
Group V	MEMP (2.5%)	15.33±0.33***++	5.02±0.35***+++	505.33±12.22***+++
Group VI	MEMP (5%)	14.66±0.42***+++	4.73±0.40***+++	521.66±13.69***+++
Group VII	CEMP (2.5%)	13.33±0.42***++	4.700.16***+++	528.00±14.22***+++
Group VIII	CEMP (5%)	12.83±0.54***+++	3.63±0.12***+++	545.16±12.30***+++

All values are mean ± SEM, n=6, ***P<0.001 Vs control, +++P<0.001 Vs respective base

Table 2. Effect of methanolic (MEMP) and chloroform (CEMP) extracts of mimosa pudica on the period of epithelization and wound contraction 50% in burn wound model.

Groups	Treatment	Burn wound	
		Epithelization period (days)	Wound Contraction-50% (days)
Group I	Control	20.00±0.36	8.13±0.34
Group II	Simple ointment	15.83±0.30***	6.23±0.27***
Group III	carbapol	16.50±0.50***	6.83±0.18***
Group IV	*Aloe vera* (5%)	13.10±0.40***	3.86±0.17***
Group V	MEMP (2.5%)	11.50±0.34***+++	3.30±0.06***+++
Group VI	MEMP (5%)	9.66±0.55***+++	3.00±0.12***+++
Group VII	CEMP (2.5%)	9.50±0.61***+++	2.70±0.12***+++
Group VIII	CEMP (5%)	8.66±0.55***+++	2.13±0.15***+++

Local application of MEMP and CEMP caused incline in breaking strength of 10 days old incision wound. High doses of both MEMP and CEMP were able to raise breaking strength of the incision wound more effectively than their respective low doses and Aloe vera extract. Moreover, increase in breaking strength in MEMP and CEMP formulation was found to be significantly different when compared to emulsifying base applied groups.

Table 3. Effect of methanolic (MEMP) and chloroform (CEMP) extracts of mimosa pudica on the period breaking strength, dry tissue weight and hydroxyproline content in dead space wound model.

Groups	Treatment	Breaking strength (ml)	Dry tissue weight (mg)	Concentration of Hydroxyproline (µg/g of tissue)
Group I	Control	308.66±17.66	77.16± 4.22	3011.81 ± 335.57
Group II	*Aloe vera* (300 mg/kg)	564.33±17.12***	206.70±12.53***	6082.45±363.81***
Group III	MEMP (100 mg/kg)	475.33±6.52***	157.50±4.68***	5955.23±272.26**
Group IV	MEMP (500 mg/kg)	570.33±13.05***	177.16±5.17***	6267.33±440.60***
Group V	CEMP (100 mg/kg)	512.83±9.97***	160.83±4.70***	6034.98±711.29***
Group VI	CEMP (500 mg/kg)	580.16±9.40***	179.16±4.06***	6559.18±480.77***

All values are mean ± SEM, n=6, ***P<0.001 Vs control, +++P<0.001 Vs respective base

Burn wound (Table 2)- Similar to excision wound model, application of MEMP (2.5%), MEMP (5%), CEMP (2.5%), CEMP (5%) and Aloe vera extract (5%) gel reduces both epithelization period and wound contraction-50% (days) to significant (P<0.001) extent compared to control. Both MEMP and CEMP formulations demonstrated fall in epithelization period and wound contraction at high as well as low doses significantly compared to their respective emulsifying bases. Dead space wound (Table 3)- The treatment of animals with MEMP (100 mg/kg, po), MEMP (500 mg/kg, po), CEMP (100 mg/kg, po), CEMP (500 mg/kg, po) and Aloe vera extract (300 mg/kg, po) promoted significantly the breaking strength of 10 days old granulation tissue. . The hydroxyproline content and dry tissue weight were significantly elevated (P<0.001) by all treatments as compared to control.

4. Discussion

The present research was done to determine the influence of the methanolic (MEMP) and chloroform (CEMP) extracts of *mimosa pudica* in promoting healing of wound in experimental animals. The observation of the current study confirms the traditional use of *mimosa pudica* roots for wound healing properties. The formulation of extracts applied locally or treated orally augments the healing process by strengthening breaking strength, promoting wound contraction and hastening epithelization period in different experimental wound models.

The important stages of healing of wound comprises of collagen formation, contraction of wound and regeneration of epithelium. There is an existence of strong relationship among inflammatory processes, fibroblast formation and collagen synthesis. Hence management of wound involves targeting any of these events leading to healing of wound. It is well known that growth hormone accelerates the healing process by promoting proliferation of epithelial cell and collagen formation. Collagen possesses number of protein that gives structural support and thus considered as main component of fibrous tissue and cartilage. The synthesis of collagen is influenced by number of growth factors [19]. It is well established that growth hormone also facilitates fibroblasts proliferation [20] which forms the granulation tissue. Treatment of animals with mimosa pudica raises granuloma tissue weight and breaking strength in dead space wound model. The exact mechanism(s) by which CEMP and MEMP raised the breaking strength of granulation tissue and granuloma tissue weight cannot be discussed with present findings.

One of the common underlying pathogenesis in burn/inflicted wound and skin ulcers is lipid peroxidation. Any drug which can prevent lipid peroxidation is said to incline the viability of collagen fibrils thereby strengthening collagen fibers by enhanced circulation. It can also prevent cell damage by promoting DNA synthesis [21].Large number of antioxidants such as metronidazole, vitamin C and vitamin E are shown to increase the wound healing [22]. There are reports that indicate the potent antioxidant properties of extracts and constituents of *mimosa pudica* [23]. Therefore it is speculated that the wound healing activity of CEMP and MEMP after both local and systemic administration may be partly due to their potent antioxidant properties.

The methanolic and chloroform extracts of mimosa pudica found to contain several common phytoconstituents such as glycoside, tannins, flavanoids and alkaloids. All these active materials may be hastening the healing process of different types of wounds by working separately or in combination. The antioxidant capacity of herbs containing flavanoids are well established, while tannins are known to have anti inflammatory, astringent, and antimicrobial activities [23]. Tannins and falvanoids could be synergistically responsible for accelerated wound healing activity. The slight non-significant increase in wound healing activity of CEMP could be attributed to the presence of additional constituent, membrane stabilizing agent, saponin [24] that was absent in MEMP.

5.conclusion

In conclusion, both CEMP and MEMP possesses potent wound healing activity when applied topically as seen in excision, incision and burn wound models as well as when administered orally as seen in dead space wound model in experimental animals. However, high doses were more effective than low doses with non-significant rise in wound healing ability of CEMP.

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