Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(8); 1143-1147
doi: 10.25258/phyto.v9i08.9623
ISSN: 0975-4873

Research Article

Evaluation of Incisional Diabetic Wound Healing Activity of Ethanolic Leave Extract of *Mimosa pudica* L. in Rats

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Received: 29th May, 17; Revised 7th July, 17, Accepted: 15th Aug, 7; Available Online:25th Aug, 17

ABSTRACT

Diabetes mellitus (DM) is a fast growing epidemic throughout the world. India is going to become the capital of DM. DM patients are at greater risk of serious infective foot ulcer or wound. The diabetic wound may be associated with late healing and septic manifestation and finally reach to limb amputation which is an overpriced incident. The herbal products are more precious in both prophylaxis as well as curative in delayed diabetic wound healing activity when compare to synthetic drugs. The present study was carried out to evaluate the diabetic incisional wound healing activity of ethanolic leave extract of *Mimosa pudica* L. (EMP) in Streptozotocin (55 mg/kg, i.p.) induced diabetic rats. A wound of 1cm incision was made on ventral side of diabetic male albino wistar rats. The two different doses (2.5% & 5%) of EMP are applied on wound b.i.d for 11 days. The initial and final fasting serum glucose levels were estimated to confirm the disease state. The breaking strength and histopathological studies of incisional healed skin was estimated. The *EMP* 2.5 % and EMP 5% have shown significant ($p<0.01$) increase in wound breaking strength as well as well epithelialization compared to diabetic control group.

Keywords: *Mimosa pudica*, diabetes, incisional wound, Streptozotocin.

INTRODUCTION

WHO projects that diabetes will be the 7th leading cause of death in 2030[1]. It was estimated that 366 million of people worldwide will enrolled in DM by 2030[2]. India is going to be the diabetes capital with 109 million individuals with DM among worldwide 592 in 2035[3]. The DM is a well recognized metabolic disorder associate with many manifestations related to physiological abnormality. The delayed wound healing activity is one of them. The unhealed foot ulcers, bed sore and wound are commonly associates with DM. This unhealed wound may leads to limb amputation and septic manifestation. The diabetic foot ulcer in particular is more difficult to treat, costing between $7,000 and $10,000 per ulcer. Many of these ulcers may ultimately require amputation of a limb, where the cost may be as high as $65,000 per person. The limb amputation is not only an overpriced process but also turn the life into a physical challenged[4]. Hence many researchers have keen interest to explore and develop better remedies for delayed diabetic wound healing. The peripheral arterial diseases (angiopathy) and peripheral neuropathy are extreme manifestation in DM which affects the blood circulation in palmar and plantar areas. Lower incidence of blood circulation is the prime cause in deprive of oxygen and nutrient in the particular area. Inconsequence to this there will be delaying in wound healing with numbness and vulnerable to infections[5]. The formation of localized free radicals and decreased immune system may be another reason in the delayed wound healing.

It has been well accepted that the herbal drugs are gaining more attention in the treatment of delayed diabetic wound healing when compared to synthetic drugs. Traditional medicines such as *Aloe vera, Bryophyllum pinnatum, Acorus calamus, Cuminum cyminum, Helianthus annuus* are well adapted in wound healing activity[6].

Therefore aqueous leaves extracts of *Mimosa pudica* L was intended here to evaluate diabetic wound healing activity in resutured incision wound model. The *Mimosa pudica* L. belongs to the genus Mimosa (Family: Mimosaceae) is popularly named as a sensitive and shy plant (touch me not). In the ancient days Charaka and Susruta have reported various properties of *Mimosa pudica* in Ayurveda such sophahara (reduces edema), yoniragahara (ameliorates vaginal disease), atisaraghna (anti-diarrhoeal), kusthahara (anti dermatoses). Maharishi Charak has categorized it as sandhaniya a healing herb[7]. The whole *Mimosa pudica* mainly grows on the hillside, jungle, glade, and roadside of Asia. Previous reports on *Mimosa pudica* revealed the presence of bioactive compounds such as flavonoids and phenolics[8]. In addition, many bioactivities of *Mimosa pudica* have also been studied such as antioxidant, antibacterial, hepatoprotective activities and so on. The leaves are bitter in taste. These are used as sudorific and tonic in the treatment of scrofula, conjunctivitis, cuts and wounds, hemorrhoids, fistula, sores and haemorrhages[9]. More recently researchers have found out a biologically active phenolic compound in the extract of *Mimosa pudica* which are useful in the

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Table 1: The fasting blood glucose level (mg/dl) of different treatment groups.

Groups	0^{th} Day	10^{th} Day
NC	71.34±4.12	80.38±3.45
DC	322±5.4	366±4.55
FRA	333±6.23	361±6.42
EMP 2.5%	319±5.8	332±6.12
EMP 5%	338±3.4	341±5.21

Table 2: Effect of treatment groups on skin breaking strength on 10^{th} day.

Groups	Skin breaking strength (gms)
ND	388.12 ± 14.87
D-CONT	238.00 ± 18.8
D-FSC	440.31±12.61**
D-MPE-2.5%	283.93±17.35*
D-MPE-5%	426.43±13.11**

maintenance and enhancement of collagen levels in the skin[10].

MATERIALS AND METHODS

Streptozotocin (STZ) was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. All the reagents and chemicals used are analytical grade and procured from Hi-media Labs, Mumbai, India.

Plant material collection & extraction

The fresh leave of *Mimosa pudica* was harvested locally from southern parts of Odisha, India in the month of October-December. The plant was identified and authenticated at the department of Plant Research Centre (PARC) Chennai. The specimen was deposited in herbarium of PARC (Voucher Specimen No: PARC/2008/128). Plant material was carefully washed with tap water and left to dryness in dark at room temperature and finally stored in well closed cellophane bags. The shade-dried and coarse powdered leave (2 kg) was subjected to defat with petroleum-ether (boiling point 40-60°C) using Soxhlet extraction apparatus (Quickfit, England). The defatted sample was air dried in order to remove solvent residue. Extract was prepared by extracting the defatted powder with ethanol solvent (80% v/v) for period of 48 h, which was then concentrated to a semisolid mass under reduced pressure (Buchi Rotavor R-200, Switzerland) for 20 min at 70°C (yield: 6.7% w/w). The extract was subjected to prepare 2.5% and 5% ointment of EMP in simple ointment base.

Mimosa pudica ointment

2.5gm of *Mimosa pudica* extract was added with 97.5gm of simple ointment base to make a MPE 2.5%. Similarly 5.0gm of *Mimosa pudica* extract was added with 95gm of simple ointment to make a MPE 5%. The prepared ointments were stored in air tight ointment pots and labeled[11].

Animals

The male albino mice (25-35gm) & rats (150-200 g) were obtained from M.K.C.G. Medical College, Berhampur, Odisha. The animals were grouped and housed in propylene cages lined with husk under standard condition (24 ±2°C temperature, 45-55% relative humidity and 12 h light: 12 h dark cycle). Animals are allowed to freely feed their standard pellet diet (Lipton India, Ltd., Mumbai) and water *ad libitum*. The mice were subjected for oral toxicity study. The rats were deprived with their food for 16-18 h before the experimental work starts but water is allowed ad libitum. The experimental procedure was performed by the approval of the Institutional Animal Ethics Committee (1275/ac/09/CPCSEA).

Oral toxicity studies

Albino wistar male mice of weight 25-35gm are selected for acute oral toxicity study and it was conducted according to the "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of EMP. Eighteen hour overnight fasted animals were subjected to oral administration of EMP at a dose of 2000 mg/kg body weight. All the animals were kept under observation for first 3 h for any changes or toxic effects like neurological, gross behavioral and lethality. The animals are observed and confirmed the absence of any toxic effects, hence two dose of EMP 2.5% and 5% ointment were prepared with simple ointment base for topical application.

Experimental protocol

The albino wistar rats are divided into 5 different groups each contain 6 animals after the acclimatization period. All the animals are subjected to induce diabetes & resutured wound except the normal control (Group I) group, which is only induced with resutured wound. The Group II is consider as disease control group and topically applied with only simple ointment base. The Group III is treated with Framycetin (2% Soframycin) ointment as standard to compare with others. The Group IV and Group V are consider as test groups which are treated with EMP 2.5% and EMP 5% respectively. The treatment was designed for 11 days after the diabetes confirmation.

Induction of diabetes

Freshly prepared single dose of Streptozotocin (55 mg/kg body weight) with citrate buffer (pH 4.00) was administered intraperitoneally induction of Diabetes to the overnight fasted rats[12]. All the animals are administered with 5% glucose water for 6hour immediately after STZ induction to avoid first phase hypoglycemic condition. The animals having fasting blood glucose level more than 250 mg/dl on 4^{th} day after STZ induction are subjected for induction of resutured incisional wound.

Induction of resutured incisional wound[13]

The animals were anaesthetized with Ketamine (100mg/kg B.w.) before the surgical procedure. The dorsal surface of each animal was shaved with a sterile blade under all aseptic measures. One linear incisional wound of whole skin thickness of length 4cm was made on back of each animal. Parallel and 1cm lateral from the vertebral column on either side. After complete hemostasis the wounds were closed by means of interrupted stitches at 1 cm gap with 4-0 silk (sterile) . All the above surgical maneuvers were done with full aseptic measure. Immediately after wounding all the rats were kept in separate spacious clean cages to avoid damage to wound and infection[14]. As the

Table 3: Histopathological studies of healed skin on 10th post days.

Observation	NC	DC	FS	MPE 2.5%	MPE 5 %
Re- epithelialization	complete	Incomplete	Complete	Incomplete	complete
Congestion	+	++	+	++	-
Edema	Mild	Mod	Mild	Mod	-
Infiltration by polymorphs	+	+ +	+	++	+
Infiltration of macrophage	+	++	+	+	+
Necrosis	Nil	Nil	Nil	Nil	Nil
Fibroblast proliferation	+ +	+ + +	+	+++	+
Collagen formation	+ +	+	++	+	+ + +
Angiogenesis	+	+ +	+ + +	+	++

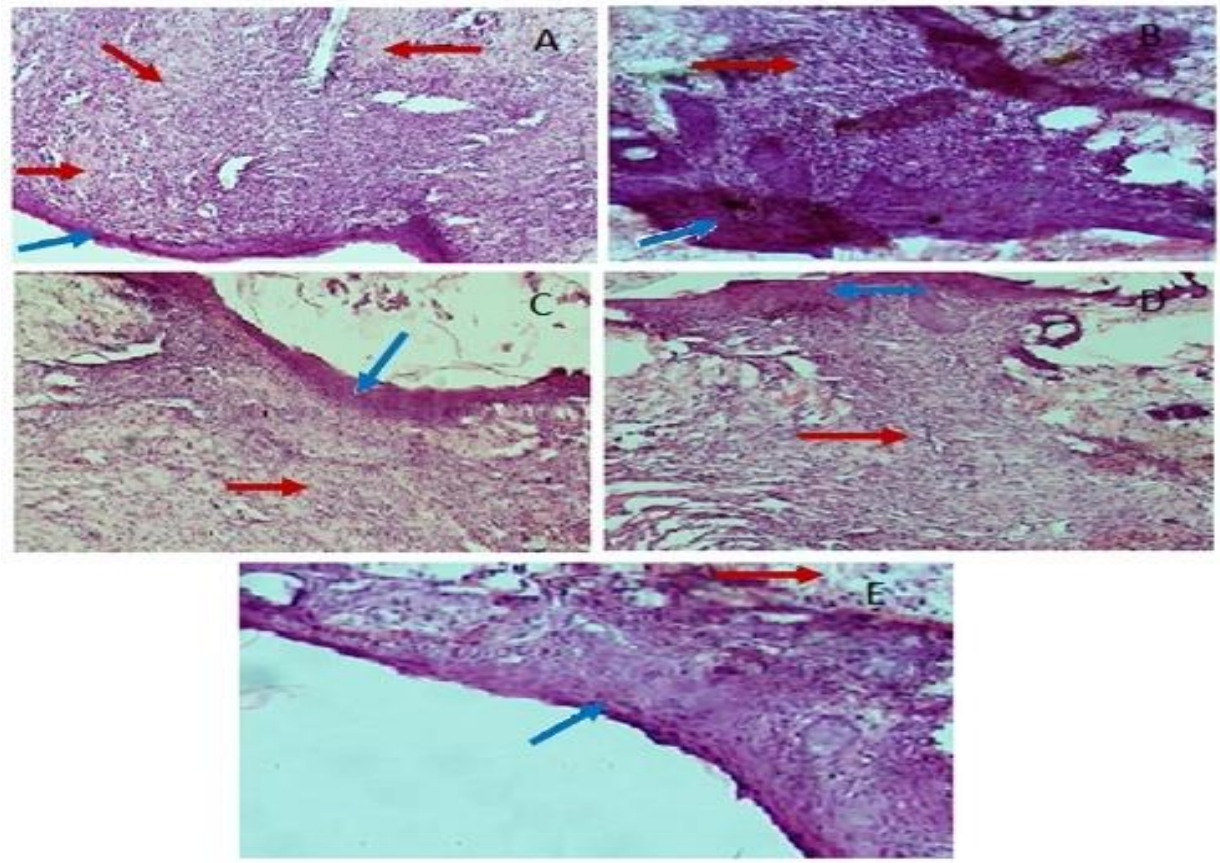

Figure 1: The histopathological study of healed skin on 10th post day.
A- Normal control; B-Disease control; C- Framycetin 2%; D-EMP 2.5%; E- EMP 5%
→ Collagenation; → Epithelialization

rats tool 5-10 minutes to come out of the effect of anesthesia, food and water was given ad libitum after 2 to 3 hours of the day of operation. No local or systemic antibiotics were given in the post operative period. The animals were inspected daily for any evidence of infection and the animals showing infection were excluded from the study. The day of wounding was referred as day-1

Measurement of healing

On 8th post wounding day each animal was anaesthetized and the sutures were removed under all aseptic measures. The treatment was continued until 10th day. Two pieces of skin was dissected out from the incision line of each healed wound for each rat to measure skin breaking strength and for histopathological studies. Tensile strength (the force required to open a healing skin wound) was used to measure healing. The instrument for this measurement is called tensiometer. Since there were no commercially made tensiometer was available, a simple procedure was followed according to method of Lee K.H 1968[15].

Histopathological Examination

A piece of skin tissue from healed wound was preserved in 10% formalin. They were examined under microscope by using hematoxylin and eosin (H&E). The histopathological observations were described below (Figure No.1 and described in Table No. 2).

Statistical analysis

All the data are expressed in Mean ±SEM. The obtained data were analyzed by one way ANOVA followed by Tukey's multiple comparison test with the help of graph

pad prism 5 soft ware. The difference mean value became significant when $p < 0.05$.

RESULT

The fasting blood sugar (FBS) levels were estimated on initial and final day of experimental protocol to confirm the diabetic state. A little change in FBS was observed (Table No.1). The data revealed that skin breaking strength of diabetic control animals were decreased to significant extent in comparison to the Non- diabetic control rats ($p<0.001$). The impact on Skin breaking strength (gms) of the Framycetin sulphate treated group was found to be highly significant ($P<0.001$) increase in wound breaking strength (440.31 ± 12.61gms) in comparison to diabetic control group. Whereas MPE 2.5% treated group did not reveal any significant change in wound breaking strength, but the rat treated with 5% MPE increased the wound breaking strength to a highly significant($P<0.001$) extent (426.43 ± 13.11gms; Table No.2). The Table No.3 described the microscopic examination of incision wound of different groups. The framycetin treated groups evidenced a significant increase in collagen deposition showing lesser macrophage & fibroblasts and complete epithelialization in comparison to others. The EMP treated groups showed complete epithelialization, mild degree of inflammatory cell infiltration and marked degree of collagenation in a dosed dependent manner, whereas the diabetic control group showed minimal and incomplete epithelialization with marked inflammatory cell infiltration, edema and congestion.

DISCUSSION

Wound healing in diabetes is a complicated and delayed process in which wound healing follows granulation, collagenation and scar formation. Hyperglycemia suppress cell proliferation collagen production[16]. In the present study the EMP showed a well incisional resutured wound healing activity in diabetic rats. The suggested mechanisms involved in the wound healing effects of the EMP are free radical scavenging, metal chelation, anti-inflammatory, astringent as well as immune modulatory property. The epithelialization is based on collagenation and anti-inflammation. Collagenation induce regaining tissue integrity and strength of the wound. In the present study the EMP has shown better collagenation and epithelialization due to its flavonoids content which are might be responsible for the localized free radical scavenging activity. The c-glycosyl flavonoid, kaempferol and quercetin are the main active constituents are believe to be responsible for the claimed property[17]. The flavonoids of EMP are believed to insist wound healing activity for its characters like reeducation in lipid peroxidation, astringent and antimicrobial effect[18,19]. The phenol and tannin containing extract of *Mimosa pudica* possesses strong biological activity, with the ability to maintain or enhance the levels of collagen in skin cells and might have produce topical free radical scavenging activity posses wound healing activity[20,21]. More specifically when provided to human fibroblasts a phenolic containing fraction of mimosa pudica can increase the collagen levels in these cells by as much as 100% or more. The *Staph aureus* and *Pseudomonas aeruginosa* microorganism are involved in infections diabetic foot ulcer[22]. The other constituents like alkaloids or tannins of *Mimosa pudica* possess antimicrobial activity[23]. Hence it is concluded that the EMP have wound healing activity in diabetes and it may be suggested for treating various types' wounds in human beings. A further study can be designed to isolate the purified constituents & depict the complete molecular mechanism action involved in wound healing activity in diabetes mellitus.

ACKNOWLEDGEMENT

Author is very much gracious at Dr. Bandana Rath, (MD), Associate Professor, M K C G medical college, Berhampur, Odisha for accomplishment of the present work.

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