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Research Article

EVALUATION OF WOUND HEALING ACTIVITY OF *PHRAGMITES VALLATORIA* LEAF ETHANOL EXTRACT IN STZ-INDUCED DIABETIC RATS

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ABSTRACT

The ethanol extract of *Phragmites vallatoria* leaf extract (400mg kg^{-1} day^{-1} for 11days) was evaluated for its wound healing activity on STZ induced diabetic rats using excision and dead space wound models. The animals were weight matched (n=6 animals $group^{-1}$) and placed into 5 groups. Animals in group A and B were normal control and normal treated with Vaseline respectively, in group C & D were the diabetic control and diabetic experimental animals treated with ethanol extract of *Phragmites vallatoria* leaf (EPVL), and group E were positive control treated with Bacitracin ointment. The EPVL extract was applied to animals of group B and D for 11days. The wound size in animals of the *Phragmites vallatoria* leaf extract treated group was significantly reduced when compare with diabetic control group. The extract treated wounds were found to epithelize faster as compared to controls. The wet and dry granulation tissue weight content significantly increases in animals treated with *Phragmites vallatoria* leaf ethanol extract (400mg kg^{-1} day^{-1} for 11days) and compared to controls. Ethanol leaf extract of *Phragmites vallatoria* promote wound healing activity within 11 days in STZ induced diabetic rats. Wound healing in diabetic rats and further evaluation of this activity in humans is suggested.

Keywords: *Phragmites vallatoria*. STZ induced diabetic rats. Wound healing activity.

INTRODUCTION

Wounds are physical injuries that result in an opening of the skin. Wound healing is an important biological process involved in tissue repair and regeneration. A wound is described as 'a break in the continuity of tissue' from violence or trauma and is regarded as healed if there is restoration of the wounded or inflamed tissue to normal condition. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin[1,2]. The exact pathogenesis of wound healing in diabetic wounds is not clearly understood. Evidence from studies involved in both human and animal reveal several abnormalities in the various stages in healing process[3]. It is an enigmatic and debilitating complication and pocesses a serious challenge in clinical practices. Mainly the healing process begins immediately following injuring when the platelets coming to contact with exposed collagen as platelet aggregation proceeds, clotting factors are released and resulting in the deposition of a fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing[4]. Many researchers have reported the improvement in the wound healing process by various plant extracts and isolated compounds in animal models in literature[5,6,7]. *Phragmites vallatoria* is belongs to the family of poaceae and it is spreaded throughout India. According to the literature, it has different types of application in medicine and agriculture products. Medicinally it has the properties of diuretic, animistic, diaphoretic, wound healing, diabetes, arthritis, rheumatism, antiemetic and febrifuges activities[8,9]. The present study has been undertaken to examine the wound healing activity of ethanol extract of *Phragmites vallatoria* leaf in experimentally induced excision and dead space wounds in diabetic rats.

MATERIAL AND METHODS

Plant material

Phragmites vallatoria[9] is obtained from Chirala (Prakasam district, Andhra Pradesh, India). Leaves were collected, shade dried and powdered. Then components present in the powdered leaves were extracted into ethanol using soxhlet apparatus. The extracts are concentrated by vacuum rotary evaporator.

Animals

Experimental Animals

The male healthy Wistar albino rats weighing 150-160 g obtained from the animal house of Sri Venkateswara agencies, Bangalore were used in this study. The animals are caged and provided with feed from Amruth laboratory animal feed (sangli) water ad libitum.

The excision and dead space models were used to evaluate wound healing activity of *Phragmites vallatoria* leaf ethanol extract. Mainly animals were distributed into five groups of 6 each in excision and 4 groups of 6 each in dead space wound models.

Animal groups

The animals were weight matched (n=6 animals $group^{-1}$) and placed into 5 groups. Animals in group A and B were normal control and normal treated with Vaseline respectively, in group C & D were the diabetic control and diabetic experimental animals treated with ethanol extract of *Phragmites vallatoria* leaf (EPVL), and group E were positive control treated with Bacitracin ointment.

Wound healing activity

Excision and dead space wound models are used to evaluate wound healing activity.

Induction of diabetes

Animals of diabetic groups were weighed and their fasting blood glucose levels were determined before inducing diabetes. The animals were injected with Streptozotocin at a dose of 55 mg kg^{-1} body weight dissolved in 0.1m citrate buffer (pH 4.5) administrated by intraperitonially[10].

Control animals were injected with normal saline. Fasting blood glucose levels were measured three days later to confirm the diabetic status of the animals. For blood glucose measurement, the blood was drawn by tail vein.

Excision wound model

Animals were anaesthetized with diethyl ether by open mask method and shaved on both sides of the back with an electric clipper.

The area of wounds to be created was outlined on the back of the animals with methylene blue using a stainless steel stencil. Animals were closely observed for any infection and those which showed signs of infection were separated excluded from the study and replaced.

Animals were divided into 5 groups of 6 in each. The normal controls group A was applied with Vaseline two times a day. Normal treated group B were applied EPVL extract two times a day. Diabetic controls group C were applied with Vaseline two times a day, diabetic experimental rats group D were applied with EPVL extract two times a day and the positive control group E an application of bacitracin ointment 2 times a day. The treatment was done topically in all cases. Wound areas were measured on day 1, 5th and 10th for all the groups using a transparency sheet and a marker.

Dead space wound models

Dead space wounds were inflicted by implanting sterile cotton pellets, one on either side in the groin and axilla on the ventral surface of each rat. The animals divided into 4 groups of 6 each the normal controls group A provided water orally. Experimental controls group B were given the extract orally in a dose of 400mg kg^{1} for 11days, diabetic controls group C were given water orally and diabetic experimental rats group D were given extract orally at a dose of 400mg kg^{-1} for 11days. On the 11th post wounding day, the granulation tissue formed on the implanted cotton pellets was removed carefully under anesthesia. Noting the wet weight of the granulation tissue the tissue was dried at 60^{0}C, for 12 hours and the weight was recorded.

Statistical Analysis

The means of wound area measured at different time intervals were statistically analyzed by (ANOVA) followed by dunnett's test as post hoc test.

RESULTS AND DISCUSSIONS

It was observed in ethanol extract of *Phragmites vallatoria* leaves (EPVL) treated rats that the excision wound model, which was carried out to study the topically applied EPVL on wound healing and its concentration, significantly increased in the wound healing activity. In diabetic animals too, the percentage of wound contraction and healing was greater in extract treated group D than in control group C animals. *Phragmitis vallatoria* having wound healing activity was proposed by earlier workers by peripheral application of crude extracts[5]. The dead space wound model was used to study the difference in matrix synthesis between drug treated and control groups. Oral administration of the leaf extract appears to increase the mass of granuloma in both normal as well as diabetic animals (Table-1).

Table 1: Effect of ethanol leaf extract of *Phragmites vallatoria* on excision wound model in STZ induced diabetic rats

Period of study (Days)	Wound area (mm^2)*				
	Group-A (NC)	Group-B (NT)	Group-C (DC)	Group-D (DT)	Group-E (PCTB)
Day – 1	215.8 ± 1.870	215.6± 1.650	216.2 ± 1.328	220 ± 1.686	215 ± 1.64
Day – 5	98.46 ± 1.23	130.8 ± 1.714	78.60 ± 1.009	105.4 ± 1.538	180.6 ± 1.65
Day – 11	48.6 ± 1.346	95.12 ± 2.15	44.6 ± 1.203	82.6 ± 1.238	139.6 ± 1.74

*The values are shown as mean ± SE (n=6 animals group^{-1}).

NC: Normal control, NT: Normal treated, DC: Diabetic control, DT: Diabetic Treated, PCTB: Positive Control Treated with Bacitracin.

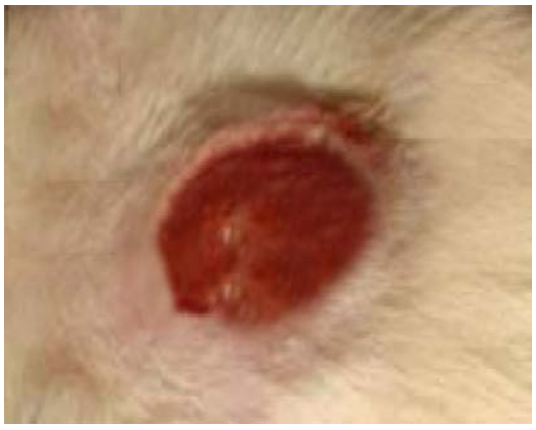
The dry granuloma weight is decreased by the leaf extract treatment, in non diabetic animals and dry granuloma mass is increased by the extract treatments in diabetic animals. Finally the conclusion is significant increase in the wound healing activity was observed in leaf extract treated rats. In excision wound model, animals of group B and D showed decrease in the epithelization period and increased percentage of wound contraction when compared with the animals of group A, C, E (Table-1). In the dead space model the extract treated animals in groups B&D showed significant increase in the dry and wet weight of the granulation tissue than the animals treated without the extract was observed (Table-2). The present study demonstrated that EPVL extract applied topically promotes healing of wound contraction in STZ induced diabetic rats where healing is delayed.

Table 2: Wound healing activity of ethanol leaf extract of *Phragmites vallatoria* in Dead space wound model on STZ induced diabetic rats

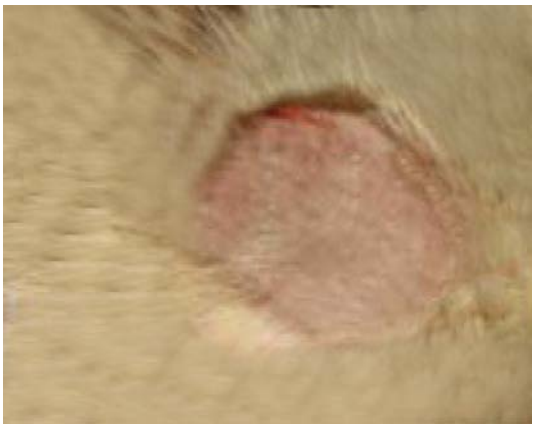
Parameter	Group-A (NC)	Group-B (NT)	Group-C (DC)	Group-D (DT)
Wet granulation Weight (mg 100g^{-1} rat)	101.23 ± 5.162	120.4 ± 6.482	86.74 ± 4.816	123.7 ± 7.106
Dry Granulation Weight (mg 100g^{-1} rat)	42.36 ± 2.162	39.84 ± 4.219	37.68 ± 1.84	62.49 ± 6.486

* The values are shown as mean ± SE (n=6 animals group^{-1}); NC: Normal control, NT: Normal treated, DC: Diabetic control, DT: Diabetic Treated

0th day excision wound



7th day excision wound



11th day excision wound

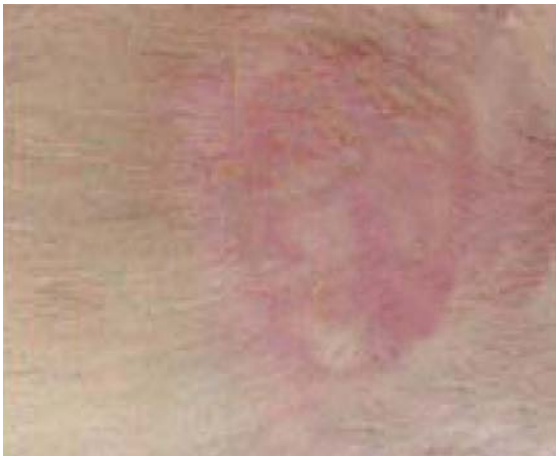


Fig. 1: Photographs showing various stages of wound healing activity of *Phragmites vallatoria* leaf ethanol extract in STZ induced diabetic rats (excision wound model)

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