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Wound healing activity of ethyl acetate fraction of Barleria noctiflora in experimentally induced diabetic rats

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ABSTRACT

Purpose: To evaluate the wound healing activity of ethyl acetate fraction of Barleria noctiflora against the STZ induced diabetic rats.

Approach: The ethyl acetate fraction of Barleria noctiflora (EAFBN) was evaluated for its wound healing activity in Streptozotocin induced diabetic rats using excision and incision wound models. In case of excision wound model, parameters like wound contraction and period of epithelialization was studied, while in incision wound model, tensile strength of the wound was measured. In incision and excision models, treatment with ointment containing 10% w/w and 5% w/w of EAFBN was done.

Findings: The excision wound area exhibited significant (P<0.01) closure of EAFBN 5% w/w and 10% w/w treated animals showed 96.6% and 95.8%, complete epithelialization was observed in same day of EAFBN (10% w/w) and standard drug. In the incision wound model treated with the topical application of EAFBN 5% w/w treated animals skin breaking strength was 290.83±3.0, EAFBN (10% w/w) and standard drug have shown more or less same significant skin breaking strength (342.5 ± 3.81and 350 ± 2.88).

Originality: The current study is the first time evaluation of the wound healing activity of Barleria noctiflora ethyl acetate fraction on STZ induced diabetes in rat. The characterization and isolation of the dynamic principle of new compound require broad research in the future.

Conclusion: The outcome of present study indicates the usage of ethyl acetate fraction of Barleria noctiflora in the management of wound healing especially in diabetes.

Keywords: *Wound healing, Barleria noctiflora, Streptozotocin, Diabetic rats.*

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INTRODUCTION

Injury of the skin induces repair mechanism that restores its functions in protecting the individual against environmental factors that might be harmful. The diabetic wounds are slow, non healing wounds that can persist for weeks despite adequate and appropriate care. Such wounds are difficult to manage[1]. The evidence from studies involving both human and animal models reveals several abnormalities in the various phases of the wound healing process. Three different phases constitute the physiologic process of wound-healing; (i) substrate phase, (ii) proliferative phase and (iii) remodeling phase. All these steps are orchestrated in controlled manner by a variety of cytokines including growth factors. Some of these growth factors like platelet

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derived growth factor (PDGF), transforming growth factor B (TFG-B), fibroblast growth factor (FGF) and epidermal growth factor (EGF) etc. have been identified in self healing wounds[2]. In chronic wounds the normal healing process is disrupted due to some unknown reasons and in such cases exogenous application of some growth promoting agents or some compounds which can enhance the in situ generation of these growth factors is required to augment the healing process. Barleria noctiflora L. (B. noctiflora), belongs to the family Acanthaceae, which is being widely used as Folk and ayurvedic medicine. It is widely distributed throughout tropical region of India, Africa, Sri Lanka and other parts of Asia. Most of the Barleria species are potent anti-inflammatory, analgesic, antileukemic, antitumor, anti-hyperglycemic, anti-amoebic, anti ulcer, virucidal and antibiotic. Many of the Acanthaceae members possess anti bacterial properties and were used for treating wounds and ulcers[3]. The wound healing properties of this Acanthaceae member has not been scientifically evaluated. Hence the present work was undertaken to evaluate the effect of ethyl acetate fraction of B. noctiflora on excision and incision wound models in rats.

MATERIALS AND METHODS

Collection of plant

B. noctiflora was collected during winter season in and around Erode District, Tamilnadu, India. It was identified and authenticated by Prof. P. Jayaraman, Director, National Institute of Herbal Science, Chennai-45, Tamilnadu, India (Ref no: PARC/2011/1015), and the voucher specimen was deposited at the same institute for future reference.

Preparation of fraction

The aerial part of the plant was shade dried, powdered and weighed. About 500 g was extracted by Soxhlet, successively with petroleum ether, chloroform and ethanol for 72 h each. The solvents were evaporated in vacuum to obtain residues of the extract. The ethanol extract was done using its solubility profile[4]. The 20 gm of ethanol extract was taken in a stopper flask, containing 200 ml of water and shaken mechanically for 1-2 h in a flask shaker. The ethanol extract was not completely soluble in water. The water insoluble portion of ethanol extract was separated using filtration and further fractionated with ethyl acetate using the same procedure. The supernatants obtained from the above fraction were concentrated and evaporated to dryness and their percent yield was determined. Fresh ointment was prepared out of ethyl acetate fraction.

Animals

Throughout the experiment rats were used and handled as per revised guidelines of CPCSEA[5]. Healthy Wistar rats (150-200g) were used for the study. Animals were kept in standard polypropylene cages and maintained under standard laboratory conditions of temperature (24±1ºC), 12 hours dark like cycles, standard diet and water ad libitum. The study protocol was approved by the institutional ethical committee (JKKMMRFCP/IAEC/ 2013/013) and all the procedures were performed for the proper care and use of laboratory animals.

Induction of Diabetes

The animals was induced in overnight fasted rats administering a single dose of freshly prepared solution of streptozotocin (50 mg/kg. b.w. i.p) in 0.1 mol/l of cold citrate buffer (pH 4.5). The STZ treated animals were allowed to drink 5% glucose solution over night to avoid hypoglycemia. After 7 days of injection of STZ, rats with moderate diabetes having persistent glycosuria and hyperglycemia (blood glucose >250 mg/dl) were selected for further experimentation[6].

Evaluation of wound healing activity

For the assessment of the wound healing activity excision and incision wound models were used. Animals were divided into five groups of 6 animals for each wound model. The Group 1 was normal control and Group 2 was diabetic control. Diabetic positive controls (Group 3) were applied with nitrofurazone ointment 0.2% w/w, and diabetic experimental rats (Group 4 and Group 5) were applied with Ethyl acetate fraction of B. noctiflora (EAFBN) 5% w/w and 10% w/w ointments.

Excision wound model

The excision wound method was carried out by Morton and Malone[7]. The rats were anesthetized under light ether anesthesia; the hair was shaved in dorsal thoracic central region. One excision wound was inflicted by cutting away a 200 mm^2 full thickness of skin from a predetermined area. The animals were closely observed without any infection. Excision wound was induced in diabetic as well as normal rats. Each animal was maintained in a separate cage till the end of study. The treatment was done topically in all the cases. Animals were kept in separate cages. The day on which wound was made consider as day '0'. Wound contraction rate was monitored by planimetric measurement of the wound area on 4^{th}, 8^{th}, 12^{th} and 16^{th} days. This was achieved by tracing the wound on a graph paper. Calculate the percentage of wound and epithelialization.

% wound contraction =Initial wound size – specific day wound size / Initial wound size X100

Incision model

The rats were anesthetized by anesthetic ether and two longitudinal parvertebral incision of 6 cm length were made through the skin and cutaneous muscle at a distance of about 1.5cm from the midline on each side of the depilated back. After the incision the parted skin was sutured 1cm apart using a surgical thread and curved needle[8]. The wound were left undressed. The drugs were topically applied to the wound once a day, till complete healing. The sutures were removed on eighth post wound day. The skin breaking strength of the 10 day

old wound was measured[9].

Tensile breaking strength measurement

Tensile breaking strength (the force required to open the healing skin) was used to measure the extent of healing. The model used for this purpose consists of wooden board with a pulley that was fixed in one side of edge of board. Two Allis forceps, one is fixed to the opposite side of pulley edge and another is tied with and hanged with rope that is attached to the pan through pulley on which the weights are placed. The weights are increased slowly till it breaks the healed wound. One day before performing this experiment the sutures are removed from the stitched wound of rats after recovery[10].

Statistical analysis

The results of study were expressed as mean ± SEM. Statistical analysis of the results was carried out using Graph pad instat software by one-way analysis of variance (ANOVA) followed by Dunnett's test. The level of significance was set at P<0.05.

RESULTS

In excision wound healing studies, significant activity was observed with EAFBN and standard drug of nitrofurazone comparable to that of the diabetic control. In EAFBN (10%w/w and 5%w/w) groups significant (P<0.01) percentage closure of excision wound area was 96.6±0.10 and 95.8±0.27, while the standard drug of nitrofurazone was 99 ±0.25 in the period of 16 days (Table 1). Wound percentage contraction was significantly delayed in diabetic control 86.93±0.27 when compared to normal control 90.1±0.27. The epithelialization period observed in EAFBN (10%w/w and 5%w/w) and standard drug and it was found to be 15.83 ± 0.16, 17.5 ± 0.34 and 15.16 ± 0.16 respectively. The complete epithelialization was observed in same day of EAFBN (10%w/w) and standard drug, hence diabetic control rats took 5 days increase in the period of epithelialization i.e. 23.66±0.55 when compare to normal control18.83 ± 0.16.

Table 1: The effects of Ethyl acetate fraction of Barleria noctiflora on Excision wound model in rats.

TREATMENT	PERCENTAGE (%) OF WOUND CONTRACTION AREA (original wound area 200 mm2)					Period of Epithelialization (days)
	0 Day	4th Day	8th Day	12th Day	16th Day	
Control	0	15.35±0.07	42.86±0.07	71.92±0.08	90.1±0.27	18.83±0.16
Diabetic control	0	13.23±0.35*	40.15±0.40*	69.77±0.33*	86.93±0.27*	23.66±0.55*
Nitrofurazone 0.2%w/w	0	24.81±0.14*	76.93±0.42*	86.45±0.50*	99 ±0.25*	15.16±0.16*
EAFBN 5% w/w	0	19.5±0.42*	64.2 ±0.34*	87.1±0.55*	95.8±0.27*	17.5±0.34*
EAFBN 10%w/w	0	19.5±0.18*	67±0.32*	85.5±0.23*	96.6±0.10*	15.83±0.16*

Values are given in mean ±SEM for groups of six animals each. *p < 0.01 denotes when diabetic control was compared with the Normal control and treated groups were compared with the diabetic control on corresponding day (One way ANOVA followed by Dunnett test). EAFBN – Ethyl acetate fraction of Barleria noctiflora.

In the incision wound model the animal treated with the topical application of EAFBN 5%w/w treated animals skin breaking strength was significantly 290.83±3.0, EAFBN (10%w/w) and standard drug have shown more or less same significant skin breaking strength (342.5 ± 3.81and 350 ± 2.88). But it was less in diabetic control 199.33±3.94 and normal control 217.5 ± 3.81(Table 2).

Table 2: The effects of Ethyl acetate fraction of Barleria noctiflora on breaking strength in incision wound model of rats

TREATMENT	Breaking strength (g)
Control	217.5±3.81
Diabetic control	199.33±3.94*
Nitrofurazone 0.2%w/w	350±2.88*
EAFBN 5% w/w	290.83±3.0*
EAFBN 10%w/w	342.5±3.81*

Values are given in mean ±SEM for groups of six animals each. *p < 0.01 denotes when diabetic control was compared with the Normal control and treated groups were compared with the diabetic control on corresponding day (One way ANOVA followed by Dunnett test). EAFBN – Ethyl acetate fraction of Barleria noctiflora.

DISCUSSIONS

Wound healing is characterized by three stages like inflammation, proliferation and remodeling. The proliferative phase typically demonstrates collagen deposition, granulation tissue formation, angiogenesis, epithelialization and wound contraction. In the stage of angiogenesis, new blood vessels grow from endothelial cell. In fibroplasias and granulation tissue formation, fibroblasts grow and form a new provisional extracellular matrix by excreting collagen and fibronectin[11]. In epithelialization, epithelial cells grow across the wound bed to cover it. Fibronectin the major glycoprotein secreted by fibroblasts has important function of chemo attraction for macrophages, fibroblast endothelial cells, promoting re-epithelization and acting as transduction agent in wound contraction. The wound contraction occurs by myofibroblasts, a grip on the wound edges bringing them in apposition[12].

Wound healing deficits in diabetes are diverse complex, multifactorial and inter related[13]. The defects are impaired blood flow and oxygen release from increased blood sugar, decreased collagen and fibronectin synthesis from protein malnutrition, impaired immune cell defenses and decreased insulin and growth hormone. Collagen, keratin and fibrin accumulate glycation which affect binding of regulatory molecules, proteolysis and decrease the ability for protein linkage[14]. The hyperglycemia affects the whole range of neutrophil functions, which include migration, chemotaxis, adherence and phagocytic bactericidal activity[15].

In the present investigation of B. noctiflora fraction promote significant wound healing activity by increasing proliferation, formation of granulation tissue, synthesis of collagen and increase the rate of wound contraction

compared with diabetic control. In incision wound study increase the breaking strength was indicate of improved collagenation, which significantly contributes to better effective wound healing. The experiments revealed that the wounds treated with ethyl acetate fraction of B. noctiflora were relatively healing of wound especially in Diabetes mellitus.

CONCLUSION

The results of study showed that the ethyl acetate fraction of B. noctiflora effectively stimulate wound contraction as compared to control groups. These finding could justify the inclusion of this plant in the management of wound healing especially in diabetes.

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