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Effect aqueous extract of Bougainvillea glabra choisy on Cutaneous Wound healing in Diabetic Rats.

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ABSTRACT

Patients with diabetes often have wounds that are difficult to heal. The initial barrier to healing is an increased blood glucose level, which causes the cell walls to become rigid, impairing blood flow through the critical area at the wound surface. The aqueous extract of *Bougainvillea glabra* choisy leaves was evaluated for its wound healing activity in alloxan induced diabetic rats. There are three models excision, incision & deadspace wound models were used to study the wound repair and regeneration in normal and diabetic rats. The aqueous extract of *Bougainvillea glabra* choisy leaves (100mg/kg/day for 10 days) was evaluated for its wound healing activity in alloxan induced diabetic rats using excision and dead space wound models. Extract treated animal's exhibit 77% reduction in wound area and found to epithelize faster as compared to controls. The wet and dry granulation tissue weight and Hydroxyproline tissue content increased with increase in tensile strength of extract treated rats.. The aqueous extract of *Bougainvillea glabra* choisy leaves treatment showed marked effect on wound healing in normal and diabetic rats. It can be a suitable part of treatment procedure of non healing diabetic and normal wounds.

Keywords: Diabetes, Hydroxyproline, Tensile strength Wound healing.

1. INTRODUCTION

Healing of wounds, a fundamental response to tissue injury occurs by a process of connective tissue repair. A fibrous scar is the end product of this process, the pre-dominant constituent of which is collagen[1]. Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. These abnormalities contribute to the impaired wound healing observed in diabetes[2].*Bougainvillea glabra* choisy (*Nyctagaginaceae*) [3], commonly known as glory of garden, is an herb that grows every where in the fields of India. The leaves of this plant contain β-Cyanins[4], Flavonoids, Saponins. Water extract of the leaves has long been used in traditional medicine to clean wounds and reducing blood glucose level[5]. Hence an attempt was made to investigate influence of Bougainvillea *glabra* choisy on Biophysical and Biochemical Parameters of Cutaneous Wounds in Diabetic Rats.

2. PROCEDURE FOR EXPERIMENTATION

Plant material & extraction:

Bougainvillea glabra choisy (*Nyctaginaceae*), leaves were collected locally from outskirts of Mandsaur district, The plant material was identified and authenticated by Head, Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur (M.P.). Dried fine powder of *Bougainvillea glabra choisy* fresh leaves were Soxhlet-extracted with water as solvent.

Animal Experimentation:

All animal experiments were ethically approved by Institutional Animal ethical committee.

1. Acute Toxicity Studies

The acute toxicity study was carried out in adult female albino rats by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.420.

2. Induction of Diabetes

Wistar albino rats (150-200 g) were made diabetic by a single injection of alloxan monohydrate (120 mg/kg, i.p.) prepared in citrate buffer (0.1 M, pH 4.5) after overnight fasting[6]. and the glucose level was estimated using Glucometer (Accu-Chek).

Surgical wound procedures and treatment

Two types of wounds were created on the 7th day after induction of diabetes, namely excision and dead space wounds were created in experimental rats.

1. Excision wounds[7]

Excision wounds were used for the study of rate of contraction of wound and epithelization, All wounds were of full-thickness type extending up to the adipose tissue. Animals were anaesthetized with 120 mg/kg (i.p.) of ketamine hydrochloride and the right side of each rat was shaved. Excision wounds sized 300 mm^2 and 2 mm depth were made by cutting out piece of skin from the shaven area. The entire wound was left open. Animals were closely observed for any infection and those which showed any sign of infection were separated, excluded from study and replaced. Animals were divided into five groups of 6 animals in each. The normal controls (Group 1) were applied with Vaseline, experimental controls (Group 2) were applied with the extract, diabetic controls (Group 3) were applied with Vaseline, diabetic experimental (Group 4) were applied with extract and the positive controls received an application of soframycin ointment (Group 5). The treatment was done topically in all the cases. The extract was applied at a dose of 100mg/kg/dayfor 10 days. Wound areas were measured on days 1, 5 and 11 for all groups, using a transparency sheet and a permanent marker. Recording of wound areas were measured on graph paper. The day of scar falling, after wounding without any residual raw wound was considered as the day of epitheliazation.

2. Dead space wounds[8]

Dead space wounds wound was used for the study of biochemical parameters and of the rate of tissue formation. Dead space wounds were created by implanting sterile cotton pellets (10 mg each), one on either side in groin and axilla on ventral surface of each rat by technique of D' Arcy *et al* as described by turner. Animals were divided into four groups of 6 animals in each. The normal controls (Group 1) were provided plain water only, experimental controls (Group 2) were given extract orally in a dose of 100mg/kg/dayfor 10 days, diabetic controls (Group 3) were provided plain water only, and diabetic experimental (Group 4) were given extract in a does of 100mg/kg/dayfor 10 days. On the 10th post wounding day the granulation tissue formed on the implanted cotton pallets was carefully removed under anesthesia. After noting the wet weight of granulation tissue the tissue was dried at 60ºC for 12 hrs and the dry granulation tissue weight was recorded to the dried tissue 5 ml 6N HCl was added and kept at 110ºC for 24 hrs. The neutralized acid hydrolisate of the dry tissue was used for the determination of hydroxyproline[9]. Additional piece of wet granulation tissue was preserved in 10% formalin for histological studies.

3. Incision wounds[10]

A longitudinal Para vertebral incision of six centimeters in length was made through the skin and cutaneous muscle on the back in anesthetized rats. After the incision, surgical sutures were applied at intervals of one centimeter. The wounds were left undressed (day

0). Animals were divided into four groups of 6 animals in each. The normal controls (Group 1) were provided plain water only, experimental controls (Group 2) were given extract orally in a dose of 100mg/kg/dayfor 10 days, diabetic controls (Group 3) were provided plain water only, and diabetic experimental (Group 4) were given extract in a does of 100mg/kg/dayfor 10 days. The sutures were removed on the 8th post wound day and the oral treatment of extract was continued. The skin-breaking strength was measured on the 11th day by tensiometer

4. Statistical analysis

The means of wound area measurement between groups at different time intervals were compared using one-way ANOVA, followed by Tukey's tests. One-way ANOVA was used to examine the mean differences in wound healing between the groups in excision and dead space wound models. Data was analyzed using the Graph Pad Software (5.0- demo version) and *P* value of < 0.05 was considered to be significant.

results

In acute toxicity studies the extract in dose up to 2000 mg/kg body weight did not produce any signs of toxicity or mortality. The animals were physically active and consumed food and water in a regular way. No abnormal behavior was noticed. Significant increase in wound healing activity was observed in leaf extract treated rats.

In excision wound model, animals of group 2 and 4 showed a decrease in the epithelialization period and an increased percentage of wound contraction when compared with the animals of group 1, 3 and 5 (Table 1). On day 11 the extract treated normal & diabetic animals (Group 2 and 4) showed high wound contraction compared with normal & diabetic control groups animals (Groups 1 & 3) (*P<0.001*). The wound contraction results of extract treated animals were comparable with positive controls. The Diabetic extract treated animals showed highest wound contraction.

In Incision wound model (Table: 2), the extract treated rats showed significant increase in wound tensile strength as compared to normal and diabetic control groups.

In the dead space wound model (Table:2), the extract treated animals in groups 2 and 4 showed significantly higher levels of hydroxyproline compared with animals in the normal and diabetic controls groups(Group 1& 3) (*P<0.001*). A significant increase was also observed in dry and wet weight of granulation tissues in the animals treated with the extract (*P<0.001*). Overall the weights of animals were did not differ for any of the study groups.In the animals that did not receive the leaf extract treatment, the wound appered tobe hard and crusty with undermined margins and were generally unclean with a biofilm glaze of the surface. In contrast the wounds in the animals treated with extract were clean and showed bright and red healthy granulation tissue. The wounds treated with soframycin showed the healthy granulation tissue.

S. No.	Groups Parameters	Normal Control	Normal Treated	Diabetic Control	Diabetic Treated
1	Final Wound Area (mm^2)	125.5 ± 0.428	66.33 ± 0.494 ***	190.5 ± 0.520	67.6 ± 0.206 ***
2	Tensile Strength (gm/mm^2)	353 ± 2.472	445 ± 1.826 ***	287± 0.412	461± 0.241 ***
3	Weight of wet Granulation Tissue(mg)	102.5 ± 0.22	153.1 ± 0.16	90.50 ± 0.16	165.6 ± 0.21
4	Weight of Dry Granulation Tissue(mg)	31.5 ± 0.22	45.10 ± 0.16	28.60 ± 0.16	42.30 ± 0.21 **
5	Weight of Hydroxyproline (mg)	55.20 ± 0.22	93.5 ± 0.22	67.50 ± 0.22	91.00 ± 0.44 **

Table. 1. Biophysical and Biochemical Parameters of Cutaneous Wounds in normal and diabetic rats.

3. DISCUSSION

Diabetes mellitus is known to be associated with a variety of alterations in connective tissue metabolism, as a result of which diabetics face the problem of poor wound healing. Loss of collagen observed in diabetes may be due to decreased levels of synthesis or enhanced catabolism of newly synthesized collagen, or both[11]. As *Bougainvillea glabra* choisy was reported to cause hypoglycemic effects, it was felt that it would be interesting to study its influence on the healing of wounds in diabetic conditions. Results obtained in the present study suggest that treatment of diabetic rats with *Bougainvillea glabra* choisy extract may have a beneficial influence on wound healing.

Collagen is the predominant extracellular protein in the granulation tissue of a healing wound and there is a rapid increase in the synthesis of this protein in the wound area soon after an injury. In addition to providing strength and integrity to a tissue matrix, collagen also plays an important role in haemeostasis. Subsequent epithelialization also requires collagen. In the present study, we have examined the influence of *Bougainvillea glabra* choisy extract on the collagen content in granulation tissues of healing full-thickness wounds in diabetic rats. Treatment of wounds with *Bougainvillea glabra choisy* extract increased the maximum levels of collagen in the granulation tissue, as compared to the untreated diabetic control.

Glycosaminoglycans and proteoglycans are synthesized by fibroblasts in the wound area. These substances form a highly hydrated gel-like ground substance, a provisional matrix on which collagen fibres are embeded. As collagen accumulates, hexosamine levels decrease[12]. Treatment with *Bougainvillea glabra choisy* extract increases the content of ground substance in the granulation tissues. It may be seen that the decrease in hexosamine content was associated with a concomitant increase in collagen content.

The wet and dry weight of granulation tissues indicates the levels of protein synthesis and cellular proliferation. Higher protein and DNA contents (compared to the untreated controls) of the treated wounds suggest that *Bougainvillea glabra* choisy extract, through an as yet unknown mechanism, stimulates cellular proliferation. The wet and dry weight ratio of the granulation tissues also suggests that *Bougainvillea glabra* choisy extract gel may increase the synthesis of collagen per cell.

The collagen molecules synthesized are laid down at the wound site and become crosslinked to form fibres. Wound strength is acquired from both, remodelling of collagen, and the formation of stable intra- and inter-molecular crosslinks. Since incisional wounds treated with the *Bougainvillea glabra* choisy extract showed greater tensile strength, it may be inferred that it not only increases collagen synthesis per cell, but also aids in crosslinking of the protein. *Bougainvillea glabra* choisy extract treated wounds also showed an increased rate of wound contraction, leading to quicker healing as confirmed by decreased period of epithelialization when compared to untreated control wounds.

The present study demonstrates that the *Bougainvillea glabra* choisy leaves extract accelerates wound healing in diabetes. The results suggest that *Bougainvillea glabra* choisy extract treatment may have a beneficial influence on the various phases of wound healing like fibroplasia, collagen synthesis and contraction resulting in faster healing. It is quite possible that the enhanced healing of wounds in diabetic rats by *Bougainvillea glabra* choisy extract is a result of its hypoglycemic activity (since a control over blood glucose levels has been shown to improve wound healing in diabetics[13] and/or its capacity to stimulate fibroblast function during the healing process.

4. CONCLUSION

The aqueous extract of *Bougainvillea glabra* choisy leaves treatment showed marked effect on wound healing in normal and diabetic rats. So it may be concluded that this can be a suitable part of treatment procedure of non healing diabetic and normal wounds. Further study is needed to establish exact mechanism of wound healing.

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