

University Grants Commission

Sanctioned

MINOR RESEARCH PROJECT

Entitled

“Evaluation of diabetic wound healing activity of some indigenous plants”

Principal Investigator

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Executive Summary of the Report

Introduction

Diabetes is a chronic disease with elevated blood glucose level and develops several secondary complications like hyperglycaemia, atherosclerosis, retinopathy, ketoacidosis, and diabetic foot formation. Due to increased blood glucose level and abnormal metabolism, it delays the normal wound healing process. In diabetic patient abnormal wound healing turns into diabetic foot ulcers and eventually leads to leg amputation. In developing countries, wounds are considered as the most severe complication. The available wound healing management modalities are expensive and limited. Natural phytoconstituents of the plants such as flavonoids have scavenging activity against free radicals; these flavonoids accelerate the process of wound healing. The treatment using medicinal plants may cut the economy loss on cost of amputation and related complications. Our present studies can explore the best alternative for promoting the diabetic wound healing. The study will be useful for development of drug from plants for treatment of diabetic wound.

Objectives

1. To assess the excision, incision wound healing activity of *Capparis aphylla* and *Dendrocalamus strictus* in normal albino rats.
2. To estimate the excision, incision wound healing activity of *Capparis aphylla* and *Dendrocalamus strictus* in experimentally induced diabetic albino rats.
3. To evaluate the Angiogenesis properties of *Capparis aphylla* and *Dendrocalamus strictus* using CAM Assay.

Plants under study:

Dendrocalamus strictus: Family: Poaceae

Capparis aphylla or *decidua* : Family: Capparaceae

Methods

Chicken ChorioAllantoic Membrane (CAM) Assay

CAM assay was used to detect angiogenic activity of aqueous and hydroethanolic extracts of *Capparis aphylla* and *Dendrocalamu strictus* extracts.

Acute dermal toxicity testing of extract of medicinal plants (OCED 402)

From the results of CAM assay, angiogenesis stimulating extracts were selected for further studies of wound healing activity.

Wound healing activity of extract using experimental animal model

Three different experimental animal models of wound healing namely, incision, excision, and dead space experimental models, were used to evaluate aqueous and hydro-ethanolic plant extracts for wound healing activity.

Incision wound assay; Dead space assay; Excision wound assay; Estimation of collagen content by hydroxyproline assay; Histology of skin tissue.

Statistical analysis was done using Graph Pad Prism (GraphPad Software, Inc.) statistical software by one way analysis of variance (one way ANOVA) with Tukey's multiple comparison post hoc tests to compare the means of more than two independent groups.

Results

The present work examines the efficacy of medicinal plants in the healing of diabetic wounds. The selected plants of the study are *Capparis aphylla* and *Dendrocalamus strictus*. These medicinal plants are traditionally used as wound healing agents and their wound healing activity had been reported in the literature. The present work is undertaken to investigate angiogenic activity and the wound healing potential of selected plants in diabetic wounds.

Aqueous extracts of *Capparis aphylla* and hydroethanolic extract of *Dendrocalamus strictus* both showed significant anti-angiogenic activity when compared to control groups. In hydroethanolic extract of *Dendrocalamus strictus* treated chick embryo, the relative number of blood vessels and branching pattern was decreased ($p < 0.05$), when compared to control of saline treated vehicle embryo.

On the contrary, aqueous extract of *Dendrocalamus strictus* and hydroethanolic extract of *Capparis aphylla* showed significantly high number of primary, secondary and tertiary blood vessels. It proved that these plant extracts has significant capability of neovascularization.

Acute Dermal Toxicity test showed that at maximum dose level of 2000 mg/kg the animals did not induce mortality within 14 days. The animals were normal with no sign of irritation, redness or itching, motility, nasal secretion and eye irritation.

In the present study, we observed significant increase in blood glucose levels in Sham (Diabetic Control) group and plant extract treated group of rats after 72 hours of STZ administration when compared to blood glucose level of initial day. During the experimental period of 14 days, the control rats appeared healthy, active and gained body weight.

The results indicates that hydroethanolic extract of *C. aphylla* significantly contracted the wound compared to control group, aqueous extract of *D. strictus* treated animal group and Sham group. Hence, the hydroethanolic extract of *C. aphylla* treated animal's showed substantial noticeable effect.

The skin and granulation tissue isolated from rats treated with hydroethanolic extract of *D. strictus* and aqueous extract of *C. aphylla* ($p < 0.05$) showed significant increase in collagen content of skin and granulation tissue than the sham group and control group.

Measurement of collagen content in control, sham and plant extracts treated wound tissues infers that hydroethanolic extract of *Capparis aphylla* and aqueous extract of *Dendrocalamus strictus* improved the production of new collagen.

Histology studies revealed that the hydroethanolic extract of *Capparis aphylla* and aqueous extract of *Dendrocalamus strictus* treated diabetic rats showed more progressive re-epithelialization, newly formed collagen fibres, refurbishment of matured granulation tissue with neovascularization and fibrosis in the dermis layer. The histopathological studies proved significant wound-healing ability of hydroethanolic extract of *Capparis aphylla* and aqueous extract of *Dendrocalamus strictus*.

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