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Original Article

Wound healing potential of *Catharanthus Roseus* on dead space wound in diabetic rats

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ABSTRACT

Catharanthus roseus L (C.roseus) is a plant that has traditionally been used to treat a wide range of illnesses, including diabetes. The researchers wanted to test if a Catharanthus flower extract might cure streptozotocin induced lesions in diabetic rat. Dead space incisions were made on the diabetic rats axillas. The rats were divided into three treatment groups at random for eight days (Group I: Normal saline; Group II: Diabetic control; Group III: *C.roseus*). On day 10, the animals were killed, cotton pellets and granuloma tissues were meticulously collected and processed for further analysis. Overall 18 rats were utilised in the experiment. During the trial, the *C.roseus* group had a substantial increase in dry and wet tissue weight when compared to the other groups. In addition, as compared to the control, *C.roseus* treatment dramatically enhanced Hydroxy proline, Hexosamines, Hexuronic acid, Tissue protein, and Lysyl oxidase. The increased hydroxyl proline content, as well as other important bio chemicals, support the use of *C. roseus* for topical wound healing treatment.

Key words: *C. roseus*, Wound healing; Diabetic; Dead space wound; Granulation tissue; Streptozotocin.

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INTRODUCTION

Wound healing is the process of repairing the skin and other soft tissues after an injury. An inflammatory reaction happens after an injury, and cells below the dermis (deepest skin layer) begin to produce more collagen (connective tissue). The epithelial tissue (outer skin layer) regenerates later. Wound healing is divided into three stages: inflammation, proliferation, and remodelling. Angiogenesis, collagen deposition, epithelialization, and wound contraction define the proliferative phase. Angiogenesis is the formation of new blood vessels by endothelial cells. Fibroblasts use collagen and fibronectin to build a new, temporary extracellular matrix during fibroplasia and granulation tissue development.[1] Wound healing is frequently hindered in people with diabetes mellitus (DM), resulting in non-healing, delayed healing, or persistent skin ulcers.[2] In diabetes, delayed wound healing can be caused by an imbalance in the inflammatory response, changed cytokine production, altered collagen synthesis, insufficient angiogenesis, extracellular matrix differentiation, lower tensile strength, or diminished growth factors.[3,4]

C. roseus L (apocyanaceae), popularly known as Vinca Rosea, is a Caribbean native that has long been used to cure a variety of ailments. The plant was utilised by European herbalists for a variety of ailments, including headaches and diabetes. It contains about 400 alkaloids, some of which are used as antineoplastic medicines to treat malignancies such as leukaemia, hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilms' tumour, and others. It has been proven to help with vascular dementia and Alzheimer's disease because of its vasodilating and memory enhancing effects.[5,6] Alkaloids and tannins are the two types of active chemicals found in Vinca.

The main alkaloid, vincamine, and its closely related semi synthetic derivative, ethyl apovincaminate or vinpocetine, which is commonly used as a medical drug, possesses vasodilating, blood thinning, hypoglycemic, and memory boosting properties.[7,8] Vinca extracts have been shown to have potent anticancer properties in a variety of cell types.[9] In certain rural cultures, extracts from dried or wet flowers and leaves of plants are used as a paste to wounds. Ayurvedic practitioners in India have utilised the fresh juice from the blossoms of *C. roseus* prepared into a tea to cure skin issues such as dermatitis, eczema, and acne. To the best of our knowledge, there is no previous report in the literature on the wound healing activities of *C. roseus* in streptozotocin-induced diabetic mellitus rats, and in this paper, we report for the first time the efficacy of *C. roseus* flower extract in the treatment of diabetic rat wounds.

MATERIALS AND METHODS

Preparation of extracts: Fresh *C.roseus* flowers were gathered, dried in the shade, and ground into a powder. For 20 hours, the fine powder (50 g) was suspended in 100 mL ethanol at room temperature. The mixture was filtered using a fine muslin cloth, followed by filter paper (Whatman No: 1). After drying the filtrate in a water bath at 40°C, the clear residue was used for the research.

Animals: Healthy Wistar rats of either sex (150–200 g) were utilised in this study with no prior pharmacological treatment. The animals were fed a commercial pellet diet and given unlimited access to water. The animals were acclimatised to laboratory hygienic conditions for 10 days prior to the start of the trial. The therapy was carried out with the approval of the animal ethics committee of King Khalid University, as well as the National Institute of Health's standards for the care and use of laboratory animals in the United States (NIH Publication No. 85-23, revised 1996). For the dead space wound model, animals of either sex were divided into three groups, each with six animals: Group I received ointment b; group II received diabetic control; and group III received *C.roseus* (100 mg/kg/day). The extracts were administered orally to the individual animal groups once a day.

Wound healing activity:

Dead Space wound model: The technique described by Rathi et al. was used to produce the dead space wounds.[10] A total of eighteen rats were divided into three groups, each consisting of six individuals. Subcutaneous dead space wounds in the vicinity of the axilla were produced by creating a pouch through a tiny nip in the skin under general anaesthesia (achieved with 10 mg/kg body weight of xylazine hydrochloride and 50 mg/kg ketamine hydrochloride). The development of granulomas was induced by implanting sterile cotton pellets (30 mg) into each axilla. Sutures were used to close the wounds, and an alcoholic swab was used to clean them up. After being grouped together, the animals were separated into individual metal cages to prevent them from biting each other's wounds.

For 8 days, the treatment groups were given extract or normal saline (1 ml/kg). Rats were euthanized on day 10 and the cotton pellets and granuloma tissues were carefully removed, dried in a 60°C oven, weighed, and compared to the control. 5mL 6 N HCl was applied to the dry tissue and maintained at 1108C for 24 hours. The hydroxyl proline, hexosamine concentration, and hexuronic acid were determined using the neutralised acid hydrolyzate of the dry tissue. For the measurement of lysyl oxidase and tissue protein, a piece of the moist granulation tissue was utilised.[11]

Induction of diabetes: Streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer pH 4.5 at a dosage of 65 mg/kg intra peritoneal (i.e., 15 minutes after intra peritoneal injection of 110 mg/kg body weight nicotinamide) was used to induce diabetes in overnight starved rats (Himedia labs Pvt. Ltd.). After 6 hours of STZ treatment, the rats were given a 10% glucose solution for additional 24 hours to prevent hypoglycemia caused by large pancreatic insulin secretion. Blood was collected from the tail veins of the rats 72 hours after the STZ injection, and rats with a fasting blood glucose level of more than 200 mg/dl were classified as diabetic and used in this investigation.[12]

Statistical analysis: The information is presented as a mean with a Standard Error Mean (SEM). The differences between means were investigated using one way analysis of variance (ANOVA), with p values less than 0.05 deemed significant. The data was analysed using one way analysis of variance (ANOVA) with a post hoc Scheffe's test in Graph Pad, and the mean and standard deviation were calculated. P values less than 0.05 were deemed statistically significant.

RESULTS

The animals treated with the *C. roseus* extract showed a substantial increase in wound-healing activity when compared to those that received placebo control treatments. The effects of the ethanolic extract *C. roseus* flower given orally at a dosage of 100 mg /kg/day for 8 days on wound healing activity in rats with dead space wounds are shown in Table 1. When compared to diabetic and control rats, the *C.roseus* treatment group had substantially higher granulation tissue breaking strength and wet and dry granulation tissue weight (table 1).

Table 1: Physical and biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Groups	Blood glucose (mg/dl)	Wet tissue weight (mg/ 100g rat)	Dry tissue weight (mg/ 100g rat)	Tissue breaking strength (g)
Wounded Control	82.3 ± 8.2	250.5 ± 14.19	34.68 ± 5.40	284.45±14.45
Diabetic Control	277.18 ± 12.1[a]	159.5 ± 11.32[a]	23.5 ± 4.60[a]	181.46±1.47[a]
C.roseus	285.38 ± 11.1[a]	282.5 ± 14.09[a]	34.5 ± 5.50[a]	317.46±14.28[a]

Values are mean ± SD of 6 replications. P values: [a]:<0.01vs control.

In diabetic rats produced by streptozotocin, the concentration of hydroxyl proline in granulation tissue was substantially lower. The experimental group had considerably lower levels of glycol saminoglycan contents such as hexuronic acid and hexosamine. When diabetic rats were compared to control rats, tissue protein content was extremely low. In the experimental group, the amount of lysyl oxidase was substantially lower. When compared to diabetic and control rats (group II), all of the above metrics considerably improved in the *C.roseus* treatment group (table 2).

TABLE 2: Biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Groups	Hydroxyproline (mg/g tissue)	Hexosamines (mg/g tissue)	Hexuronic acid (mg/g tissue)	Tissue protein (mg/g tissue)	Lysyl oxidase (SFU)
Wounded control	13.72 ± 5.12	13.49 ± 2.47	13.11 ± 3.19	42.58 ± 3.80	1712 ± 67
Diabetic Induced	12.38 ± 4.10[a]	8.1 ± 1.30[a]	9.6 ± 1.42[a]	27.5 ± 2.60[a]	1125 ± 46[a]
C.roseus	15.72 ± 4.52[a]	14.49 ±2.47[a]	14.31 ±3.29[a]	45.58 ±3.89[a]	1916 ± 63[a]

Values are mean ± SD of 6 replications. (SFU- Spctroflourimetric units), P values: [a]:<0.01 vs control.

DISCUSSION

The current study was a worthwhile attempt to determine the efficacy of the plant *C. roseus* in diabetic wound healing, since the plant has been known to have substantial anti diabetes and wound-healing properties. Streptozotocin is commonly used to cause diabetes in a number of animals by causing pancreatic β-cell degeneration and necrosis. Similarly, the current investigation utilized STZ induced diabetes and a dead space wound model to assess wound healing capacity.

Wound healing is a complicated and dynamic process that restores injured tissue's cellular structures and tissue layers as closely as possible to their original condition. It has three phases: inflammatory, proliferative, and maturational, and is determined by the type and amount of injury, the host's overall health, and the tissue's ability to mend. Hemostasis and inflammation define the inflammatory phase, which is followed by epithelization, angiogenesis, and collagen deposition in the proliferative phase. The wound contracts in the maturational phase, the last step of wound healing, resulting in a decreased quantity of visible scar tissue.

Granulation tissue is made up largely of fibroblasts, collagen, edema, and new tiny blood vessels and forms in the last stages of the proliferative phase. Higher protein content is suggested by the rise in dry granulation tissue weight in test treated animals. The hydroxyl proline concentration of granulation tissue increased significantly after treatment with an ethanol extract of *C.roseus*, indicating enhanced collagen turnover. Collagen is made primarily of the amino acid hydroxyl proline, which has been utilized as a biochemical marker for tissue collagen. It is the main component that builds and maintains extracellular tissue.[13]

The floral extract included tannins, triterpenoids, and alkaloids, according to early phytochemical investigation. The wound healing action of *C. roseus* might be attributed to any of the phytochemical components found in the plant. Recent research has found that phytochemical components such as flavanoids and triterpenoids[14,15] enhance wound healing, owing to their astringent and antibacterial characteristics, which appear to be responsible for wound contraction and higher epithelialization rates. The previous research revealed that Cecropia peltata [16] and Pentas lanceolata contain triterpenoids, which are responsible for the plants wound-healing abilities.[17] In the *C.roseus* treatment group, the levels of hydroxyl proline, hexuronic acid, and hexosamine increased. Enhanced lysyl oxidase activity in our study might lead to increased granulation tissue crosslinking and breaking strength. The wound-healing ability of *C. roseus* might be ascribed to the phyto-components found in the plant, and the faster wound healing process could be a result of the individual or cumulative actions of the phyto-constituents. We intend to undertake more research on the phytochemical elements of *C. roseus* that contribute to its pharmacological efficacy in diabetic rat.

Conclusion: The current study found that an ethanol extract of the *C. roseus* flower possesses characteristics that enable it to promote faster wound healing in diabetic rats when compared to placebo controls. Increased hydroxyl proline, hexosamines, hexuronic acid, tissue protein, and lysyl oxidase promote future research into *C. roseus* as a topical wound therapy.

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