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Original Article

Calendula Officinalis enhance the wound healing potential in diabetic rats

Saravanan VS[1], Kalpana Krishnaraju[1], Manimekalai Pichaivel[2], Krishnaraju Venkatesan[3*], Premalatha Paulsamy[4], Divya Kuppan[5]

[1]Erode College of Pharmacy, Veppampalayam, Erode, India
[2]Professor & Head Department of Pharmacology, SVCP, Tiruchengode,Tamilanadu, India
[*3]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, KSA
[4]King Khalid University, Khamis Mushayit, Asir Province, Saudi Arabia.
[5]JKK Nataraja College of arts and science, Kumarapalayam, Namakkal District, India

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ABSTRACT

The goal of this study was to see how well *Calendula.officinalis (C.officinalis)* could treat dead space wounds in rats in vivo. On each axilla of diabetic rats, dead space incisions were created. For eight days, the rats were randomly assigned to one of three treatment groups (Group I: Normal saline; Group II: Diabetic control; Group III: Tannin). Animals were euthanized on day 10, and cotton pellets and granuloma tissues were carefully collected and processed for further estimates. Tissue breaking strength, dry and wet weight, and biochemical markers including hydroxyproline, hesosamine, and tissue protein were used to assess healing capacity. The extract was taken orally and had a favourable effect on the wound. The *C.officinalis* extract shows considerable wound healing activity in diabetic wounds, based on the preceding findings.

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

INTRODUCTION

Wounds are clinical entities that occur often in everyday life. A wound is a break in the continuity of live tissue caused by an injury. Wounds are inconvenient, and they are more prone to infection and other problems. Some illnesses, such as immune compromised states, ischaemia, and malnutrition, ageing, local infection, and local tissue injury, cause wound healing to be delayed.

Wound healing is a complex process in which the skin heals itself following an injury. Inflammatory, proliferative, and remodelling phases are the three stages of wound healing. Increased blood flow, increased capillary permeability, and increased leucocyte migration in the affected area characterise the inflammatory phase. Granulation, contraction, and epithelisation are all characteristics of the proliferative phase.

Address for Correspondence: Dr. Krishnaraju Venkatesan, Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, KSA; Email: kvenkatesan@kku.edu.sa

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The strength and appearance of the healed region are determined during the remodelling phase.[1] *Calendula.officinalis (C.officinalis)* often known as pot marigold, is a common Asteraceae plant that is native to southern Europe. Phyto-chemicals found in the plant species include carbohydrates, phenolic compounds, lipids, steroids, tocopherols, terpenoids, quinones, and carotenoids [2-5] all of which have various health effects[6-8] and [9]Triterpendiol esters, saponins, and flavonoids such as rutin and hyperoside are among the plant's most active components. This plant is utilised in the form of infusions, tinctures, liquid extracts, lotions, and ointments for medical purposes. This factory also produces skin care items that are sold all over the world.

The goal of this study was to see how effective aqueous extracts of *C.officinalis* were at healing wounds on excision-wounded rats. Wound healing is frequently hindered in people with diabetes mellitus (DM), resulting in non-healing, delayed healing, or persistent skin ulcers.[10] 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.[11,12] Incision and excision wound models were used to investigate the effects of tannin on wound healing activities. The tannin impact on a diabetic wound model, on the other hand, is unknown. As a result, the goal of this study is to see how *C.officinalis* affects wound in streptozotocin (STZ) induced diabetes in rats.

MATERIALS AND METHODS

Preparation of extract: *C.officinalis* leaves (100g) were coarsely pulverized. In a Soxhlet extractor, the powdered materials were loaded and defatted using petroleum ether (40-60ºC). The marc was dried and extracted three times with ethanol (50 percent v/v) in the same extractor. Finally, using a rotary evaporator under vacuum, the extracts were condensed to a semi solid mass. The solvent was removed from the dried extract by placing it in a desiccator.

Animals: Healthy Wistar rats of either sex (150–200 g) were utilised in this study, and no prior pharmacological therapy was given to them. The animals were fed a commercial pellet diet and given unlimited water. The animals were given a 10 day acclimatisation period before starting the experiment. The therapy was carried out with the approval of the animal ethics committee of King Khalid University and in compliance with 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- Normal control; group II-diabetic control; and group III received *C.officinalis* (100 mg/kg/day). The extracts were administered orally to the individual animal groups once a day.

Wound healing activity:

Dead Space wound model: Rathi et al. described a technique for creating dead space wounds.[13] Eighteen rats were divided into three groups of six individuals each. Subcutaneous dead space wounds were produced in the area of the axilla 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) in each axilla. Sutures were used to close the wounds, which were then cleaned with an alcoholic swab. After being grouped together, the animals were placed individually in a metal cage 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. The hydroxyproline, 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.

Induction of diabetes: The overnight starved rats were given a newly produced solution of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer pH 4.5 at a dosage of 65 mg/kg intraperitoneally (i.p.) 15 minutes after receiving 110 mg/kg body weight nicotinamide (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 those with a fasting blood glucose level of more than 200 mg/dl were classified as diabetic and used in this investigation.[14]

Statistical analysis: The data is presented as a mean with a standard deviation (SEM). The differences between means were investigated using one way analysis of variance (ANOVA), with p values less than 0.05 being 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.

RESULTS

Animals given the *C.officinalis* extract showed a substantial increase in wound-healing activity when compared to those given sham treatments. The effects of C.officinalis, given orally at a dosage of 100 mg kg-1 day-1 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.officinalis* treatment group's granulation tissue breaking strength and wet and dry granulation tissue weight were considerably higher (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	82.1 ± 6.0	245.5 ± 14.09	30.38 ± 5.20	275.19±15.17
Diabetic Control	276.38 ± 14.1[a]	169.5 ± 10.32[a]	26.5 ± 4.40[a]	166.41±1.30[a]
C.officinalis	281.18 ± 12.1[a]	279.5 ± 12.19[a]	30.5 ± 4.20[a]	313.19±13.37[a]

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

In diabetic rats produced by streptozotocin, the concentration of hydroxyproline in granulation tissue was substantially lower. The experimental group had considerably lower levels of glycosaminoglycan 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. In comparison to diabetic and control rats (group II), all of the following metrics significantly increased in the *C.officinalis* 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	13.42 ± 4.12	11.39 ± 2.47	11.03 ± 3.19	44.68 ± 3.70	1714 ± 59
Diabetic Induced	10.28 ± 2.20a	8.1 ± 1.30a	8.5 ± 1.42a	26.5 ± 2.40a	1128 ± 37a
C.officinalis	14.72 ± 4.12[a]	13.49 ± 2.47[a]	14.01 ±3.19[a]	43.48 ± 3.70[a]	1910 ± 68[a]

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

DISCUSSION

The levels of hydroxyproline and hexosamine, as well as the tissue protein content of the granulation tissue of the dead space injured animals, are shown in Table 2. When normal control injured rats were compared to positive control rats, the amounts of hydroxyl proline, hexosamine, and tissue protein were considerably lower. These levels were raised after treatment with *C.officinalis*. Collagen is a key extracellular matrix protein that helps for wound strength. Collagen not only gives the tissue matrix strength and stability, but it also aids in wound healing by promoting homeostasis and epithelialization.[15] Hydroxyproline is a rare amino acid found in granulation tissue collagen fibres. After applying herbal ointment topically, researchers discovered a rise in hydroxyproline concentration, which indicates enhanced cellular proliferation and hence higher collagen production. Hexosamine and hexuronic acid are matrix molecules that serve as the starting point for the production of new extracellular matrix. Collagen fibres are known to be stabilised by glycosaminoglycans, which improve electrostatic and ionic interactions with them and may influence their eventual alignment and size. They've been discovered as key determinants of cellular response in development, homeostasis, and illnesses because of their capacity to bind and modify protein interactions.[16]

Hexosamine concentrations were substantially higher in the herbal ointment treated groups than in the excision wound control groups in the current investigation, indicating that collagen fibres were stabilised.[17] As a result, damaged tissue with increased hydroxyproline and hexosamine production has the ability to heal. Protein is required for wound healing and granulation tissue formation.

The low protein concentration in excision injured controls indicates that wound healing is delayed due to a prolonged inflammatory phase, fiber plasia

inhibition, and remodelling phase. The animals treated with herbal ointment had a simultaneous rise in total protein content, indicating active synthesis and deposition of matrix proteins in the granulation tissues, which improved wound healing.[18] Streptozotocin has been frequently used to cause diabetes in a number of animals by causing pancreatic β-cell degeneration and necrosis. Similarly, the current investigation comprised STZ induced diabetes followed by an assessment of wound healing capacity utilising a dead space wound model.

Conclusion: Finally, it is reasonable to infer that *C.officinalis* has considerable wound healing activity as a consequence of enhanced collagen production, wound contraction, and biochemical marker changes, based on the findings of this study. In the future, *C.officinalis* isolated components will be utilised in wound models.

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