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Research Article

Medicine for novel therapy

Evaluation of Wound Healing Activity of *Catharanthus Roseus* Aqueous Extract in Adult Albino Rats

A M Satish[1]*, Jayanthi M. K[1], Totagi Vinod Gangadhar[1] and Ramith Ramu[2]

[1]*Department of Pharmacology, JSS Medical College, JSS Academy of Higher Education and Research (JSS AHER) Mysuru – 570015, Karnataka, India*

[2]*Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research (JSS AHER) Mysuru-570015, Karnataka, India.*

Abstract: Use of plant extracts in the treatment of several ailments is a science known to the mankind since time immemorial. Yet, its popularity is limited owing to the lack of recorded experimental evidence to determine their potential health benefits. *C. roseus* is a traditional medicinal plant with over 100 identified alkaloids and several other phytochemicals known for various health promoting activities. The aim of the present study was to evaluate *C. roseus* aqueous extracts' wound healing potential using incision, excision, and dead space wound models on healthy male albino rats. To assess this, the tissue breaking strength for incision wound model, percent epithelialization and period to complete epithelialization for excision wound model and granuloma break strength, dry weight, and hydroxyproline content for dead space wound model were evaluated after the treatment of the extract in comparison with the standard drug sucralfate. The results obtained demonstrated that the treatment of extract on the wound significantly enhanced tissue breaking strength and reduced the time required for complete epithelialization and was on par with that of the standard drug sucralfate. In the incision wound model, an elevated breaking strength was observed compared to that of the control group. Further, in the dead space wound model, the granuloma dry weight and breaking strength were remarkably elevated along with a concomitant increase in the hydroxyproline content suggesting the enhanced rate of wound contraction rate, collagen synthesis and lowered healing period. In conclusion, the present study demonstrates that *C. roseus* is effective in wound healing, as shown in the three wound models and thus, further studies to formulate this into a commercial product should be carried out.

Keywords: *Catharanthus roseus;* wound healing; aqueous extract; incision wound model; excision wound model; dead space wound model

***Corresponding Author**

A M Satish , Department of Pharmacology, JSS Medical College, JSS Academy of Higher Education and Research (JSS AHER) Mysuru – 570015, Karnataka, India

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1. INTRODUCTION

A cut or break caused on the skin surface leads to disruption of normal integrity of the skin, in turn causing impairment in the anatomic and cellular functionality.[1] They are mainly classified based on their symptoms, aetiology, location, depth and clinical appearance of the wound.[2] It involves complex biological processes such as inflammation, matrix synthesis and deposition, coagulation, angiogenesis, fibroplasia, epithelialization, contraction, and remodelling working in tandem to bring about wound healing.[3] Delayed wound healing has important clinical implications in patients with diabetes, anaemia, obesity, and other comorbidities. It can be caused due to various factors such as malnourishment, ischemia, immunosuppressive drugs, reactive oxygen species, and so on. Wounds that fail to heal under normal circumstances reach a stage of pathological inflammation owing to uncoordinated healing. Such chronic wounds have affected several million people across the globe, with over 85% occurring in patients over the age of 65.[4] Agents with enhancing wound healing potential are intended to cause speedy recovery, thereby preventing amputation and other complications. Attempts to identify such chemical and herbal agents in the past have led to identification of several herbal remedies. Indian medicinal system relies on traditional medicinal formulations for the treatment of several diseases. Over 80% of the population depend upon herbal medicines to treat ailments as simple as cold and cough to chronic conditions such as diabetes[5]. *Catharanthus roseus* is a commonly known medicinal plant belonging to the family of Apocynaceae. It is commonly called periwinkle, this ornamental plant is known for several health benefits[6]. Over 100 alkaloids and many other phytochemicals belonging to the class of flavanoids, phenolics and others have been identified from this plant. Studies carried out to identify the biological role of these alkaloids led to the identification of two popularly known compounds vincristine and vinblastine from the class of terpenoid indole alkaloids[7]. Use of plant extracts enriched with these bioactive compounds in traditional formulations has given way for understanding their enthnomedicinal potential in the treatment of several health ailments. The alkaloids from this plant are identified and approved as antineoplastic agents in diseases ranging from malignant lymphomas, leukemia, Wilms tumor, Hodgkin's disease, and many other cancer types.[8]Vincamine is one such alkaloid with potential blood thinning, vasodilating, memory enhancing and hypoglycaemic properties.[9] Traditional records have shown the application of wet flower and leaf extract from the plant on wound surfaces. Ayurvedic physicians prepared juice from flower prepared in the form of tea was administered for the treatment of skin ailments such as acne and eczema[8]. Previously, *C.atharanthus roseus* flower extract was evaluated for its wound-healing activity in Sprague Dawley rats.[7] This study demonstrated that the tensile strength and wound contraction were remarkably increased that corresponded to an increase in the hydroxyproline content. This wound-healing property of *C. roseus* may be attributed to the phytoconstituents present in the plant. The quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. With this background, the objectives of the present study were to evaluate the wound healing properties of *C. roseus* flower extract on cutaneous wound models of adult albino rats using sucralfate as a standard.

2. MATERIALS AND METHODS

2.1. Plant Material Preparation

C. roseus flower was collected from a local vendor and its authentication was performed by the Department of Horticulture, Government of Karnataka, Mysore, India, and its voucher specimen (No. 749732) was deposited at the herbarium. The freshly obtained flowers were dried under the shade and ground into fine powder using a kitchen blender. The fine powder was suspended in water with the help of gum acacia overnight at room temperature to obtain a homogenous solution. The obtained mixture was filtered using Whatman No. 1 filter paper and the filtrate obtained was placed on a water bath to dry at 40°C to obtain a waxy residue. This extract was dissolved in water using gum acacia to the desired concentration and used for further studies.

2.2. Animals

Healthy, male, adult albino rats procured from the central animal house were used to study the various parameters of wound repair. After availing the animal ethical committee clearance (JSSMC/IAEC/02/July 2013 dt. 23/12/2013, the body weight of these animals varied from 150 to 250 gms and the age ranged from 8-10 months. All the animals were kept in the laboratory for about a week and the backs of the animals were depleted prior to the day of experimentation without causing any injury. They were then placed in individual cages after recording the respective body weights. The animals were starved with water ad libitum overnight prior to wounding. The excision, sutured incision and dead space wounds were inflicted aseptically under mild dose of ether anaesthesia.

2.3. Incision Wound Model

The back of the anesthetized animals were shaved using sterile blades. Two parallel straight incisions of 6 cm were inducted using a sterile scalpel and stitched back with a black silk at an interval of 1 cm using a curved needle and surgical thread. The threads were tightened on either ends for optimal closure of the wounds. The plant extract was topically applied for treatment of the wound for 10 days along with sucralfate as a standard. For this treatment, the animals were divided into three groups with six in each group. The first group was treated with gum acacia, the second with sucralfate as a standard and the third group was treated with *C. roseus* aqueous extract (CE). The day of introduction of wound was treated as day 0 whereas the sutures were removed after 10 days of wounding. The weight required to break open the wound was measured using tensiometer on day 10 and represented in grams.[10]

2.4. Excision Wound Model

The anaesthetised animal was secured to the operation table in the natural position. An impression was made on the dorsal intercapular region 5 mm away from the ears using a circular seal of 2.5 cm diameter as described by Morton and Malone (1972).[11] Full thickness skin from the demarcated area

was excised to get a wound area of approximately 500 mm^2. After achieving haemostasis by blotting the wound with a cotton swab soaked in normal saline, the animal was placed in its individual cage. The physical attributes of healing namely, wound closure (contraction), epithelization time and scar features were studied in this model.

2.5. Dead Space Wound for Granuloma Studies

Physical, mechanical and biochemical changes in the granuloma tissues were studied[l]. Subcutaneous dead space wounds were inflicted in the region of the axilla and groin aseptically by implanting either sterile cotton pellets weighing 10 mg or cylindrical grass piths measuring 25 x 3 mm in diameter under mild dose of ether anaesthesia. The wounds were sutured and excision of the granuloma from the surrounding tissue was performed on the eleventh post-wounding day under ether anaesthesia. Cotton pellet granuloma excised from dead space wounds were dried overnight at 60° C in an incubator so as to obtain a constant dry weight. Their weights were noted and expressed as mg/100 gm body weight as suggested by Dipasquale and Meli (1965).[12] After the tabulation of dry weight, they were subjected for the assessment of hydroxyproline content using standard procedure as described by Nasir et al. (2016).[13]

3. STATISTICAL ANALYSIS

The experiments were performed in triplicates. Results were expressed as mean ± SE. Statistical comparisons between the treatment groups and control were performed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS Software (version 21.0, Chicago, USA). [14-15]

4. RESULTS

4.1 Incision Model

The treatment with CE led to a remarkable increase in the breaking strength of the incision wound (193.66 g) when compared with that of the control group (186.66 g). The results were comparable with that of the standard drug treated group (208.66 g). It was noted that the increase in breaking strength was statistically significant ($P < 0.05$) as shown in Figure 1.

4.2 Excision Model

The study showed that there was a remarkable increase in the wound contracting ability exerted by CE when compared with that of the control group. The wound closure was tabulated from day 4 (19.07, 18.87 and 18.03 for control sucralfate and *C. roseus*, respectively) to day 18 (86.27, 92.65 and 91.27 for control sucralfate and *C. roseus*, respectively), which demonstrated a noteworthy improvement. The closure time was lowered by the application of CE along with the reduction in epithelialization that was observed in the excision model (Figure 2). The overall progression of wound healing after the administration of the extract is represented in Table 1.

4.3 Dead Space Model

The study revealed that after 10 days the tissue granulation (Figure 3) was significantly promoted by improving the breaking strength after the treatment with CE (226.66 g) when compared with that of the control and sucralfate treated groups (193.33 and 220.66 g, respectively). In addition, there was an elevation of dry tissue weight after the treatment (27.98 g). Similarly, the biochemical estimation performed by evaluating the hydroxyproline content revealed that the treatment of CE led to a noteworthy increase in its content suggesting the beneficial effects of the extract on wound healing (Figure 4). Overall, the findings from the study indicate the ability of the extract in improving the collagen content and in turn improvement in the breaking strength.

Table 1 Wound contraction percentage and epithelialization period in excision wound model

Groups	% Closure of Incision Wounds [x]					Time for complete epithelialization (days)
	Day 4	Day 8	Day 12	Day 16	Day 18	
Control	19.07±4.31[b]	47.78±4.18[a]	78.13±5.03[a]	84.12±5.43[a]	86.27±5.31[a]	19.17±1.17[a]
Sucralfate	18.87±2.10[b]	54.38±7.25[c]	85.67±2.42[c]	89.67±4.28[c]	92.65±3.30[c]	18.33±1.03[a]
Catharanthusroseus	18.03±1.79[a]	51.20±5.27[b]	84.62±2.41[b]	89.35±4.18[b]	91.27±3.45[b]	18.50±0.55[a]

[x]Values are expressed as mean ± SD. Means in the same column with distinct superscripts are significantly different ($p \leq 0.05$) as separated by Duncan's multiple range test

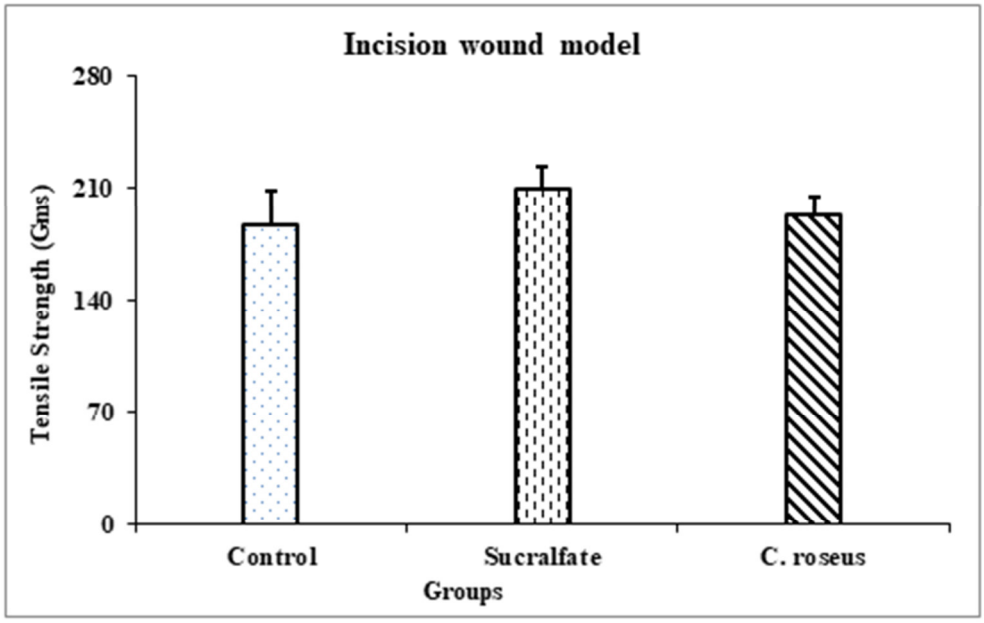

Fig 1: Measurement of breaking strength in incision model

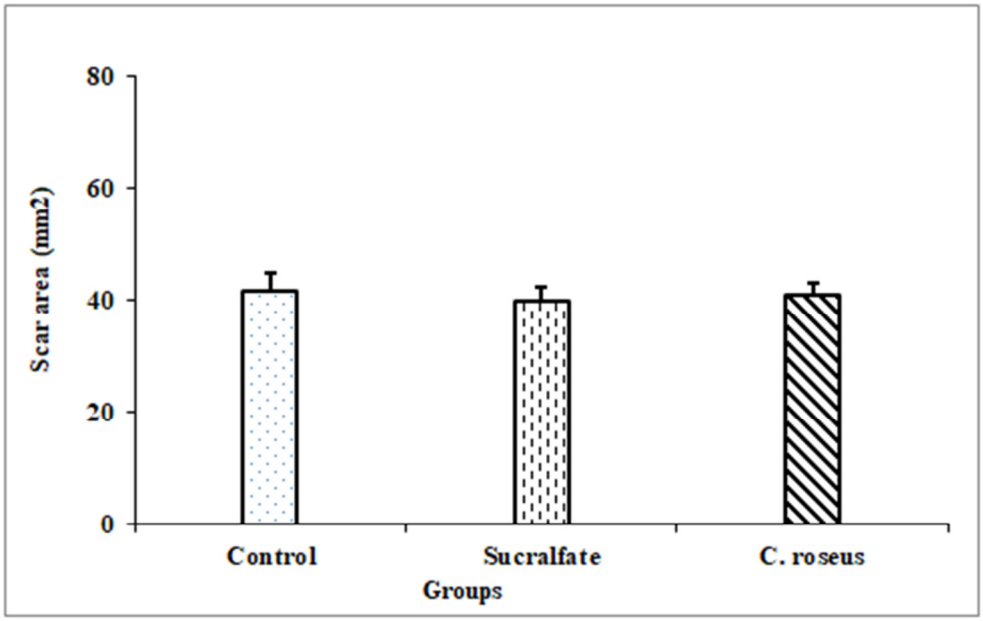

Fig 2: Measurement of scar area (mm^2) on complete epithelialization

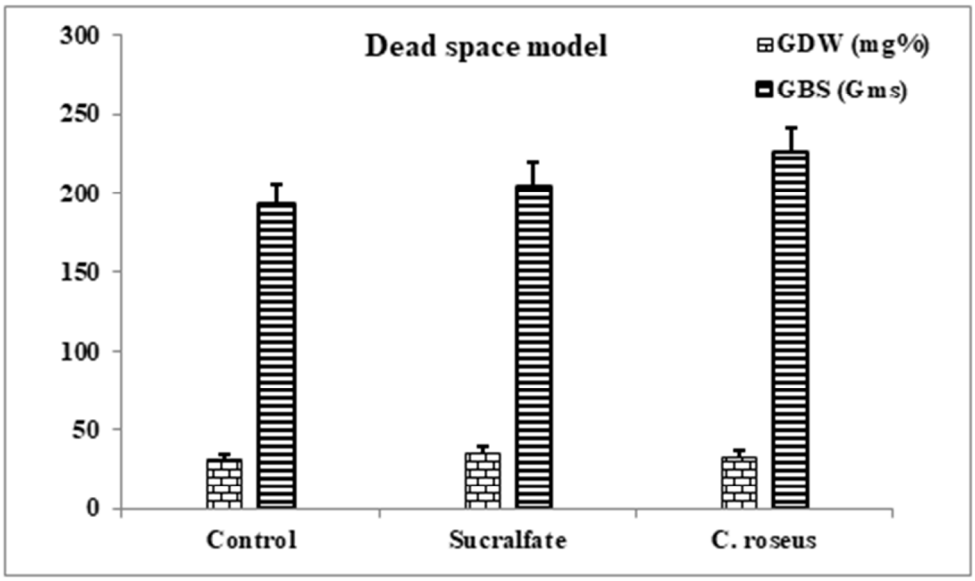

Fig 3: Measurement of granuloma dry weight and granuloma breaking strength in dead space model

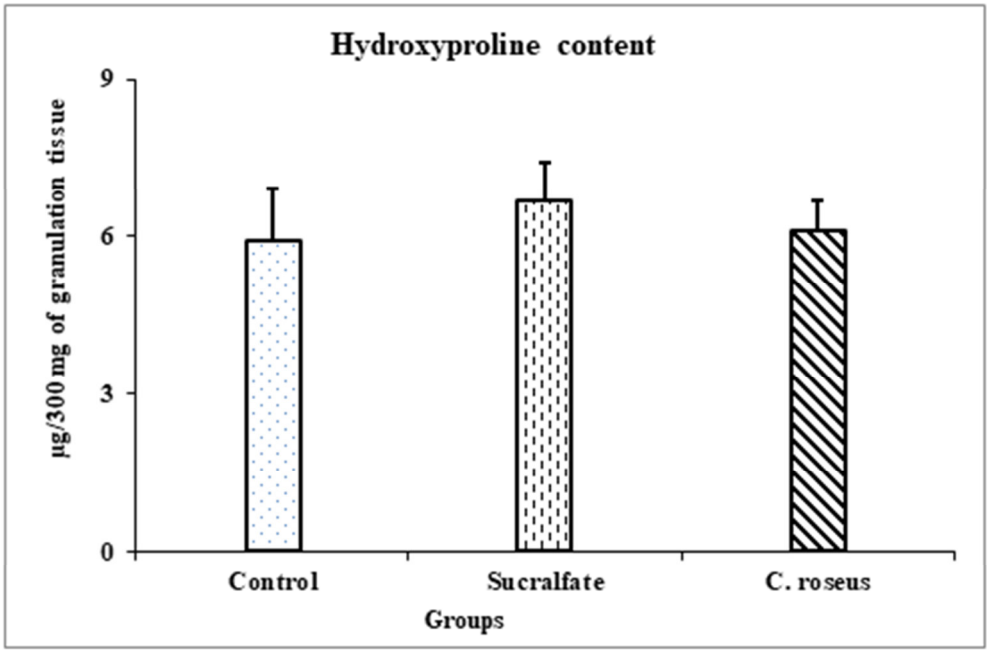

Figure 4: Measurement of hydroxyproline content

5. DISCUSSION

Wound healing primarily deals with the restoration of damaged tissues to its normal or near-normal state, which is determined by factors such as the general health of the injured tissue, type and extent of damage. Epithelialization, contraction and deposition of connective tissue are the main processes of wound healing.[15] Wound healing process begins when the fibroblasts are formed from the mesenchymal cells at the wound margin, which then combine with fibrin strands to cover the wound gap16. While the intracellular tissues are repaired through the formation of fibrin strands, the healing of the extracellular tissues is occurred by the cross-linking of the collagen. Consequently, the wound healing process is a result of inflammation, collagen maturation and scar formation that work in tandem yet independently of each other. Any injury to the tissues is more often repaired without any external aid. However, in conditions such as diabetes, immunodeficiency, local infections causing a reduced blood supply and presence of foreign particles can lead to a more chronic state, thus delaying wound healing. Such a condition is highly dangerous as improper or delayed wound healing can under many circumstances prove fatal. Under such instances, external agents need to be applied to ensure and hasten this process. Several studies have reported the efficacy of synthetic agents such as omeprazole and sucralfate in enhancing wound healing.[17-20] However; they have failed to be effective in one type of wound whereas shown effects on another. For instance, while omeprazole was found most effective on wound contraction in gastric ulcers, it was ineffective on cutaneous wounds probably owing to its short half-life and quick metabolism.[21,22] Likewise, sucralfate was effective in healing burn-type of wounds owing to its topical effects on prostaglandins and growth factors.[23] Therefore, a potent agent to enhance wound healing is always on the lookout and therefore, the present study was performed to assess the same on different wound models namely, incision, excision and dead space wound models. In our study, the breaking strength evaluated for the incision wound model showed an elevation when compared with that of the control group. Breaking strength/ wound strength is a resultant of stable collagen cross-linking and remodelling. Our studies are in agreement with several others that reported an elevated breaking strength probably due to the elevated collagen synthesis.[24,25] Similarly, in our study the extract showed promising potential in improving wound healing in the excision wound model. The results obtained for CE was comparable to that of the standard sucralfate showing increased percentage of wound closure and reduced time for complete epithelialization. This reduced duration for wound healing can be due to the release of one of the inflammatory chemokine-interleukin-8 that plays a significant role in the recruitment of inflammatory cells such as keratinocytes and fibroblasts[26]. This in turn leads to an elevation in the intracellular communication within the fibroblasts.[27] Collagen being the predominant protein in the extracellular matrix is important for tissue granulation that occurs during the wound healing process. Collagen turnover in terms of liberated hydroxyproline is a good indicator to determine the extent of wound healing[28]. In our study, the dead space wound model evaluated this biochemical indicator showing an elevation in the hydroxyproline content and a corresponding increase in the breaking strength of the treated wounds. In addition, in this wound model the granuloma dry weight and granuloma breaking strength were evaluated as an indicator of tissue granulation. It was observed from the study that both the parameters were on par with that of the standard drug sucralfate. Several studies have proven that higher the breaking strength better is the wound healing ability, which agrees with the present findings.[27-29]

6. CONCLUSION

The present study evaluated the wound healing potential of *C. roseus* using three different wound models. The study evaluated the wound healing properties of the extract by observing the time taken for epithelialization, breaking strength, tissue granulation and hydroxyproline content. Overall, it was observed that findings were comparable to that of the standard sucralfate, suggesting the beneficiary properties of CE in promoting wound healing. Further studies on preparation of a standard formulation for the optimal application of the extract on the wound are essential in order to transform it as a product for commercial preparations.

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8. AUTHOR'S CONTRIBUTION

Concept and design: A M SATISH; Data acquisition**:** Jayanthi M K &TotagiVinodGangadhar; Critical revision of manuscript: RamithRamu. The manuscript was completed with equal contributions from all the authors in terms of preparing the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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