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B-GLUCAN ENHANCED THE WOUND-HEALING ACTIVITY OF *CATHARANTHUS ROSEUS* FLOWER EXTRACT IN WISTER ALBINO RATS

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ABSTRACT

Catharanthus roseus L (Catharanthus roseus) is a plant that has been used to cure a variety of ailments, including diabetes. The goal of this study is to determine the effect of biological response modifier β-glucans in improving the wound healing ability of *Catharanthus roseus* flower extract in rats. Excision and incision wounds models were used to assess wound healing activities in rats following administration (100mg/kg/day) of the ethanol extract of *Catharanthus roseus* flower and *Catharanthus roseus* flower + β-glucans. In each model, the animals were separated into three groups of six. In the excision model, group 1 animals were given carboxymethyl cellulose as a placebo, group 2 received a topical application of *Catharanthus roseus* ethanol extract at a dose of 100mg/kg body weight/day, and group 3 received a topical application of *Catharanthus roseus* ethanol extract at a dose of 100mg/kg body weight/day + β-glucans 80mg/kg.

KEYWORDS

Albino rats, *Catharanthus roseus* and β-glucans.

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INTRODUCTION

In an incision model, Group 1 animals were given normal saline, while group 2 received the extract orally at a dose of 100mg kg-1 day-1, and group 3 received the extract orally at a dose of 100mg kg-1 day-1 + β-glucan 80mg/kg. The rate of wound contraction, period of epithelization, tensile strength (skin breaking strength), and granulation tissue weight were all used to determine healing. In the

incision wound model, *Catharanthus roseus* alone and in combination significantly increased wound breaking strength when compared to controls ($p<0.001$). In comparison to control wounds, the combination treated wounds epithelialized faster and wound contraction rate was significantly increased ($p<0.001$). The use of *C. roseus* +β-glucans in the topical management of wound healing is supported by increased wound contraction and tensile strength.

Background

Traditional herbal medicine practitioners have highlighted the therapeutic efficacy of several indigenous plants for a variety of ailments[1]. Synthetic and traditional herbal medication can both be found in natural items. In some regions of the world, they are still the predominant health-care system[2]. 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 characterise the proliferative phase. Angiogenesis is the formation of new blood vessels by endothelial cells. *Catharanthus roseus* contains over 400 alkaloids[3,4].

In some rural cultures, extracts from dried or wet flowers and leaves of plants are applied as a paste to wounds. Ayurvedic practitioners in India have utilised the fresh juice from the blooms of *Catharanthus roseus* prepared into a tea to cure skin disorders such as dermatitis, eczema, and acne. The biological response modifier β-glucans, whether particulate or soluble, has been found to improve immune functions by acting as an anti-infective, anti-tumor, and immunomodulatory agent. One promising area of β-glucan application is dermatology, including wound care[5]. The activation of immunological and cutaneous cells by β-glucan molecules promotes moist wound healing and repair. Homeostasis, re-epithelization, granulation, tissue creation, and extracellular matrix remodelling are all part of the wound healing process[6]. As a result, a multi-modal therapeutic method may help the wound heal faster. As a result, the purpose of this study is to determine the effect of β-glucan in improving the wound healing activity of *Catharanthus roseus* flowers.

METHODS

Plant material and extract preparation

Catharanthus roseus fresh flowers were collected, shade dried and processed into a powder. The fine powder (50g) was suspended in 100mL ethanol at room temperature for 20 hours. A fine muslin cloth was used to filter the mixture, followed by filter paper (Whatman No.1). The clear residue was employed for the investigation after the filtrate was dried in a water bath at 40°C.

Animals

The study used healthy inbred gender-matched Wister albino rats weighing 200-220g. They were housed separately and fed ad libitum normal food and water. Before and after the experiment, the animals were weighed on a regular basis. Ketamine anaesthesia (120mg/kg) was used to perform the surgical procedures in sterile settings. Animals were carefully monitored for signs of infection, and those that showed signs of infection were removed from the trial and replaced. The study used healthy inbred gender-matched Wister albino rats weighing 200-220g. The treatment was carried out in accordance with the consent of King Khalid University's animal ethics committee and the National Institute of Health's guidelines for the care and use of laboratory animals in the United States (NIH Publication No.85-23, revised 1996).

Wound-healing activity

The wound-healing activity of *Catharanthus roseus* alone and in combination with β-glucan was assessed using excision and incision wound models. Prior to and throughout the development of the wounds, the animals were anaesthetized. Morton and Malon[7]. Detailed how the rats were given excision wounds. The animals' dorsal fur was shaved with an electric clipper, and a circular stainless steel stencil was used to define the predicted area of the wound to be produced on the backs of the animals with methylene blue. The rats had been afflicted. Using toothed forceps, a surgical blade, and pointed scissors, a full thickness excision wound of 2.5cm in length and 0.2cm in depth was produced following the markings. The wound was left completely exposed[8]. The animals were split into two groups, each with six animals.

As a placebo control, group 1 animals were given carboxymethyl cellulose (100mg/kg/day) topically. Group 2 got an ethanol extract of *Catharanthus roseus* topically at a dose of 100mg/kg/day, whereas group 3 received the extract orally at a dose of 100mg/kg/day + βglucans 80mg/kg until complete epithelization. A graph paper was used to measure the wound areas that were recorded. The period of epithelization was determined by the number of days required for falling off the dead tissue without any residual raw wound.

Incision wound model

Rats were anaesthetized before and during the wound formation, same like in the previous model. The animals dorsal fur was shaved with an electric clipper. Ehrlich and Hunt *et al*[9]. described a six-centimeter-long longitudinal paravertebral incision into the skin and cutaneous muscle on the back. Surgical sutures were put to the parted skin at one-centimeter intervals after the incision. The wounds were not treated at all. The group-2 rats were given floral extract (dissolved in drinking water) orally at a dose of 100mg/kg/day, group 3 was given the extract orally at a dose of 100mg/kg/day + β-glucans 80mg/kg, and group 1 was given normal saline. On the eighth post-wound day, the sutures were removed and the treatment proceeded. On the tenth day, the skin-breaking strength was determined using Lee's method[10].

Statistical analysis

Results, expressed as mean ± SD were evaluated using Student's t-test and significance was set at ($p < 0.05$).

RESULTS AND DISCUSSION

The animals treated with the *Catharanthus roseus* extract and combination showed a considerable increase in wound-healing activity when compared to those that received placebo control treatments. The effects of the ethanolic extract *Catharanthus roseus* flower alone and in combination in the incision wound model are shown in Table No.1, where a significant increase in wound breaking strength was noted when compared to the controls. *Catharanthus roseus* treated animals alone and in combination demonstrated a substantial reduction in wound area ($P < 0.001$) and epithelization duration in the excision wound model (Table No.2).

Discussion

Wound contracture is a condition that develops throughout the healing process, beginning with the fibroblastic stage, in which the wound shrinks. It has three phases: inflammatory, proliferative, and maturational, and is determined by the type and level of injury. Hemostasis and inflammation characterize the inflammatory phase, which is followed by epithelization, angiogenesis, and collagen deposition in the proliferative phase. The wound contracts in the maturational phase, the ultimate phase of wound healing, resulting in a lower quantity of visible scar tissue.

The floral extract contains tannins, triterpenoids, and alkaloids, according to early phytochemical investigation. The wound healing action of *Catharanthus roseus* could be attributed to any of the phytochemical elements found in the plant. Recent research has found that phytochemical elements such as flavanoids[11] and triterpenoids[12] improve wound healing, owing to their astringent and antibacterial qualities, which appear to be responsible for wound contraction and higher epithelialization rates. The wound-healing ability of *Catharanthus roseus* might be related to the phytoconstituents found in the plant, and the faster wound healing process might be due to the individual or cumulative actions of the phytoconstituents. The tannin phytoconstituent of *C. roseus* from the astringent action, which has been documented earlier[13] may have contributed to the early tissue approximation and improved tensile strength of the incision wound seen in our work.

β-glucans have a wide range of biological activities that improve human immunity. The use of β-glucans for topical treatments is on the rise, thanks to their pluripotent qualities. During wound healing, the main target cells of β-glucans are macrophages, keratinocytes, and fibroblasts. β-glucans aid wound healing by promoting macrophage infiltration, which promotes tissue granulation, collagen deposition, and re-epithelialization. β-glucan wound dressings are a good wound healer because they are stable and resistant to wound proteases.

Table No.1: Wound healing effect of *Catharanthus roseus* + β-glucans in Incision wound model

S.No	Parameter	Placebo control	*Catharanthus roseus*	*Catharanthu roseus*+ β-glucans
1	Skin breaking strength (g)	319.13± 3.23	420.0 ± 4.43**	470.0 ± 4.43**

N = 6, Values are expressed as mean ± SD

*p < 0.05 and **p < 0.001 vs. control. Independent *t*-test

Table No.2: Wound healing effect of *Catharanthus roseus* + β-glucansin excision wound model

S.No	Parameter	Placebo control	*Catharanthus roseus*	*Catharanthus roseus* + β-glucan
	Wound area (mm^2)			
1	Day 1	226.3± 23.80	232.50 ± 14.7	235.50 ± 12.7
2	Day 5	183.6 ± 22.8	183.16 ± 31.58	172.16 ± 32.58
3	Day 15	129.8± 25.90	65.40 ± 23.8 **	60.40 ± 24.8 **
4	Period of epithelization (day)	14.6 ± 0.10	10.20 ± 0.14**	8.20 ± 0.14**

N = 6, Values are expressed as mean ± SD

**P < 0.001 vs. control. Independent *t*-test

CONCLUSION

The current investigation found that an ethanol extract of *Catharanthus roseus* flower+ β-glucan possesses features that make it capable of stimulating wound healing activity faster than placebo controls. Further research into the topical treatment and management of wounds with *Catharanthus roseus* + β-glucan is needed due to wound contraction and enhanced tensile strength.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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