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THE ENHANCED WOUND-HEALING ACTIVITY OF *CENTELLA ASIATICA L* BY Β-GLUCANS IN WISTER ALBINO RATS

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ABSTRACT

Centella asiatica is a well-known medicinal herb in Indian medicine that is used to treat a variety of skin disorders. The goal of the research presented in this article was to assess the wound healing capacity of the plant's ethanolic extract alone and in combination with β-glucans. Incision and excision wounds were used in the investigation on Wistaralbino rats. When compared to controls, the extract of *Centella asiatica* containing β-glucans significantly increased wound breaking strength in an incision wound model ($p<0.001$). When compared to control wounds, the combination treated wounds epithelized faster and had a significantly higher rate of wound contraction ($p<0.001$). In a rat model, the results showed that the plant extract with β-glucans improves wound healing considerably.

KEYWORDS

Centella asiatica, β-glucans, Wound healing, Incision wound and Excision wound.

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INTRODUCTION

Wound healing is a complex, orderly process involving the induction of an acute inflammatory response, parenchymal cell regeneration, migration and proliferation of both parenchymal and connective tissue cells, synthesis of extracellular matrix proteins, connective tissue remodelling, and wound strength acquisition[1]. Collagen is required to mend tissue that has been disturbed by damage and to restore anatomic structure and function. *Centella* *asiatica L* (*Centella asiatica*) has been used to improve wound healing both topically and systemically. Scientific research is only now begun to confirm efficacy and investigate mechanisms of action for these botanicals[2]. Indian pennywort is the common name for *Centella asiatica.* Asia, Africa and North and South America are all home to the plant[3]. This plant is said to aid wound healing and defend against the formation of ulcers, as well as having antibacterial characteristics. Topical and systemic administration of the plant's extracts has been found to hasten wound healing[4]. Sedative, antidepressant, analgesic and anticonvulsive properties have been observed in pharmacological tests of this plant's preparations[5]. *Centella asiatica* is well known in Indian medicine for its use in the treatment of chronic and persistent eczema, psoriasis, syphilis and leprosy[6].

The titrated extract of *Centella asiatica* contains three main ingredients: asiaticoside, asiatic acid, and made cassicacid, all of which have been shown to be clinically useful in the treatment of scleroderma, aberrant scar formation and keloids[6]. This extract greatly reduces the time it takes for a wound to heal by acting on the immediate healing process[7]. The components primary positive effect appears to be the encouragement of scar maturation through type I collagen formation, as well as the resultant reductions in inflammatory reaction and myofibroblast formation[8].

The major active ingredient, asiaticoside, is isolated from *Centella asiatica* and displays considerable wound healing activity in both normal and delayed healing models. The anti-healing effects of corticosteroids are difficult to overcome. 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[9]. As a result, a multi-modal therapeutic method may help the wound heal faster. The current study will look into the effects of ethanol extract of *Centella asiatica* on various wound healing parameters with and without β-glucan.

MATERIAL AND METHODS

Centella asiatica leaves were pulverised after being dried in the shade. The powder (75g) was extracted in a soxhlet device at 60^{o}C to 75^{o}C with 700mL of 95 percent ethanol and concentrated. The extract was kept cool in the fridge. The study employed healthy Wistar albino rats of either sex and around the same age, weighing between 150 and 250g. Rats were randomly assigned, individually housed and kept in clean polypropylene cages, where they were fed commercially pelleted rat chow and free access to water. 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).

Experimental Procedure

The animals were placed into four groups, each of which contained six animals.

Group 1: Control group with wound and standard saline treatment.

Group 2: Wounds in the test group were treated with extract.

Group 3: Wounds in the test group were treated with extract and β-glucan.

The wounding techniques were performed on ketamine-anesthetized rats (1mL/kg body weight) in three separate wound types. For 10 days, the rats were administered plant extract and /or β-glucan orally in the case of incision. Extraction and or β-glucan were given to the rats every day until epithelization day in the case of excision wounds[10].

Incision Wound Model

On either side of the rat's spinal column, two paravertebral incisions (6cm long) were made through the full thickness of the skin[11]. Wounds were closed with 1cm apart interrupted sutures. On the seventh day, the sutures were removed. On the tenth post-wounding day, wound-breaking strength was tested[12].

Determination of Wound Breaking Strength

Each anaesthetized animal was strapped to the operating table and a line was drawn 3mm away from the wound on either side. Allice forceps were used to grab this line, one at each end, opposite to each other. One forceps was firmly supported, while the other was connected to a freely suspended light weight polypropylene container through a line that was run across to a pulley. Water was allowed to flow slowly but consistently into the graded container from a reservoir. A progressive rise in weight due to a rise in water volume was communicated to the wound site, forcing the incision edges apart.

The water flow was halted when the wound was just opening up and the volume of water collected in the container was measured in grams as a measure of breaking strength. For each incision wound, three readings were taken and the technique was then repeated on the opposite wound. The group's average reading was used to calculate an individual's breaking strength. The breaking strength of a group is determined by the mean value.

Excision Wound Model

A circular chunk of full thickness (about 500mm 2) wound was created on back of rats in a designated place. Wounds were traced on 1mm 2 graph paper on the day of wounding and thereafter on alternate days until complete healing. The rate of wound contraction was determined by calculating changes in wound area. The period of epithelization was determined by the number of days required for to fall without any remaining raw wound.

Statistical analysis

Student's t-test was used to examine the results, which were represented as mean SD, with a significance level of ($p<0.05$).

RESULTS AND DISCUSSION

The animals given the *Centella asiatica* extract and combination showed a considerable increase in wound healing activity when compared to those given the placebo control treatments. The results of *Centella asiatica* alone and in combination in the incision wound model are shown in Table No.1, where a significant increase in wound breaking strength was seen when compared to controls. *Centella asiatica* treated animals, both alone and in combination, demonstrated a significant reduction in wound area ($p<0.001$) and epithelization duration in the excision wound model (Table No.2).

Discussion

Granulation, collagen maturation, and scar formation are some of the many phases of wound healing, which run concurrently but independently of each other. The use of a single model is inadequate and no reference standard exists that can collectively represent the various phases of wound healing. For this reason, two different models were used in this study to assess the effect of *Centella asiatica* alone and in combination on the various phases of wound healing. The results of this study demonstrate that the ethanolic extract of *Centella asiatica* alone and in combination promoted healing. An increase in collagen content as well as fibrestabilization may be responsible for the reported increases in wound breaking strength in treated wounds[13]. In rat experimental wounds, triterpenes from *Centella asiatica* increase extracellular matrix formation and glycosaminoglycans synthesis, according to scientific investigations.

The only component responsible for collagen production stimulation was asiatic acid[14]. Free radicals in oxygen play a key part in the failure of ischemic wound healing and antioxidants have been shown to help repair ischemic skin wounds[15]. Other research have demonstrated that the active component of *Centella asiatica* increases the levels of antioxidants such as vitamin E, vitamin C, superoxide dismutase, catalase and glutathione peroxidase[16]. The antioxidant properties of *Centella asiatica* leaf extract are likely to have influenced the quicker wound healing seen in this rat model. 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, scollagen deposition and reepithelialization. β-glucan wound dressings are a good wound healer because they are stable and resistant to wound proteases. Before such discoveries can be applied to the human model, where most wound-healing issues are chronic, there are several long stages to take. However, these promising results point to the necessity for further research and clinical trials to determine *Centella asiatica* safety and efficacy in combination with β-glucans.

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

S.No	Parameter	Placebo control	*Centella asiatica*	β-glucan+*Centella asiatica*
1	Skin breaking strength (g)	318.13± 3.23	422.0 ± 4.44**	471.0 ± 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 *Centella asiatica*+ β-glucans in Excision wound model

S.No	Parameter	Placebo control	*Centella asiatica*	β-glucan +*Centella asiatica*
Wound area (mm^2)				
1	Day 1	226.3± 23.70	234.50 ± 14.7	225.50 ± 12.7
2	Day 5	178.6 ± 22.8	184.16 ± 31.58	162.16 ± 32.58
3	Day 15	128.8± 25.90	67.40 ± 23.8 **	65.40 ± 24.8 **
4	Period of epithelization (day)	14.7 ± 0.10	12.23 ± 0.14**	11.50 ± 0.14**

N = 6, Values are expressed as mean ± SD

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

CONCLUSION

The current investigation shows that *Centella asiatica* with β-glucan has features that make it capable of enhancing wound healing activity better than placebo controls. Further research into the topical treatment and management of wounds with *Centella asiatica* and 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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