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WOUND HEALING ACTIVITY OF *ACHYRANTHES ASPERA* IS ENHANCED BY BETA-GLUCANS

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ABSTRACT

Achyranthes Aspera Linn (Amaranthaceae) is a plant that is widely available in India. Tamil Nadu residents have traditionally used this herb to treat cuts. Wound healing properties have been reported for aqueous extracts of *Achyranthes aspera* (*Achyranthes aspera*) leaves. β-Glucans are a type of medication that comes from a range of sources, including yeast, grain and fungus. They belong to the biological response modifiers class of medications. They have a wide range of biological activities that boost human immunity. Dermatology, particularly wound treatment, is one promising application for β -Glucans. The goal of this study is to determine the effect of biological response modifier β -Glucans in improving *Achyranthes aspera* wound healing. Two wound models, an excision wound model and an incision wound model, were used to study wound healing activity. The extracts were further tested for free radical scavenging activity using two methods: DPPH radical scavenging activity and superoxide scavenging activity. In both wound types studied, the extracts and their combination showed a substantial response. In the two models investigated, the plant also had a strong antioxidant impact, reducing the generation of free radicals. These findings point to the possibility of using this combination to treat wounds.

KEYWORDS

Beta-glucans, *Achyranthes aspera Linn* and Biological response.

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INTRODUCTION

Wounds are physical injuries that result in the skin being opened or broken. The repair of compromised anatomical continuity and compromised functional status of the skin requires proper wound healing. It's the result of multiple cell types combined response to damage. Wound healing is a multifaceted process that involves the contraction and closure of the

wound, as well as the restoration of a functional barrier. Inflammation, proliferation and migration of diverse cell types are all part of the process of repairing wounded tissues. Because of their damaging effects on cells and tissues, reactive oxygen species (ROS) are detrimental to wound healing.

Free-radical-scavenging enzymes (FRSE) are a class of cytoprotective enzymes that play a key role in the reduction, deactivation and elimination of ROS as well as wound healing. Furthermore, self-generated autocoids and hormones act in a systematic synchronisation to promote wound healing in this complicated phenomena[1].

Plants are high in phytochemicals, which have anti-inflammatory and antioxidant properties. In folklore Indian medicine, several indigenous medications have been documented for the treatment of cuts, bruises, burns and wounds. *A. aspera* also known as apamarga, is a plant that grows wild and abundantly in India. Folk healers and locals in Karungal village, Kanyakumari district, Tamil Nadu, India, utilise the plant's leaves to treat wounds. Wound healing, hepatoprotective, cancer-fighting, antimicrobial and immunomodulatory properties have all been documented for *Achyranthes aspera*[2,3]. β-Glucans, which are glucose polymers derived from a range of sources such as yeast, grain and fungus, belong to the biological response modifiers class of pharmaceuticals.

The plant's ability to heal wounds may be attributed to its free radical scavenging and immune-enhancing properties[1]. 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. Dermatology, particularly wound care, is one promising area of β-Glucans application[2]. By activation of immunological and cutaneous cells by β-Glucans, the molecules promote moist wound healing and repair. Homeostasis, re-epithelization, granulation, tissue creation and extracellular matrix remodelling are all part of the wound healing process[1]. As a result, a multi-modal therapeutic method may help the wound heal faster. Hence, the purpose of this study is to see how β -Glucans, can help *Achyranthes aspera* heal wounds faster.

MATERIAL AND METHODS

Preparation of extracts

Shade-dried, coarsely powdered leaves (500g) were defatted with petroleum ether (60-80ºC) for 72h. The defatted drug was Soxhlet-extracted with distilled water. The obtained extracts were evaporated in vacuum to give residues and percentage yield of the extracts were found to be 10.1% and 5.5%, respectively. The extracts were used in the form of ointment. Plant extract ointments (10% w/w) were prepared by mixing the extracts separately in yellow soft paraffin[4].

Animals

The present experiments used healthy wistar rats of either sex (150-200g) with no prior pharmacological treatment. The animals were fed a commercial pellet diet and were allowed to drink as much as they wanted. Before beginning the experiment, the animals were acclimatised to laboratory sanitary conditions for 10 days. 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).

Animals of either sex were placed into four groups for incision and excision wound models, each with six animals: Group I - ointment base; group II - 0.2 percent nitrofurazone ointment; group III - aqueous extract of *Achyranthes aspera;* group IV - *A. aspera*+ β -Glucans. The extracts and the standard ointment were applied twice daily to the various animal groups.

Wound healing activity

Excision wound model

Throughout the surgical operations, the animals were kept under light ether anaesthesia. After leaving at least 5mm space between the ears, a 500mm 2 impression was produced, as described by Nagappa and Binu (2001)[5]. The skin of the impressed area was carefully removed to its

maximum thickness, resulting in a 500mm 2 wound. A normal saline solution was used to establish hemostasis. Topically, ointment base, nitrofurazone ointment and extracts in ointment form were administered until the wound healed completely.

The physical characteristics of wound healing were noted, including wound closure (contraction), epithelization and scarring. Throughout the surgical operations, the animals were kept under light ether anaesthesia. After leaving at least 5mm space between the ears, a 500mm 2 impression was produced, as described by Nagappa and Binu (2001). The skin of the impressed area was carefully removed to its maximum thickness, resulting in a 500mm^2 wound. A normal saline solution was used to establish hemostasis. Topically, ointment base, nitrofurazone ointment and extracts in ointment form were administered until the wound healed completely. The physical characteristics of wound healing were noted, including wound closure (contraction), epithelization and scarring.

Tracing the raw wound area on translucent paper on days 0, 8 and 16 until complete epithelization occurred was used to study wound contraction. The creation of a scar with no raw wound area was established as the criterion for complete epithelization. Using mm 2 scale graph paper, the wound area was measured planimetrically. The wound contraction is calculated by the following Wilson's formula.

% wound contraction = (wound area on day zero - wound area on particular day / wound area on day zero) × 100

The period of epithelization was calculated as the number of days required for falling off the dead tissue remnants without any residual raw wound.

Incision wound model

Ehrlich and Hunt described the incision wound model, which was investigated (1969)[3]. The animals were fixed to an operation table in their normal position under light ether anaesthesia. A scalpel blade was used to make two paravertebral straight incisions of 6cm each on either side of the spinal column. To the spinal column, incisions were made at least 1cm apart. With the use of a straight round-bodied needle, the wounds were closed with sutures at equidistant spots of 1cm apart by silk thread of zero grade.

Cotton swabs soaked in 70% alcohol were used to clean the wounds. Topically, the animals were given ointment base, nitrofurazone ointment and extracts in ointment form. After 8 days, the sutures were removed. A constant water supply technique was used on both sides to measure the wound's tensile strength[6].

The sedated animals were fixed to the operating table using the continuous constant water delivery technique. All of the forceps were firmly attached to lines that were parallel to each other. The forceps on one side were attached to a metal rod to keep them in place, while the forceps on the other side were connected to a polythene reservoir by a string that was pulled through a pulley. Under controlled conditions, water was allowed to flow at a consistent rate into a polythene reservoir and the pulling force required to break the wound was progressively created.

The water flow was controlled using an occlusion clamp on polythene tubing that was linked to the reservoir and raised to the appropriate height. The water flow was turned off as soon as the gaping of the incision was developed. To avoid additional wound opening, the pulling tension of the wound was promptly removed by lifting up the polythene reservoir. The volume of water accumulated in the reservoir was measured and the weight was translated using the density of water as 1.

Statistical analysis

The data is given as a mean with a Standard Error Mean (SEM). One-way Analysis of Variance (ANOVA) was used to examine the differences between means, with p values less than 0.05 considered significant.

RESULTS AND DISCUSSION

Table No.1 displays the results of the excision wound model. When *Achyranthes aspera* leaf extracts were tested for wound healing activity, they

demonstrated considerable wound healing activity when compared to a control group. At various time periods, the percentage closure of the initial wound area was calculated. The measurement on day 8 showed that the percentage closure of the original excision wound area was found to be 54.23 (standard), 57.20 (aqueous extract) and 67.04 (aqueous extract+ β-Glucans). The tested extracts significantly (P <0 percentage wound closure was 97.62 (standard), 84.13 (aqueous extract) and 96.55 (aqueous extract+ β-Glucans).

Tensile strength or wound breaking strength of granulated tissue is the most essential and often used metric to measure wound healing. Table No.2 shows the results of the incision wound model. When compared to the extracts and regular ointment treated groups, the mean tensile strength of granulated tissue in control animals was lower. The prohealing effect of the II, III and IV groups is confirmed in the incision wound model, as it was in the excision wound model (Table No.1).

Discussion

Homeostasis, re-epithelization, granulation, tissue creation and extracellular matrix remodelling are all part of the wound healing process. Although the healing process is self-sustaining and does not require much assistance, different risk factors such as infection and delayed healing have drawn attention to the need to enhance it[1].

The wound healing activity of *Achyranthes aspera* leaves + β -Glucans was investigated in this work. In an excision wound healing model, topical administration of aqueous extracts of leaves + β -Glucansto the wound site resulted in considerable (P 0.001) wound healing activity. The healing of excision wounds can be tracked by taking measurements of wound area changes (closure rate) at predetermined time intervals. Complete epithelization was also recorded and monitored. For complete wound closure, epithelization can be directly measured in days.

Furthermore, experiments with an animal model revealed a faster rate of wound contraction and a shorter healing time, which could be attributable to improved epithelization[1]. Tensile strength was assessed indirectly in an incision model to quantify collagen composition and maturation. During the healing process in treated rats, an increase in protein and collagen content is responsible for increased migration of fibroblast cells, epithelial cells and formation of extracellular matrix, including collagen[4].

Table No.1: Effect of extracts of *A. aspera* on excision wound model

S.No	Treatments	Remaining of original excision wound area (mm^2)			Epithelization time (d)
		Day 0	Day 8	Day 16	
1	Control	484.00 ±14.80	311.00 ± 14.97 (35.75)	151.40 ± 8.10 (68.64)	23.30 ± 1.34
2	Nitrofurazone	480.30 ± 11.33	219.80 ± 14.55** (54.23)	11.40 ± 0.48** (97.62)	16.50 ± 0.51*
3	Aqueous extract of *Achyranthes aspera*	479.00 ± 11.59	204.50 ± 10.96** (57.30)	76.00 ± 0.84** (84.13)	20.10 ± 0.63*
4	Aqueous extract of *Achyranthes aspera* + β-Glucans	488.30 ± 13.49	160.90 ± 13.59** (67.04)	16.80 ± 3.14** (96.55)	4.09 ± 0.89*

Values are expressed as mean ± SEM for five observations. * P < 0.01, **P < 0.001 versus control; values in parenthesis are percentage closure of original excision wound area.

Table No.2: Effect of extracts of Achyranthes aspera on incision wound model

S. No	Treatment	Incision wound breaking strength (g)
1	Control	131.20 ± 9.67
2	Nitrofurazone	433.30 ± 18.13*
3	Aqueous extract of *Achyranthes aspera*	418.60 ± 19.24*
4	Aqueous extract of *Achyranthes aspera* + β-Glucans	450.69 ± 19.17*

Values are expressed as mean ± SEM
* $P < 0.001$ versus control.

CONCLUSION

The biological response modifier β-Glucans greatly increased the wound healing activity of the aqueous extracts of *Achyranthes aspera* according to the findings of this investigation. This study's findings highlight the traditional usage of *Achyranthes aspera* for wound healing.

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CONFLICTS OF INTEREST

"The authors state that they have no competing interests. The funders had no involvement in the study's design, data collection, analysis, or interpretation, manuscript preparation, or the decision to publish the findings."

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