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B-GLUCANS ENHANCE THE WOUND HEALING ACTIVITY OF TANNININ ALBINO RATS

Krishnaraju Venkatesan[*1], Rajalakshimi Vasudevan[1], Absar Ahmed Qureshi[1], Ester Mary Pappiya[2], Premalatha Paulsamy[3], Rama Ramaiah[3], Kalpana Krishnaraju[4]

[1*]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Asir Province, Saudi Arabia.
[2]Directorate of General Health Affair, Ministry of Health, Najran, Saudi Arabia.
[3]King Khalid University, Khamis Mushayit, Asir Province, Saudi Arabia.
[4]Department of Pharmacy, Erode College of Pharmacy, Veppampalayam, Erode, Tamilnadu, India.

ABSTRACT

Tannin was isolated from *Palas* gum. The precipitated tannin was extracted with alcohol, which was confirmed by calculating the tannin as Tannic Acid Equivalent. The beneficial effect of adding β-glucan to it was examined in light of the known wound healing effects of *Palas* tannin on test animals (albino wistar rat) in the literature. The wound healing abilities of *Palas* tannin + β-glucan were studied in depth on two groups of animals utilizing the excision and incision wound models. In comparison to the usual control group, the tannin + β-glucan showed substantial wound healing activity, according to the study.

KEYWORDS

Palas, β-glucan, Butea gum, Tannin, Wistar rats and Wound healing.

Author for Correspondence:

Krishnaraju Venkatesan,
Department of Pharmacology,
College of Pharmacy, King Khalid University,
Abha, Asir Province, Saudi Arabia.

Email: kvenkatesan@kku.edu.sa

INTRODUCTON

Butea (*Palas*) is an Indian native that may be found all throughout the country. It is commonly referred to as 'dhak' or '*Palas*'. It is also known as the 'Flame of the Forest' because of its red-colored blossoms. *Palas* is found across India, Burma, and Ceylon, with the exception of the most arid regions. It can be found in abundance on open meadows and scattered across mixed forests. Plantations can be grown on both irrigated and non-irrigated land. *Palas* is an essential medicinal tree whose diverse

components are utilized to treat a variety of clinical conditions in traditional medicine[1].

Tannins are said to offer a variety of medicinal qualities[2]. Tannins are phenolic compounds with molecular masses ranging from 500 to 3000 that bind to biomolecules and precipitate proteins and other organic substances such as amino acids and alkaloids[3]. *Terminalia chebula* tannins have been claimed to have the ability to heal wounds[4]. The tannins are fantastic for treating burn wounds[5].

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-glucan application. The purpose of this study is to determine the effect of β-glucan in improving *Palas* tannin wound healing ability.

MATERIAL AND METHODS

Animals

Albino male wistar rats that were 10 months old and weighed 150-200g were used. 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).

Isolation of *Palas* tannin

The tannin was isolated from the *Palas* gum by dissolving it in boiling water, then filtering the cool filtrate and treating it with saturated brine solution. The precipitated tannin was then filtered, rinsed with brine solution, and dried. The powdered material was extracted with alcohol and then treated with ether. The tannin was refined by ether precipitation on a number of occasions.

Wound healing study

For this investigation, healthy adult male albino Wistar rats (Rattus norvegicus) weighing 150-200g and aged 10 months were used. All rats that passed the veterinarian health test were given a 5-day acclimatisation period before starting therapy. The *Palas* tannin dose was chosen based on literature[6]. For each wound model, a total of 18 animals were divided into three groups, each with six animals. The control group received no treatment, the second group received a topical application of 20% *Palas* tannin, and the third group received a topical application of 20% *Palas* tannin + β-glucan 80mg/kg, po. Excision and incision wound models were used to study wound healing in animals.

Wound-healing activity

The wound-healing efficacy of *Palas* tannin alone and in combination with was evaluated using excision and incision wound models. Prior to and throughout the development of the wounds, the animals were anaesthetized[7]. 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. 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 entire wound was left open[8]. The wound closure rate was assessed by tracing the wound on days 1, 5 and 15 post wounding using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of dead tissue without any residual raw wound gave the period of epithelization.

Incision wound model

Statistical analysis

As in the prior experiment, rats were anaesthetized before and during wound development. An electric clipper was used to shave the animals dorsal fur. A six-centimeter-long longitudinal paravertebral incision into the skin and cutaneous muscle on the back was made. Following the incision[9] surgical sutures were placed at one-centimetre intervals on the separated skin. The sutures were removed on the eighth post-wound day, and the treatment continued. The skin-breaking strength was evaluated on the ninth day using Lee's method[10]. 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

Animals treated with the *Palas* tannin alone and in combination with β-glucan showed a significant increase in wound-healing activity when compared to those that received placebo control treatments. The results of the *Palas* tannin alone and in combination in the incision wound model are shown in Table No.1, where a considerable increase in wound breaking strength was seen when compared to the controls. The wound area and epithelization duration were significantly reduced in the excision wound model when *Palas* tannin treated mice were used alone or in combination (Table No.2).

The contraction was faster in the case of the combination, according to the experiment and wound healing observations up to 15 days. Despite the fact that tannin alone had a lower percent contraction than the combination, both wounds recovered consistently and steadily.

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, the host's overall health, and the tissue's ability to mend.

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.

Tannins are said to offer a variety of medicinal qualities, including wound healing[5]. β-glucan have a wide range of biological activities that improve human immunity. The use of β-glucan for topical treatments is on the rise. During wound healing, the main target cells of β-glucan are macrophages, keratinocytes and fibroblasts. β-glucan 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. Based on the aforesaid separate properties of Tannins and β-glucan, combining the two in a multi-modal treatment method dramatically improved wound healing.

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

S.No	Parameter	Placebo control	*Palas* tannin	*Palas* tannin + β-glucans
1	Skin breaking strength (g)	321.13± 3.23	422.0 ± 4.33**	472.0 ± 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 *Palas* tannin+ β-glucans in Excision wound model

S.No	Parameter	Placebo control	*Palas* tannin	*Palas* tannin+ β-glucans
	Wound area (mm^2)			
1	Day 1	227.3± 23.80	233.50 ± 13.7	237.50 ± 11.7
2	Day 5	183 ± 22.8	184.16 ± 31.58	176.16 ± 32.58
3	Day 15	130.8± 24.90	65.40 ± 22.8 **	65.40 ± 23.8 **
4	Period of epithelization (day)	14.8 ± 0.10	10.272 ± 0.14**	9.24 ± 0.14**

N = 6, Values are expressed as mean ± SD

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

CONCLUSION

In comparison to the usual control group, the 20% *Palas* tannin + β-glucan demonstrated considerable wound healing activity. As a result, our findings suggest that *Palas* tannin has potent wound healing properties.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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