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Original Article

Wound healing potential of *tannin* on dead space wound in diabetic rats

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ABSTRACT

Butea or Palas gum is a tree bark exudate that comes from the stems naturally or as a result of the plant being wounded. *Tannin* was isolated from palas gum by dissolving it in boiling water and then treating the cool filtrate with a saturated brine solution to create a precipitate. The precipitated *Tannin* was extracted with alcohol, yielding 42 percent, which was confirmed by calculating the *Tannin* as Tannic Acid Equivalent. *Tannin*'s effect on the healing of rat dermal wounds was studied in diabetic rats utilising an in vivo dead space wound model. On each axilla of diabetic rats, dead space incisions were created. For eight days, the rats were randomly assigned to one of three treatment groups (Group I: Normal saline; Group II: Diabetic control; Group III: *Tannin*). Animals were euthanized on day 10, and cotton pellets and granuloma tissues were carefully collected and processed for further estimates. When compared to the control, the levels of hydroxyl proline, hexuronic acid, tissue protein, and lysyl oxidase were considerably higher. These findings substantiate *Tannin*'s positive benefits in the speeding up of the healing process. As a result, the current study backs up the plant's wound healing claims in diabetic wounds.

Key words: *Tannin*, Wound healing; Diabetic; Dead space wound; Granulation tissue; Streptozotocin

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INTRODUCTION

Butea (Palas) is a native of India that may be found all throughout the nation. 'Dhak' or 'Palas' are two prevalent names for it. Because of its red-colored blooms, it is also known as the 'Flame of the Forest.' Palas may be found across India, Burma, and Ceylon, with the exception of the driest areas. It grows in large numbers on open meadows and is dispersed across mixed woods. Irrigated and non-irrigated land can both be used to develop plantations. Palas is an important medicinal tree whose many components are used in traditional medicine to treat a number of clinical conditions.[1] *Tannins* are thought to have a wide range of therapeutic properties.[2] *Tannins* are phenolic compounds with molecular weights ranging from 500 to 3000 that bind to bio molecules and precipitate proteins, amino acids, and alkaloids.[3] *Tannins* from Terminalia chebula have been claimed to have wound-healing properties.[4] *Tannins* are excellent for healing burn wounds.[5]

Wound healing is typically hindered in people with diabetes mellitus (DM), leading to non-healing, delayed healing, or persistent skin ulcers.[6] An imbalance in the inflammatory response, changed cytokine production, altered collagen synthesis, insufficient angiogenesis, extracellular matrix differentiation, lower tensile strength, or diminished growth factors may all contribute to delayed wound healing in diabetics.[7,8] Incision and excision wound models were used to test the effects of *Tannin* on wound healing activities. In a diabetic wound model, however, the *Tannin* impact is unknown. As a result, the goal of this research is to see how *Tannin* affects diabetic wounds.

MATERIALS AND METHODS

Isolation of *Palas Tannin*: By dissolving the Palas gum in boiling water, sifting the cold filtrate, and treating it with saturated brine solution, the *Tannin* was extracted. After that, the precipitated *Tannin* was filtered, washed with brine, and dried. Alcohol was used to remove the powdered substance, which was subsequently treated with ether. On some cases, ether precipitation was used to improve the *Tannin*.

Animals: Healthy wistar rats of either sex (150–200 g) were utilised in this study, and no prior pharmacological therapy was given to them. The animals were fed a commercial pellet diet and given unlimited water. The animals were given a 10 day acclimatisation period before starting the experiment. The therapy was carried out with the approval of the animal ethics committee of King Khalid University and in compliance with the National Institute of Health's standards for the care and use of laboratory animals in the United States (NIH Publication No. 85-23, revised 1996). For the dead space wound model, animals of any sex were divided into three groups, each with six animals: Group I-Normal control; group II- diabetic control; and group III was given *Tannin* (200 mg/kg/day). The extracts were given to the different animal groups orally once a day.

Wound healing activity:

Dead Space wound model: Rathi et al. described a technique for creating dead space wounds.[9] Eighteen rats were divided into three groups of six individuals each. Under general anaesthesia (achieved with 10 mg/kg body weight of xylazine hydrochloride and 50 mg/kg ketamine hydrochloride), subcutaneous dead space wound was established in the area of the axilla by creating a pouch by a tiny nip in the skin. The development of granulomas was induced by implanting sterile cotton pellets (30 mg) in each axilla. Sutures were used to close the wounds, which were then cleaned with an alcoholic swab.

After grouping the animals, they were placed individually in a metal cage to prevent them from biting each other's wounds. For 8 days, the treatment groups received extract or normal saline (1 ml/kg). At the end of day 10, the rats were euthanized, and the cotton pellets and granuloma tissues were carefully removed, dried in a 60°C oven, weighed, and compared to the control. The dry tissue's neutralised acid hydrolyzate was utilised to determine hydroxyproline, hexosamine concentration, and hexuronic acid. For the measurement of lysyl oxidase and tissue protein, a piece of the moist granulation tissue was utilised.[10]

Induction of diabetes: The rats were given a newly produced solution of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer pH 4.5 at a dosage of 65 mg/kg intraperitoneally (i.p.) 15 minutes after receiving 110 mg/kg body weight nicotinamide (HiMedia labs Pvt. Ltd.). The rats were given a 10% glucose solution after 6 hours of STZ treatment for additional 24 hours to prevent hypoglycemia owing to large pancreatic insulin secretion. Blood was collected from the tail veins of the rats 72 hours after the STZ injection, and rats with a fasting blood glucose level of more than 200 mg/dl were deemed diabetic and used in this investigation.[11]

Statistical analysis: The information is presented as a mean with a Standard Error Mean (SEM). The differences between means were investigated using one-way Analysis of Variance (ANOVA), with p values less than 0.05 deemed significant. The data was analysed using one way analysis of variance (ANOVA) with a post hoc Scheffe's test in Graph

Pad, and the findings were presented as mean + SD. P values of less than 0.05 were deemed statistically significant.

RESULTS

Animals given *Tannin* extract had much higher wound healing activity than those given placebo control treatments. The effects of *Tannin*, given orally at a dosage of 200 mg kg^{-1} day^{-1} for 8 days, on wound healing activity in rats with dead space wounds are shown in Table 1. When compared to diabetic and control rats, *Tannin* therapy rats had considerably higher granulation tissue breaking strength and wet and dry granulation tissue weight (table1).

Table-1: Physical and biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Groups	Blood glucose (mg/dl)	Wet tissue weight (mg/ 100g rat)	Dry tissue weight (mg/ 100g rat)	Tissue breaking strength (g)
Wounded Control	79.1 ± 8.2	241.5 ± 14.09	33.58 ± 5.50	287.49±15.47
Diabetic Control	266.38 ± 15.1[a]	170.5 ± 11.32[a]	21.5 ± 4.40[a]	174.51±1.70[a]
Tannin	284.08 ± 13.1[a]	283.5 ± 11.09[a]	34.5 ± 4.45[a]	317.19±12.37[a]

Values are mean ± SD of 6 replications. p values: [a]:<0.01vs control.

In streptozotocin induced diabetic rats, the concentration of hydroxyproline in granulation tissue was dramatically reduced. The experimental group's glycosaminoglycan contents, such as hexuronic acid and hexosamine concentration, were considerably lower. When diabetic rats were compared to control rats, tissue protein content was quite low. The level of lysyl oxidase in the experimental group was considerably lower. When compared to diabetic and control rats (group II), all of the following metrics rose considerably in the *Tannin* therapy group (table-2).

Table 2: Biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Groups	Hydroxyproline (mg/g tissue)	Hexosamines (mg/g tissue)	Hexuronic acid (mg/g tissue)	Tissue protein (mg/g tissue)	Lysyl oxidase (SFU)
Wounded control	13.72 ± 4.12	12.09 ± 2.57	12.91 ± 3.19	43.18 ± 3.20	1713 ± 49
Diabetic Induced	12.36 ± 2.13a	7.9 ± 1.20a	9.4 ± 1.30a	26.3 ± 2.40a	1127 ± 46a
Tannin	14.02 ± 4.22	13.49 ± 2.07	12.31 ± 3.19	42.08 ± 3.40	1921 ± 61

Values are mean ± SD of 6 replications. (SFU- Spctroflourimetric units), P values: [a]:<0.01 vs control.

DISCUSSION

The findings suggest that pure tannic acid might be a useful agent for wound healing.[12-14] Tannic acid improved the stability of the collagen matrix against collagenolytic breakdown in another investigation, resulting in considerable wound healing outcomes in rats. The collagen-based polymer and tannic acid were both found to be bio degradable; suggesting that they might be used in tissue engineering and drug entrainment.[15] Tannic acid has essential characteristics that allow it to be used in a number of technical applications, particularly in the pharmaceutical area as a wound healer.[16]

Tannins have been shown to increase nitric oxide production and relax arterial segments that have been pre contracted by norepinephrine. *Tannins* have antibacterial, wound healing, astringent, hypotensive, antioxidant, and wound healing properties.[17] Because the plant has been known to have substantial wound healing activities, the current study looked at the efficacy of *Tannins* in diabetic wound healing. Streptozotocin has been frequently used to cause diabetes in a number of animals by causing pancreatic β-cell degeneration and necrosis. Similarly, the current investigation comprised STZ induced diabetes followed by an assessment of wound healing capacity utilising a dead space wound model.

Granulation tissue is largely made up of fibroblasts, collagen, edoema, and new tiny blood vessels, and it forms near the end of the proliferative phase. The test treated animals' dry granulation tissue weight increased, indicating a greater protein content. *Tannins* enhanced the hydroxyproline level of the granulation tissue, indicating accelerated collagen turnover. Collagen is made up of the amino acid hydroxyproline, which has been utilized as a biochemical marker for tissue collagen. Collagen is the main component that builds and supports extracellular tissue.[18] The hydroxyproline content, hexuronic acid and hexosamine levels was found to increase in *Tannins* treatment group. The increased lysyl oxidase activity in our study may result in the

increased cross linking and greater breaking strength of the granulation tissue.

Conclusion: In this study, we discovered that *Tannin* promoted wound healing in diabetic rats with a dead space wound model. In comparison to chemical medications, herbal medicines have shown to be less expensive, have less side effects, and have lower drug resistance. Our findings showed that natural *Tannin* may be used as a safe and effective therapy for clinical damage repair.

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