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Original Article

Effect of *Terminalia Arjuna* on dead space wound in diabetic rats

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ABSTRACT

The effects of *Terminalia arjuna (T.arjuna)* on the healing of rat cutaneous wounds were 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: B.lanzan). Animals were euthanized on day 10, and cotton pellets and granuloma tissues were carefully collected and processed for further estimates. The tensile strength of the dead space wounds increased statistically significantly, according to the findings. When the hexosamine content of granulation tissue produced from dead space wounds was compared to the control, the hexosamine concentration was found to be higher. In addition, as compared to the control, the levels of hydroxyproline, hexuronic acid, tissue protein, and lysyl oxidase were considerably higher. These findings support the use of *T.arjuna*, which is primarily composed of tannins, to speed up the healing process. As a result, the current study backs up the plant's wound healing claims in diabetic wounds.

Key words: *T.arjuna,* Wound healing; Diabetic; Dead space wound; Granulation tissue; Streptozotocin

INTRODUCTION

Therapeutic properties have been found for *Terminalia arjuna* (Combretaceae). The bark of the *T.Arjuna* tree has been used in Ayurvedic medicine for over three centuries, notably as a heart tonic. It is also claimed to enhance the skin and cleanse the blood. *T.Arjuna* bark has previously been utilised to aid wound healing in rat incision and excision wound models.[1,2] The researchers discovered that

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the tensile strength of incision wounds had increased, and the rate of epithelialization. This encouraged us to investigate *T.arjuna's* involvement in diabetic wound healing in rats.

Wound healing is frequently hindered in people with diabetes mellitus (DM), resulting in non-healing, delayed healing, or persistent skin ulcers.[3] In diabetes, delayed wound healing can be caused by an imbalance in the inflammatory response, changed cytokine production, altered collagen synthesis, insufficient angiogenesis, extracellular matrix differentiation, lower tensile strength, or diminished growth factors.[4,5] *T. arjuna* is commonly used for the treatment of cardiovascular illnesses, such as heart disease and associated chest discomfort, high blood pressure, and high cholesterol, based on the existing literature data. Urinary tract disorders are treated with it. *T. arjuna's.* Usefulness as an anti-ischemia drug, a powerful antioxidant in preventing LDL, reperfusion ischemic injury to the heart, and its ability to lower atherogenic lipid levels has been established in several experimental and clinical trials.[6] After oral or topical administration in the form of a hydrogel, the effects of an ethanolic extract of *T. arjuna* bark and tannins extracted from the bark on wound healing activity in incision and excision wound models were investigated. *T. arjuna's* impact on a diabetic wound model, on the other hand, remains unknown. As a result, the goal of this study is to see how *T. arjuna* affects diabetes caused by streptozotocin.

Materials and Methods

Preparation of extracts: The bark of the plant was coarsely crushed in a hammer mill and extracted for 18 hours using a soxhlet extractor and 50% ethanol as a solvent. The extract was concentrated under decreased pressure on a water bath at a temperature below 50°C to a syrupy consistency after the solvent was distilled away. After that, the dessicator was used to dry it out.

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 either sex were divided into three groups, each with six animals: Group I normal control; group II received diabetic control; and group III received *T. arjuna* (400 mg/kg/day). The extracts were administered orally to the individual animal groups once a day.

Wound healing activity:

Dead Space wound model: Rathi et al. described a method for creating dead space wounds.[7] Eighteen rats were divided into three groups, each with six individuals. Subcutaneous dead space wounds in the area of the axilla were produced under general anaesthesia (10 mg/kg body weight xylazine hydrochloride and 50 mg/kg ketamine hydrochloride) by creating a pouch by a tiny nip in the skin. The development of granulomas was induced by implanting sterile cotton pellets (30 mg) on each axillae. Sutures were placed in 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.

The extract or normal saline (1 ml/kg) was given to the treatment groups over an 8 day period. After the rats were euthanized on day 10, the cotton pellets and granuloma tissues were carefully removed, dried in a 60°C oven, weighed, and compared to the control. Hydroxy proline, hexosamine concentration, and hexuronic acid were measured using the neutralised acid hydrolyzate of dry tissue. Lysyl oxidase and tissue protein were determined using a sample of moist granulation tissue.[8]

Induction of diabetes: The overnight starved 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.e) 15 minutes after receiving 110 mg/kg body weight nicotinamide (HiMedia labs Pvt. Ltd.). After 6 hours of STZ treatment, the rats were given a 10% glucose solution for additional 24 hours to prevent hypoglycemia caused by large pancreatic insulin secretion. The rats' blood was taken from their tail veins 72 hours after the STZ injection, and those with a fasting blood glucose level of more than 200 mg/dl were deemed diabetic and used in this investigation.[9]

Statistical analysis: The information is presented as a mean with a Standard Error Mean (SEM) (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 mean and standard deviation were calculated. P values less than 0.05 were deemed statistically significant.

RESULTS

Animals given the *T. arjuna* extract showed a substantial increase in wound-healing activity when compared to those given sham treatments. The effects of *T. arjuna*, given orally at a dosage of 100 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, *T. arjuna* treatment rats had substantially higher granulation tissue breaking strength and wet and dry granulation tissue weight (table-1).

TABLE1: 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	78.1 ± 6.2	238.5 ± 12.09	30.48 ± 4.90	281.39±13.07
Diabetic Control	276.38 ± 14.1[a]	169.5 ± 10.32[a]	22.5 ± 4.50[a]	178.51±1.20a
T. arjuna	274.38 ± 14.1[a]	280.5 ± 13.09[a]	33.5 ± 4.50[a]	315.49±14.37a

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

Hydroxyproline concentration of granulation tissue was significantly decreased in the streptozotocin induced diabetic rats. Glycosaminoglycan contents like hexuronic acid and hexosamine concentration was significantly decreased in the experimental group. Tissue protein concentration was very low in the case of diabetic rats when compared to control. Lysyl oxidase level was significantly decreased in the experimental group. All the above parameters increased significantly in increased in *T. arjuna* treatment group compared to diabetic and control rats (group II) (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	12.52 ± 5.12	11.59 ± 3.37	14.21 ± 3.19	42.68 ± 3.90	1709 ± 61
Diabetic Induced	11.28 ± 3.10[a]	8.1 ± 1.30[a]	9.6 ± 1.42[a]	28.5 ± 2.60[a]	1126 ± 37[a]
T. arjuna	16.72 ± 4.12[a]	13.49 ±2.37[a]	14.20 ±3.09[a]	45.58 ± 3.30[a]	1913 ± 66[a]

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

DISCUSSION

Tannins have been shown to increase nitric oxide production and relax arterial segments that have been pre-contracted by norepinephrine. The bark of *T. arjuna* has yielded a range of tannins in addition to flavonoids. Around fifteen different kinds of tannins and related chemicals were extracted from *T. arjuna* bark, and their structures were deduced using spectrum analysis. From the bark of *T. arjuna*, hydrolyzable tannins such as castalagin, casuariin, casuarinin, punicalagin, pyrocatechols, punicallin, terchebulin, and terflavin C were extracted. Tannins have antibacterial, wound healing, astringent, hypotensive, antioxidant, and wound-healing properties.[6]

Because the herb *T. arjuna* has been known to have substantial wound healing activities, the current study looked at its efficacy in diabetic wound healing. Streptozotocin is commonly used to cause diabetes in a number of animals by causing pancreatic β-cell degeneration and necrosis.[9] Similarly, the current investigation utilised STZ induced diabetes and a dead space wound model to assess wound healing capacity. Granulation tissue is made up largely of fibroblasts, collagen, oedema, and new tiny blood vessels and forms in the last stages of the proliferative phase. Higher protein content is suggested by the rise in dry granulation tissue weight in test treated animals. The hydroxyl proline content of the granulation tissue was significantly enhanced by the ethanol extract of *T. arjuna*, indicating accelerated collagen turnover. Collagen is made primarily of the amino acid hydroxyl proline, which has been utilised as a biochemical marker for tissue collagen. It is the main component that builds and maintains extracellular tissue.[10]

In the *T. arjuna* treatment group, the levels of hydroxyl proline, hexuronic acid, and hexosamine all increased. Enhanced lysyl oxidase activity in our study might result in increased granulation tissue cross linking and breaking strength. *T. arjuna's* wound healing ability might be related to the phyto-constituents found in the plant, and the faster wound healing process could be due to the individual or cumulative actions of the phyto-constituents. We intend to undertake more research

on the phyto-chemical elements of *T. arjuna* that contribute to its pharmacological efficacy in diabetic rats.

Conclusion: Due to the presence of a significant number of bio active chemical components, almost every portion of the plant has a high ethno-pharmacological value with a wide range of traditional as well as pharmaceutical applications. Wound healing, cardio protective, hepato-protective, antioxidant, anti-cancerous, anti-inflammatory, analgesic, antidiabetic, antihelminthic, antibacterial, antiviral, and molluscicide properties have all been established in an experimental research. The current study found that an ethanol extract of *T. arjuna* possesses characteristics that enable it to promote faster wound healing in diabetic rats when compared to placebo controls. However, more comprehensive clinical study is needed to fully understand the therapeutic potential of *T. arjuna* different components in order to establish it as a standard medication.

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