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Original Article

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

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ABSTRACT

The goal of this study is to see how effective *Achyranthes Aspera (A.aspera)* is at healing dead space wounds in diabetic rats. 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: *A.aspera*). Animals were euthanized on day 10, cotton pellets and granuloma tissues were carefully collected and processed for further estimates. Overall, 18 rats were utilized in the experiment. During the trial, the *A.aspera* group had a substantial increase in dry and wet tissue weight when compared to the other groups. In addition, as compared to the control, *A.aspera* therapy dramatically enhanced Hydroxyproline, Hexosamines, Hexuronic acid, Tissue protein, and Lysyl oxidase. The use of *A.aspera* extract improves wound healing activity in diabetic rats, according to the results of this investigation. The phytoconstituents in *A.aspera* are responsible for the plant's wound healing ability.

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

INTRODUCTION

Wound healing is a multi-phased process that involves bleeding, coagulation, inflammation, proliferative, and remodelling.[1,2] Wound healing is typically hindered in diabetes mellitus (DM), resulting in non-healing, delayed healing, or persistent skin ulcers.[3] More than 371 million people are believed to have diabetes globally, and the number is rising significantly every year in virtually all nations.[4] Delayed wound healing in diabetes may be due to an imbalance in either

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inflammatory response, changed production of cytokines, altered collagen synthesis, insufficient angiogenesis, differentiation of the extracellular matrix, lower tensile strength or diminished growth factors.[5,6] As a result of the altered reaction described above, an increased risk of infection, inadequate wound contraction, and lower wound breaking strength may occur, resulting in extended hospitalisation and a higher death rate.[7] The migration of inflammatory cells to the wounded area is delayed in diabetic wounds, resulting in chronic inflammation, which leads to a failure of fibroblast growth and collagen synthesis, delaying healing processes.[6] Plants are high in phyto-chemicals, which have anti-inflammatory and antioxidant properties. Several indigenous medicines have been described in folklore Indian medicine for the treatment of cuts, bruises, burns, and wounds.

A.aspera, commonly known as apamarga, is a wild and prolific plant found in India. The plant's leaves are used to cure wounds by folk healers in Tamil Nadu, India. *A.aspera* has been shown to have wound healing, hepato protective, cancer fighting, antibacterial, and immunomodulatory activities.[8,9] Free radical scavenging and immune enhancing characteristics of the plant may explain its capacity to heal wounds. As a result, the goal of this research is to investigate if *A.aspera* can help diabetic wounds heal quicker.

MATERIALS AND METHODS

Preparation of extracts: The leaves were defatted in petroleum ether (60–80°C) for 72 hours after being shade-dried and roughly pulverized. The defatted drug was extracted using Soxhlet and distilled water. The obtained extracts were evaporated in vacuum to produce residues, and the extract percentage yields were determined to be 10.1 percent and 5.5 percent, respectively.

Animals: Healthy wistar rats of either sex (150–200 g) were utilised in this study, with no prior pharmacological treatment. The animals were fed a commercial pellet meal and were given unlimited water. The animals were acclimatised to laboratory hygienic conditions for 10 days before the trial began. The therapy was carried out with the approval of King Khalid University's animal ethics committee and in compliance with the National Institute of Health's standards for the care and use of laboratory animals in the US (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 was given ointment b; group II was given diabetic control; and group III was given *A.aspera*. 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 technique for creating dead space wounds.[10] Eighteen rats were divided into three groups of six individuals each. Subcutaneous dead space wounds were produced in the area of the axilla by creating a pouch through a tiny nip in the skin under general anaesthesia (achieved with 10 mg/kg body weight of xylazine hydrochloride and 50 mg/kg ketamine hydrochloride). 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 being grouped together, the animals 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. 5mL 6 N HCl was applied to the dry tissue and stored at 1108C for 24 hours. Hydroxyproline, 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.[11]

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.p.), 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. 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.[12]

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. The results were analyzed using one way analysis of variance (ANOVA) with post hoc Scheffe's test using Graph Pad and were expressed as the mean ±SD. P values less than 0.05 were considered as statistically significant.

RESULTS

The levels of blood glucose in the control and experimental animal groups are shown in Table 1. When compared to the control group, streptozotocin treated rats had considerably higher blood glucose levels. When compared to diabetic and control rats, the *A.aspera* therapy group 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	81.1 ± 8.2	244.5 ± 16.09	32.18 ± 5.50	286.46±14.47
Diabetic Control	274.38 ± 15.1[a]	167.5 ± 10.22[a]	23.5 ± 4.40[a]	176.56±1.27[a]
A.aspera	277.38 ± 15.1[a]	288.5 ± 14.09[a]	35.5 ± 5.50[a]	316.46±14.38[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 increased considerably in the *A.aspera* 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	15.72 ± 5.02	11.49 ± 2.47	13.11 ± 3.19	42.58 ± 3.90	1712 ± 69
Diabetic Induced	11.38 ± 2.20a	7.6 ± 1.50[a]	9.8 ± 1.42[a]	28.5 ± 2.60[a]	1129 ± 47[a]
A.aspera	16.72 ± 4.12[a]	13.49 ± 2.57[a]	15.11 ±3.19[a]	45.58 ± 3.90[a]	1915 ± 69[a]

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

DISCUSSION

In diabetic rats, the results of this investigation clearly indicated that *A.aspera* had improved wound healing activities. Streptozotocin has a clear anti healing impact as well as a hyperglycemic effect. Hyperglycemia is one of the most common clinical symptoms of diabetes mellitus, and it's linked to both micro and macro vascular problems.[13] Diabetes causes collagen alteration such as advanced glycation and cross linking, both of which are essential in the pathophysiology of diabetes mellitus.[14]

Diabetes wound healing deficiencies are multifaceted, complicated, and interconnected, and are thought to be caused by decreased blood flow and oxygen release as a result of high blood sugar levels.[15] Hyper glycosylation of locally produced cellular fibronectin is thought to be the cause of wound healing defects.[16] In the *A.aspera* therapy group, the hydroxyproline content increased. It's possible that a drop in hydroxyproline content in diabetic wounds is related to a decrease in collagen concentration and fibre stability. Low protein content and collagen bundle production were indicated by the reduction in wet and dry granulation tissue weight.

Wet and dry granulation weights, on the other hand, increased after *A.aspera* therapy. Skin, joints, eyes, and many other tissues and organs include glycosaminoglycans as a key component of the extracellular matrix. Despite its basic structure, it has exceptional visco elastic and hygroscopic characteristics that are important for cutaneous tissue function. Collagen fibres are known to be stabilised by glycosaminoglycans, which enhance electrostatic and ionic interactions with them and may influence their eventual alignment and size. Hexuronic acid and hexosamine levels were significantly reduced in our research. Hexuronic acid and hexosamine levels increased in *A.aspera* treated animals, which was unexpected. Lysyl oxidase is responsible for the maturation of collagen fibrils. This enzyme is involved in the production of cross-links and hence plays a critical part in the maturation and wound healing processes. Enhanced enzyme activity may result in increased cross linking and breaking strength of the granulation tissue in our investigation. Overproduction of reactive oxygen species causes

oxidative stress, which leads to cytotoxicity and slowed wound healing.

Streptozotocin is extensively utilised for the development of experimental diabetes mellitus because it destroys pancreatic b-cells by producing free radicals. Streptozotocin has been shown to cause lipid peroxidation and DNA breakage in pancreatic cells.[17] In streptozotocin-induced diabetes mellitus, Prakasan et al found increased lipid peroxidation and decreased antioxidants.[18] Another cause for poor wound healing patterns in sterptozotocin-induced diabetic rats might be this. In contrast to the above-mentioned characteristics, *A.aspera* has a stronger wound-healing action.

Conclusion: These results suggest that *A.aspera* promotes significant wound healing in diabetic rats. The results of this experiment warrant further investigation to validate it's suitability for humans.

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