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WOUND HEALING ACTIVITY OF *Azima tetracantha* Lam. ON ALBINO RATS

T. Nargis Begum[1], M. H. Muhammad Ilyas[2], R. Prasana[1], A. Jaswanth[3] and R. Krishnamoorthy[4]

[1]Department of Biochemistry, Pavendar Bharathidasan College of Arts and Science, Trichy-24
[2]Post Graduate Department of Botany, Jamal Mohammed College, Tiruchirapalli - 20 (India)
[3]Department of Pharmacology, Periyar College of Pharmaceutical Sciences for Girls,Trichy-21 (India)
[4]Department of Biotechnology, Pavendar Bharathidasan College of Arts and Science, Trichy-24 (India)

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ABSTRACT

In the present study, *Azima tetracantha Lam* was taken to investigate its wound healing property, excision and dead space wound model in albino rats, were used to study the healing efficiency. Formation (5% & 10% ointment) of ethanolic extract of *Azima tetracantha* (leaves) was applied topically over the excision wound. Formation of (100 mg/kg & 200 mg/kg) ethanolic extract of *Azima tetracantha*(leaves) was applied orally for dead space. It was found that ointment treated rats showed accelerated healing than control in excision wound. In dead space, the tensile strength was also found to increased in extract treated groups.

Key words: *Azima tetracantha*, ethanolic extract, wound healing activity, nitrofurazone.

INTRODUCTION

Wound healing is a complex (but orderly) phenomenon involving a number of processes, including induction of an acute inflammatory process by wounding, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of Extracellular Matrix (ECM) proteins, remodeling of connective tissue and parenchymal components, acquisition of wound strength.[1] In chronic wounds, the normal healing process is disrupted due to some unknown reasons and in such cases exogenous application of certain growth promoting agents or compounds which can enhance the *in situ* generation of these growth factors is required to augment the healing process.

Azima tetracantha, Family Salvadoraceae, is a rambling spinous shrub, flowering throughout the year, found in peninsular India, in Orissa and West Bengal. The leaves are considered stimulant and are given to women immediately after confinement. Leaves are eaten with food in rheumatism and their juice is given to relieve cough of phthisis and asthma. The leaves are reported to have been used in treating ulcers, especially after smallpox[2,3] . The present study was undertaken to evaluate the wound healing activity of ethanolic extract of *Azima tetracantha* and is reported in this paper.

MATERIALS AND METHODS

Fresh leaves of *Azima tetracantha Lam* were collected in Ponnamaravathi (Pudukottai District). The collected plants were Botanically identified and dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. The powdered materials were extracted with ethanol using Soxhlet extraction apparatus. This ethanolic extract was then concentrated and dried under reduced pressure. The ethanol free semi-solid mass (ethanol free) thus obtained was used for the experiment[4]. Two types of formulations were prepared from the extract Viz., 5% w/w and 10% w/

w, where 5g and 10g of extract was incorporated in 100g of simple ointment base B.P. [5] 0.5 g of each extract ointment and simple ointment were applied once daily to treat different groups of animals, respectively. Nitrofurazone ointment (0.2% w/w, Smithkline-Beecham) was used as a standard drug for comparing the wound healing potential of the extract.

Animals used

Wistar albino rats (150-180 g) were selected for the experiment. Six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water ad libitum.

Excision wound model

Four groups with six animals in each groups were anaesthetised by the open mask method with an aesthetic ether. The rats were depilated on the back. One excision wound was left undressed to the open environment. Then the drugs, i.e. the reference standard, (0.2% w/w) Nitrofurazone (NFZ) ointment, Simple ointment B.P, *Azima tetracantha* ethanolic extract (ATC) ointment (5% W/W) and (10% w/w) were administered till the wound was completely healed[5]. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day.

Dead space wound

Three groups of wistar albino rats (150-200g) were used. Dead space wounds were made by implanting, subscutaneously, a 2.5x2.5 cm polypropylene tube beneath the dorsal paravertebral lumbar skin. Control animals received 2ml of 1% carboxy methyl cellulose (CMC), orally, while the test groups received *Azima tetracantha* (100 mg/kg & 200 mg/kg) orally once daily for 10 days. On the 11th post-operative day, the granuloma tissue formed on the dead space wound was excised. Wet weight was recorded and the granuloma was dried in an over at 60^{o}C and dry weight noted [6]. The tensile strength was measured by continuous water flow technique[7]. In the preliminary phytochemical evaluations of the ethanolic extract of *Azima tetracantha* revealed of all the phytoconstituents tested i.e., alkaloids, reducing sugar, phytosterol, fixed oil and fats, phenolic compounds, tannins, proteins and amino acids, gums and mucilage, flavonoids, terpenes, lignins and saponins. The *Azima tetracantha* showed the presence of alkaloids reducing sugar, phytosterol, fixed oil and fats, phenolic compounds and tannins, proteins and amino acids, gums and mucilage, flavanoids, lignins and saponins. The above said plant products have been shown to possess a good therapeutic potential as an anti-inflammatory agents and promoters of wound healing due to presence of active terpenes, alkaloids and flavonoids[8,9]. The leaves of *Azima tetracantha* yield alkaloids including three dimeric piperidine alkaloids, viz., aziminie, azcarpine and carpaine. Friedelin, glutinol, lupeol and â-sitosterol have been isolated from the leaves of *Azima tetracantha*[10]. A glycosidal mixture extract of *Centella asiatica* has been reported to be responsible for enhanced repair only in incised wounds[11] and in stimulating collagen in human skin fibroblast cells[12]. These findings indicate that the wound healing potential of the *Azima tetracantha* appears due to the presence of its active principles, which, as growth promoting agents, may accelerate the healing process and confer breaking strength to the healed wound.

RESULTS AND DISCUSSION

The measurements of the progress of the wound healing induced by the NFZ ointment (0.2% w/w), Extract ointment (5% w/w and 10% w/w) and the control group in the excision wound model are shown in Table-1. After treatment from 2nd day to 6th the wound healing process was found to be maximum in 0.2% and 5%w/w respectively. Whereas on 8th day onwards, this was reverse and the wound was completely healed on 16th day in 10%w/w treatment. This may be due to the increased concentration of the leaf extract and age of the animal in synthesizing more collagen. It is observed that the wound contracting ability of the extract ointments were significantly greater than that of the control, which was comparable to that of the reference standard, NFZ ointment. The extract ointment produced complete healing at 18th day

Table - 1: Effect of *Azima tetracantha* leaf extract and Nitorfurazone on excision wound model

Post Wounding days	Wound Area (mm^2) Simple ointment (Control)	Nitrofurazone Ointment (o.2% w/w)	Extract ointments (5% w/w each)	Extract ointments (10% w/w each)
0	530 ± 33.6 (0)	516 ± 36.8 (0)	505 ± 23.0 (0)	518 ± 39.8 (0)
2	509 ± 18.6 (3.9)	458 ± 36.8 (11.2)	363 ± 19.8 (28)	435 ± 14.8 (16)
4	465 ± 13.8 (12.2)	318 ± 12.6 * (38.3)	343 ± 18.9 * (32)	389 ± 18.6 * (24)
6	424 ± 30.1 (20.0)	270 ± 14.7 * (47.6)	275 ± 14.3 * (45)	297 ± 19.4 ** (42)
8	389 ± 14.8 (26.6)	193 ± 11.4 ** (62.5)	260 ±11.5 **(68)	143 ± 9.8 ** (72)
10	345 ± 23.6 (34.9)	110 ± 8.6 ** (77.3)	105 ± 8.6 ** (79)	94 ± 5.9 ** (81)
12	269 ± 14.3 (49.2)	79 ± 6.3 ** (84.6)	62 ± 5.4 ** (87)	43 ± 2.1 ** (91)
14	215 ± 11.3 (59.4)	36 ± 1.6 ** (93.0)	32 ± 2.8 ** (93)	16 ± 1.3 ** (96)
16	189 ± 14.3 (64.3)	10 ± 1.9 ** (98.0)	13 ± 0.04 * (97)	0.0 ** (100)
18	171 ± 15.1 (67.7)	0.0 ** (100)	0.0 ** (100)	

Values are mean ± S.E of 6 animals is each group figures in parentheses indicates percentage of wound contraction.

p < 0.01., ** p< 0.001 Vs respective control by students t - test.

and 16th day by 5% w/w and 10% w/w respectively.

In Dead space wound studies, the ethanolic extract of *Azima tetracantha* at (100 mg/kg & 200 mg/kg produced a significant increase in the wet granuloma tissue as well as in the dry weight. The tensile strength were also found to be increased (P<0.001) in the extract treated groups (Table-2). The water content of the animal tissue in control is found to be lesser when compared to the treated animals. It was 369.8 mg in 100 mg/kg 387.8 mg in 200 mg/kg. As the retention of water content is maximum the tensile strength is also high (Table. -2). Wound healing was a correlation with the tensile strength. From this experiment it is clear that feeding the animal with the leaf extract of *Azima tetracantha,* made the tissue to retain water, which may trigger the wound heading metabolism.

Table - 2: Effect of leaf extracts of A*zima tetracantha* on dead space wound in rats (Mean ± S.E., n = 6)

Treatment	Wet granuloma weight (mg)	Dry granuloma weight (mg)	Tensile strength (g)
Control (1% CMC, 2ml, P.O)	210.3± 11.2	34.6 ± 2.1	380 ± 26.1
Leaf extract (100 mg/kg, P.O)	476.6 ± 21.4 *	106.8 ± 73 *	543.7 ± 33.7 *
Leaf extract (200 mg/kg, P.O)	515.8 ± 26.7 *	128 ± 8.2 *	668 ± 38.6

Data are expressed as mean ± S.E., n=6.

• P< 0.001 Vs control by student's "±" – test

The process of wound healing occurs in four phases:(i) coagulation, which prevents blood loss (ii) inflammation and debridement of wound (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition. Any agent, which accelerates the above process, is a promoter of wound healing. Plant products have been shown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing, due to the presence of active terpenes, alkaloids and flavonoids[13]. The wound healing property of the leaves extract of *Azima tetracantha* appears to be due to the presence of its active principles which accelerates the healing process and confers breaking strength to the healed wound and present work confirm previous report on the presence and activity of active substances.

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