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Research Article

WOUND HEALING ACTIVITY OF ALCOHOLIC EXTRACT OF *NYCTANTHES ARBORTRISTIS* L. IN WISTAR RATS

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ABSTRACT

Healthy wistar rats of either sex were chosen and were divided in to for group (n=60. They were administered single dose of alcoholic extract of *N. arbortristis* orally The wound healing effect of alcoholic extract of *Nyctanthes arbortristis* (Family. Oleaceae) and its effect in dexamethasone suppressed wound healing was studies in wister rats. There wound models viz.incision,and excision wounds were used in this study. The parameters studied were breaking strength in case of incision wounds. epithelization and wound contraction in case of excision wound. The dexamethasone treated group showed a significant ($p<0.001$) reduction in the wound breaking strength when compared to control group in incision type of wound model co administration of *Nyctanthes arbortristis* with dexamethasone had significantly ($P<0.001$) increased the breaking strength of dexamethasone treated group. In excision wound model, the percentage of the wound contraction was significantly ($p<0.05$) increased by *Nyctanthes arbortristis* only on 16th day and also it reversed the dexamethasone suppressed wound contraction on the 16th day. *N.arbortristis* significantly ($P<0.001$) reduced for epithelization and reserved the epithelazation delaying effect of dexamethasone significantly ($P<0.001$).

Keywords: *Nyctanthes arbortristristis*, Dexamethasone, Wound contraction, Wound breaking strength.

INTRODUCTION

Wound is a breach in the normal tissue continuum, resulting in a variety of cellular and molecular sequelae . The basic principles of optimal wound healing which include minimizing tissue damage, debriding non-viable tissue, maximizing tissue perfusion and oxygenation, proper nut ration and moist wound healing environment have been recognized for many years 1. A number of drugs ranging from simple non- expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound affect healing either positively or negatively. Aspirin, Indomethacin, Cytotoxic agents and immunosuppressant have been proved experimentally to effect healing negatively. [2, 4, 5, 6]

Medicinal herbs are an indispensable part of traditional medicine. The leaves of *Nyctantes arbortristis* finds an important place in indigenous medicine as an asthma, piles, and fever. It is used for the treatment of various skin disease. The however to the best of our knowledge a systematic study on wound healing activity of *N.arbortristis* has not been undertaken. Hence, The present study was undertaken to evaluate the wound healing property of alcoholic extract of *N. arbortristis* leaves and to study its influence on Dexamethasone suppressed wound healing on various animal wound model in wistar rats.

MATERIALS AND METHODS

Plant material collection and preparation

Nyctanthes arbortristis fresh leaves were collected from Vidisha (M.P.) India, in month of July . The plant was identified and authenticated by Dr. P. G. Diwakar, Joint Director, Botanical Survey of India, Pune,(M.H.). India, where a voucher specimen (No. BSI/WRC/Tech/2010-Nyct ARMP1) of the plant has been kept in the herbarium.

Plant extract preparation

Could extraction method was followed. 100g of the fresh and dry samples were weighed in to 1000ml conical flask and 1000ml of alcoholic or water was added and left for 48h. The mixtures were filtered under vacuum pressure and the filters were concentrated using rotary evaporator and subjected for the various activity studies.

Chemicals

All chemical and regents used were of the analytical grade purchased from BDH, Merck, Qualigens. and Ranbaxy.

Animal care and Handling

This was done as per the guidelines set by the Vogel (2002). Twelve week-old healthy Wister rats (150-200g) of either sex bred carried out experimental work National Toxicology Center Pune (M.H.). They were housed under controlled conditions of temperature of 23±2ºc, humidity of 50±5%and 10-14 h of light and dark cycles respectively. The animal were housed individually in polypropylene cases containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food (animal chow) (M/s Hindustan Lever Ltd.) and water *ad libitum* . The study was undertaken after obtaining the approval of Institutional Animal Ethical Committee (IEAC approval letter No. IEAC/RP.77.2009-2010.Dated July 23-2010).

Study Design

The animals were randomly allocated in to four groups of six animals each for the three experimental animal wound model.

Group 1 received 2ml of gum acacia 2% (E. Merck India Ltd.) po through intragastric tube

Group 2nd received *N.arbortristis* .300mg/kg po. The dose selection was based on the toxicity studies.

Group 3rd received Dexamethasone. 0.17mg/kg(13) (Cadila Healthcare, Mumbai) Im.

Group 4th received Dexamethasone (0.17 mg/kg im) & N.arbortristis (300mg/kg) po.

The suspension of the alcoholic extract of *N. arbortristis* was made in 2% gum acacia. Studies in group 4th extract of *N.arbortristis* was administered immediately after intramuscular injection of Dexamathasone.

Acute Toxicity Studies

Healthy wistar rats of either sex were chosen and were dived in to four groups (n=6). They were administered single dose of alcoholic extract of *N.arbortrisis* orally with increasing doses of 100, 300, 1000, 3000, mg/kg body weight respectively. The doses up to 300mg/kg was well tolerated without producing any sings of toxicity and mortality. 10% of the maximum tolerated dose i.e. 300 mg/kg was selected for the study.

Dosing Schedule

N.arbortristis extract and Dexamethasone were administered orally and intramuscularly respectively once daily from 0 day to 9th in the incision wound models from 0 day to the day of complete healing or the 31st postoperative day, whichever occurred earlier in the excision wound model. In group 4th *N.arbortristis* extract was given after in the injection of Dexamethasone.

Wound models

All wounding procedures were carried out under light ether anesthesia. In the present study no animal showed visible sings of infection.

Incision wound

On the depilated backs of the animals, two Para vertebral incisions 6cm in length were made, cutting through the full thickness of the skin. Interrupted sutures, 1cm apart, were placed to approximate the cut edges of the skin[14].

The studies were removed on the 7th post wound day and skin breaking strength was measured on the 10th day by continuous water flow technique of lee [3].

Excision wound

An Excision wound was inflicted by cutting away 500 mm^2 full thickness of ± pre-determined area on the depilated back of the rat. Epithelization period was noted as the number of days after wounding, required for the scar to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of the wound area on the alternate days. This was achieved by tracing the wound on the graph paper. Reduction in the wound area was expressed as percentage of the original wound size[17].

Statistical analysis

Results were analyzed by one way analysis of variance (ANOVA) followed by Scheffe's test using SPSS computer package vession-11.

RESULTS

Incision wound model

The mean breaking strength in the control group was 348.27± 7.8g . The alcoholic extract of *N.arbortristis* did not after the breaking strength when compared to control. In the dexamethasone treated group the mean breaking strength was 166.03±7.45 g which was significantly (P<0.001) less compared to control group. co administration of *N.arbortristis* with Dexamethasone has significantly (P<.001) increased the breaking strength to 292.6±11.72g (Table 1).

Table 1: Wound breaking strength in incision wound model.

Drug	Dose/route	Breaking strength (g) Mean ±S.E
Gum acacia	2 ml oral	348.27±7.8
N.arbortristis	300mg/kg im	349.78±9.13
Dexa	0.17 mg/kg	166.03±7.45e
Dexa + *N.arbortristis*	0.17 mg/kg im+300mg/kg oral	292.6±11.72d

Dexa =Dexamethasone

aP<o.o5 Vs Dexamethasone. Oneway ANOVA, F=5.004,df=3, 28

bP<o.o5Vs Control, oneway ANOVA, F=2.939,df=3,28

cP<0.001Vs Control, Oneway ANOVA, F= 88.249, df=28

dP<0.001 Vs Dexamethasone, Oneway ANOVA,F=88.249,df,28.

Table 2: Effect of *N.arbortristis* on excision wound parameter

Drugs	Dose /route	4th day	8th day	12th day	16th day	Periodof epithelization (days) Mean ± S.E
Gum acacia	2 ml; oral	27.75 ± 4.38	47.15±5.25	59.45 ± 2.77	68.67 ± 1.28	16.75± 0.75
N.arbortristis	300mg/kg; oral	21.2 ± 3.21	48.25±4.46	67.55 ± 3.48	82.1±2.22	11.12± 0.47c
Dexa	0.17mg/kg; im	23.4 ± 3.32	39.57±3.58	55.85 ± 2.39	67.02±2.12	17.25±0.75
Dexa+*N.arbortristis*	0.17mg/kg; im+300mg /kg oral	26.25 ± 3.32	37.5±2.64	65.77 ±0.93	76.22 ± 1.03	12.75±0.77d

Dexa =Dexamethasone

aP<0.001 Vs Control, Oneway ANOVA, F=5.004, df=3,28

bP<0.001 Vs Dexamethasone, Oneway ANOVA, F=2.939,df=3,28.

cP<0.001 Vs Control, Oneway ANOVA, F=18.483, df=3.28

dP<0.001 Vs Dexamethasone, Oneway ANOVA, F=18.483, df=3, 28.

Excision Wound

The percentage of wound contraction was 27.75±4.38, 47.15 ± 5.25, 59.45 ± 2.77 and 68.67± 1.28 as measured on the 4th,8th, 12th , and 16th day respectively in the control group. The wound contraction rate was not altered significantly in any of the test groups on 4th, 8th and 12th day as compared to control group at same time. Apart from this, we also noted a positive trend wound contraction rate in *N.arbortristis.* treated group and negative trend in wound contraction rate in dexamethasone treated group even through they were not statistically significant on and 12th day.

However wound contraction rate was significantly increased in *N.arbortristis* treated group compared to the control group on 16th day (p<.001) (82.1 ± 2.22) similar observation was also made in the dexamethasone & *N.arbortristis* treated group when compared to

the dexamethesone treated group where it increased from 67.02 ± 2.12 to 76.22 ± 1.03 on 16th day (P<0.001) (Table 2nd). The mean period of epithelization in the control group was 16.75 ± 0.75 days. It was significantly (p<0.001) red used to 11.12 ± 0.47 days in *N.arbortristis* treated group. The mean period of epithelialization in dexamethesone treated group was 17.25 ± 0.75 days which was significantly (P<0.001) reduced to 12.75± 0.77 days in the group treated with both dexamethasone and *N.arbortristis* (Table 2nd).

DISCUSSION

Granulation, collagen maturation and scar formation are some of the many phases of wound healing which run concurrently, but independent of each other. The use of single model is inadequate and no reference standard exits that can collectively represent the various phases of wound healing. Hence three different model have been chosen in our study to assess the effect of *N.arbortristis* on wound healing. The increase in weight in Dexamethasone treated group could be due to high protein concentration add collagen bundle formation [2]. It is difficult to explain the effect of *N.arbortristis* along with the Dexamethasone as there was a slight increase in breaking strength and dry weight of granulation tissue in the Dexamethasone alone treated group compared to control group.

Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to due to the activity of myofibroblast [5]. Since *N.arbortristis* enhanced wound contraction, it wound have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited in to the wound area. In excision wound model *N.arbortristis* hastened the period of epithelization significantly and the co- administration of *N.arbortristis* with dexamethasone hastened of the epithelization in dexamethasone group. Even though only during later part, *N.arbortristis* showed significant increase in wound contraction we have observed the positive trend in the initial stages concomitant administration of *N.arbortristis* along with dexamethasone had also significantly increased the wound contraction on 16th day. Hence it appears that *N.arbortristis* has prohealing effect as evidenced by the above finding. It also appears that *N.arbortristis* was able to promote epithelization either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells. It is difficult to draw any conclusion from the study regarding the dexamethasone & *N.arbortristis* effect in dexamethasone suppressed wound model.

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