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Effect of root extract of Aegle marmelos on dermal wound healing in rats

A Jaswanth, S. Sathya, S. Ramu, A. Puratchikody, and K. Ruckmani.
Department of Pharmacology, Periyar college of Pharmaceutical sciences for girls, Sathanoor Main Road K.K. Nagar Post, Tiruchirappalli – 620 021.

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ABSTRACT: *The wound healing effect of methanolic extract of the root of aegle marmelos was evaluated in the form of an ointment with two different concentrations (5% and 10% w/w in simple ointment base) in excision wound model and incision wound model in rats. In both the concentrations, the extract ointment produced a significant response in both the wound types tested, as evidenced by its wound contracting ability, wound closure time and increase in the tensile strength. The results were also comparable to those of a standard drug nitrofurazone*.

INTRODUCTION

Aegle marmelos (Rutaceae) is a moderate-sized slender, aromatic tree 6.0-7.5 m in height and 90-120cm in girth, growing wild throughout the deciduous forests of India1. The leaves are used externally to heal wounds, boils and cuts2,3. The expressed juice of the leaves are used in eye infections[4]. The unripe fruit is regarded as astringent, digestive and stomachic. It is beneficial in cases of diarrhoea and dysentery. The ripe fruit is sweet, aromatic and cooling[1]. Te root is sweet, cures fevers, due to "tridosha" pain in the abdomen, palpitations of tee art, urinary troubles hypocheondriasis, melancholia; removes "Vata", pitta" and " Kapha"[4] Te root was used for shake bite[5]. The present study was undertaken to evaluate the wound healing potential of the root extract of this plant.

MATERIALS AND METHODS
PLANTMATERIALS

Te root of Aegle marmeios were collected at Gobichettipalayam, Tamilnadu, The roots were shade dried, pulverized b a mechanical grinder, sieved through 40 mesh and then stored in a well closed container for further use. Extracts and standard used.

The powdered roots were extracted with methanol using a soxhlet extraction apparatus. This methanol extract was then concentrated and dried under reduced pressure. The semi solid mass (methanol free) thus obtained was used for the experiment. Two types of ointment formulations were prepared form the extract; 5% (w/w) and 20% (w/w) where 5 g and 10g simple ointment base B.P6 respectively. Nitrofurazone ointment (0.2% (w/w) smithkline-Beecam) was used as a standard drug for comparing the wound healing potential of the extract.

ANIMALSUSED

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

EXCISION WOUND MODEL7

Four groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. One excision wound was inflicted b cutting away a 500 mm^2 full thickness of skin from the depilated area, the wound was left undressed to open environment. Then the drugs, i.e the reference standard, (2.0% /w) nitrofurazone (NF) ointment, simple ointment B.P. Aegle marmelos root extract (AMRE) ointment (10% /w) were applied once daily till the wound was completely ealed8. This model was used to monitor wound contraction and wound closure time,. Wound contraction was calculated as percent education on wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day.

INCISION WOUND MODEL

Four groups with six animals in each group were ana3stetised and two paravertebral-long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5cm intervals; surgical threads (N0.000) and a curved needle (N0.11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed, all the groups were treated in the same manner as has already described above/. The were administered once daily for 9 days; when wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured with a tensiomener[9].

MEASUREMENT OF HEALING

Tensile strength, the force required to open a healing skin wound, was used to measure healing. Te instrument for his measurement is called tensiometer was designed on the same principle as the tread tester used in the textile industry. It consisted of a 6x12 inc board with one post of 4 inc long fixed on each side of the longer ends. The board was placed at the end of a table. A pulley with bearing was mounted on the top of one of the posts. An alligator clamp wit 1 cm width, was tied on the tip f the post without pulley b a piece of fishing line (20-lb test monofilament) so that the clamp could react e middle of the board. Another alligator clamp was tied on a piece of fishing line wit a 1-L polyethylene bottle tied on the other end. Before testing, the animal was anesthetized with ether in an open mask. The sutures of the wound were cut out with a pair of scissors, The animal was then placed on a stack of paper towels on the middle of the board. The amount of the towels could be adjusted so that the wound was on the same level of the tips of the posts. The clamps were then carefully clamped on the skin of t opposite sides of the wound at a distance of 0.5 cm away from the wound. The longer piece of fishing line was placed on the pulley, and the position of the board as adjusted so that the polyethylene bottle was freely hanging in the air. Water was but constant rate by siphon from a large reservoir (20-L bottle) until the wound began to open up. The amount of water in the polyethylene bottle was weighed and considered as the tensile strength of the wound.

STATISTICALANALYSIS

Data are expressed as mean ± SEM and subjected to student's t-test by comparing with the control.

pages 111 - 114

RESULTS AND DISCUSSION

Te measurements of the progress of the wound healing induces by the NF ointment (2% w/w), AMRE ointment (5% w/w), AMRE ointment (10% w/w) and the control group (i.e. simple ointment in the excision wound model are sown in Table 1. It is observed that the wound contracting ability of t AMRE ointments were significantly wound healing from the fourth day onwards, which was comparable to that of the reference standard, NFZ ointment.

In the incision wound studies, there was a significant increase in tensile strength of the 10-day old wound due to treatment with the (5% w/w & 10%w/w) and the reference standard NEF ointment when compared with the control group. The measurements of the tensile strength are shown in Table 2. The tensile strength of the AMRE ointment (10% w/w) treated group were almost the same to that of the NFZ ointment treated group AMRE ointment 5% w/w sowed a lesser but significant increase in the tensile strength compared to the control group.

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 would healing. Plant products have been sown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing, due to the presence of active terpenes, alkaloids and flavonoids [10]. The roots contained xanthotoxin, 6,7-dimethod coumarin, scopoletin, tembamide, umbelliferone, marmesin, marmin skimmianine and a glycoside skimmin[11]. A glycosidal mixture extract of Centella asiatica has been reported to be responsible for enhanced repair only in incised wounds[12] and in stimulating collagen inhuman skin fibroblast cells [13]. Te wound healing property of the root extract of Aegle marmelos appears to be due to the presence of its active principles which accelerates the healing process and confers breaking strength to the healed wound.

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Table-1
EFFECT OF AEGLE MARMELOS ROOT EXTRACT AND NITROFURAONE ON EXCISION WOUND MODEL

Post Wounding Days	Wound Area (mm2)			
	Simple ointment (Control)	Nitrofurazone ointment (0.2% w/w)	Extract ointment (5%w/w)	Extract ointment (10% w/w)
0	530 ± 33.6(0)	516 ± 36.8(0)	521±23.0 (0)	534± 39.8(0)
2	509 ± 18.6 (3.9)	458 ± 36.8 (11.2)	476±19.8 (8.6)	411±14.8 (20.4)
4	495±13.8(12.2)	318 ± 12.6*(38.3)	349±18.6 (33.0)	326±18.6* (34.7)
6	424 ± 30.1 (20.0)	270 ± 14.7*(47.6)	288±14.3 *(44.7)	199±19.5 **(60.8)
8	389 ± 14.8(26.6)	193 ± 11.4**(62.5)	199±11.5 **(61.8)	134±9.8** (74.0)
10	345 ± 23.6(34.9)	110 ± 8.6**(77.3)	118±8.6** (77.3)	89 ± 5.9**(83.3)
12	269 ± 14.3 (49.2)	79 ± 6.3**(84.6)	79±5.4** (84.8)	48± 2.1**(91.0)
14	215 ± 11.3 (59.4)	36 ± 1.6**(93.0)	40±2.8** (92.3)	29± 1.3**(94.5)
16	189 ± 14.3 (64.3)	10± 1.9**(98.0)	15±0.4** (97.1)	11**(97.9)
18	171 ± (67.7)	0.0**(100)	0.0**(100)	0.0**(100)

Values are mean ± S.E of 6 animals in each group. Figures in parentheses indicates percentage of wound contraction *P<0.01.,**P<0.001 Vs respective control by student's t-test.

Table-2

EFFECT OF AEGLE MARMELOS ROOT EXTRACT AND NITROFURAONE ON INCISION WOUND MODEL

GRUP	TREATMENT	TENSILESTRENGTH (g)
1	Simple ointment (control)	341 ± 14.6
2	Extract ointment (5%w/w)	526 ± 15.8*
3	Extract ointment (10%w/w)	565 ± 13.9*
4	Nitrofurazone ointment (0.2%w/w)	558 ± 16.9*

Values are mean± S.E of 6 animals in each group. Tensile strength measured at the end of 9^{th} day. *P<0.001 Vs respective control by student's t-test.