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Wound Healing Activity of *Aegle marmelos*

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Effects of topical and intraperitoneal administration of methanolic extract of *Aegle marmelos* ointment and injection was studied respectively on two types of wound models in rats, (i) the excision and (ii) the incision wound model. Both the injection and the ointment of the methanol extract of *Aegle marmelos* produced a significant response in both of the wound types tested. In the excision model the extract-treated wounds were found to epithelialise faster and the rate of wound contraction was higher, as compared to control wounds. The extract facilitated the healing process as evidenced by increase in the tensile strength in the incision model. The results were also comparable to those of a standard drug nitrofurazone.

Aegle marmelos (Rutaceae) is a moderate-sized slender, aromatic tree 6.0-7.5 m in height and 90-120 cm in girth, growing wild throughout the deciduous forests of India[1]. The root is sweet, cures fever, pain in the abdomen, palpitations of the heart and urinary troubles[2]. The unripe fruit is regarded as astringent, digestive and stomachic. It is beneficial in cases of diarrhea and dysentery. The ripe fruit is sweet, aromatic and cooling[1]. The expressed juice of the leaves is used in eye infections[2]. The leaves are used externally to heal wounds, boils and cuts[3,4]. Based on its use in wound healing in traditional practices, the present study was initiated to evaluate the wound healing activity of an extract of *Aegle marmelos*.

EXPERIMENTAL

Plant material:

Fresh leaves of *Aegle marmelos* were collected at Gobichettipalayam, Tamilnadu, India. The leaves were shade-dried, powdered and sieved through 40 mesh and then stored in a well-closed container for further use.

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Preparation of extracts:

The powdered leaves 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 formulations were prepared from the extract: (i) 5% (w/w) ointment where 5 g of extract were incorporated in 100 g of simple ointment base BP[5]. The extract ointment and the simple ointment 0.5 g each was applied once daily to treat different groups of animals, respectively. (ii) intraperitoneal injection (200 mg/kg) of the extract in (2%w/v) Tween 80 aqueous solution was given to another group. Nitrofurazone (NFZ) ointment (0.2%w/w, Smithkline-Beecham) was used as a standard drug for comparing the wound healing potential of the extract. One simple ointment treated group and another 2% w/v Tween-80 aqueous solution treated groups served as controls for the extract ointment and injection-treated groups of animals, respectively.

Effect on excision wounds[6]:

Wistar albino rats (150-180 g) were selected for these studies. Six rats were taken for each group. The rats were

used after an acclimatization period of 7 d to the laboratory environment. They were provided with food and water *ad libitum*. Five groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. One excision wound was inflicted by 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, (0.2% w/w) NFZ ointment, simple ointment BP., *Aegle marmelos* methanol extract (AME) ointment (5% w/w), and AME injection (200 mg/kg, i.p.) were administered till the wound was completely healed.[7]. 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.

Effect on incision wounds:

Five groups with six animals in each group were anaesthetized 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[8]. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5 cm intervals; surgical threads (No.000) and a curved needle (No.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. They were administered once daily for 9 d; when wounds were cured completely the sutures were removed on the day 9 and tensile strength was measured with a tensiometer[9].

Statistical Analysis:

Data are expressed as mean±SEM and subjected to student's t-test and the level of significance was set at $P<0.001$.

RESULTS AND DISCUSSION

The measurements of the progress of the wound healing induced by the NFZ ointment (0.2% w/w), AME

TABLE 1: EFFECT OF *AEGLE MARMELOS* EXTRACT AND NITROFURAZONE ON EXCISION WOUNDS

Post Wounding Days	Wounding Area (mm^2) Simple ointment (control)	Nitrofurazone ointment (0.2%w/w)	Extract ointment (5%w/w)	Injection vehicle (control)	Extract Injection (200 mg/kg, i.p.)
0	525±21.1(0)	516±36.8(0)	522±22.3(0)	520±38.6(0)	513±39.8(0)
2	505±18.6(3.8)	458±36.8(11.2)	461±19.8(11.7)	501±15.6(3.6)	408±14.8(20.4)
4	465±13.8(11.4)	318±12.6(38.3)	369±18.9(29.3)	470±31.3(9.6)	335±18.6(34.7)
6	405±30.1(22.8)	270±14.7(47.7)	286±14.3(45.2)	400±25.6(23.0)	201±19.4(60.8)
8	373±14.8(28.9)	193±11.4(62.5)	190±11.5(63.6)	384±16.8(26.1)	103±9.8(79.9)
10	311±23.6(40.7)	110±14.6*(78.6)	100±8.6*(80.8)	345±20.8(33.6)	73±5.9*(85.7)
12	288±14.3(45.1)	79±6.3*(84.6)	73±5.4*(86.0)	273±16.1(47.0)	31±2.1*(93.9)
14	211±11.3(59.8)	36±1.6*(93.0)	28±2.8*(94.6)	209±16.5(59.8)	12±1.3*(97.6)
16	188±14.3(64.1)	10±1.9*(98.0)	8.0±0.4*(98.4)	191±13.4(63.2)	0.0* (100)
18	159±15.1(69.7)	0.0*(100)	0.0*(100)	184±12.8(64.6)	0.0* (100)

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

TABLE 2: EFFECT OF *AEGLE MARMELOS* EXTRACT AND NITROFURAZONE ON INCISION WOUNDS

GROUP	TREATMENT	TENSILE STRENGTH (g)
1	Simple ointment (control)	405 ±16.9
2	2% w/v Tween 80 aqueous solution (Injection Control)	411 ±13.6
3	Extract ointment (5% w/w)	585 ±11.8*
4	Extract injection (200 mg/kg, i.p.)	612 ±16.9*
5	Nitrofurazone ointment (0.2% w/w)	598 ±14.9*

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

ointment (5% w/w), AME injection (200 mg/kg, i.p.) and the respective control groups (i.e. simple ointment and 2% w/v Tween 80 aqueous solution treated groups) in the excision wound method are shown in Table 1. It is observed that the wound contracting ability of the AME ointment and injection were significantly greater than that of the control. The AME injection treated group showed much greater contraction of wounds from the sixth day onwards than those treated with the reference standard, NFZ ointment. The time to wound closure of the AME injection (200 mg/kg) was less than in the other groups (16±2 d). In the case of NFZ ointment- and AME ointment-treated groups it was found to be 18±2 d.

In the incision wound studies, there was a significant increase in tensile strength of the 10-d old wound due to treatment with AME injection, AME ointment and the reference standard NFZ ointment when compared with the respective control. Measurements of the tensile strength are shown in Table 2. The tensile strength of the NFZ ointment- and the AME ointment-treated groups were almost the same but the tensile strength of the AME injection-treated group was much greater than that of the NFZ ointment-treated 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[10]. Any agent, which accelerates the above process is a promoter of wound healing. Plant products have been shown to possess good therapeutic potential as antiinflammatory agents and promoter of wound healing, due to the presence of active terpenes, alkaloids and flavonoids[11,12]. A phenolic base containing oxazole and pyridine moieties have been isolated from the leaves of *Aegle marmelos*. The leaves contained tannins, phlobatannins, flavan-3-ols, leucoanthocyanins, anthocyanins and flavonoid glycosides. An essential oil from the leaves had broad-spectrum antifungal activity comparable to that of 0.5% hamycin[13]. A glycosidal mixture extract of *Centella asiatica* has been reported to be responsible for enhanced repair only in incised wounds[14] and in stimulating collagen in human skin fibroblast cells[15]. The wound healing property 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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