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RESEARCH ARTICLE

INVITRO WOUND HEALING ACTIVITY OF THE AERIAL PARTS OF *Urena Lobata* L.

Mr. R.Thirumalaikumaran[1]*, Dr.D.Chamundeeswari[2], Dr.S.Seethalakshmi[3], Dr.V.Gopal[3]

1 -Department of Pharmacognosy, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai, India-600116.
2- Department of Pharmacology, Employee State Insurance Medical College and Research Institute, Chennai,
3- Mother Theresa Institute of Post Graduate and Pharmaceutical Sciences, Pudhuchery.

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Abstract:
Restoration of normal cell structures is favoured through the healing of wounds which is depending upon migration and proliferation. Collagen formation, angiogenesis and migration of cells is an intricate process of wound healing through fibroblasts. Currently there is less evidence for the wound healing at cellular level. Alcoholic extract of the aerial parts of *Urenalobata*was used to study the fibroblast cell migration and proliferation using scratch wound assay procedure. The present study provides the scientific rationale of the the ethanolic extract of plant in the management of wounds.

Keywords: *Urenalobata*, Malvaceae, Scratch assay and fibroblast proliferation

Introduction

Wounds are dermal changes in which opening or breaking of the skin may be resulted due to accidental or intentional trauma. Anatomical stability may be restored through the proper healing of and the skin functional status. There may be a sequence of events for the repairing of the tissues such as inflammation, proliferation and migration of cell types [1].Healing is also completed by knitting of collagen.The treatment and cure of various diseases in Indian traditional medicine is based on the herbal origin [2].Management of wound by herbal medicine involve debriment, providing moist environment and disinfection [3].*Urenalobata*also known as Ceaser weed is an erect, branched shrub of 0.6m to 2.5m high. It is very variable and more or less hairy. Its stem is often with reddish branches. The leaves are pale beneath, heart shaped at the base, toothed or somewhat lobed or angled, [4].

The flowers are pink or purplish, about 1.7mm in diameter and borne singly in the axil of the leaves, [5]. The fruits are rounded, but flattened and about 7mm in diameter with carpels covered with barbed spines, [6]. Traditionally the *leaves* are used as demulcent,vulnerary,against gonorrhoea,as a haemostat,contraceptive,for liver disease and applied as a poultice to ulcers.Flowers are expectorant .Bark juice is used against dysentery.Seeds are potent vermifuge.Stems furnish a textile fibre.[7]The present study evaluates the wound healing effect of the aerial parts of *Urenalobata* by 3T3 fibroblast cell migration and proliferation by scratch assay which mimics in *in vivo* migration cells.

Urenalobata is one of the useful medicinal plants that is used extensively in ethno medical practices It is an erect, branched shrub of 0.6m to 2.5m high.It is very variable and more or less hairy. Its stem is often with reddish branches. The leaves are pale beneath, heart shaped at the base, toothed or somewhat lobed or angled,[8]. The flowers are pink or purplish, about 1.7mm in diameter and borne singly in the axil of the leaves, [9]. The fruits are rounded, but flattened and about 7mm in diameter with carpels covered with barbed spines, [10].Different extracts of the leaves were used in herbal medicine to cure diverse ailments. The medicinal potentials of this shrub as claimed by traditional medical practitioners range from the cure of rheumatism, gonorrhea, toothache and wounds to fungal infections (ringworm) expulsion of worms, intestine cleansing. In South Africa it is used for high blood pressure, fever, asthma, syphilis; [11, 12].

MATERIALS AND EQUIPMENTS

1. Ethanolic extract of *Urenalobata*
2. Human Keratinocyte Cell Line (HaCaT)
3. Mouse fibroblast 3T3 cells
4. Dulbecco's Modified Eagle Medium, DMEM (Invitrogen #10313-021)
5. Fetal Bovine Serum (ATCC #30-2020)
6. PBS (Invitrogen #14190-144)
7. BD Falcon 24-well tissue culture plate (Fisher #08-772-1H; BD #353226)
8. Rainin pipet tips, 1 ml (Rainin #GPS-L1000)
9. Glutaraldehyde (Sigma-Aldrich #G6257)
10. Ethanol (Sigma-Aldrich #459836)
11. Crystal violet (Sigma-Aldrich C3886)

Cell culture incubator: 37 °C and 5% CO_2.

PLANT MATERIAL

Aerial parts of the plant *Urenalobata* was collected from Thirunelveli district during the month of February 2010 and authenticated by the Dr.Chelladurai,Taxonomist.

PREPARATION OF ETHANOLIC EXTRACT OF *URENA LOBATA*

2.5 kg of shade dried powder of the plant was extyracted using ethanol and concentrated in rotary evaporator at 50°-60°C under reduced pressure leaving a dark brown residue which was stored in air tight container at 4° C till further Use.

PROCEDURE

Wound healingScratch assay

For the in vitro scratch assay [13,14], HaCaT cells were seeded in six-well plates (Nunc, Wiesbaden, Germany) in a density of 5 10^5 cells/well in growth medium. Cells were grown until they had reached a confluence of about 80%. Then a scratch was made through each well using a sterile 200-ll pipet tip. Cells were washed twice with PBS pH 7.4 and the medium was changed. As a positive control TGF-b1 (1 ng/ml) was added to the medium; the test ethanolic extract of *Urenalobata*, which was added in a concentration of 50µg/ml. Scratches were documented under the microscope (x150) immediately after the wounding procedure and once more when kept at 37 LC, 5% CO_2 for 48 h. Pictures were taken exactly at the same position beforeandafter the incubation to document the repair process. The experiments were repeated twice and representative picturesareshown.

Cell proliferation assay

Fibroblast 3T3 (103) cells were seeded in a 96-well culture plate in a humidified 5% CO2 atmosphere. Cells were serum starved for 24 hours and then incubated with 5, 10, 25 and 50µg/ml of test ethanolicextract of *Urenalobata* and further incubated for 24 hours. After 24 hours 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added and formazan product was dissolved in 100µl of DMSO. Absorbance at 570 nm was measured with microtiter plate reader and cell viability was determined by tryphan blue exclusion method.(15)

RESULTS

Cell migration assay

Test extract treatment enhanced the cell migration of HaCaT cells when tested by scratch wound assay. After 12hr of treatment with 50µg of test extract, the migratory nature of the fibroblast could be seen by microscopic examination. Complete covering of the wound was observed within 24 hours of treatment, similar to that of EGF. This response was not recorded either in the cell culture plate maintained as untreated control or placebo treatment **(Figure 1)**

Cell migration assay shows the movement of cells (indicated by arrow lines towards the imaginary line drawn in the center of the image) captured at 12 and 24 h after incubation using phase-contrast microscope

Fibroblast proliferation assay

An increased proliferation of fibroblast in response to treatment with test extract was observed. The rate of proliferation was directly proportional to the concentration. However, concentration above 50µg/ml did not greatly influence the proliferation rate. 30% increase in the proliferation of fibroblast in response to test extract at a concentration of 50µg/ml was demonstrated, whereas in the positive control, EGF showed 70% increase in cell proliferation. Cell proliferation was insignificant in untreated and placebo treated control **(Table 1 and Figure 2)**

DISCUSSION

The present study suggests that test extract of *Urenalobata*enhances the cell proliferation and migration of fibroblasts. Besides the keratinocytes the 3T3 fibroblasts play a role in healing process. Wound healing effect of the test extract was evidenced by enhanced cell proliferation and cell migration of fibroblast.

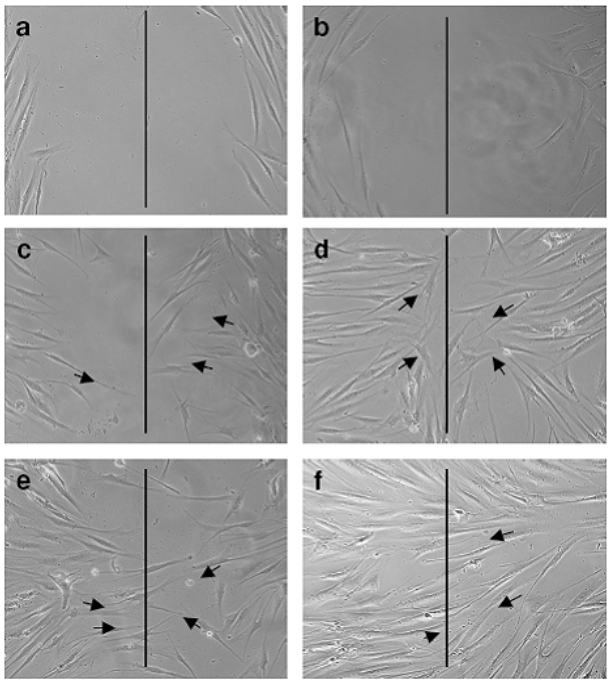
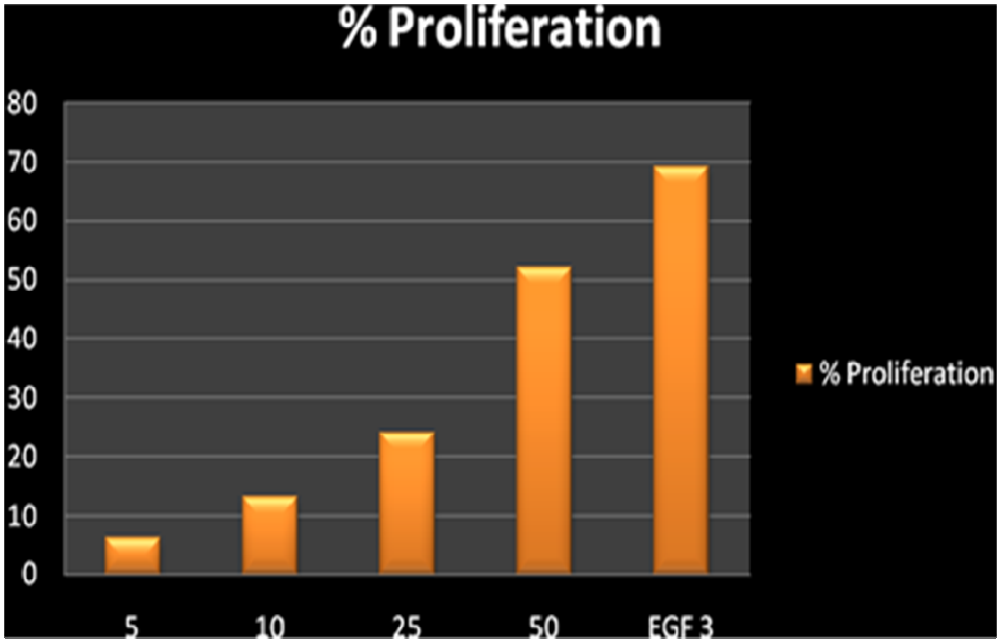


Fig1: Cell Migration Assay
a. Control- 12hours, **b.** Control- 24hours, **c.** Test Extract-12hours, **d.** Test Extract-24hours, **e.** EFG-12hours, **f.** EFG-24hours

Table 1

Sl.No	Concentration (µg/ml)	% Proliferation
1.	5	6
2.	10	13
3.	25	24
4.	50	52
5.	EGF 3	69

Figure 2



CONCLUSION

Management of wounds can be possible by using the ethanolic extract of *Urena lobata* which promotes the cell proliferation and migration.

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