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Evaluation of Wound Healing Potentials of the Methanolic Leaf Extracts of Some Indigenous Plants

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ABSTRACT

The present work objective are to investigate the wound healing activity of some indigenous plant's (*Ipomoea Carnea* and *N.Oleander* Linn) leave extracts in Wister Albino Rats using two different models Viz., excision and incision model. In the form of an ointment with two concentrations (5% and 10% w/v ointment of methanolic leave extract in simple ointment base). Higher concentration of both concentrations of the methanolic extracts showed significant response in both the wound types tested when compared with the control group. From both the plants 10 % w/v ointment of plant *Ipomoea carnea* leave extracts on topically showed more significant activity than other test compounds. Nitrofurazone ointment (0.2%w/w) used as standard.

Keywords- Methanol extract, *I. Carnea*, *N. Oleander* Linn, Excision, Incision, Nitrofurazone.

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INTRODUCTION

Plants have been rich source of medicines because they produce a host of biomolecules, most of which probably involved as chemical defenses against predation or infection. The world health organization has defined traditional medicine as comprising therapeutic practice that has been in existence for hundreds of years. More than 80% of the world's population depends upon traditional medicines for various skin diseases¹. Recently, the traditional use of plants for wound healing has received attention by the scientific community². Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3 % of modern drugs³. *Ipomoea carnea* (family: Convolvulaceae) is a perennial shrub, 1–5 m tall, with 10–25 cm heart-shaped leaves and funnel-shaped pink flowers. Native to Mesoamerica, *I. carnea*, which was introduced into some regions as an ornamental plant, is a globally distributed invasive shrub found in tropical and subtropical regions. Preliminary qualitative phytochemical screening of *I. carnea* revealed the presence of phenolic compounds, terpenoids, flavonoids and steroids. Some of them have antioxidant and antimicrobial activities. The most common biologically active constituents from these plants are ergoline alkaloids, indolizidine alkaloids, nortropane alkaloids, phenolics compounds, coumarins norisoprenoids, diterpene, isocoumarin and benzenoids flavonoids and antocianosides, It used as aphrodisiac, purgative and cathartic⁴. *Ipomoea* - a class of medicinally important plant species is reported in literature for their antimicrobial, anticancer, anti-inflammatory and for many other medicinal activities⁵. *Nerium oleander L.* is an important Chinese folk medicine. It is a vegetatively propagated ornamental plant, *Nerium oleander L.* is an evergreen shrub reaching up to four meters in height and belongs to the family Apocynaceae, is a shrub or occasionally tree distributed in tropical Asia. *Nerium oleander L.* is cultivated worldwide as an ornamental plant. It is native to the Mediterranean region^{6, 7} and is also found in Southern Europe and Southwest Asia, but is naturalizing very easily and in many areas the plant is sub-spontaneous. Leaves are 10 to 22 cm. long, narrow, acute in the apex, shortly petiolate, with a coriaceous dark green blade narrow, untoothed, short-stalked and dark or grey- green in color. There are large numbers of secondary metabolic compounds found in plants. Phytochemical technique mainly applies to the quality control of herbal medicine of various chemical components, such as saponins, alkaloids, flavanoids and anthraquinones. The entire plant, including the milky white sap, is toxic and any part of the plant can cause an adverse reaction, when applied to the living organisms. The leaves and the flowers are cardiogenic, diaphoretic, diuretic⁹, anticancer, antibacterial⁸, anti fungal⁹ and

expectorant. A decoction of the leaves has been applied externally in the treatment of scabies and to reduce swellings. This is a very poisonous plant, containing a powerful cardiac toxin and should only be used with extreme caution. The root is powerfully resolvent, is used in the form of plasters and is applied to tumors because of its poisonous nature it is only used externally. Oil prepared from the root bark is used in the treatment of leprosy and skin diseases of a scaly nature. Seeds are Poisonous, abortifacient and alternative. They used as purgative in dropsy and rheumatism. The whole plant is said to have anticancer properties ¹⁰. Wound healing is a complex process characterized by homeostasis, reepithelization, and granulation tissue formation and remodeling of the extracellular matrix ¹¹. Reports about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, collagenation, epithelization and wound contraction are abundant in the scientific literature ^{12,13}. The work was aimed at the scientific validation of the ethno pharmacological claim of the treatment of wounds healing effect of the plant by using both excision and incision animal models.

MATERIALS AND METHODS

Plant materials and preparation of extracts

The leave of *I. Carneia* (Convolvulaceae), is large, diffuse or straggling shrub with milky juice, leaf ovate cordate, entire, acuminate, flower large campanulate, pale rose, pink or light violet in lax, dichotomously branched axillary and terminal, pedunculate cymes. *Nerium oleander* L. (family – Apocynaceae) is a large glabrous evergreen shrub that produces milky juice. It is native to Iran, the Mediterranean region, as well as India. The leaves are in pairs of three, shortly stalked, coriaceous, 10 - 15 cm long, linear lanceolate with dark green colour. The dried leave materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used. Powdered leaves of the plant were extracted successively using soxhlet extractor with petroleum ether (60-80°C), to defat it and then macerated with ethanol with constant stirring.

Animals

Male Wistar rats (180-200 g) of 2-3 months of age were used. The animals were housed in standard environmental conditions of temperature $25 \pm 1^\circ\text{C}$, relative humidity $55\% \pm 5\%$ and lighting 08:00 20:00 h). The animals were fed on a pellet diet and water *ad libitum*. The animals were transferred to the laboratory at least 1 h before the start of the experiment. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Wound healing activity

Screening for wound healing activity was performed by Excision wound model and incision wound model respectively. The animals were starved for 12h prior to wounding. Studies were carried out using ether-anaesthetized rats. The hair on the skin of back surface of animals was removed by using a suitable depilatory. The selected animals were divided into six groups of six animals in each. All the test samples were applied topically.

Excision Wound Model

Circular wounds of approximately 8mm diameter were inflicted on the cleared skin by cutting under mild ether anesthesia. The areas of the wound were measured (sq. mm) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. This was taken as the initial wound area reading. Group – I served as negative control, which received simple ointment I.P. Group – II served as positive control to which nitrofurazone (0.2% w/v in simple ointment I.P.) was applied topically. Group – III and Group-IV animals were treated with the methanolic extract of leaves of *I.Carnea*. Group – V and Group-VI were treated with the methanolic extract of leaves of *N.Oleander* Linn. All the test samples were applied once daily. The wound area of each animal was measured on 1st, 4th, 8th, 12th and 16th post wounding day. The percentage of wound contraction was calculated from the days of measurement of wound area^{14, 15}

Incision Wound Model

Light incisions were made on the cleared surface by cutting the skin of the animals under mild ether anaesthesia^{16,17}. The wounds were created at a length of about 2cm. after the incision, the parted skin was kept together and stitched with black silk at both the end of the created wound. The test samples were applied as above in similar manner. The tensile strength was measured by using tensiometer on 16th post wounding day^{18, 19}.

Statistical Analysis

All the results were analyzed by One-way Analysis of Variance (ANOVA) followed by dunnett's test. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Healing is the body response to injury in an attempt to restore normal structure and function. Regeneration when healing takes place by proliferation of paranchymal cells and usually results in complete referation of the original tissue. Repair response takes place by participation of mensenchymal cells (consisting of connecting tissue, steam cells fibrocytes and histrocytes) endothelial cells, macrophages, platelets and parenchymal cells of the injured organ²⁰.

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. This is mainly achieved by the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength. Tannins promote the wound healing through several cellular mechanism; chelation of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels²¹ and fibroblasts and including keratinocyte proliferation, but do not act on the differentiation towards cornified cells²². The measurement of the progress of the wound healing induced by the Nitrofurazone ointment, and extract ointment as control and simple ointment in the excision wound model shown in the Table-1. It is observed that the wound contracting ability of the extract ointment was significantly greater than that of the control as well as the reference standard, NFZ ointment. The 10% (w/w) extract ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e. nitrofurazone ointment treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 10% w/w extract ointment treated group (18±1 days for 100% contraction which was almost similar to that of the nitrofurazone treated group). The 5%(w/w) extract ointment treated group of animals showed significant wound contraction from the eighth day onwards and achieved 100% with the wound closure time of 20 ±2 days. In incision wound model the measurement of the effect of the extract and standard drug on the tensile strength is shown in Table 2. The tensile strength of the 10% extract treated group and the nitrofurazone ointment treated group were comparable to each other. The 5% extract ointment of *I. Carnea* treated group showed a lesser but significant increase in the tensile strength compared to the control group but 5% extract ointment of *N. Oleander* Linn treated group has not producing the significant activity. Thus 10% w/v concentrations of the *I. Carnea* leave extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound. The results of the present study revealed that both concentration (5% and 10%w/w) of methanolic extract of *I. Carnea* leave have significant wound healing activity in both incision as well as excision wound models. The wound healing property of the leaf extract of “*N. Oleander* Linn and *Ipomoea Carnea*” appears to be due to the presence of its active principles, which accelerate the healing process and confer tensile strength to the healed wound. The animals treated with methanolic extract of (10 % W/V) preparation plant shows highest wound healing potency treatment was continued up to 20th days, no raw wound left after 16th days. The present studied showed methanolic extract possesses a good wound healing activity, on the 16th day of

post healing and results were compared to control and standard group are significant. The single model of wound healing is inadequate because the wound healing process involved various phases and no *in vitro* experiment exists that collectively represent the various components of wound healing. The increase in tensile strength of the granulation tissue indicated enhanced collagen maturation by increase crosslinking. However, our results revealed that tannins are one of the important phytoconstituents responsible for wound healing mainly due to their astringent and antimicrobial property. Hence, it can be inferred that the wound healing activity of the leave of the plant. *I. Carnea* is due to its high tannin content, which seems to be responsible for wound contraction and increased rate of epithelization.

Table – 1: Effect of methanolic extracts of different plants on Excision wound Model

Group	Treatment	Remaining of Original excision wound area (mm ²).mean ± SEM				
		0 Day	4 th Day	8 th Day	12 th Day	16 th Day
1	10 ml/kg	468.3 ± 6.49	395.5 ± 6.09	333.5 ± 7.91	209.5 ± 4.6	138 ± 5.7
2	Nitro furazone	474.6 ± 4.91	278 ± 9.2 ^c	206.3 ± 8.39 ^c	96.3 ± 4.03 ^c	27.8 ± 4.94 ^c
3	M.E.I.C. (5%)	482.6 ± 6.98	375.6 ± 7.6	301.6 ± 4.96 ^a	188.1 ± 7.18 ^a	114.8 ± 3.3 ^b
4	M.E.I.C. (10%)	462 ± 4.97	289.6 ± 4.6 ^c	226.6 ± 9.38 ^c	115.3 ± 4.24 ^c	52.3 ± 4.42 ^c
5	M.E.N.O. (5%)	464.3 ± 5.81	387.3 ± 7.06	307.3 ± 10.76	198.3 ± 6.99	124.8 ± 4.23 ^a
6	M.E.N.O.(10%)	460 ± 9.26	369.5 ± 6.8 ^a	299.1 ± 7.81 ^a	180.6 ± 4.27 ^b	102.3 ± 4.42 ^c

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t- value denotes statistical significance at ^ap<0.05, ^bp<0.01 and ^cp<0.001 respectively, in comparison to group-I).

Animal: Albino rats

Treatment: Topical

Table – 2: Effect of methanolic extract of different plants on Incision wound Model

Animal: Albino rats

Treatment: Topical

Group	Treatment	Tensile
1	10 ml/kg	329 ± 4.91
2	Nitro furazone	548 ± 7.55 ^c
3	M.E.I.C. (5%)	353.8 ± 5.01 ^a
4	M.E.I.C. (10%)	529.6 ± 9.34 ^c
5	M.E.N.O. (5%)	332.8 ± 8.44
6	M.E.N.O. (10%)	359.8 ± 5.28 ^b

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at ^ap<0.05, ^bp<0.01 and ^cp<0.001 respectively, in comparison to group-I).

CONCLUSION

On the basis of the results finding in the present investigation, it is concluded that the methanolic extract of *I. Carnea* and *N. oleander* Linn leaves has wound healing activity as compared to the solvent extracts. The wound healing activity of the methanolic extract may be due to the

individual or combined effect of the above phytochemicals. Comprehensive evaluation on the plants with wound healing activity on the basis of traditional medicine may possibly give new compounds that could be used as prominent drugs in wound healing therapy. Further investigations are needed for identification of active principles responsible for the wound healing activity. Enhanced wound contraction and histological observations suggest that *I. Carnea* has potential in the management of wound healing and suggests further study.

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