Vol 3 | Issue 1| 2012 | 42-49.

e - ISSN – 2249-7552
Print ISSN – 2229 7502

Research Article

WOUND HEALING PROCESS AND EFFECT OF PETROLEUM ETHER EXTRACT OF *CROSSANDRA INFUNDIBULIFORMIS* L. LEAF EXTRACT IN EXCISION WOUND MODEL

***K. Sumalatha**

*Assistant professor, Department of pharmacognosy, Bhaskar Pharmacy College, Banjara Hills, Hyderabad - 500 034.

ABSTRACT

Crossandra infundibuliformis is an important horticultural plant and belongs to the family Acanthaceae. In India, the plant is used for healing wounds; it is ornamental and bears red flowers which have a high value in the Indian flower market. Hence, the present study was aimed to evaluate its scientific validity. The petroleum ether extract investigated for the evaluation of its healing efficiency on excision wound model in rats. The effect produced by extract, in terms of wound contracting ability, wound closure, decrease in surface area of wound, and tissue regeneration at the wound site were significant ($p < 0.01$) in treated rats. The present study thus provides a scientific rationale for the traditional use of this plant in the management of the wounds.

Keywords: *Crossandra infundibuliformis*, Wound healing, Excision wound.

INTRODUCTION

A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue [1]. Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Healing is a complex and intricate process initiated in response to an injury that restores the function and integrity of damaged tissues. Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phases Viz. inflammation (0–3 days), cellular proliferation (3–12 days) and remodeling (3–6 months) [2]. Healing requires the collaborative efforts of many different tissues and cell lineages. It involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization. Healing is not complete until the disrupted surfaces are firmly knit by collagen [3]. The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part [4]. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue [5]. In spite of tremendous advances in the pharmaceutical drug industry, the availability of drugs capable of stimulating the process of wound repair is still limited. Moreover, the management of chronic wounds is another major problem due to the high cost of therapy and the presence of unwanted side effects [6].

Corresponding Author: **K. Sumalatha** Email: sumampharmacy@gmail.com

PHASES OF WOUND HEALING

1. Inflammatory Phase

In this phase the immediate response to injury is vasoconstriction of the small vessels and capillaries in the surrounding area of the wound. Vascular occlusion occurs at the point of injury tending to control haemorrhage. This response remains for immediate 5-10 minutes, following this vasodilation occurs. This vasodilation involves all elements of local autocoids.

2. Repair Phase

The repair processes begin almost immediately after the injury. Polymorphonuclear (PMN) leucocytes are the first cells to appear in large number at the site of wound. PMN leucocytes are important in wound healing because in case of infection at the site of wound, PMN leucocytes phagocytize the bacteria. They are also known to clear the wound area of dead cells and debries and clear the area for the regeneration of new cells. Fibroblast also migrate in to the wound area and deposit the collagen.

3. Fibroblastic phase

Shortly after injury undifferentiated mesenchymal cells begin to change into miograting fibroblast migrate to the wound area, after PMN leucocyte clear the wound area of the debries. Fibroblast secrete the emzymes, which converts the fibrinogen to fibrin. This fibrin act as haemostatic barrier and provides frame work for the other elements of wound repair. Fibroblast secretes proteins, polysaccharides and various other glycoproteins which make up the ground substance. Mucopolysaccharides of the matrix secreted surrounds the fibroblast and immobiles them. It influences the aggregation and orientation of collagen. The collagen is synthesized by the fibroblast, it utilizes hydroxyproline and hydroxylysine. These processes are usually occurs after the 4th day post wounding. Fibroblastic phase of wound healing usually remains for 2-4 weeks. Capillaries begin to progress and collagen rate of synthesis declines which is the halmark of the end of fibroblastic phase.

4. Epithelization phase

Epithelial cell migration is one of the vital process of wound healing. The stem cells of epithelium must detach from the edges of the wound and migrate into wound. Normally dermal basal cells adhere to each other and to the underline basal layer of the dermis. Following mobilization, epithelial cells begin to enlarge and migrate down and across the wound. Transected hair follicles also contribute to the number of migrating epithelial cells (Santoro *et al.*, 2005). Epithelial cell migrating across wound usually move along the basal lamina or fibrin deposits, this phenomenon is called contact guidance and is an important factor in epithelial migration. Epithelial migration is followed by increased mytosis of epithelium. Recent evidence suggest that a water soluble heatlabile substance called chalone which is secreted at the wound site is responsible for regulation for mytosis.

5. Contraction phase

Wound Contraction is defined as the process by which the size of a full thickness open wounds diminished and is characterised by centripetal movement of the own thickness of the surrounding skin. All evidence points to contraction as a cell mediated phenomena. Electron microscopy have shown that, some of the fibroblast in the contracting phase of the wounds have the appearance of smooth muscle cells. These fibroblasts are called myofibroblast. They have features of both smooth muscle and fibroblast. In vitro pharmacological studies demonstrated that strips of granuloma tissue contracts or relax as do strips of smooth muscles. In vivo studies showed that wound contraction can be inhibited by topical application of smooth muscle relaxants. It is very clear that myofibroblast play key role in wound healing. Observation reveal that myofibroblast have cell to cell attachement and adherent processes of the cell, attach them to the wound bed, the penicularis and dermis of the wound edge. Once contraction beings it continues until wound edges meet contraction inhibition halts the process are until the tension in surrounding skin equals or exceeds the force of contraction. During contraction the skin of surrounding wound is streched and thinned under tension. However this state does not persist long. Gradually new collagen is laid down in the dermis and new epithelial cells are formed, this process is continued until the full thickness of the stretched skin is restored. The process is called as intussusceptive growth.

6. Remodeling phase

This phase is divided in to two stages viz early phase and late phase. In the early phase the wound strength plays a key role, it is mainly due to formation of fibrin clot with in the wound. Epithalization across the wound also contribute significantly the early wound strength. In addition to it, embeding new capillaries in to the wound area also contribute to the wound strength. In sequence to the early phase the wound strength increases profoundly at 14 to 16th post wound day. This increase in wound strength during the period of rapid fibroblastic and parallel the synthesis and deposition of the collagen. Hydroxyproline which is one among the precursor for synthesis of collagen.

In the late phase which continues to stabilize a new collagen fibers are laid down. Excess cells are found in the wound area are digested and are removed by the scavenging effect of enzyme collagenase. The wound strength continues to increase in strength even after wound collagen content stabilizes. It is to be recalled here that the collagen fibers with in the scars also have an effect on

tensile strength [7].

WOUND REPAIR PARAMETERS

Physical attributes

Physical attributes like wound contraction, epithelization and scar remodeling can be monitored by measuring the total wound area, open wound area, and noting the physical changes in the scar e.g. size, shape and colour etc. Excision wound is ideal to study these attributes. The area measurement not only gives the rate of healing, but can distinguish between contraction and epithelization. The extent of epithelization is determined by measuring the raw wound, bound by hairless belt intervening between wound margin and then by deducting the raw wound area from total wound area. Different methods for measuring the areas are available. These may be traced on a paper, weighed and compared with that of a reference piece of same thickness and unit area or the same can be retraced on a graph paper to directly measure the area. The completion of epithelization can be assessed by noting the time for complete covering of the raw surface of the wound. "Thorotrast" a sophisticated technique with the electron opaque marker is reported for the identification of migrating epithelial cells. Granuloma study is another physical attribute of wound healing study which can be assessed by quantifying the granuloma itself by noting its overnight dried weight [3].

Mechanism of Wound contraction

The wound starts contracting after 2-3 days and the process is completed by the 14th day. During this period, the world is reduced by approximately 80% of its original size contracted would results in rapid healing since lesser surface area of the injured tissue has to be replaced.

In order to explain the mechanism of wound contraction, a number of factors have been proposed. These are as under.

1. Dehydration as a result of removal of fluid by drying of wound was first suggested but without being substantiated.
2. Contraction of collagen was thought to be responsible for contraction but wound contraction proceeds at a stag when the collagen content of granulation tissue is very small.
3. Discovery of myofibroblasts appearing in active granulation tissue has resolved the confroversy surrounding the mechanism of wound contraction. These cells have features intermediate between those of fibroblasts and smooth muscle cells. Their migration into the would area and their active contraction decreases the size of the defect. The evidences in support of this concept are both morphological as well as functional characteristics of modified fibroblasts or myofibroblasts as under.

i. Fibrils present in the cytoplasm of these cells resemble those seen in smooth muscle cells.
ii. These cells contain action-myosin similar to that found in non-striated muscle cells.
iii. Cytoplasm of these modified cells demonstrates immunofluorescent labeling with anti-smooth muscle antibodies.
iv. Nuclei of these cells have inforldings of nuclear membrane like in smooth muscle cells.
v. These cells have basement memrane and desmosomes which are not seen in ordinary fibroblasts.
vi. Drug response of granulation tissue is similar to that of smooth muscle.

Factors Influencing Healing

Two types of factors influence the wound healing; those acting locally, and those acting in general.

A. Local Factors

1. Infection is the most important factor acting locally which delays the process of healing.
2. Poor blood supply to wound slows healing e.g. injuries to face heal quickly due to rich blood supply while injury to leg with varicose ulcers having poor blood supply heals slowly.
3. Foreign bodies including sutures interfere with healing and cause intense inflammatory reaction and infection.
4. Movement delays wound healing.
5. Exposure to ultraviolet light facilitates healing.
6. Type, size and location of injury determines whether healing takes place by resolution or organization.

B. Systemic factors

1. Age. Wound healing is rapid in young and somewhat slow in aged and debilitated people due to poor blood supply to the injured area in the letter.
2. Nutrition. Deficiency of constituents like protein, vitamin C (scurvy) and zinc delays the wound healing.
3. Systemic infection delays wound healing.
4. Administration of glucocorticoids has anti-inflammatory effect.
5. Uncontrolled diabetics are more prone to develop infections and hence delay in healing.
6. Haematologic abnormalities like defect of neutrophil functions (chemotaxis and phagocytosis), and neutropenia and bleeding disorders slow the process of wound healing [8].

Effects of drugs on wound healing

Apart from various wound healing factors, which are naturally present (cytokines and growth factors). There are many substances which are known to intervene and stimulate or depress the wound healing process. These substances may be acting as stimulants or depressants of synthesis of various growth factors which appears in wound healing process.

The drugs like aspirin, phenylbutazone and indomethacin are likely to depress the wound healing process. For examples large doses of aspirin decreased wound tensile strength in rats.

Cortisone and its derivatives decrease the rate of protein synthesis, stabilize lysosomal membranes and inhibit the normal inflammatory processes. Generally steroids are known to causes delay in wound healing. Vitamin A has been found to stimulate fibroblasts and the accumulation of collagen, however there is no evidence that administration of vitamin A alters the wound healing rate in animals [9].

Vitamin E, which stabilizes the membrane, is known to retard wound healing and collagen production. Role of vitamin C in wound healing is established. Vitamin C is necessary for synthesis of collagen. Without vitamin C the collagen molecules remain incomplete and may not be secreted by fibroblast. Deficiency of vitamin C delays wound healing process [10].

Administration of zinc to the zinc deficient animals restore the wound healing. Normal epithelial and fibroblastic proliferation requires the zinc dependant enzymes viz DNA polymerase and reverse transcriptase without sufficient zinc levels, although epithelial cells and fibroblast may migrate to wound area but they cannot multiply. Thus epithelization gets slower and collagen synthesis also becomes inadequte to hold the wound together. Cytotoxic drugs are known to inhibit wound healing on local application 5-fluororacil and meclorethamine are the few examples [11].

PDGF : Platelet derived growth factor.
TGF : Transforming growth factor.
IL : Interleukin.
GM-CSF : Granulocyte macrophage colony stimulating factor.
FGF : Fibroblast growth factor.

Plants have provided a source of inspiration for novel drug compounds and plant medicines have made large contributions to human health and well being. *Crossandra infundibuliformis* is an important horticultural plant and belongs to the family Acanthaceae. In India, the plant is used for healing wounds; it is ornamental and bears red flowers which have a high value in the Indian flower market. Micropropagation is being used extensively for the rapid clonal propagation of many fruits, nuts and ornamental trees. Therefore, the present study was performed to verify the folklore claim of wound healing activity of *Crossandra infundibuliformis (L.)* on excision wound model in albino wistar rats.

MATERIALS AND METHODS

Plant collection

The Plant material of *Crossandra infundibuliformis (L.)* was collected from Tirupati, Andhra Pradesh, in the month of August 2009. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, West Tambaram, Chennai. The voucher specimen (PARC/2009/350) of the plant was deposited at the college, for further reference.

Preparation of extracts

The leaves of the plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (80gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of petroleum ether extract of *Crossandra infundibuliformis (L.)* was found to be 22.5 % w/w.

Animals

Adult wister albino rats weighing between 200-250 gm of either sex. They were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The animals were maintained under normal laboratory condition and kept in standard polypropylene cages at room temperature of 30° ± 2° and 60 to 65% relative humidity and provided with standard diet and water *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 01 / SVCP / 2009 - 2010). The rats were then divided into four groups of six animals each.

Excision wound model

Animals were anaesthetized (light ether) prior to and during creation of the wounds according to the method of Morton and Malone, 1972 (Morton *et al.,* 1972). The hairs on the skin of back surface of the animals were removed by wiping with a suitable depilatory (Anne-French hair removing cream) with the help of a cotton swab. A circular wound of about 500sq mm was made on depilated dorsal thoracic region of animals by cutting the skin of the animals by using forceps and scissors. The entire wound was left open. The observation of percentage wound closure was made on 1^{st}, 4^{th}, 7^{th}, 10^{th}, 13^{th} and 16^{th} days post wounding days [13]. The area of the wound was marked by placing a transparency sheet over the wound. The wound areas recorded were measured in square millimeter by using graph paper. This was taken as the initial wound area healing.

Experimental Design

Group – I : Control group with simple ontiment base(I.P).

Group – II : Standard group received topical application of 0.2% w/w nitrofunazone ointment.
Group – III : Received PECI 200mg/kg topically.
Group – IV : Rreceived PECI 400mg/kg topically.

The simple ointment, standard drug and test drug was applied on wound everyday up to 16th day. The wound area of each animal was measured on the 1st, 4th, 7th, 10th, 13th and 16th days in square millimeter by using graph paper. They were observed thoroughly for epithelization and contraction of wound. The complete epithelization was observed by fall of scab without any raw wound area. Number of days required for falling of scab without any residual raw wound gave the period of epithelization [14]. The post wounding day and epithelization period are tabulated in the table. The percentage protection was calculated on the 16th day by using the following formula and tabulated in table [15].

$$\text{Percentage protection} = \frac{100 - (\text{Final} \times 100)}{\text{Initial}}$$

Statistical Analysis

The results are presented as mean ± SEM and subjected to "One-way ANOVA followed by Dunnett's post test of three animals in each group. The values of $p<0.05$ were considered significance.

RESULTS

The effect of petroleum ether extract of leaves of *Crossandra infundibuliformis* L. extract ointment on excision wound model, the wound healing contracting ability in different concentration was significantly greater than that of the control (i.e., simple ointment treated group).

Excision wound healing by contraction (wound closing) and epithelization, the percentage of wound closure or closure rate studied by recording the changes in wound area at fixed intervals of time. Nitrofurazone showed more potent significant wound healing activity compare to that of control group rats. PECI showed significant dose dependent wound healing activity at the doses of 200 and 400 mg/kg body weight.

It was further found that all the four groups showed decreasing of wound area from day to day. However on 16th day, the Group – I showed 63.15% protection, (which may be due to self-immunity of animals) where as the Group – II (i.e., standard) showed 98.40% protection. On the other hand Group – III (i.e., the 200mg/kg treated group) showed appreciable wound healing activity of 85.69% protection as compared to standard group, where as Group – IV (i.e., the 400mg/kg treated group) exhibited 96.83% protection, which is closer to that of standard Nitrofurazone indicating significant wound healing activity.

Fig 1. Pathology of wound

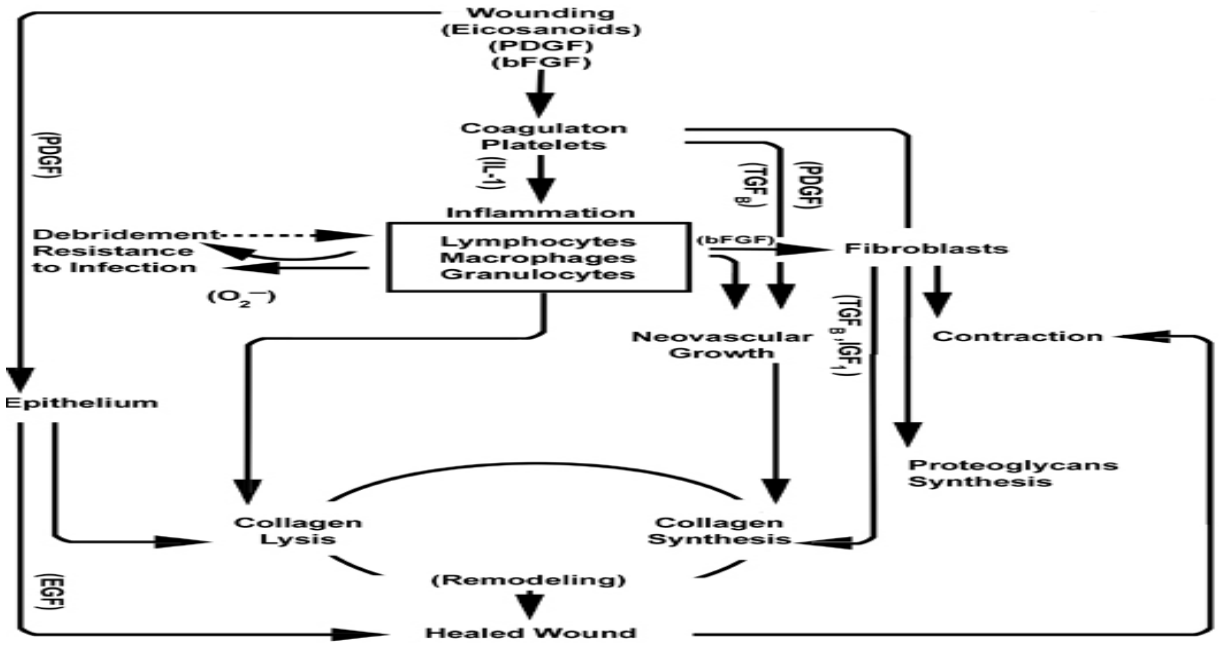

Fig 2. Effect of petroleum ether extract of *Crossandra infundibuliformis* L. leaf in Excison wound model

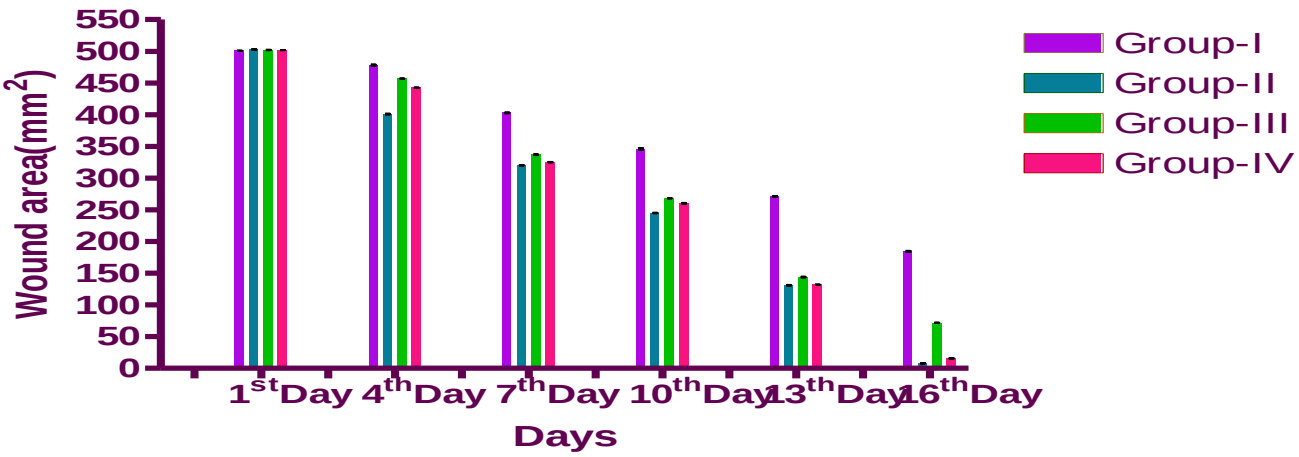

Fig 3. Effect of Epithelization period of petroleum ether extract of *Crossandra infundibuliformis* L. leaf in Excison wound model

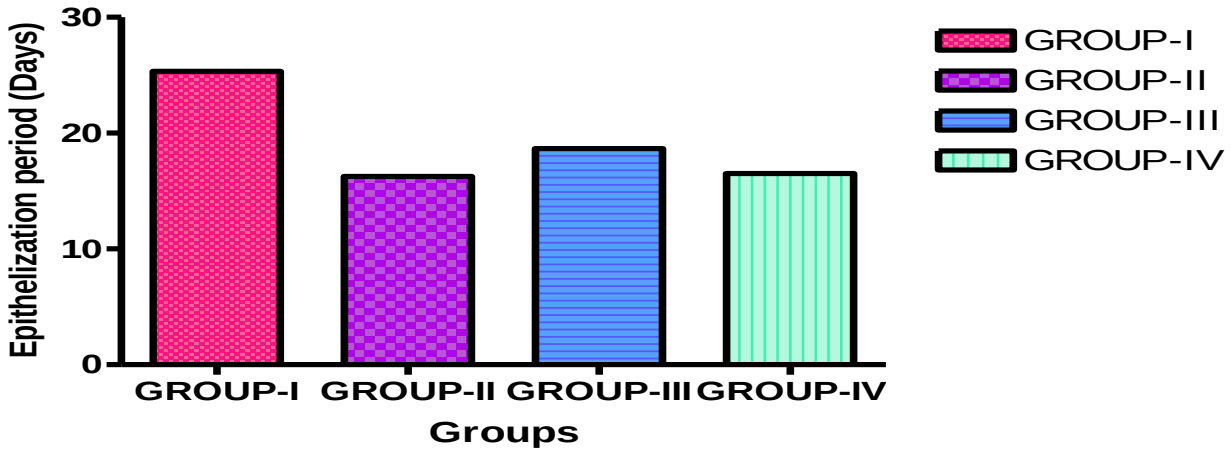

Fig 4. Woumd healing activity of *Crossandra infundibuliformis* L. leaf Showing excision wound (Topical treated) at different days

0 Day **8th Day** **16th Day**

Table 1. Growth factors&cytokines on wound healing [12]

Sl. No.	Name	Pharmacological action	Clinical application
1.	PDGFBB	Helps in reepithelization	Pressure and diabetic ulcers
2.	TGFβ2	Increases collagen containing granulation tissue	Heeling of venous stasis ulcers
3.	TGFβ3	Increased wound healing	Wound healing of chronic ulcers
4.	Basic FGF	Increased role of reepithelization	Pressure ulcers
5.	IL 1β	Wound healing of infected open wound	Pressure ulcers
6.	GM-CSF	Wound healing of incisions	Chronic ulcers
7.	Acidic FGF	Increase neovessels and matrix information	Diabetic and venoustasis ulcers
8.	FGF	Increased reepithelization	In chronic ulcers

Table 2. Effect of Topical Application of petroleum ether extract of *Crossandra infundibuliformis* L.(Acanthaceae) by excision wound model (from 1st day to 16th day)

Group	Treatment	Post wounding day Wound area in square millimeter						Percentage protection	Period of Epithelization
		1st Day	4th Day	7th Day	10th Day	13th Day	16th Day		
I.	Control	501 ±0.33	478±0.964	403±0.76	346±1.24	271±0.954	184.6±0.918	63.15	25.336
II.	Nitrofurozone (0.2% w/w)	503±0.5164	401.1±0.703*	320±0.494*	245±0.365*	131±0.73*	8±0.258*	98.409	16.25
III.	PECI 200mg/kg	502±0.417	457±0.614	337±0.614	268.16±0.307	143.8±0.609*	71.833±0.30*	85.691	18.671
IV.	PECI 400mg/kg	502±0.307	442.6±0.33	325.33±0.426*	260.5±0.670*	132±0.447*	15.88±0.3816*	96.836	16.522

Values represent the mean ±SEM, n=6, *P<0.05 compared with control, Dunnet's test by ANOVA. The values of P<0.005 were consider significance. Comparison between Group-I vs Group-III & IV, Group-I vs Group-II.

DISCUSSION AND CONCLUSION

Traditionally, medicinal plants have been used for many years as topical and internal preparations to promote wound repair. Current researches are devoted to validating their efficacy and to uncover the mechanisms responsible for this activity. Medicinal plants have great potentials and have been shown to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort, and scarring to the patient [7]. Some of these plants owe their effects to direct effect on the wound healing process.

In this study, topical application of *Crossandra infundibuliformis* L. petether extract on the infected wound of the rats caused a significant (P<0.05) and faster rate of wound closure and reduced the epithelialization period. Wound healing is a natural process of regenerating dermal and epidermal tissue. Whenever there is a wound, a set of overlapping events takes place in a predictable fashion to repair the damage [16]. The process has been conveniently categorized into phases such as the inflammatory, proliferative, and remodeling phases [17]. In the inflammatory phase, bacteria and debris are phagocytosed and removed and factors are released that cause the migration and division of cells involved in the proliferative phase. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. In epithelialization, epithelial cells crawl across the wound bed to cover it. The wound is closed by a combination of all these and by the process of wound contraction. During wound contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis. Although wound treatment with the ointment containing the extract of *Crossandra infundibuliformis* L. showed a wound healing activity, the exact step and mechanism in wound repair processes affected by the extract was not established [18].

Topical application of petroleum ether extract of *Crossandra infundibuliformis* L. showed a significant dose dependent wound healing activity due to increased rate of wound contraction at wound site in excision wound model. The phyto constituents present in the leaves of *Crossandra infundibuliformis* L. may be responsible for the wound healing activity. The observations and results obtained in this study showed that the petroleum ether extract of *Crossandra infundibuliformis* L. produced significant wound healing activity.

REFERENCES

1. Ramzi SC, Vinay K and Stanley R. Pathologic basis of Diseases. WB Saunders company, Philadelphia, 5, 194, 86.
2. Martin AA. The use of antioxidants in healing. *Dermatological Surgery*, 22, 1996, 156–160.
3. Buffoni F, Bancheli G., Cambi S, Ignesti G., Irisind R., Raimondi L, Vannelli G. Skin wound healing: some biochemical parameters in Guinea pig. *Journal of Pharmaceutics and Pharmacology*, 45, 1993, 784–790.

4. Pierce GF, Mustoe TA. Pharmacologic enhancement of wound healing. *Annual Review of Medicine*, 46,1995, 467-481.
5. Phillips GD, Whitehe RA, Kinghton R. Initiation and pattern of angiogenesis in wound healing in the rat. *American Journal of Anatomy*, 192, 1991, 257–262.
6. Porras-Reyes BH, Lewis WH, Roman J, Simchowitz L, Mustoe TA. Enhancement of wound healing by the alkaloid taspine defining mechanism of action. *Society of Experimental Biology and Medicine*, 203, 1993, 18–25.
7. Nguyen DT, Orgill DP, Murphy GF. The pathophysiologic basis for wound healing and cutaneous regeneration bio materials for treating skin loss. CRC Press (U.S), 2009, 25-27.
8. Harsh Mohan. Text book of pathology,6th edition, Jaypee Brother Medical Publishers Pvt Ltd., 2010,165-172.
9. Douglas M, Alan LM. Nutritional Support for wound healing. *After Med Rev*, 8, 2003, 359-77.
10. Blee TH, Coybill TH, Lambert PJ. Hemorrtage associated with vitamin C deficiency in surgical patients. Surgery, 131, 2002, 408-412.
11. Suffness M & Dourous J. Drugs of plant origin, in methods in cancer research, Vol. 16, edited by VTDe vita and H. Bush, 79, 1979.
12. Lawrence WJ, Diegelmann RF. Growth factors in wound healing. *Clin Dermatol*, 12, 1994, 157-69.
13. Kumara Swamy HM, Krishna V, Shankarmurty K, Abdul Rahiman B, Mankani KL, Mahadevan KM, Harish BG, Raja Naika H. Wound healing activity of embelin isolated from the ethanol extract of leaves of *Embelia ribes* Burm. *Journal of Ethanopharmacology*, 109, 2007, 529-534.
14. Anuradha R,Sukumar D,Madhava S. Wound healing activity of quercimetrin from *Tithonia iversifolia* Linn flower extract. *Int.Journal of pharmacol & biological science*, 2 (1), 2008, 109-111.
15. Agrahari AK, Panda SK, Meher A, Padhan AR. Screening of Wound Healing Activity of *Curculigo orchioides* Gaertn Root Tubers' Methanolic Extract. *International Journal of Pharmaceutical and Applied Sciences*, 1 (1), 2010, 91-95.
16. Iba Y, Shibata A, Kato M., Masukawa T. Possible involvement of mast cells in collagen remodeling in the late phase of cutaneous wound healing in mice. *Int. Immunopharmacol*, 4 (14), 2004, 1873-1880.
17. Stadelmann WK, Digenis AG, Toxbin GR. Impediments to wound healing. *Am J Surg* 176, 1998, 39-47.
18. Midwood KS, Williams LV, Schwarzbauer JE. Tissue repair and the dynamics of the extracellular matrix. *Int J. Biochem Cell Biol*, 36 (6), 2004, 1031-1037.