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A STUDY OF *IN-VITRO* WOUND HEALING ACTIVITY IN THE METHANOL EXTRACT OF *PROTOREASTER LINCKII*

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ABSTRACT: A wound is an injury of living tissue or a break in the epithelial integrity of the upper layer of skin. This may lead to disturbance of skin anatomical structure and functions. Nowadays, wound healing is a challenging clinical problem. So, effective wound management is required. Different *in-vitro*, *in-vivo* and *ex-vivo* models have been developed to evaluate wound healing activity. The present study aims to evaluate the *in-vitro* wound healing potential of methanol extract of starfish *Protoreaster linckii* on the Human L-32 Cell line through scratch assay. The wound-healing properties of the *Protoreaster linckii* extract at different concentrations (25µg/ml, 50µg/ml and 100µg/ml) were assessed. The wound closure and migration of cells were directly related to the concentrations of the extract. The extract concentration increased from 25µg/ml to 100µg/ml and the percentage of wound healing (40% - 98%) also increased. The results showed that the methanol extract of *P. linckii* has potential wound-healing activity and it can serve as an alternative therapy against synthetic drugs for wound healing.

INTRODUCTION: The class Asteroidea of marine benthic animals, which is part of the *Phylum echinodermata* includes starfish. They have a large number of tube feet, pentameral symmetry, and excellent regeneration. Numerous physiologically active substances and chemicals in starfish have pharmacological qualities such as antifungal, anti-inflammatory, antibacterial and wound-healing effects [1]. The wound is the interruption of the continuity in the tissue resulting from the opening or break of the skin.

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The wound's healing is essential for restoring tissue continuity and disturbed skin status [2]. Wound healing is essential for the restoration of the barrier function of the skin. During this process, cells at the wound edges proliferate and migrate, leading to re-epithelialization of the wound surface [3]. Healing a wound involves inflammation, proliferation, repair, and regeneration.

Human society has so far paid a high price in terms of health and finances due to a lack of prompt repairs. Therefore, in contemporary understanding, the natural therapy modalities and the application of biological science are given significant thought in healing all types of wounds, shortening healing time, and avoiding infection. Meanwhile, Sea has opened up a wide range of natural medicines for us. If new pharmacological findings show positive

results from aquatic effects such as starfish, sea cucumbers and sea urchins [4]. The therapy of wounds can occasionally be difficult, especially when they are chronic wounds, which have an incidence of 4.5 per 1000 people [5]. Despite significant advancements in pharmaceutical technology, the availability, cost and various negative side effects of pharmaceuticals make them still unsatisfactory [6, 7].

The scratch wound healing assay has been widely used and adapted to examine how mammalian cells migrate and proliferate in response to various experimental settings, such as gene knockdown or chemical exposure [8]. This assay is easy to perform and reasonably priced, and the experimental setup may be quickly changed to suit various needs. A high throughput screen can also be performed using the assay. The present study was undertaken to examine the wound-healing activity of the methanol extract of *P. linckii* using *in-vitro* models.

MATERIALS AND METHODS:

Wound Healing Activity: *In-vitro* wound-healing scratch assay was carried out according to the method described by Yarrow and Perlman [9]. In the present study, the cell density (put comma near study) of "2×10^5 cells" was seeded into each well of a 24-well plate and incubated with a complete medium at 37°C and 5% CO_2. After 24 hours of incubation, the monolayer confluent cells were scrapped horizontally with sterile pipette tips. The debris was removed by washing with Phosphate buffered saline (PBS). The cells were treated with methanol extract of *P. linckii* with various concentrations (25 µg/mL, 50 µg/mL and 100µg/mL) by diluting with serum-free DMEM. The cells without treatment and with treatment (100 µg/ml) were used as the control and positive control, respectively.

The induced scratch represented the wound and was photographed using phase-contrast microscopy at ×40 magnification (0 hours). After 24 hours of incubation the second set of images was photographed. To determine the migration rate, the images were analyzed using "image J" software, and the percentage of the closed area was measured and compared with the value obtained at 0 hours. An increase in the percentage of the closed area indicated the migration of cells.

RESULTS:

Wound Healing Activity: In the present study, the methanolic extract of *Protoreaster linckii* was tested for wound healing activity by using *in-vitro* scratch assay in the Human L-32 Cell line. The migration rate was measured from the distance between the wound edge at 0 hours and 24 hours and cell migration was reported as a percentage in terms of wound closure. Results show that the wound closure and migration of cells were directly related to the concentrations.

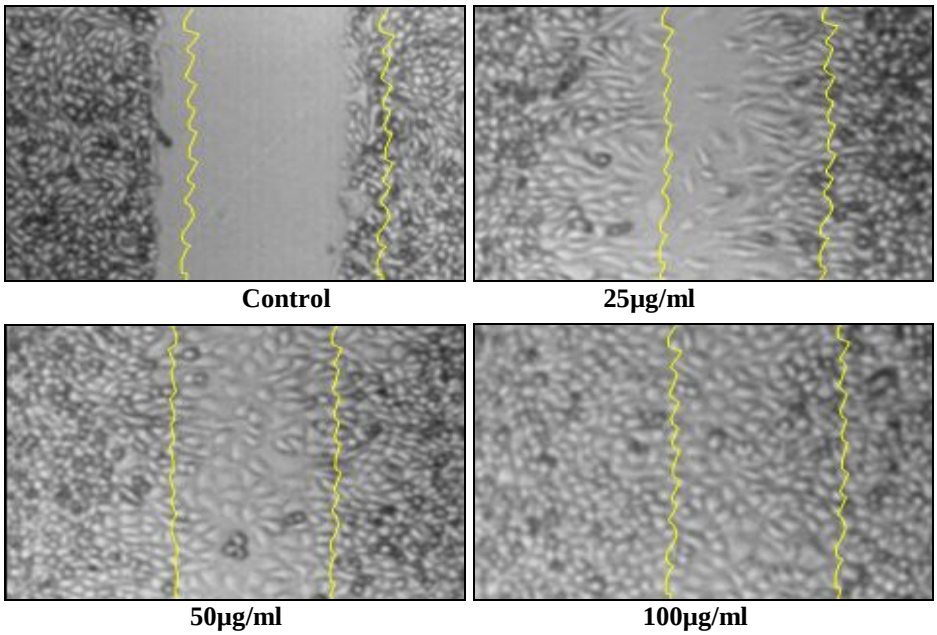

FIG. 1: *IN-VITRO* SCRATCH ASSAY- MIGRATION OF CELLS AT DIFFERENT CONCENTRATIONS

As the concentration of the extract increases from 25 µg/ml to 100 µg/ml the percentage of wound healing and the migration of cells (human L-32 cells) also increases. At 25 µg/ml the percentage of migration was 40%. At 50 µg/ml and 100 µg/ml, the migration was 76% and 98%, respectively. The results were compared with the control. **Fig. 1** shows the microscopic images of untreated and treated Human L-32 cell.

DISCUSSION: The breakdown of tissue integrity caused by various intrinsic and extrinsic sources, such as physical, chemical, or microbiological damages, is known as a wound. Coordinated cellular and biochemical activities alter the structural and functional continuity of the skin. Activities like proliferation, migration of endothelial and epithelial cells towards the wound bed, extracellular matrix deposition, and remodeling occur in a tightly controlled phenomenon through growth factors and cytokines stimuli [10]. It is believed that cell migration and proliferation are the rate-limiting elements in skin regeneration [11]. Since, fibroblast cells are involved in creating collagen, their proliferation is essential for wound healing. The scratch assay is used to evaluate the capacity of fibroblast cells to proliferate and be stimulated in an *in-vitro* wound [12].

In the present study, the methanolic extract of *Protoreaster linckii* was tested for wound healing activity by using in vitro scratch assay in the Human L-32 Cell line. The migration rate was measured from the distance between the wound edge at 0 hours and 24 hours and cell migration was reported as a percentage in terms of wound closure. The wound closure and migration cells were directly related to the concentrations. The concentration of extract increases from 25 – 100µg/ml, and the percentage of wound healing is (40% - 98%) also increased.

Similar observations have been reported by Baveja *et al*. [13], the sea star coelomic fluid (SCF) extracted from the sea star *Astropecten indicus.* The wounds were created on A-549 cells in six-well plates *in-vitro*. The abilities of different concentrations of SCF to heal the wounds were observed at every 24 hours up to 72 hours post-treatment. Wounds were completely healed by SCF within 72 hours of treatment. Results are expressed as a percentage of wound healing. It is noted that the wound area decreased significantly in cells treated with 2.5 and 5µg/ml of SCF as compared to that in control (untreated cells). This indicated a significant wound-healing activity of SCF in A-549 cells.

Gupta *et al*. [14] investigated the healing efficacy of aqueous–methanolic extract of *Pentaceraster regulus* on cutaneous wounds in guinea pigs. Nur Afiqah Bahram *et al.* [15] observed the sulfated glycosaminoglycans extracted from *A. planci* have a wound-healing effect in the excision wound model. Ben Khadra *et al.* [16] reported wound repair during arm regeneration in the red starfish *Echinaster sepositus* in this study proves red starfish *Echinaster sepositus* has natural wound healing potential. Esther Elsie and Thilaga [17] analysed the wound-healing activity of the methanolic extract of *Turbinella pyrum* and the results showed a significant dose-dependent in both excision and incision wound models. These studies corroborate the present work.

CONCLUSION: In the present study, the methanol extract of *P. linckii* exhibited strong wound-healing activity *in-vitro*. This findings can be considered an important basis for the formulation of drug products. Further studies are recommended to determine the potential-wound healing activity of *P. linckii* in the animal model and identify the responsible bioactive compounds.

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CONFLICTS OF INTEREST: The author declares no conflict of interest.

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