Effect of Probiotics on Cutaneous Wound Healing

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INTRODUCTION
PREVIOUS STUDIES


WOUND HEALING

- A **wound** is a type of injury in which skin is torn, cut or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin.

- Different chemical agents have been used for wound healing but each agent has negative side effects. Appropriate treatment and care is essential to accelerate the healing process. A therapeutic agent selected for the treatment of wounds should ideally improve one or more phases of healing.
Probiotics and its exopolysaccharide, were both tested for antimicrobial and healing activities in chronic ulcers but though, they have many strains and species the only study on the use of probiotics on cutaneous wound healing was done by Rodrigues et al. in 2004 (Rodrigues et al., 2004).
These bacteria have been known as beneficial factors that have many effects on human health like reduction of serum cholesterol, stimulation of immune system and prevention or treatment of human infections.
Probiotic bacteria produce exopolysaccharides (EPS) that can be connected to a cell’s surface or that can discharge into the environment. EPS have many roles like immunostimulatory, anti-tumor activity. Also, phosphate groups in EPS play an important role in activating macrophages and lymphocytes.
MATERIALS & METHODS
Male Wistar rats weighting 250–280 g were housed under controlled conditions of light, room temperature and humidity.
The animals were separated into five groups \((n = 7)\) which contain negative control, control and three experimental groups for the days 1, 3, 7, 14 and 21.

- **Negative control group**: 35 mice were kept as negative controls, where the wounds were left untreated.
- **Control group**: A second group of 35 mice were used as control, where the wound was treated with the eucerin alone.
- **Experimental 1 group**: In the third set of 35 mice as experimental 1 groups, the wound was treated with the eucerin contain live *Lactobacillus casei*.
- **Experimental 2 group**: In the forth set of 35 mice as experimental 2 groups, the wound was treated with the eucerin contain dead *Lactobacillus casei*.
- **Experimental 3 group**: In the fifth set of 35 mice as experimental 3 groups, the wound was treated with the eucerin contain *exopolysaccharide*. 
OINTMENT PREPARATION

- *Lactobacilli* MRS agar, for 48 h at 37 °C
- by sterilized spoon, bacteria on the surface of culture were collected.
- In order to prepare ointment for experimental 1 and 2 groups, 1 ml bacteria that collected every day after 48-hour culture were added to 4 ml eucerin for 5 mice per as a preservative and mix thoroughly until a uniform income.
- Powder from 20 mm of the culture medium was mixed with 80 mm of eucerin.
The animals were anesthetized by a mixture of ketamine and xylazine (i.p.; 15 and 20 mg/kg, respectively).

IP injection for faster absorption of injection material.
- shaving the dorsal hair
- Ethanol 60%
- One open excision full-thickness wound approximately $1.5 \times 1.5$ cm long and 6 mm deep wounds were created in the back of each mice using shablon.
- Full-thickness wound

- Digital photographs of wounds were taken at days 1, 3, 7, 14 and 21 and the maximum length and width of each wound was measured with calipers in tertian.
HISTOLOGICAL STUDIES

- The mice were killed at 1, 3, 7, 14, 21 day after wounding. Paraffin embedded sections were prepared from skin samples including the wound and the surrounding skin were harvested using surgical blade.

- Cut perpendicular to the skin surface and included the whole thickness of the skin. Serial sections were stained with H&E.
RESULTS & DISCUSSION
The percent of wound healing and inflammation in experimental groups as compared to control and negative control groups were significant (p≤0.05). The rate of wound healing in experimental group 1 compared with the experimental groups treated with dead bacteria and exopolysaccharide showed a significant increase (p≤0.05). The current study showed a significant reduction in inflammation and a significant acceleration in wound healing on the rats treated by live form of *Lactobacillus casei* as compared to other groups. Further studies are required for detail mechanism of *Lactobacillus* during wound healing.
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Wound healing percentage

Days

Day 1  Day 3  Day 7  Day 14  Day 21

Negative Control
Control
Experimental 1
Experimental 2
Experimental 3
Thank you for your attention.