

**Immunohistochemical Evaluation of The Effect
of 790-805nm Diode Laser on Interlukin-1 β ,
Epidermal, Fibroblast, Vascular Endothelial
and Platelet Derived Growth Factors
In Wound Healing**

(An Experimental study on mice)

A Thesis

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Background: wound healing represents one of the challenging aspects in medical field due to its importance to reduce suffering and pain and facilitating recovery time. The induction of repair in wounds represents a dynamic process that involves the integrated actions of many cell types, extracellular matrix and chemical mediators. Wound healing includes subsequent, biological events includes inflammatory ,proliferative and maturation phase .These events are controlled by certain biological molecules called growth factors and cytokines which are produced and secreted under complicated control system of the nucleus.

Low-level laser therapy has been extensively applied to improve wound healing due to some biostimulatory properties presented by laser arrays which apparently able to accelerate the repair of soft tissue injuries. However, many controversial results have been reported in the literatures, probably as a result of the wide sort of different protocols of photobiomodulation employed in those experiments.

Aims of the study: the goals of this study were to investigate:

1. Histological evaluation of the effect of low level laser therapy (790-805nm diode laser) on the healing of incisional cutaneous wound in mice.
2. Immunohistochemical estimation of low level laser therapy (790-805nm) on the molecular events in wound healing by the cellular expression of growth factors and cytokines: platelet derived growth factor, vascular endothelial growth factor, fibroblast growth factor, epidermal growth factor and IL-1 β in the control and lased group.
3. Comparison of the histological and immunohistochemical results between the control and lased group.

Materials and Methods: ninety-six white albino mice had been used throughout the study exposed to recommended laboratory environments.

A surgical incision wound with full skin thickness and 1.5 cm length had been done on the cheek of the animals for an ideal wound model on mice. Then the animals were divided into two main groups; the control and lased group(48 mice for each) which then subdivided into 4 subgroups according to healing intervals into 1,3,7, and 14 days (12 mice each).

The experimental group was exposed to laser irradiation using low energy laser (780-805nm diode laser) immediately after the incision procedure as single dose of 360 joules deposit. The specimens were taken and prepared for the histological and immunohistochemical examination. Histological sample were assessed regarding the count of (neutrophil, lymphocyte, macrophage and eosinophil count in addition to epithelization thickness measurement).

While immunohistochemical stained sections for the growth factors platelet derived, vascular endothelial, fibroblasts and epidermal growth factors in addition to interleukin -1 beta had been done on tissue sections of formalin fixed paraffin embedded type. The expression assessed by using certain scoring system depending on the percentage of positively stained cells. The positive stain localized extracellularly for interlukin-1 beta; membranous for platelet derived, epidermal growth factors, and cytoplasmic localization for vascular endothelial and fibroblasts growth factors.

The results were confirmed statistically by using t-test (independent and paired) in addition to correlation test and ANOVA test.

Results: the histological findings for the lased group showed a higher count numbers of all estimated inflammatory cells in addition to higher epithelial thickness in comparison to control group at all healing day's intervals.

Immunohistochemical findings revealed higher level of expression for all growth factors. In addition to interleukin -1 beta in lased group for

both epidermal and stromal expressions in comparison to the control group at all day's intervals.

There was a significant correlation between eosinophil count and epidermal expression of vascular endothelial, fibroblast and platelet derived growth factor in both control and lased group significantly ($P > 0.05$); while neutrophil count correlated with interleukine-1 beta and vascular endothelial growth factor in lased group significantly ($P > 0.05$). Lymphocyte count correlated with epidermal growth factor in the control group; while correlated to fibroblast growth factor in lased group significantly ($P > 0.05$). Macrophage count correlated to vascular endothelium and fibroblast growth factor in lased group significantly ($P > 0.05$). Finally; epithelization parameter correlated significantly to vascular endothelium, interleukine-1 beta and fibroblast growth factor in both groups significantly ($P > 0.05$).

Conclusions: the application of low level laser therapy has a great effect on wound healing by activation of (neutrophils, macrophages, lymphocytes and eosinophil that involve in wound healing by secretion of effective growth factors (platelet derived growth factor, vascular endothelial, fibroblasts and epidermal growth factors. In addition to interleukin -1 beta increased significantly indicating certain molecular changes in the epidermal and stromal layer of wound area in comparison to control group.