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Original article

Accelerating wound healing and skin loss sealing using low level laser therapy

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Abstract

Many therapeutic aids are used to accelerate wound healing and promote the healing processes; the knowledge base about the role of Low Level Laser Therapy L.L.L.T. in regeneration processes continues to grow especially in the fields of dermatology and cosmetic surgery. The aim of the current study was to present an overview of the interrelationship between the hormones involved in wound healing and irradiation with Low Level Lasers. The experiment was conducted on twenty adult male New Zealand rabbits, they were divided into two groups with 10 rabbits each: group 1 (induced wound group) and group 2 (lost skin group).The animals of the 1st. group underwent a surgical operation on the lateral aspect of the left thigh; a surgical wound with 7cm length was made and then closed by simple interrupted stitches using surgical silk 3-0, while the 2nd. group operation involved removing of a whole thickness skin square graft of (10x10 mm) dimensions. The animals of each group were divided into two subgroups (control and treated with laser irradiation). The laser used was diode with wave length 820nm and output of 200 mW. Irradiation began after the operation and continued for three days in the animals of the induced wound subgroup and seven days in the skin loss subgroup animals with 1.2 minute /session daily. Irradiation with the laser was done by directing the beam (1cm) distance from the wound or around the square area of the lost skin. Blood samples were collected at days (0, 1. 3 & 7) from the animals of the 1st. group and (1, 3, 7 &10) in the animals of the 2nd. group. The samples were taken from the marginal ear vein from all the animals and sent for examination with Enzyme-Linked Immunosorbent Assay - ELISA to determine the levels of Prostaglandin E2 (PGE2), Prostaglandin F2α (PGF2α), Growth hormone (GH) and cyclic Adenosine Monophosphate (cAMP). All the readings got from the study were tested statistically using Minitab and SPSS regression test**.** Clinically, the animals of the 1st. group showed significant variations in the time of healing being about four days in the treated subgroup and eight days in the control one. The stages of the skin defect's contraction and sealing, was faster in the animals of the treated subgroup taking nine days, while it took fifteen days in the control subgroup. Statistical evaluations revealed significant variations in the values of PGE2, PGF2α, cAMP and GH, between the two subgroups of the 1st. group, $P > 0.05$. Hormonal assessment of PGE2, PGF2α, cAMP, GH and the diameter of the skin defect for the animals of the 2nd. group showed significant variations between the two subgroups $P > 0.05$. Conclusion can be done that treating the surgical wounds and skin disorders with low level laser radiation is efficient to promote and accelerate the primary healing.

Key words: Laser, Wounds, Skin loss, Regeneration

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Introduction

The three phases of wound-healing process: inflammation, proliferation, and remodeling are integral to the completion of the process, and may occur at various rates, (1). There are a number of hormones involved with anabolism or protein synthesis, and catabolism or protein breakdown. The balance of

anabolic and catabolic hormones affects wound healing both indirectly by the status of overall net protein synthesis and directly by improving the wound healing process. Cell proliferation is accentuated as is overall protein synthesis and new tissue growth, (2). The role of prostaglandins in promoting human diseases has been widely studied particularly in inflammatory disorders, After initial vasoconstriction, the classic signs of inflammation manifest from increased vascular permeability .This reaction is followed by vasodilatation, mediated by Prostacyclin (PGI2), prostaglandin A (PGA), prostaglandin D (PGD), and prostaglandin E (PGE). These changes are potentiated by prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) and allow the ingress of inflammatory cells into the area of injury, (3). Activation of the enzyme adenylcyclase catalyzes the formation of cyclic Adenosine Mono Phosphate cAMP, which has multiple effects inside the cell to control cell activity. cAMP is called a second messenger because it is not the hormone itself that directly institutes the intracellular changes; instead, cAMP serves as a second messenger to cause these effect , (4). The range of L.L.L.T.'s medical applications in tissue stimulation continues to increase as new devices are constructed. Continued research into tissue biostimulation has revealed that L.L.L.T. has a beneficial effect on living organisms, (5). L.L.L.T. has been used for treatment of wounds for over two decades in many medical facilities of the world. It proved to be useful and efficient because the primary healing was stimulated (6). The aim of the current study is to evaluate the role of L.L.L.T. in surgical aseptic wound management based on clinical examination, (estimating the timing of the healing necessary for healing of the wounds and sealing of skin defects) and hematological examination,(studying the effect of the laser irradiation on the hormones and the enzyme which interlaced with wound healing, especially those from global specialized laboratories, such as ; PGE2, PGF2α, cAMP and GH and the effect of each one of them on the others.

Materials and Methods

Twenty adult white New Zealand male rabbits with 1.5 -2 Kg body weight each used in the study, they were divided into two groups with 10 rabbits each: group 1 (induced wound group) and group 2 (lost skin group).The animals of the 1st. group underwent a surgical operation on the lateral aspect of the left thigh; a surgical wound with 7cm length was made and then closed with simple interrupted stitches using surgical silk 3-0, while the 2nd. group operation involved removing of a whole thickness skin square graft of (10x10 mm) dimensions. The animals were anesthetized a mixture of Xylazine[1] 5 mg /B.W and Ketamine hydrochloride[2] 10 mg /kg B.W injected i/m, the animal reached to the stage of surgical anesthesia after 5 minutes and continued for 45 minutes, then the animal was placed on the surgical stage and fixed in a manner so that the lateral aspect of the left thigh faced to the surgeon. In the animals of 1st. an incision with 7cm length was done in the lateral aspect of the thigh involving the whole thickness of the skin and closed by simple interrupted stitches using 3-0 silk[3], then the animal injected with systemic antibiotics, Penicillin[4] 1000i.u. kg. B.W. and Streptomycin[5] 10 mg/kg .B.W. i/m for 3 days post the operation , while the animals of the 2nd. group underwent surgical operations to remove a square peace of a whole thickness skin with 10mm² diameter, then the site of the operation in all the animals was treated with antibiotic spray. Both of the groups were subdivided in to two subgroups; (control & treated) with five rabbits in each, the difference between the two subgroups

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that the treated subgroups was irradiated with diode laser. The animals of the 1st. (induced wound group) were examined daily looking for the signs and symptoms often accompany wounds like bleeding or oozing of blood, redness, swelling, pain, tenderness, heat, loss of function of the organ, oozing pus, foul smell, also they were examined looking for the type of approximation of the wound's edges and the healing processes. While the animals of the 2nd. group (skin loss group) were examined every three days using a very precise caliper to determine the diameter of the skin defects

The laser[1] used was diode with wave length 820 nm, maximum output of 200 mW, power density, 1.6 W/ cm² energy density 115.2 J/cm², and pulsing frequency 1-10 Hz. Irradiation began after the operation directly and continued for three days in the animals of the induced wound subgroup and seven days in the skin loss subgroup animals with 1.2 minute /session daily. Irradiation with the laser done by directing the beam (1 cm) distance from the wound or around the square area of the lost skin. Blood samples were collected at regular intervals from the marginal ear vein right after disinfection using 70% alcohol, the amount of blood drawn was 3 cc, the samples were collected using syringes of 3 ml , then the serum was separated by centrifugation at 2500 cycles / minute for 15 minutes. Serum was used to measure the values of; PGE2, PGF2α, cAMP and GH. All the results were evaluated statistically using Minitab and SPPS regression tests.

Results

Clinical examinations of the animals of the first group showed significant variations between the two subgroups of animals starting the second postoperative day, when the laser irradiated subgroup's animals had a perfect wound edges approximation, partial epithelization with no serous, bloody or suppurative exudates investigated. Two animals from the treated subgroup showed clinical wound healing within three days while the remainder showed complete healing and stitches removing at the fourth postoperative day. Wound healing in the animals of the control group took approximately eight days, some of the animals showed clinical healing at the seventh days, as shown in Table 1 . No complications were seen associated with the healing processes in the animals of the both subgroups. The skin defects of the animals of the second group were measured using a fine measurement caliber looking for the stages of defect's contraction and sealing, it was faster in the animals of the treated subgroup taking (nine days), while it took fifteen days in the control subgroup, the defects filled with epithelial tissue and little scaring.

No complications accompanied the wound contraction in both subgroups, Table 2 shows the skin defect diameter in the animals of both subgroups of the 2nd. group there is a significant variations $P > 0.05$ between the two subgroups. The results of the ELISA test for the hormones showed significant variations in the values of PGE2, between the two subgroups of the 1st. group, $P > 0.05$, Figure 1 and Table 3 .

1 Omega laser system.UK.

Table 1 : Clinical wound healing of the both subgroups of the 1st. group (wound healing group).

Subgroups	Animals	Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day10
Control C	1							√			
	2							√			
	3								√		
	4							√			
	5								√		
Treated T	1				√						
	2				√						
	3				√						
	4			√							
	5			√							

Table 2 : Diameter of the skin defect in the animals of the 2nd. group estimated in cm²

Subgroups	Animals	Day 0	Day 3	Day 6	Day 9	Day12	Day 15
Control C	1	1	0.99	0.85	0.72	0.32	sealed
	2	1	0.96	0.81	0.69	0.31	sealed
	3	1	0.93	0.81	0.61	0.27	sealed
	4	1	0.98	0.87	0.73	0.34	sealed
	5	1	0.94	0.82	0.67	0.24	sealed
Mean		1	0.96	0.832	0.684	0.296	sealed
Treated T	1	1	0.81	0.34	sealed		
	2	1	0.78	0.41	sealed		
	3	1	0.74	0.35	sealed		
	4	1	0.69	0.36	sealed		
	5	1	0.65	0.37	sealed		
Mean		1	0.73	0.37	sealed		
Subgroups	Animals	Day 0	Day 3	Day 6	Day 9	Day12	Day 15
Control C	1	1	0.99	0.85	0.72	0.32	sealed
	2	1	0.96	0.81	0.69	0.31	sealed
	3	1	0.93	0.81	0.61	0.27	sealed
	4	1	0.98	0.87	0.73	0.34	sealed
	5	1	0.94	0.82	0.67	0.24	sealed
Mean		1	0.96	0.832	0.684	0.296	sealed
Treated T	1	1	0.81	0.34	sealed		
	2	1	0.78	0.41	sealed		
	3	1	0.74	0.35	sealed		
	4	1	0.69	0.36	sealed		
	5	1	0.65	0.37	sealed		
Mean		1	0.73	0.37	sealed		

Results of regression test showed that there were a relationship between the two variants (time & PGE2 level), any additional day passed lead to increase in the level of the hormone, till the 3rd. day when it began to decrease by time, the correlations between the two subgroups was high reaching 0.99. At zero time, the level of hormone in the treated subgroup was double when compared with the control one; the clearing rate of the PGE2 hormone in the treated subgroup was higher than that of the control one , Figure 1 . The values of hormonal assessment of PGF2α for the animals of both subgroups of the 1st. group showed significant variations between the two subgroups $P > 0.05$; see Figure 2 and Table 3 . Results of regression test showed that at the zero time, the level of the hormone was higher in double in the treated subgroup compared with the control one, and the clearing rate of the hormone in the treated subgroup was higher than that of the control one. The values of hormonal assessment of cAMP for the animals of both subgroups of the 1st. group showed significant variations between the two subgroups $P > 0.05$; Table 3 .

Table 3: Values of PGE2 ,PGF2α , cAMP and GH in the animals of the 1st. group.

Samples	Days	Subgroups					Mean
		Con. of PGE2 (pg/ml)					
Control C		1	2	3	**4**	**5**	
	0 Day	241.1	227.7	239.4	1.5	1.0	1.18
	1st. Day	331.0	335.7	330.5	1.7	2.5	2
	3rd. Day	400.7	420.0	415.7	4.0	3.5	3.74
	7th. Day	279.5	280.5	282.9	4.5	3.9	3.76
Treated T	0 Day	507.9	506.9	503.7	505.3	510.7	506.9
	1st. Day	539.1	542.7	540.8	542.2	536.9	540. 34
	3rd. Day	580.9	579.5	581.2	580.3	583.8	581.14
	7th. Day	246.1	241.7	240.9	243.8	239.9	242.48
		Con. of PGF2α (pg/ml)					
Control C	0 Day	186.5	190.4	184.9	181.6	187.2	186.12
	1st. Day	276.3	270.6	273.9	279.3	271.4	274.3
	3rd. Day	347.4	345.9	349.6	341.9	343.5	345.66
	7th. Day	293.5	293.1	290.3	289.9	295.5	292.46
Treated T	0 Day	232.3	229.9	235.6	233.8	234.5	233.22
	1st. Day	298.7	294.4	299.5	295.3	293.9	296.63
	3rd. Day	473.9	472.6	479.1	470.8	478.0	474.88
	7th. Day	391.9	395.8	397.3	389.8	390.6	393.08
		Con. of cAMP (pg/ml)					
Control C	0 Day	363.3	360.6	361.1	369.1	362.7	363.36
	1st. Day	437.2	432.6	439.4	436.6	434.9	436.14
	3rd. Day	462.9	460.6	465.5	463.4	467.7	464.02
	7th. Day	380.7	379.9	384.6	381.1	377.8	380.82
Treated T	0 Day	576.9	575.5	579.8	571.6	573.7	575.5
	1st. Day	581.1	579.8	580.0	583.3	582.4	581.32
	3rd. Day	711.3	706.7	710.9	712.4	714.5	711.18
	7th. Day	672.6	670.5	679.2	675.5	677.5	675.06
		Con. of GH (ng/ml)					
Control C	0 Day	0.6	1.2	0.9	1.0	1.5	1.04
	1st. Day	1.7	1.5	1.2	2.5	2.8	1.94
	3rd. Day	2.5	3.4	3.0	2.9	3.5	3.06
	7th. Day	3.7	4.0	4.3	4.5	4.8	4.26
Treated T	0 Day	1.5	2.0	2.9	2.2	2.5	2.22
	1st. Day	2.5	2.7	2.0	3.0	2.9	2.62
	3rd. Day	3.3	3.5	4.0	3.9	3.0	3.45
	7th. Day	4.5	4.9	5.3	5.0	5.6	5.06

The values of hormonal assessment of GH for the animals of both subgroups of the 1st. group showed significant variations between the two subgroups P > 0.05, Table 3 . The relationship between the two variants (time and GH level) shows that the increase in the level of the hormone was continuous in the treated subgroup but ceased on the 7th. day in the control one. The values of hormonal assessment of PGE2 for the animals of both subgroups of the 2nd.group showed significant variations between the two subgroups P > 0.05, Table 4 .

Regression test showed that the relationship between the two variants has a slow rate, it's value was 0.5680 and 0.515 in both subgroups respectively.

The values of hormonal assessment of PGF2α for the animals of both subgroups of the 2nd. group showed significant variations between the two subgroups P > 0.05, Table 4 .

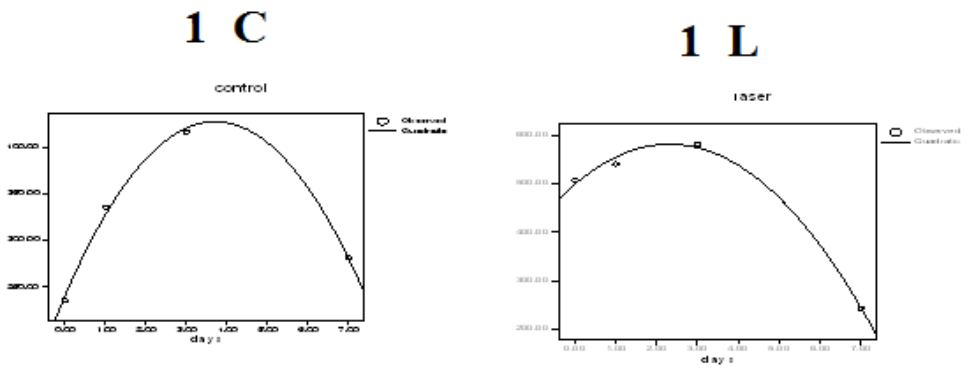

Figure 1: SPSS regression test for the animals of both subgroups for PGE2.
- The Vertical axis represents the concentration of PGE2 estimated in (pg/ml).
- The Horizontal axis represents the days.

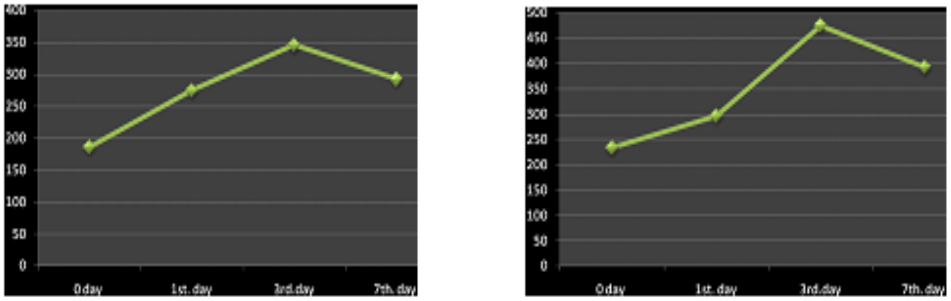

Figure 2: Average values of PGF2α in both control (L) and treated (R). subgroups of the 1st. group.

Regression test showed that the level of the hormone stopped increasing after a several days then begin to decrease. At zero time the level of the hormone in the control subgroup was higher than the treated one, as was the percentage of hormonal increase. The values of hormonal assessment of cAMP for the animals of both subgroups of the 2nd. group showed significant variations between the two subgroups P > 0.05, see Figure (3) and Table 4 .

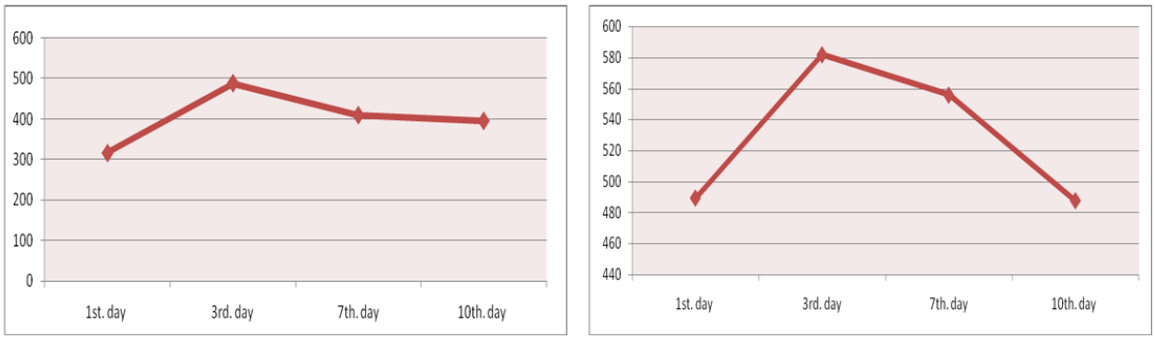

Figure 3: Average values of cAMP in both control (L) and treated (R) subgroups of the 2nd. group.

The values of hormonal assessment of GH for the animals of both subgroups of the 2nd. group showed significant variations between the two subgroups P > 0.05, see Table 4 . The relationship between the two variants (time and GH) was identical, and the r^2 degree was 0.99 for the laser treated subgroup and 0.98 for the control subgroup, but the degree of the hormonal increase was higher in the treated subgroup.

Table 4: Values of PGE2, PGF2α , cAMP and GH in the animals of the 2nd. group.

Samples	Days	Subgroups					Mean
Con. of PGE2 (pg/ml)							
Control C		1	2	3	4	5	
	1st. Day	254.6	241.0	248.9	250.5	255.2	250.4
	3rd. Day	418.6	410.5	420.7	415.1	422.8	417.54
	7th. Day	303.4	300.5	299.7	311.5	301.5	3030.32
	10th. Day	257.8	241.9	247.9	245.2	256.3	249.82
Treated T	1st. Day	418.7	401.2	411.3	420.7	415.6	413.5
	3rd. Day	516.6	510.8	520.7	515.0	522.8	517.18
	7th. Day	439.7	434.5	329.9	448.0	430.0	416.42
	10th. Day	399.6	388.9	395.8	386.4	386.4	391.42
Con. of PGF2α (pg/ml)							
Control C	1st. Day	243.2	241.8	246.5	242.2	244.3	243.6
	3rd. Day	406.5	411.6	408.3	405.5	410.6	408.5
	7th. Day	357.6	358.6	353.3	360.2	355.5	357.04
	10th. Day	342.3	341.0	339.9	348.5	340.2	342.38
Treated T	1st. Day	363.6	360.3	368.7	361.9	366.2	364.14
	3rd. Day	453.3	451.4	459.3	450.0	451.5	453.1
	7th. Day	390.2	394.9	391.6	397.7	395.8	394.04
	10th. Day	387.5	385.4	389.8	386.1	384.6	386.68
Con. of cAMP (pg/ml)							
Control C	1st. Day	317.4	315.5	310.8	320.2	317.9	316.36
	3rd. Day	489.9	485.6	487.8	488.1	486.9	487.66
	7th. Day	409.6	405.9	415.3	411.6	404.8	409.44
	10th. Day	396.6	394.5	390.4	397.8	396.7	395.2
Treated T	1st. Day	489.0	488.9	495.3	489.5	483.7	489.28
	3rd. Day	581.4	579.8	585.9	583.4	578.7	581.84
	7th. Day	556.9	559.6	552.3	551.4	558.7	555.78
	10th. Day	490.5	485.5	487.7	480.9	493.5	487.62
Con. of GH (ng/ml)							
Control C	1st. Day	1.9	1.0	0.5	1.5	1.0	1.18
	3rd. Day	2.3	2.0	1.5	1.7	2.5	2
	7th. Day	3.7	3.0	4.5	4.0	3.5	3.74
	10th. Day	2.3	3.7	4.4	4.5	3.9	3.76
Treated T	1st. Day	2.5	2.5	2.0	2.7	2.5	2.44
	3rd. Day	3.7	3.5	2.5	2.0	3.9	3.12
	7th. Day	4.5	4.9	5.0	5.2	4.0	4.72
	10th. Day	5.4	4.7	4.8	5.0	5.5	5.08

Comments

Many alternative treatments are available to help heal wounds that do not respond for treatments, among these treatments are using ultrasound, electrical stimulation and use of magnets. A large number of remedies used to accelerate wound healing while hormones were used successfully in experiments and clinically to accelerate wound healing , (7) . Literature indicates that laser photobioactivation of mast cells proliferate and lose their granules which in turn release chemical mediators which helps in healing process, accelerate epithelial hyperplasia of normal epithelial cells , (8). Low-level laser emission increased tissue oxygenation, morphofunctional activity, and substantial expansion of the microcirculatory bed. They, in turn, accelerated the restoration of functions, stimulation of adaptational ability, and stabilization of the hormonal status, (9). Prostaglandins are produced by all the cells in the body and are released when there is any disruption of cell membrane

integrity. Certain prostaglandins further contribute to long-term vascular vasodilation. The fibrin plugs that clot in the wound to seal leakage also form in the lymphatic vessels. The blocking of the lymphatic flow not only seals the wound but also helps to stop the spread of infection. They remain closed until later in the healing process, (10). Prostaglandins initiate the inflammatory reaction in both subgroups but, within the current framework the laser radiation shots sparks of the Prostaglandin E2 (PGE2) concentration in the treated subgroups (of both the wound healing or skin loss sealing groups), twice higher than that of the control one. Reaching the 3rd. day the concentration of the hormone reached it`s highest level in both subgroups; yet it is still higher in the treated subgroup and then the clearing stage started being carried out faster in the treated subgroup. These results showed that the PGE2 was stimulated by the laser irradiation , if these results compared with those of the Prostaglandin F2α (PGF2α), it will be easy to conclude that the level of the hormone reached the peak in the 3rd. day regardless which subgroup or the group and ceased after that in the wound healing group and the 7th. and 10th. day in the defect sealing group which means that the PGF2α level starts increasing after the PGE2 ceased. PGF2α have a classic model of a pro-inflammatory lipid mediator, also has anti-inflammatory effects that are both potent and context dependent. Thus, accumulating data suggest that PGs not only participate in initiation, but may also actively contribute to the resolution of inflammation, (11). Cyclic Adenyl Mono Phosphate cAMP system appears to be involved in action of prostaglandins as modulators , production of a large amount of cAMP activate cAMP-dependent protein kinase (PKA), which may in turn activate cAMP response element binding protein (CREB), a transcription factor, in the nucleus,(12).

A closer look to the values of the cAMP in the animals of the 1st. group leads to a conclusion that the level of this enzyme increases with the inflammatory process because the enzyme acts as the second messenger which triggers the 3rd. messenger and the 3rd. one triggers the 4th. and so on; thus the level of the enzyme remains high till the healing process closes to completion in both groups. A rapid induction of PGE2 in the early inflammatory phase of wound healing followed with PGI2 and PGF2α induction in the late resolution phase of wound healing when extracellular matrix gene induction declines, (13). Laser irradiation has a number of biochemical responses that can have a positive clinical effects including ; enhancement of ATP synthesis, increased histamine and vasodilation, NO and serotonin, increase leukocyte activity and prostaglandin synthesis, reduction in Interleukin-1 levels and increased angiogenesis, the light increase ATP synthesis which is the substrate for adenyl cyclase, and therefore the ATP level controls the level of cAMP.

Laser can be used for initiation intrinsic resistance against the extrinsic factors (antigens) through increase the viability of monoclonals or isotypes of the antibodies directly or indirectly by releasing of mediators such as ; Enkephalins , Endorphins ,Prostaglandins and others, (14). The results of current study strongly agreed with those found from experiments on rat and mice, which review identified 47 relevant studies in the mouse (n=8) and in the rat (n=39). Findings from these consistently demonstrated the ability of laser or monochromatic light therapy to photobiomodulate (typically stimulate) wound healing processes in experimental wounds in the rat and mouse, and strongly support the case for further controlled research in humans, (15). The important role of locally acting cytokines and growth factors in wound repair. However, crucial functions of endocrine acting hormones have been recognized. One of the hormones, which are thought to influence the repair process, is growth hormone GH. In the second group (the skin lost group) the defect is closed in the treated subgroup after nine days while they took fifteen days to be closed in the control subgroup, these results are very

encourage if compared with those got by a team of authors in their work on pigs , they induced full-thickness, round skin defects 4 cm in diameter were created on the back of each pig, and divide the animals into two groups ,the treated wounds were dressed with the GH-containing cream and foam dressing while the control wounds left without GH administration ,(16). A significant reduction in the wound sizes of the GH-treated group was observed as compared with the control group ($P < 0.05$). The wound size of the experimental group decreased significantly more than the control group each week. In the later weeks, the ratio of wound area reduction between the two groups increased. The healing rate in the GH-treated group was faster than that of the control group,(16). The increase in the level of cAMP in the subgroups treated with L.L.L.T. is agreed with those approved by Lutho Innocent Zungu , from his work on human fibroblast , he founded that the irradiation with laser increases the level of cAMP , mitochondrial intracellular calcium ion ca^{+2} , Mitochondrial Membrane Potential MMP and Adenine Triphosphate ,(17). The results got from the second group (the group of skin loss) are satisfied with those got by Nayak, et al. using excision wounds in Wister rat model. The parameters studied were wound area, period of epithelization. Significant reduction in the wound size was observed in the treated group when compared to controls. Significant epithelization was noticed also. The treated wounds were, on average, fully healed by the 15^{th}. day, whereas the control group healed, on average by $22^{nd.}$ day, (18). The accelerating of wound healing in the current study is more significant than that achieved by the application of an exogenous electrical stimulus to chronic wounds with the aim of instigating electrotaxis, (19), or an external herbal formulation, (20).

Conclusions

1- L.L.L.T. accelerates wound healing and the skin defects by approximately half the time needed for normal healing procedures.
2- There is a close relationship between the wound healing processes and the hormones and enzymes which control the homeostasis, initiate the inflammatory processes, precipitate the granulation tissue and interlace with the remodeling stage.
3- There is a complementary relationship or synergy between each of the PGE2, PGF2α, cAMP, and GH with each other during the healing process. L.L.L.T. has a stimulatory effect on the hormones which intervene with wounds and skin defects healing.
4- Application of L.L.L.T. in wound healing was safe, with no side effects reported.

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