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Original article

Effect of 915nm diode laser on some hormones &minerals concerning with fracture healing

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Abstract

Present the interrelationship between laser radiation and stimulation the hormones and minerals involved in fracture healing. The role of L.L.L.T. in regeneration grows especially in the fields of fracture care. Twenty four rats used in this study, they were divided into two groups with twelve rats each. (control and treated with diode laser). A fracture was induced in the tibia using two sterile artery forcipes. Blood samples were collected from the animals and sent for examination with Elisa to determination the levels of growth hormones (GH), Parathyroid hormone (PTH) and Calcitonin hormone (CTH) and with spectrophotometer to determine the levels of Calcium (Ca), Phosphorus (P) and Magnesium (Mg). The readings obtained from the laboratory estimations tested statistically using Minitab test. Statistical evaluations revealed significant variations in the values of GH, PTH, CTH, Ca, P and Mg in the blood samples between the two groups $P< 0.05$. L.L.L.T. has a stimulatory effect on the hormones and minerals which intervene with fracture healing.

Key words: Laser, Minerals, Fractures.

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Introduction

Laser therapy is a new approach applicable in different medical fields, (1), Low energy laser irradiation has positive effects on bone fracture healing low level energy treatment accelerated the deposition of bone matrix and increases vascularization after seven days of irradiation. The periosteum, endosteum and bone marrow provided cells that proliferate and differentiate into osteoblasts, chondroblasts and fibroblasts, which contribute to new bone formation, (2).There are several hormones work in fracture on bone, Growth hormone can enhance the osteogenic potential of the bone marrow, (3). Parathyroid glands are sensitive to blood calcium level, when it falls below normal, parathyroid hormone will be secreted, and this will lead to increase calcium absorption from the gut and from the bone, (4). Parafollicular cells of the thyroid gland also monitor calcium ion levels in the plasma, when the calcium ion level become elevated, these cells secrete calcitonin, a polypeptide hormone, that activates receptors on osteoclasts inhibiting them from resorbing bone, (5). Bone consisted roughly of 70% minerals, Calcium is the most abundant mineral in the human body, (6), total body stores of approximately 1,000 g: 99% in bone, 0.9%

intracellular, and 0.1% extracellular, fractures can heal normally independent of dietary calcium and indeed it has been found that during the first few weeks of healing, calcium is drawn from the skeleton for fracture healing, (7) About 85% of phosphorus in the body can be found in bones and teeth, Phosphorus is needed for the growth, maintenance, and repair of all tissues and cells, (8). Magnesium is an essential element for bone and plays a major role in bone and mineral homeostasis by functioning on all phases of skeletal metabolism, (9).

Materials & methods

The experiment was conducted on twenty four adult white male rats with (350 -400) g body weight each, they were divided into two groups with 12 rats each: group 1 (induced fracture group) and group 2 (control group). The animals of the two groups underwent a surgical operation by breaking the tibia of left leg in each animal,(under general anesthesia using a mixture of (Xylazine 3 mg / Kg) B.W and Ketamine hydrochloride (10 mg /kg B.W) injected i/m) ,fracture was induced using two sterile arty forcipes, one of them to fix the tibia from its center and the second one to bend the distil end so that the fracture was induced, two pieces of cotton used to circle the thigh proximal and distal to the fracture site then gypsum used to fix the fracture beginning from the piece of the cotton below to that above leaving a small window in the animals of the treated group for laser irradiation after the operation directly and for 7 days later ,while the second group remain as the control group. The laser used was diode 915nm wave length, with maximum output of 100 mW, density 32 J/cm², pulsing frequency 10 KHz, with 2.40 minute /session daily and direct on the window of gypsum. Blood samples were collected at days (1, 2, 3, 5,7,10 and 14) from the animals of the both groups. The samples were taken from the heart from all the animals and sent for examination with Elisa to measure the values of Growth hormones (GH), Parathyroid hormone (PTH) and Calcitonin hormone (CTH), and with spectrophotometer to measure the values of calcium (Ca), phosphorus (P) and magnesium (Mg).

Results

1. **Clinical examinations**

   Clinical examinations of the animals showed significant variations between the two groups of animals. The animals of the control group showed clinical healing approximately at the twelve to fifteenth postoperative day while those of the treated group showed clinical fracture healing and began using the fractured limb within seven to ten days. These observations are explained in Tables; 1.

2. **Hormonal examinations**

   The results of the Elisa test for the hormones showed significant variations in the values of PTH, CTH and GH between the two groups of animals $P < 0.05$, which measured in pg/ml.

   a. Parathyroid hormone (PTH)

   PTH concentrations raised in the blood in the second day and reach to 10.89 ± 1.174 pg/ml in treated group versus 9.025 ± 1.13 pg/ml in control group at $P<0.05$ and began to decrease in the third day in both groups but in the treated group the are faster and continue to the fifth day to the lower value 6.938 ± 1.007 pg/ml after that increased and returned gradually to normal values in the seventh and tenth day in 8.292 ± 1.005 pg/ml . In the control group PTH concentrations decreased until reach in the seventh day to the lower value 5.867 ± 0.624 pg/ml, then raised and returned proximately to the normal values in the fourteenth day 6.963 ± 0.953 pg/ ml, as shown in Table;2 and Figure;1.

Table 1: The different in clinical fracture healing of both groups.

Day	Control group	Treated group
7		6
8		
9		3
10		3
11		
12	1	
13	5	
14	4	
15	2	
Mean of healing's day	13.5	8.25

Table 2: Mean Differences in PTH concentration in both groups (pg/ml).

group / day	Treated group Mean ± S.D. n = 12	Control group Mean ± S.D n = 12
1	8.192 ± 0.99	7.061 ± 1.137 *
2	10.89 ± 1.174	9.025 ± 1.13 *
3	9.671 ± 0.922	8.1 ± 1.074 *
5	6.938 ± 1.007	7.354 ± 1.005
7	7.908 ± 0.91	5.867 ± 0.624 *
10	8.292 ± 1.005	6.608 ± 0.79 *
14	8.783 ± 1.189	963. 0.953 *

* The significant difference in hormone values between the two groups in same day.

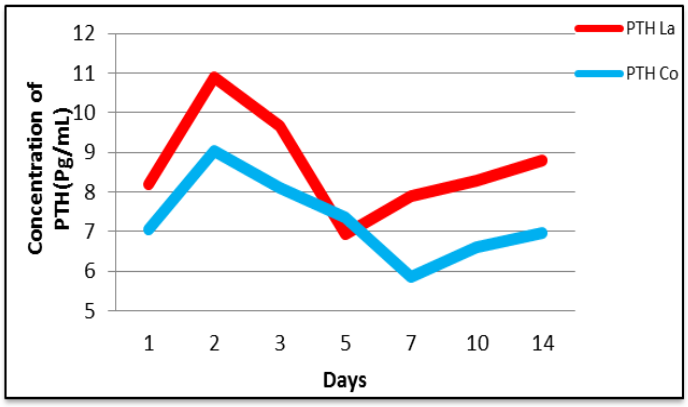

Fig. 1 : The variation in values average of PTH concentration between the two groups.

During these days of healing the PTH correlated significantly with other parameters that evaluated in the current study. There are significant relationship of the PTH with each of CTH, Ca, Mg in control group and in addition to this PTH and P in the treated group, Table; 8 & 9.

b. Calcitonin hormone (CTH)

The results showed that CTH values decreased in the second day at 10.2 ± 0.7 pg/ml in the treated group versus 8.7 ± 0.833 in the control group at P<0.05 and began rising in the third day in the two groups but in the treated group they are faster and reaching to the higher level in fifth day at 13 ± 0.89 after that returned gradually to normal values in tenth days 11.6 ± 0.75 pg/ml. In the control group CTH values raised gradually until reach to the higher value in seventh day at 11.71 ± 0.708 then returned proximately to the normal values in fourteenth days 11.02 ± 0.632 pg/ml, as shown in Table;3 and Figure;2. During these days of healing the CTH correlated significantly with other parameters that evaluated in the current study. There are significant relationship of the CTH with each of GH, PTH, Ca, Mg, P in control and treated groups, Table; 8 & 9.

c. Growth hormone (GH)

The concentration of GH hormone in serum of the treated group rose in the second day and continued to third day at 1.521 ± 0.402 pg/ml then decreased something and returned gradually to normal values in tenth day 0.895 ± 0.122 pg/ml at P<0.05. In the second group it increased slowly until the higher value in seventh day 0.91 ± 0.055 pg/ml then decreased and returned to the normal values proximately in the fourteenth day 0.79 ± 0.05 pg/ml, it`s shown in Table; 4. During these days of healing the GH correlated significantly with other parameters that evaluated in the current study. There are significant relationship of the GH with each of P, CTH, PTH, Ca^{+2}, Mg in control and treated group, Table; 8 & 9 and Figure;3.

3. The biochemical evaluations

The results of the Spectrophotometer test for the minerals significant variations in the values between the two groups of animals P < 0.05, which measured in mmol/L.

a. Calcium (Ca^{+2})

Ca^{+2} concentration increased in second day to the higher levels 2.44 ± 0.376 pg/ml in the treated group,

Table 3: Mean Differences in CTH concentration in both groups (pg/ml).

group / day	Treated group Mean ± S.D. n=12	Control group Mean ± S.D. n=12
1	11.4 ± 0.84	11.03 ± 0.774
2	10.2 ± 0.71	8.7 ± 0.833 *
3	12 ± 0.73	9.8 ± 1.133 *
5	13 ± 0.89	10.58 ± 0.921 *
7	12 ± 1.1	11.71 ± 0.708
10	11.6 ± 0.75	11.09 ± 0.738
14	11.6 ± 0.69	11.02 ± 0.632 *

* The significant difference in hormone values between the two groups in same day.

Table 4 : Mean Differences in GH concentration in both groups. (pg/ml).

group / day	Treated group Mean ± S.D. n=12	Control group Mean ± S.D. n=12
1	0.863 ± 0.083	0.79 ± 0.05 *
2	1.047 ± 0.248	0.813 ± 0.052 *
3	1.521 ± 0.402	0.832 ± 0.054 *
5	1.248 ± 0.318	0.857 ± 0.058 *
7	1.029 ± 0.269	0.91 ± 0.055
10	0.895 ± 0.122	0.815 ± 0.041 *
14	0.89 ± 0.085	0.79 ± 0.05 *

* The significant difference in hormone values between the two groups in same day.

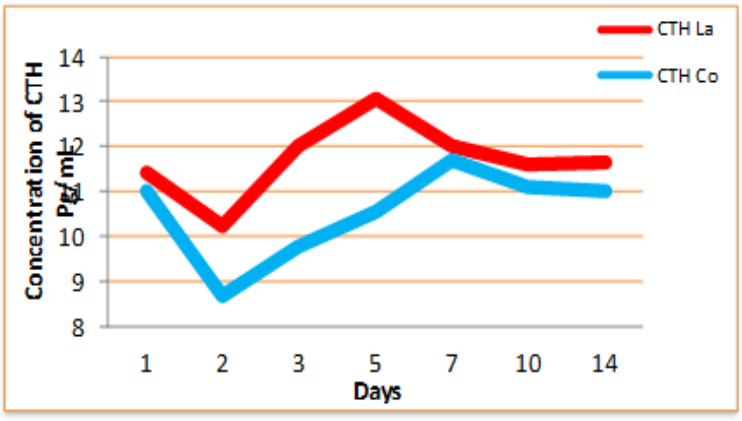

Fig. 2 : The variation in values average of CTH concentration between the two groups. .

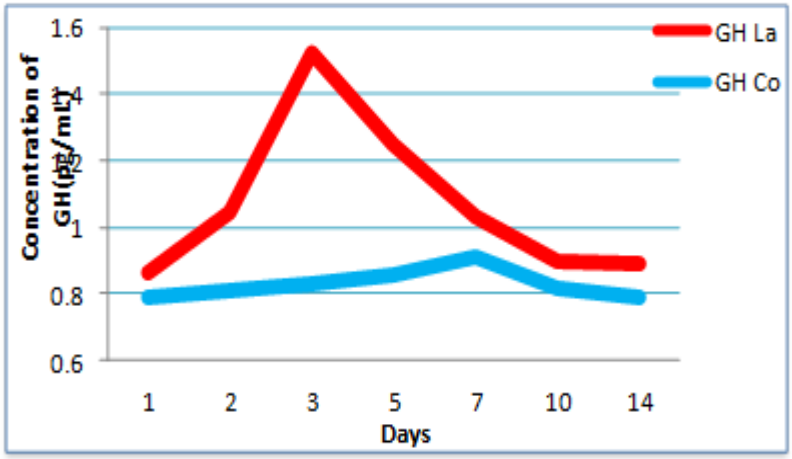

Fig. 3 : The variation in values average of GH concentration between the two group

1.8283 ± 0.3322 in the control group at P< 0.05. It began to decrease in the third day in the two groups but in the treated group it is faster and continues to the lower level in fifth day 1.357 ± 0.177 pg/ml, after

that returned gradually to the normal values in seventh day .In the second group it is decreased gradually until reach to the lowest value in the tenth day 0.9454 ± 0.1916 then returned proximately to the normal values, Table; 5. During these days of healing the Ca^{+2} correlated significantly with other parameters that evaluated in the current study in the treated and control group, Table; 8 & 9 and Figure;4.

b. Phosphorus (P)

In phosphorus test, phosphorus decreased in the blood of both groups in the second day but in the treated group it continued to the third day as lower value 2.02 ± 0.284 pg/ml at P<0.05 then incresed gradually to natural value in the tenth day post operation at 3.375 ± 0.6 pg/ml while in the second group it decreased until reached the lower value in the fifth day 1.647 ± 0.579 then increased gradually to the normal values. This is explained in Table: 6. During these days of healing the PTH correlated significantly with other parameters that evaluated in the current study. There are significant relationship of the PTH with each of CTH, Ca, Mg in control group and in addition to this PTH and P in the treated group, Table; 8 & 9 and Figure;5.

Table 5 : Mean Differences in Ca^{+2} concentration in both groups. (mmol/L)

group / day	Treated group Mean ± S.D. n=12	Control group Mean ± S.D. n=12
1	1.578 ± 0.523	1.1067 ± 0.2926
2	2.447 ± 0.376	1.8283 ± 0.3322
3	2.113 ± 0.309	1.605 ± 0.2139
5	1.357 ± 0.177	1.4925 ± 0.205
7	1.667 ± 0.397	1.2342 ± 0.2372
10	1.688 ± 0.413	0.9454 ± 0.1916
14	1.981 ± 0.346	1.1842 ± 0.2407

*The significant difference in mineral values between the two groups in same day.

Table 6: Mean Differences in P concentration in both groups. (mmol/L).

group / day	Treated group Mean ± S.D. n=12	Control group Mean ± S.D. n=12
1	3.462 ± 0.59	3.039 ± 0.642 *
2	2.608 ± 0.473	2.506 ± 0.686
3	2.02 ± 0.284	2.042 ± 0.581
5	2.581 ± 0.332	1.647 ± 0.579 *
7	3.029 ± 0.507	1.985 ± 0.628 *
10	3.375 ± 0.6	2.262 ± 0.64 *
14	3.483 ± 0.573	2.62 ± 0.62 *

* The significant difference in mineral values between the two groups in same day.

c. Magnesium (Mg)

Mg values in both groups had smaller rising in second day to the higher levels 1.948 ± 0.182 pg/ml in the treated group versus 1.426 ± 0.153 pg/ml in the control group at P<0.05. They gradually decreased in the treated group until the lower value in the seventh day 0.855± 0.22 after that return to the normal level in the tenth day 1.412 ±0.193 pg/ml. In the control group, they decreased slowly and reached to the lower 0.637 ± 0.076 pg/ml after that increased gradually to the normal value. This is explained in Table: 7. During these days of healing the PTH correlated significantly with other parameters that evaluated in the current study in the treated and control groups, Table; 8 & 9 and Figure;6.

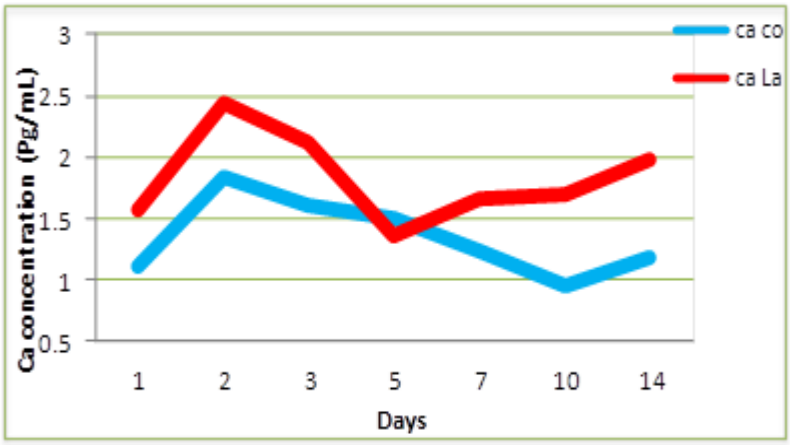

Fig. 4: The variation in values average of Ca^{+2} concentration between two groups.

Table 7: Mean Differences in Mg concentration in both groups. (mmol/L).

group / day	Treated group Mean ± S.D. n=12	Control group Mean ± S.D. n=12
1	1.481 ± 0.222	1.22 ± 0.169 *
2	1.948 ± 0.182	1.426 ± 0.153 *
3	1.413 ± 0.326	1.008 ± 0.112 *
5	1.082 ± 0.311	0.878 ± 0.068 *
7	0.855 ± 0.22	0.746 ± 0.078
10	1.412 ± 0.193	0.637 ± 0.076 *
14	1.592 ± 0.271	1.013 ± 0.107 *

* The significant difference in mineral values between the two groups in same day.

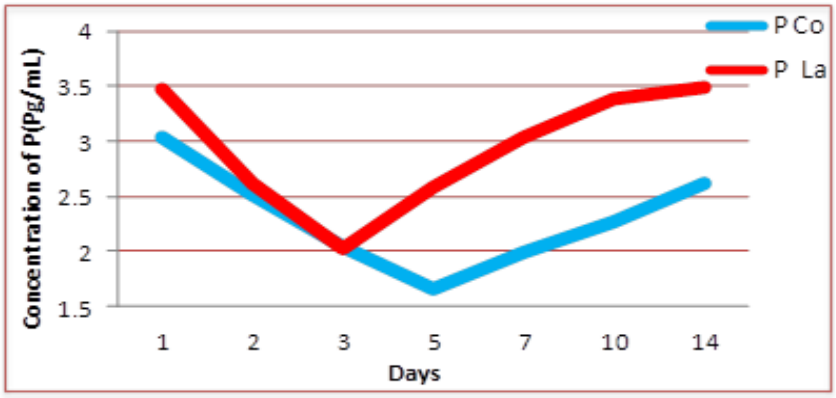

Fig. 5: The variation in values average of P concentration between the two groups.

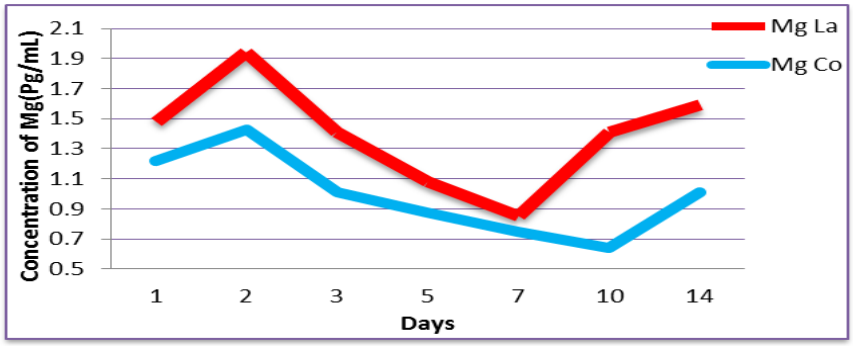

Fig. 6: The variation in values average of Mg concentration between the two groups.

Table 8: Correlation between the parameters of the study in treated group.

Correlations						
treated		GH	PTH	CTH	Ca	P
PTH	Correlation	0.088				
	P	0.426				
CTH	Correlation	0.159	-.322**			
	P	0.148	0.003			
Ca	Correlation	0.155	.424**	-.392**		
	P	0.159	0	0		
P	Correlation	-.410**	-.295**	-0.035	-.252*	
	P	0	0.007	0.754	0.021	
Mg	Correlation	-0.036	.449**	-.528**	.449**	0.029
	P	0.744	0	0	0	0.797
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

Table 9: correlation between the parameters of the study in control group.

Correlations						
control		GH	PTH	CTH	Ca	P
PTH	Correlation	-.280**				
	p	0.01				
CTH	Correlation	-0.001	-.375**			
	p	0.994	0			
Ca	Correlation	0.177	.592**	-.534**		
	p	0.11	0	0		
P	Correlation	-0.012	-0.158	-0.188	-0.212	
	p	0.914	0.151	0.086	0.054	
Mg	Correlation	-.285**	.422**	-.568**	.402**	.262*
	p	0.009	0	0	0	0.016
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

Comments

Infrared lasers causes high activity of the source of osteoblasts and mesenchymal stem cells of autologous bone marrow for direct formation of bony material to fill the gap of fracture line instead of cartilage or fibrocartilage tissue formation as seen in common stages of fracture healing, this variation of period between these both groups may be due to the action of combination of autologous bone marrow and infrared laser which accelerate the osteoblasts and osteoclasts activity to deposition and resorption of bone matrix, (10). It has been reported that laser photonic energy is absorbed by the mitochondria in the cell in which this energy is converted to chemical kinetic energy and finally leads to more production of ATP. ATP is the source of energy in the cell which is necessary for cell activities such as synthesis of DNA, RNA and proteins that are important in cellular proliferation, PTH is likely to play a role in the early stages of fracture healing after some hours of operation and it's value raising in second day, Parathyroid hormone (PTH) is a major regulator of bone metabolism. It increases calcium in the extracellular fluid by activating osteoclasts, increasing tubular reabsorption of calcium in the kidney and indirectly promoting intestinal absorption of calcium in the gut,(11). Serum Ca^{+2} may increase from Ca^{+2} influx from intestinal absorption or bone resorption and decrease with Ca^{+2} efflux into bone mineralization sites, secretion into the intestinal lumen, or filtration at the renal glomerulus and secretion along selected segments of the nephron. PTH increases Ca^{+2} influx into the extracellular space through enhanced renal tubule reabsorption of filtered Ca^{+2} that occurs within minutes; increased osteoclastic and osteocytic mediated bone resorption that appears within minutes to hours; and stimulation of intestinal Ca^{+2} absorption indirectly through increased renal proximal tubule 1,25(OH)2D synthesis that appears by 24 h after PTH secretion, (12). The results of current study agreed with, (13), who discovered that PTH dependent actions, blood calcium concentration rises and blood phosphate concentration declines. Mg is a major constituent of bone; it is not a consistent component of the hydroxyapatite crystal structure. Mg is primarily on the crystal surface, and a portion is in equilibrium with ECF Mg. Mg is the most abundant divalent cation in the intracellular compartment PTH increases Mg reabsorption, but in primary hyperparathyroidism, the hypercalcemia reduces Mg reabsorption,(14). Human body works hard to maintain homeostasis, which means "steady state," with regard to different parameters. For instance, your body tries to maintain a constant temperature and acidity level. Similarly, you need stable levels of calcium in your blood to maintain normal function, (15). Calcitonin is a hormone that increases the amount of calcium in the bones and lowers the calcium level in the blood; Calcitonin plays a role in regulating the level of calcium stored in your body. Important for both bone strength and nerve conduction, calcium levels in the blood must be tightly regulated. The main functions of calcitonin are preventing calcium release from bones and encouraging calcium to be filtered by the kidneys. The sum effect of these two processes lowers the total level of calcium in the blood, (16). According to that when blood calcium levels increased in the second day stimulating calcitonin secretion from thyroid gland therefore increasing in its values after fracture. Osteoclasts are cells in the bones that function to break down the bones, leading to release of calcium into the blood stream. When osteoclasts detect calcitonin, it causes them to separate from the bone and to stop releasing calcium into the blood, decreasing the blood calcium level; it causes them to release phosphorus into the urine. Phosphorus is another mineral that is important for bone formation. Along with the phosphorus release, calcitonin also triggers the release of some calcium into the urine. The net effect is to lower the blood levels of

phosphorus and calcium, (17). Thyroid hormones are known to influence GH levels and they act via the receptors on the cell nuclei by stimulating the transcription of GH (18) ,GH is effective on the growth plate and the osteoblasts both directly and indirectly, through the IGF system, the indirect effects of GH are exerted primarily through IGF-I, in the liver and other tissues (e.g., the epiphyses and the muscle), GH increases the synthesis of IGF- I, IGF binding protein (IGFBP)-3 and the acid labile subunit (ALS). The direct effect of GH is mostly on the adipose tissue. GH and IGF-1 levels also show increase and decrease respectively, (19).Analyzing the values obtained from the animals of both groups showed that the GH level started increasing slowly from the 1st to the 3rd day in the treated group while followed by a significant increase after that reaching to the $7^{th.}$ day in second group; after that the level of the hormone takes a semi constant level till the end of the healing process in the $1^{st.}$ and $2^{nd.}$ groups, these results contribute to the anabolic role of this hormone which started from the point of terminating the inflammatory process.

Conclusions

Better understanding of the physiology of fracture healing will eventually contribute to progress in the treatment of fracture:

1. L.L.L.T. has a stimulatory effect on the hormones and minerals which intervene with fracture healing.
2. L.L.L.T. accelerates fracture healing approximately half the time needed for normal healing procedures.
3. There is a close relationship between the fracture healing processes and the hormones and minerals which control the homeostasis. There is a complementary relationship or synergy between each of the GH, PTH, CTH, Ca, P and Mg with each other during the healing process.
4. Application of L.L.L.T. in fracture healing was safe, with no side effects reported.

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