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# Effects of topical growth hormone on mitochondrial function during facial skin wound healing in rabbits

Lubna Anmar Tohala[1*], Faehaa Azher Al-Mashhadane[2], Ayad Abdulrahman AL-Saraj[3]

Department of Dental Basic Sciences, University of Mosul, Mosul, Iraq[1,2,3]

Corresponding author: 1*

**ABSTRACT**— There are evidences supporting the use of growth hormone (GH) for cellular growth and wound healing. Also GH was found to act indirectly on mitochondrial biogenesis, likely in synergy with other growth factors. To examine the effects of GH on facial skin wound healing in rabbits and evaluate its effect on mitochondrial function. Thirty healthy rabbits included in this study classified into two groups group A: euthanized at day 7 and group B: euthanized at day 14 of study period, then each group subdivided into three subgroups: negative control group (normal rabbits), positive control group: full –thickness square shaped, 1 $cm^2$ wounds were excised in the forehead skin for each rabbit without any medication, treatment group: full –thickness square shaped 1 $cm^2$ wounds will excised in the forehead skin of each rabbit, then 0.1ml [1.2mg /3.6 IU] of GH will be injected subcutaneously around the wound area every other day. Following euthanasia, blood samples (5 mL) were collected from rabbits in all groups for analysis using lactate, pyruvate and L- carnitine detection kits. The clinical observations show that the wound closure rates were similar in the negative control, and treatment groups. On the day $14^{th}$, the wound closure rate in the treatment group was faster than other groups. Both serum Pyruvate and Lactate at the end of the $7^{th}$ and $14^{th}$ days showed a significant decrease in the treatment group when compared to co positive control group but its higher than in negative control group. While L- carnitine level showed a significant increase in the treatment group compared to positive control group but its less than negative control group. Local GH can affect mitochondrial function and accelerates facial skin wound healing.

**KEYWORDS:** growth hormone, Mitochondria, Wound Healing, Rabbits

## 1. INTRODUCTION

Growth hormone is a 191 amino acids protein, manufactured and secreted by the anterior pituitary gland under the positive control of growth hormone releasing hormone (GHRH) and the negative control of somatostatin [1]. It is an anabolic hormone which controls growth by both hypertrophy and hyperplasia. It results in tissue differentiation, cellar proliferation, and synthesis of proteins. It acts by both direct effect on the tissues and indirect effect induced by its mediators such as IGF-1 [2].

Mitochondria, a dynamic organelles with double membrane-bound organelles, that generate adenosine triphosphate (ATP) through oxidative phosphorylation can play important role in cellular energy homeostasis and metabolism [3], [4]. Both GH and its receptors (GHR) act together in the GH–GHR–IGF1 signaling pathway and have impact on mitochondrial function. GH can control mitochondria by the Box 1 region of the GHR gene [5].

Wound healing consists of several cellular and biosynthetic processes, which all necessitate energy in the form of ATP, amino acids, and other different precursor molecules to substitute injured tissues [6]. The healing process occurs in overlapping stages consisting of inflammation, formation of the granulation

tissue, angiogenesis, tissue re-epithelialization, and remodeling [7].

Studied were identify a strong association between GH and the control of cellular growth. Also it was found that GH and IGF-1 have indirect effects on mitochondrial biogenesis, likely in cooperation with other growth factors that have a role in healing process [8]. The current study aimed to evaluate effect of topical growth hormone on the facial skin wound healing and to study mitochondrial function during healing period.

## 2. Material and Method

The current research was approved by the Research Ethics Committee and Scientific Committee/Department of Dental Basic Science/College of Dentistry/University of Mosul. And the approval number is UoM.Dent/A.L.16/22

Thirty apparently healthy mature male rabbits of (11-12) months old and a body weight of about 1.5 Kg were included in this study. Animals were housed indoors in animal houses according to national and institutional guidelines for the care and use of animals. The animals were given access to a standardized diet with water supply and with care and clinical examination daily by the veterinarian until euthanized.

The animals were classified randomly into two groups according to day of euthanization:
Group A: 15 rabbits euthanized at day 7 after surgical procedure.
Group B: 15 rabbits euthanized at day14 after surgical procedure.
Each group is subdivided into 3 groups (5 rabbits/subgroup)

Group I: (Negative control) the rabbits in this group not undergo surgical procedure and not receive any treatment.
Group II: (Positive control) the rabbits in this group undergo surgical procedure but not receive any treatment.
Group III: (Treatment) the rabbits in this group undergo surgical procedure, and each rabbit, treated by 0.1ml [contain 1.2mg /3.6 IU] of growth hormone as subcutaneously injection at the surgical area, the injection process was carried out every other day till the euthanizing day.

### *2.1 Drugs used*

1-Growth hormone injection Genotropin® Pfizer company.
prefilled pen contain 1ml/ (12 mg) 36 IU
2- Xylazine base solution,20 mg/ml (Interchemie, Holland) and ketamine hydrochloride solution 100 mg/ml (Dutch Farm, Holland) for general anesthesia.

### *2.2 Surgical procedure*

On the first day of study, the rabbits in both group II (positive control group) and group III (treatment)were anesthetized by given IM dose of a mixture of xylazine hydrochloride and ketamine hydrochloride at 5, 50 mg/Kg respectively. The forehead of the animal were shaved, washed with water and disinfected with povidone–iodine solutions to be ready for operation.

A circle with 1 cm in diameter was measured on the skin of each animal's forehead using a ruler, and full-thickness circular excision was carefully done.

Then, the animals in group III(treatment group) were treated topically by 0.1ml [contain 1.2mg /3.6 IU] of

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growth hormone as subcutaneously injection at the surgical area, the injection manner was carried out every other day till the euthanizing day(7 days group A and 14 days group B).

### *2.2.1 Blood sampling*

For analysis of biochemical parameters, fresh blood was collected from each rabbit at time of euthanizing, and he serum was then separated by centrifuge, stored at (-20C°) till analysis by using Pyruvate Assay Kit, Lactate Assay Kit and L-Carnitine Assay Kit (SIGMA Life Science) MAK065, (SIGMA Life Science) MAK332 and (SIGMA-ALDRICH) MAK063.

### *2.3 Statistics*

The results were identified as mean ± SD, variances were statistically analyzed by one way analysis of variance "ANOVA" followed by Duncan test. P values ≤ 0.01were considered significant [9].

## 3. Results

The clinical observations show that the wound closure rates were similar in the negative control group, and treatment groups. On the day 14th, the wound closure rate in the treatment group was faster than other groups.

Comparisons of Pyruvate, Lactate and L-Carnitine among all study groups.

- The 1st week(7th day)

ANOVA test showed a highly significant difference among all study groups in serum Pyruvate, Lactate and L-Carnitine as shown in table (1)

**Table (1):** Comparison of serum Pyruvate, Lactate and L-Carnitine among study groups at the end of the 1st week

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| **Test** | **S.O.V.** | **Sum of Squares** | **d.f.** | **Mean Square** | **F** | **Sig.** |
| **Pyruvate** | **Between Groups** | 28860.133 | 2 | 14430.067 | 79.026 | 0.000** |
| | **Within Groups** | 2191.200 | 12 | 182.600 | | |
| | **Total** | 31051.333 | 14 | | | |
| **Lactate** | **Between Groups** | 4597320.000 | 2 | 2298660.00 | 70.888 | 0.000** |
| | **Within Groups** | 389120.000 | 12 | 32426.667 | | |
| | **Total** | 4986440.000 | 14 | | | |
| **Carnitine** | **Between Groups** | 477.733 | 2 | 238.867 | 102.371 | 0.000** |
| | **Within Groups** | 28.000 | 12 | 2.333 | | |
| | **Total** | 505.733 | 14 | | | |

** High Significe at P ≤ 0.01

Duncan's Multiple Range Test of both serum Pyruvate and Lactate at the end of the 1st week showed a significant decrease in Pyruvate and Lactate level in the treatment group when compared to positive control group but its higher than in negative control group. figure (1) and (2).

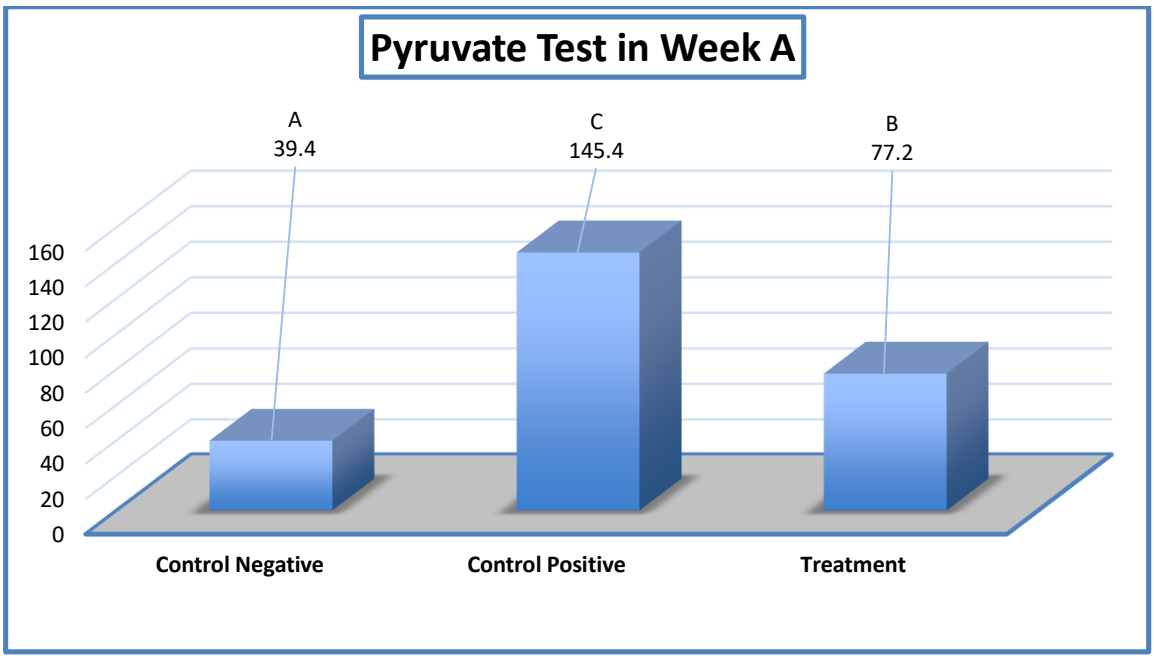


**Figure (1):** Duncan's Multiple Range Test for Pyruvate measurements among study groups at the end of the 1st week

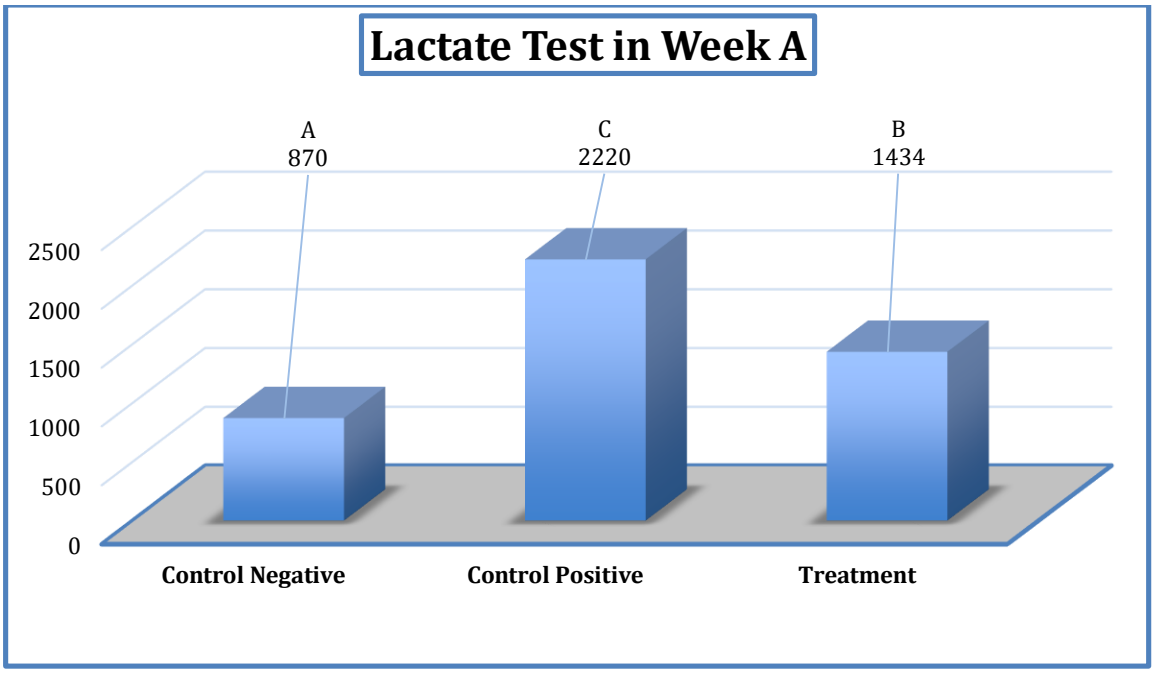


**Figure (2):** Duncan's Multiple Range Test for Lactate measurements among study groups at the end of the 1st week

Duncan's Multiple Range Test of the serum L- Carnitine at the end of the 1st week showed a significant increase in treatment group compared to positive control group but its less than its level in negative control group. figure (3)

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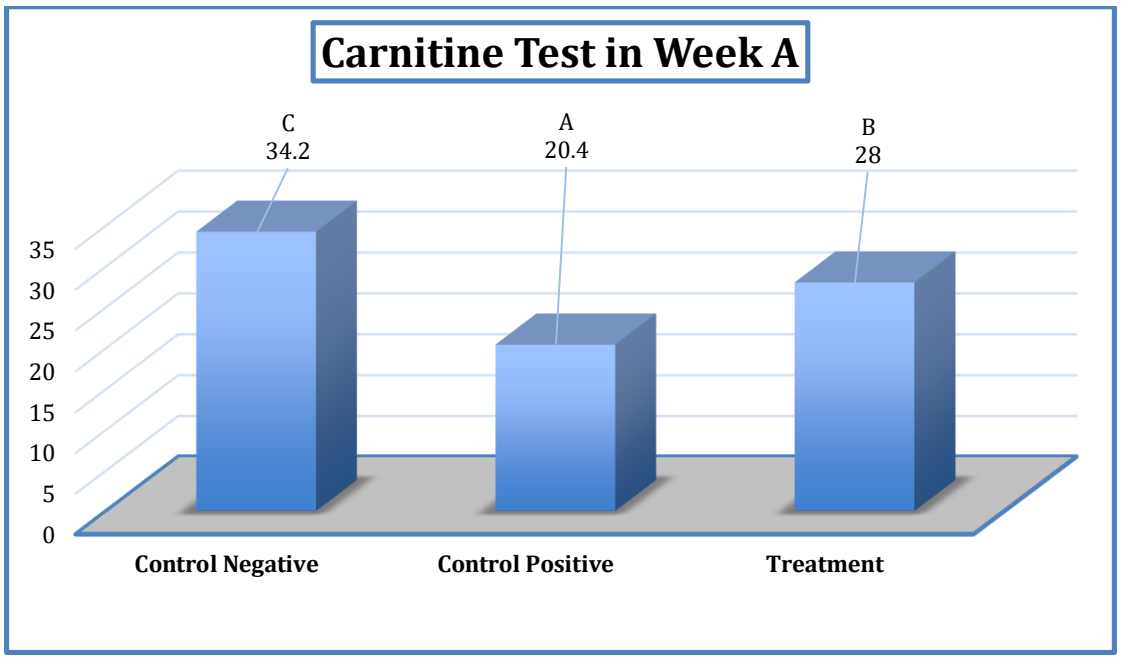


**Figure (3):** Duncan's Multiple Range Test for L –Carnitine measurements among study groups at the end of the 1st week

- The 2nd week (14th day)

ANOVA test showed a highly significant difference among all study groups in serum Pyruvate, Lactate and L-Carnitine as shown in table (2)

**Table (2):** Comparison of serum Pyruvate, Lactate and L-Carnitine among study groups at the end of the 2nd week

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| **Test** | **S.O.V.** | **Sum of Squares** | **d.f.** | **Mean Square** | **F** | **Sig.** |
| **Pyruvate** | **Between Groups** | 8906.912 | 2 | 4453.456 | 51.762 | 0.000** |
| | **Within Groups** | 1032.441 | 12 | 86.037 | | |
| | **Total** | 9939.353 | 14 | | | |
| **Lactate** | **Between Groups** | 1811080.000 | 2 | 905540.000 | 29.005 | 0.000** |
| | **Within Groups** | 374642.000 | 12 | 31220.167 | | |
| | **Total** | 2185722.000 | 14 | | | |
| **Carnitine** | **Between Groups** | 122.533 | 2 | 61.267 | 17.340 | 0.000** |
| | **Within Groups** | 42.400 | 12 | 3.533 | | |
| | **Total** | 164.933 | 14 | | | |

** Highly Significant at $P \leq 0.01$

Duncan's Multiple Range Test of both serum Pyruvate and Lactate at the end of the 2nd week showed a significant decrease in Pyruvate and Lactate level in the treatment group when compared to positive control

group but its higher than in negative control group. figure(4) and (5).

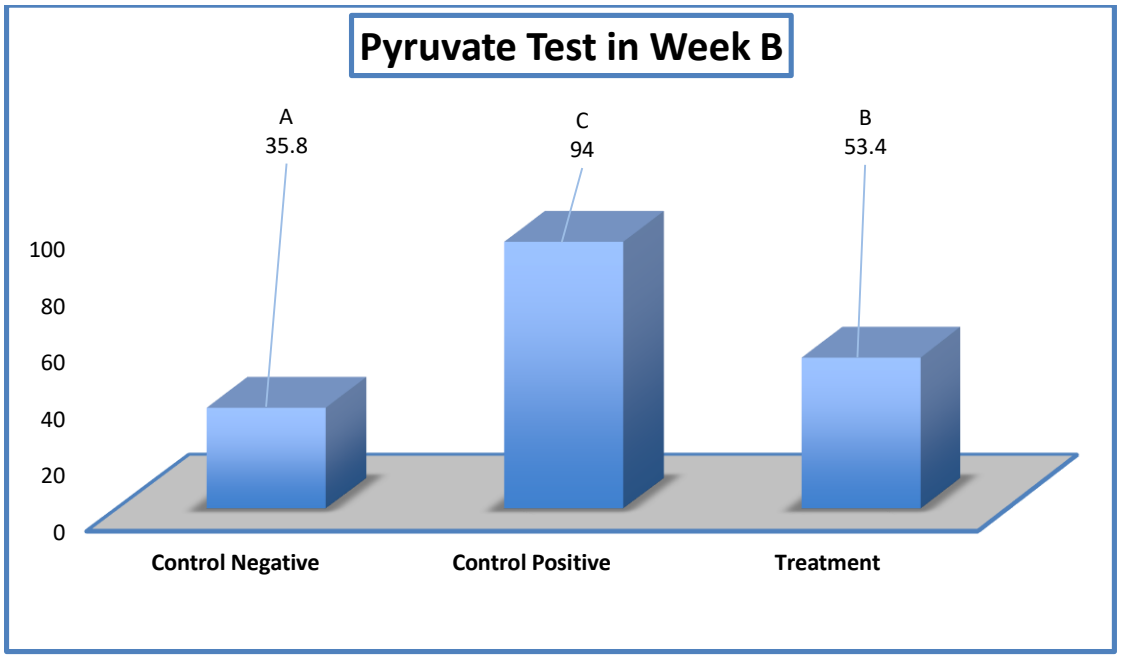


**Figure (4):** Duncan's Multiple Range Test for Pyruvate measurements among study groups at the end of the 2nd week

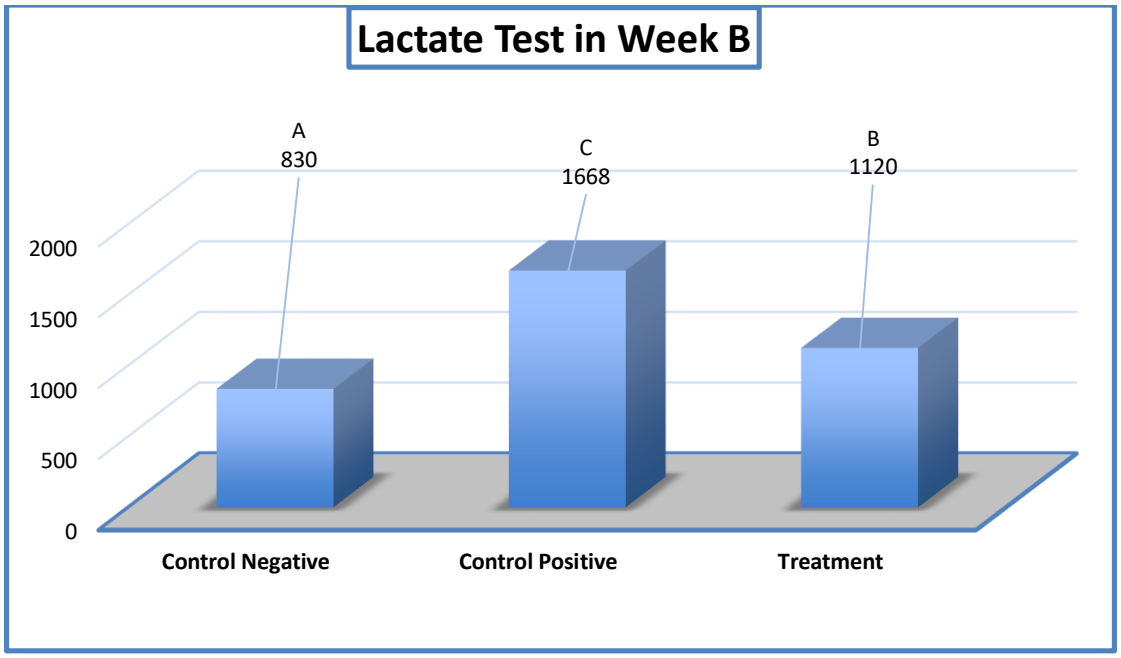


**Figure (5):** Duncan's Multiple Range Test for Lactate measurements among study groups at the end of the 2nd week

Duncan's Multiple Range Test of the serum L- Carnitine at the end of the 2nd week showed a significant increase in L- carnitine level in treatment group compared to positive control groups but its less than its level in negative control group. figure (6)

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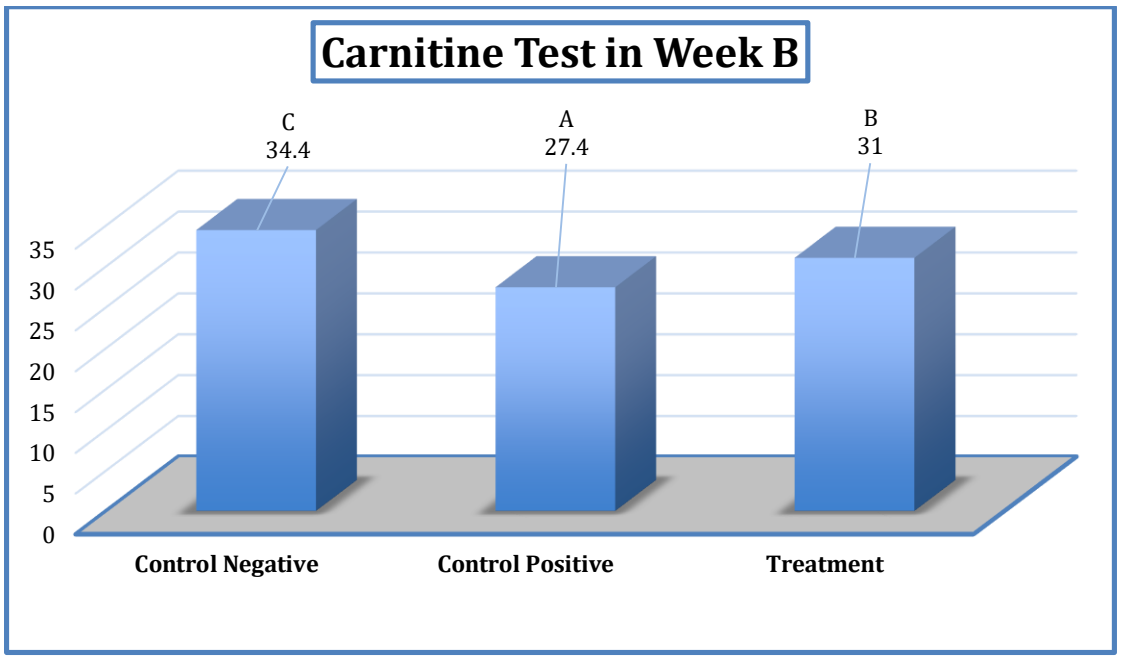


**Figure (6):** Duncan's Multiple Range Test for L- Carnitine measurements among study groups at the end of the 2nd week

**4. Discussion**

Wound healing is an overlapping process that normally ends with closure of the wound and repair of the tissue layer and tissue remodeling [10]. The epithelial healing is characterized by the accumulation of actin and non-muscle myosin II at the cell margins that expressed in the wound face, forming an actomyosin cable, then by its contraction it can makes cells adjacent together, which leads to the hole closure [11]. According to recent research, mitochondria may be essential role in tissue healing. It can stimulate wound healing by modulating actin and myosin at the wound edges, either through Rho GTPases or through cell-cell junction remodeling [12].

In the early 1970s, the roles of the GH/IGF-1 axis in mitochondrial biogenesis were noticed. GH has a role in mitochondrial protein synthesis. Human GH injection to rats showed that the mitochondrial protein synthetic capacity of the liver, significantly increased in GH-treated rats [13], [14].

Results of the current study showed both serum pyruvate and lactate levels showed a significant decrease in the treatment group when compared to the positive control group. Lactate levels increase in the presence of a local wound environment and decrease when the wounds start to heal. Their rise is due to poor blood supply to the tissue, poor oxygenation, bacterial colonization, and immune system activation [15], [16]. Mitochondrial function is complex in response to cellular energy homeostasis and metabolism. The control of cellular growth, apoptosis, and free radical production by mitochondria could describe its role in wound healing.

GH receptors are found in almost all tissue cells. They can stimulate many pathways involved in cellular growth, proliferation, differentiation, survival, metabolism, gene transcription, and protein translation. The "PI3K/AKT" pathway appears to be critical for controlling cell metabolism and cell fate "apoptosis", both of which involve mitochondrial function and integrity [17].

In the present study, there was a significant increase in L-carnitine levels in the treatment group when

compared to the positive control group. L-carnitine has the ability to support the antioxidant and mitochondrial defense systems and can eliminate free radicals from various cellular sites [18]. In extreme trauma, such as severe burns, carnitine excretion will be increased in order to enhance tissue repair [19].

In addition to ATP production, mitochondria also produce ROS as a by-product of ATP synthesis. ROS role in wound healing is complicated. they contributes to the oxidation that help to kills bacteria, and also acts as a cell signal to enhance cell proliferation, which is necessary in wound repair, they are also very potent molecules and can cause DNA, lipids, and protein damage. High levels of ROS cause chronic wounds, tissue damage, extreme inflammation, and delayed healing. Moreover, high levels of oxidative stress influence the mitochondrial morphology and positioning within cells, leading the clustering of the mitochondria around the nucleus as a defensive strategy [20- 22]. The association between GH and mitochondria in regulating of cellular growth and replication makes the studies of their effects on wound healing and tissue repair persistent need. The exact effects of GH on the mitochondria of inflammatory cells at wound site are still underexplored [17].

## 5. Conclusion

Local GH can affect mitochondrial function and accelerates facial skin wound healing. Further studies is still to be done. We think that our results could open the door for more research to confirm these findings.

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