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Effect Of 820nm Diode Laser On Some Hormone And Enzyme Concerning With Wound Healing And Skin Loss Sealing

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By

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تأثير الليزر ثنائي الصمام بطول موجي 820nm على بعض الهرمونات والأنزيمات التي تتداخل مع التئام الجروح وسد الثغرات الناشئة عن التحام فقدان الجلد

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Certification

We Certify that that this thesis entitled "Effect Of 820nm Diode Laser On Some Hormones And Enzyme Concerning With Wound Healing And Skin Loss Sealing " was prepared by " Dunnia Abdullah Barakat AL-Moussawi " under our supervision in the Department of life Sciences, Faculty of Science, AL-Muthanna University, as a part of the fulfillments of the Master degree of Science in Biology / Zoology .

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Reference to the above recommendations submitted by the supervisors to transmit this letter to the Commission for Consideration and Debate statement of opinion

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ADP	**adenosine diphosphate**
TGF-ß	tissue growth factor beta
PDGF	platelet-derived growth factor
TGF-b	transforming growth factors
PMNs	polymorph nuclear leukocytes
FGF	fibroblastic growth factor
C3	complements
C	Carbon
IL	interleukin
GAGs	glycosaminoglycans
CW	continuous Wave
LLLT	Low Level Laser Therapy
ATP	adenosine tri phosphate
AMP	adenosine mono phosphate
LED	light emitting diodes
PDT	photodynamic therapy
HpD	hematoporphyrin derivative
He-Ne	helium–neon
cAMP	Cyclic adenosine monophosphate
GHRH	Growth hormone - releasing hormone
GH	Growth hormone
IGF-1	insulin-like growth factor-1
MPE	Maximum Permissible Exposure Level
ECM	Extracellular Matrix
PGs	Prostaglandins
PGG	Prostaglandin G
PGH	Prostaglandin H
PGA	Prostaglandin A
PGE2	Prostaglandin E2
PGF2α	Prostaglandin F2α
PGD	Prostaglandin D
PGI	Prostacyclin
TXA	Thromboxane A
λ	Wavelength (lambda)
r^2	Coefficient of determination
HETE	Hydroxy Ecosatetranoic acid
NO	Nitrous oxide
PDT	Photodynamic Therapy

bFGF	basic Fibroblast growth factor
HGF	Hypatocyte growth factor
HGH	Human growth hormone
VEGF	Vascular endothelial growth factor
TGF-ß	Tissue growth factor -beta
IU	International unite
IM	Inter muscular

(Tables of content)

Tables and Figures

الخلاصة :

تهدف هذه الدراسة إلى التعرف على تأثير العلاج بالليزر واطئ الطاقة على تكاثر خلايا الأنسجة الطلائية والرابطة (الضامة) في التئام الجروح القطعية أو بعد فقدان جزء من الجلد و تحديد استجابة الخلايا وتطبيعها بعد التشعيع بالليزر من الناحية الفسلجية بالاعتماد على مسح مخبري بجهاز الأليزا لمصل الدم لبعض الهرمونات الأكثر تداخلا مع عمليات التئام الجروح .

استخدمت في هذه التجربة عشرين أرنبا نيوزلنديا من الذكور البيضاء البالغة ، كان معدل أوزانها يتراوح بين ١,٥-٢ كيلوغرام ، تم تقُسيمها إلى مجموعتين تتكون كل مجموعة من ١٠ أرانب ; المجموعة الأولى هي مجموعة إحداث الجروح ، أما المجموعة الثانية فهي مجموعة فقدان الجلد.

خضعت حيوانات المجموعة الأولى لعملية جراحية على الجانب الوحشي من الفخذ الأيسر و لغرض إحداث جرح جراحي بطول ٧ سم ثم أغلق الجرح بغرز من النوع المتقطع البسيط باستخدام السلك الجراحي قياس ٣-٠ ، أما حيوانات المجموعة الثانية فقد خضعت هي الأخرى لعمليات جراحية على الجانب الوحشي من الفخذ الأيسر ، تضمنت رفع رقعة مربعة من الجلد بكافة طبقاته بقياس (ا سم x اسم) .

تمت معالجة موضع العملية لكل الحيوانات برذاذ المضاد الحيوي ثم حقنت بمضادات حيوية جهازية ; البنسلين (١٠٠٠ وحدة دولية / كغم) و الستربتومايسين (١٠ ملي غرام / كيلوغرام من وزن الجسم) بالعضلة لمدة ٣ أيام بعد التجربة .

تم تقسيم حيوانات كلا المجموعتين إلى مجموعتين ثانويتين (السيطرة والمعالجة بأشعة الليزر). الليزر المستخدم هو ثنائي الصمام بطول موجي ٨٢٠ نانومتر ، بأقصى خرج مقداره ٢٠٠ ملي واط ، كثافة ٨ جول / سم^2 ، التردد النبضي ١-١٠ هيرتز . بدأ التشعيع بعد العملية مباشرة و لمدة ٥ أيام في حيوانات المجموعة الثانوية (مجموعة إحداث الجروح) و ٧ أيام في حيوانات المجموعة الثانوية (مجموعة فقدان الجلد بمعدل ١,٢ دقيقة / جلسة يوميا .التشعيع بالليزر تم بتوجيه الشعاع بمسافة ١سم من الجرح أو حول محيط المنطقة المربعة ذات الجلد المفقود.

تم جمع نماذج الدم في الأيام (صفر ، واحد ، ثلاثة و سبعة) من حيوانات المجموعة الأولى و (واحد، ثلاثة، سبعة و عشرة) في حيوانات المجموعة الثانية ، العينات أُخذت من الوريد ألأذني الهامشي لكل الحيوانات وأرسلت إلى المختبر حيث تخضع للفحص بالا ليزا لتحديد مستويات البروستاكلاندين اي٢ (PGE2) ، بروستاكلاندين أف ٢ ألفا (PGF2α)، هرمون النمو (GH) و الأدينوسين الأحادي الفوسفيت الحلقي (c AMP).

أظهرت نتائج الدراسة الحالية وجود زيادة ملحوظة عالية إحصائيا في مستويات PGE2، PGF2α,هرمون النموGH وال c AMP في عينات دم المجاميع الثانوية المعالجة بالمقارنة مع تلك السيطرة ،مع عودة سريعة إلى الوضع الطبيعي واستقرار الهرمونات بعد إجراء العملية التشعيع.

يمكن أن نستنتج بأن معالجة الجروح الجراحية وإصابات الجلد بأشعة الليزر الواطئة الطاقة مفيدة وفعالة حيث إن الالتئام يتحسن وتتزايد سرعته . انتهاء عمليات الالتئام ورفع الغرز كان في اليوم الرابع في حيوانات المجموعة الثانوية التي شععت بالليزر في حيوانات مجموعة إحداث الجروح فيما احتاجت حيوانات المجموعة الثانوية المشععة بالليزر إلى ٧ أيام في مجموعة فقد الجلد .

النتائج المستحصلة من هذه الدراسة تعزى إلى تحسين الخواص السائلية للدم ، زيادة الشعيرات الدموية ، تدفق الدم ،انخفاض المقاومة الوعائية و الشد الوعائي وهي التي تؤدي إلى زيادة سرعة وتدفق السوائل من المساحات بين الخلوية إلى الجهاز اللمفاوي . الاحتياج للطاقة هو لتنشيط بعض العمليات في الخلية لتسبب السلسلة المذكورة أعلاه من التفاعلات والتي بدورها تعزز إعادة البناء و تسرع التئام الجروح.

لقد اختبرنا النتائج إحصائيا بواسطة البرنامج الإحصائي SPSS ، ووجدنا أن نتائج اختبار الاليزا للهرمونات أظهرت اختلافات كبيرة في قيم PGE2، PGF2α ، cAMP، وGH،بين المجموعتين الثانويتين لمجموعة التئام الجروح $P > 0.05$.

ووجد أيضا أن قيم التقييم الهرموني لـ PGE 2، PGF2α، cAMP, GH وقطر الجلد المفقود في مجموعة الحيوانات المجموعة الفرعية لمجموعة الجلد المفقود. قد أظهرت اختلافات جوهرية بين مجموعتين,$P > 0.05$.

Introduction

1-1-Introduction

Wound healing is a complex and carefully regulated physiologic response to a traumatic injury. Deregulation of this coordinated process can lead to exuberant scar formation, In some individuals, an aberrant healing process results in excessive scar formation that may extend beyond the original boundaries of the wound, resulting in a significant and troubling cosmetic defect.(Robles and Berg , 2007)

There are three distinct biological phases in the wound-healing process of restoring cellular structure and tissue layers: inflammation, proliferation, and remodeling or maturation. All three phases are integral to the completion of the healing process, and they may occur at various rates. Scar formation may occur from excessive fibroblastic proliferation or inadequate healing and local and systemic factors that impede wound healing. Factors that impede wound healing can be divided into local which include dry environment, edema, incontinence, infection, necrosis, and pressure and systemic which include age, body build, chronic diseases, nutritional status, vascular insufficiencies, and radiation and immunosuppressant therapy . (Koh-Knox and Sussman, 2007)

Although various categories of wound healing have been described, the ultimate outcome of any healing process is the repair of a tissue defect. Primary healing, delayed primary healing, and healing by secondary intention are the three main categories of wound healing. Even though different categories exist, the interactions of cellular and extracellular constituents are similar. A fourth category is the healing that transpires with wounds that are only partial skin thickness. (Mercandetti and Cohen, 2008)

There are a number of hormones involved with energy production, 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, for example the Human Growth Hormone HGH has a number of metabolic effects. The most prominent is it`s anabolic effect.HGH increases the influx of amino acids into the cell and decreases the efflux. Cell proliferation is accentuated as is overall protein synthesis and new tissue growth.(Demling,2005)

The role of prostaglandins in promoting human disease 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 PGE2 and prostaglandin F2α (PGF2α) and allow the ingress of inflammatory cells into the area of injury **.(** Torre and Chambers, 2008)

Activating of enzyme adenylyl cyclase catalyzes the formation of cyclic Adenosin 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, the cAMP serves as a second messenger to cause these effect ;when it is activated, it activates a second enzyme, which activates a third, and so forth. The importance of this mechanism is that only a few molecules of activated adenylyl cyclase immediately in the enzyme is activated inside the cell membrane can cause many more molecules of the next enzyme to be activated, and so on. In this way, even the slightest amount of hormone acting on the cell surface can initiate a powerful cascading activating force for the entire cell. (Guyton and Hall, 2006)

The relationship between nutrition and wound healing after injury or surgical intervention has been recognized in the experimental works and the clinic, adequate carbohydrate, fat, and protein intake is required for healing to take place, but researches in the laboratory has suggested that other specific nutritional interventions can have significant beneficial effects on wound healing. Experimental evidence for the use of arginine, glutamine, vitamins, and micronutrient optimize the relationship between nutrition and wound healing. (Arnold and Barbul ,2006)

Laser irradiation was first used in medicine when a pioneer laser apparatus was built by T. Maiman in 1960. The first low-level laser for tissue biostimulation was applied by E. Mester in 1969. Despite initial doubts concerning its efficiency, low level laser therapy (L.L.L.T.) has been used for thirty years, and it has occupied a prominent place in medicine. 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. (Chyczeweski, et al.,2010)

Surgical wounds may be superficial or deep on skin or mucous membranes due to a surgical intervention using a scalpel to cut through skin

or mucosa and the underlying tissue. Low Level Laser Therapy 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 stimulate. The process comes to an end in the 10th postoperative day. (Calin, et al.2010)

The goal of the current study is to evaluate the role of Low Level Laser Therapy in surgical aseptic wound management based on the hematological examination in search for the levels of the hormones which parcipitate in the wound healing like Prostaglandins E2 & F2α and Growth hormone and cyclic Adenosin MonoPhosphate cAMP.

1-2-Objective of the study:

1- Accelerating the healing of the wounds and the skin defects using the L.L.L.T..
2- Studying the effect of the laser irradiation on the hormones and the enzymes which interlaced with wound healing , especially the ones which we could obtain from the global specialized laboratories , specifically the PGE2,PGF2α,cAMP,and GH.
3- Studying the relationship between these hormones and the enzymes and the effect of each one of them on the others.
4- Estimating the timing of the healing necessary for healing of the wounds and sealing of skin defects.

2-1-The wounds:

A wound is a cut in the integrity of the skin**.** Most wounds affect the skin, the first line of defense against infection causing minor wounds. Wounds that have failed to progress through the normal stages of healing enter a state of pathologic inflammation, the healing process is delayed, incomplete, and does not proceed in a coordinated manner, resulting in a poor anatomical and functional outcome,(Menke , et al. 2007).

2-1-1-Classification and types of wounds:

There are many different ways in which wounds can be classified. In many cases a wound may consist of a combination of different classification types, figure 1, below designed depending upon the table of wound classification cited by, (Nagan, 2005).

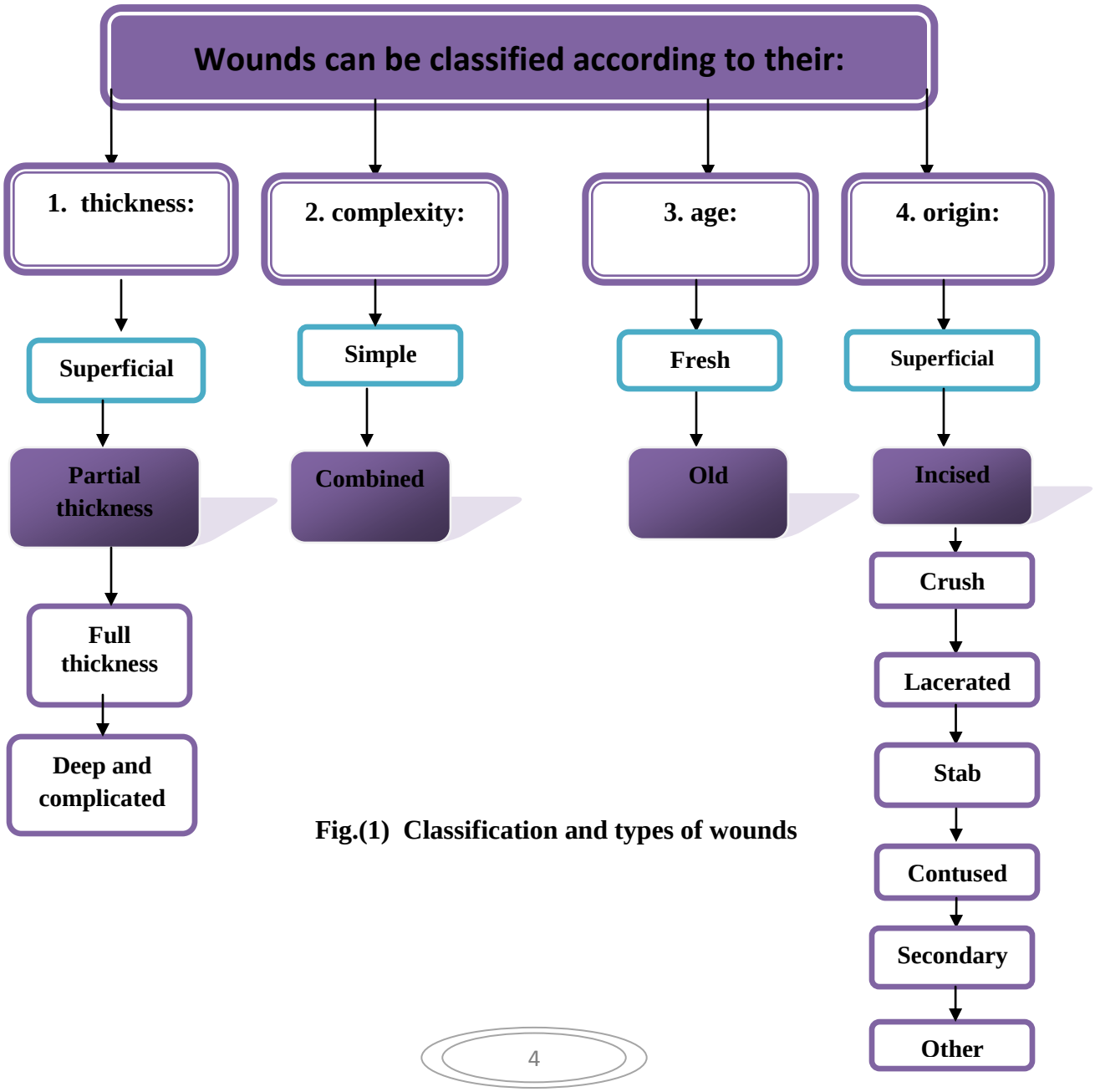


Fig.(1) Classification and types of wounds

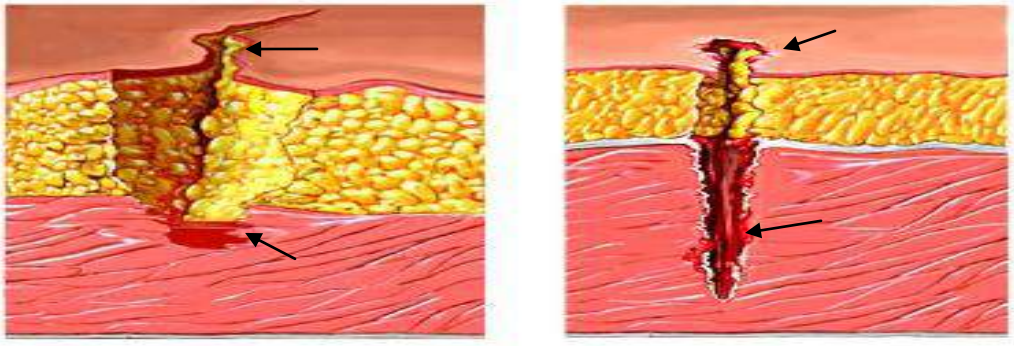
Based on the nature and depth, wounds can be classified as, (Nayeem and Karvekar, 2010):

❖ **Open wounds :**

Open wounds are those wounds in which the wounded area is entirely exposed to external environment and highly susceptible to infection, (Honnesh,2010).

Open wounds are classified based on the object that caused the wound, types of open wounds are: (Nayeem and Karvekar,2010).

- **Incisions** or **incised wounds**, any sharp cut in which the tissues are not severed; a clean cut caused by a keen cutting instrument – the wound may be aseptic or septic, depending on the circumstances, (Mallefet and Anthony, 2008).
- **Lacerations**, blunt forces tear, shear or crush skin and soft tissues, producing lacerations. The wound edges are irregular and often abraded or contused as are the surrounding tissues. A laceration wound is usually contaminated with bacteria, (Robertson and Mckeown, 2007).
- **Abrasions** ,superficial wounds in which the topmost layer of the skin (the epidermis) is scraped off. Abrasions are often caused by a sliding fall onto a rough surface., also called scrapes, which occur when the skin is rubbed away by friction against another rough surface. (Mallefet and Anthony, 2008)
- **A puncture,** sharp points or object such as a nail, animal teeth, or a tack, produce puncture wounds in which the greatest dimension is the depth, when the wound penetrates a body, it is penetrating ,figure 2 shows the lacerated and puncture wounds , cited by , (Watson,2007).



Fig(2): types of open wounds ; R, lacerated, L, Puncture wound,

- **Penetration wounds**, in which the skin is broken and the agent causing the wound enters subcutaneous tissue or a deep lying structure or cavity (the agent might be a nail, splinter or spike). (Mallefet and Anthony, 2008).
- **Gunshot wounds**, caused by a bullet or similar projectile driving into or through the body. There may be two wounds, one at the site of entry and one at the site of exit, such is generally known as a through-and-through, (Watson , 2007).

❖ **Closed wounds:**

Closed wounds are those in which the wound occurs below the skin due to blunt force or trauma resulting in the damage of tissue and blood vessels .Sometimes these wounds are also formed by extreme amount of force applied over a long period of time, (Honnesh,2010).

Closed wounds have fewer categories, but are just as dangerous as open wounds. The types of closed wounds are:

- Contusions,
- Hematomas,
- Crush injury,

2-1-2-Causes of wounds:

Accidents or injuries usually cause wounds, but they may be due to any of the following causes:

- Blunt or penetrating trauma
- Surgery
- Chemical injury
- Thermal injury
- Temperature extremes (for example, burns or frostbite)
- Radiation. (Watson, 2007)

2-1-3- Treatment of wounds:

1- Stopping the bleeding: minor cuts and scrapes usually stop bleeding on their own. If they don't, a gentle pressure should be applied with a clean cloth or bandage. If bleeding persists or the blood spurts or continues to flow after several minutes of pressure, the bleeding part is elevated above the level of the heart-for example, by raising a limb, immediately after an injury the

body will initiate processes to prevent blood loss. The importance of blood to the maintenance of blood pressure is self-evident.

Large wounds causing massive blood loss can send a person into a state of shock where blood pressure can fall to the point where life is threatened. Thus, the first task after an injury is to halt blood loss.

Because tourniquets shut off all blood flow to a body part and deprive it of oxygen, they are used only for very severe injuries, such as combat casualties.

Through the process of angiogenesis, vasculogenesis or arteriogenesis, the vascular network regenerates and viable tissue fills the wound bed, (Stavrou , 2008).

2- Cleaning the wound: rinsing the wound well with clear water, using tweezers cleaned with alcohol to remove small, superficial particles without leaving any debris.

Thorough wound cleaning reduces the risk of tetanus. To clean the area around the wound, use soap and a clean cloth. If a wound is clean with little exudates and is healthily granulating, no cleansing is required. In chronic wounds it is recognised that sterility of the wound surface is not necessary for healing nor is it possible.

Wounds should only be cleaned prior to the application of a dressing to provide optimum local conditions for wound healing; i.e.: to remove excess exudates , cell debris and bacterial burden,(Dale and Hood,2010).

3- Applying an antibiotic: Infected wounds produce malodorous and produce copious exudates. Selecting appropriate antibiotics can alleviate these symptoms and improve the patient's quality of life within a short time period.after cleaning the wound, a thin layer of an antibiotic cream or ointment must be applied to help keep the surface moist. These products don't make the wound heal faster, but they can discourage infection and allow the body to close the wound more efficiently.(Alavi, et al., 2010)

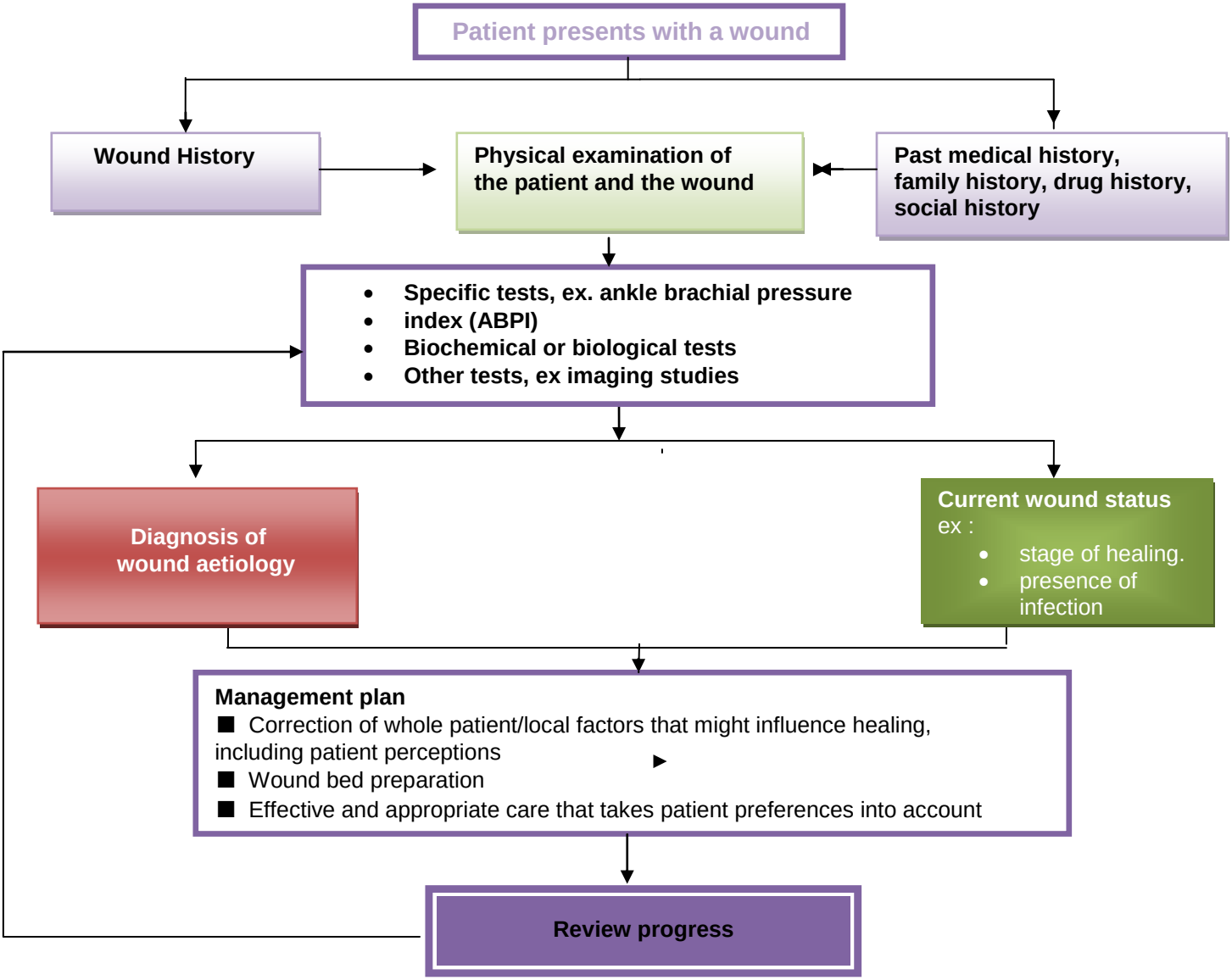
4- Changing the dressing at least daily or whenever it becomes wet or dirty. If the patient is allergic to the adhesive used in most bandages, free dressings or sterile gauze and hypoallergenic paper tape, which don't cause allergic reactions, must be used. Bacteria can grow unchallenged within the dressing environment, so an antimicrobial dressing can limit this bacterial growth. (Lipp,et al. 2010)

5- Daily watching and exposure of the wound is necessary looking for any sign of infection; if the wound doesn't heal or if a redness, drainage, warmth or swelling has been determined, it must be examined by a physician, quite ventilation is very important to speed healing. (Gottrup, et al., 2010)

6- Warming the wounds make them heal with fewer complications. In addition, warming for only two hours immediately after surgery may provide similar benefits to seven days of warming,(Melling and Leaper 2006).

2-1-4-Diagnosis

The process of diagnosis identifies a disease or medical condition from the patient's signs and symptoms, and from any tests performed. In the effective treatment of patients with wounds, the diagnostic process will be as shown in figure 3 , cited by. (Harding,2008) :



Fig(3): Steps of diagnosis of wounds.

Once the management plan has been implemented, repetition of elements of the diagnostic and assessment process, e.g. re-examination and repetition of tests, can assist in monitoring healing progress and detecting complications such as infection . (Harding,2008)

2-2-Wound Healing

Wound healing, or wound repair, is an intricate process in which the skin (or some other organ) repairs itself after injury,(Mughrabi ,et al.,2011).

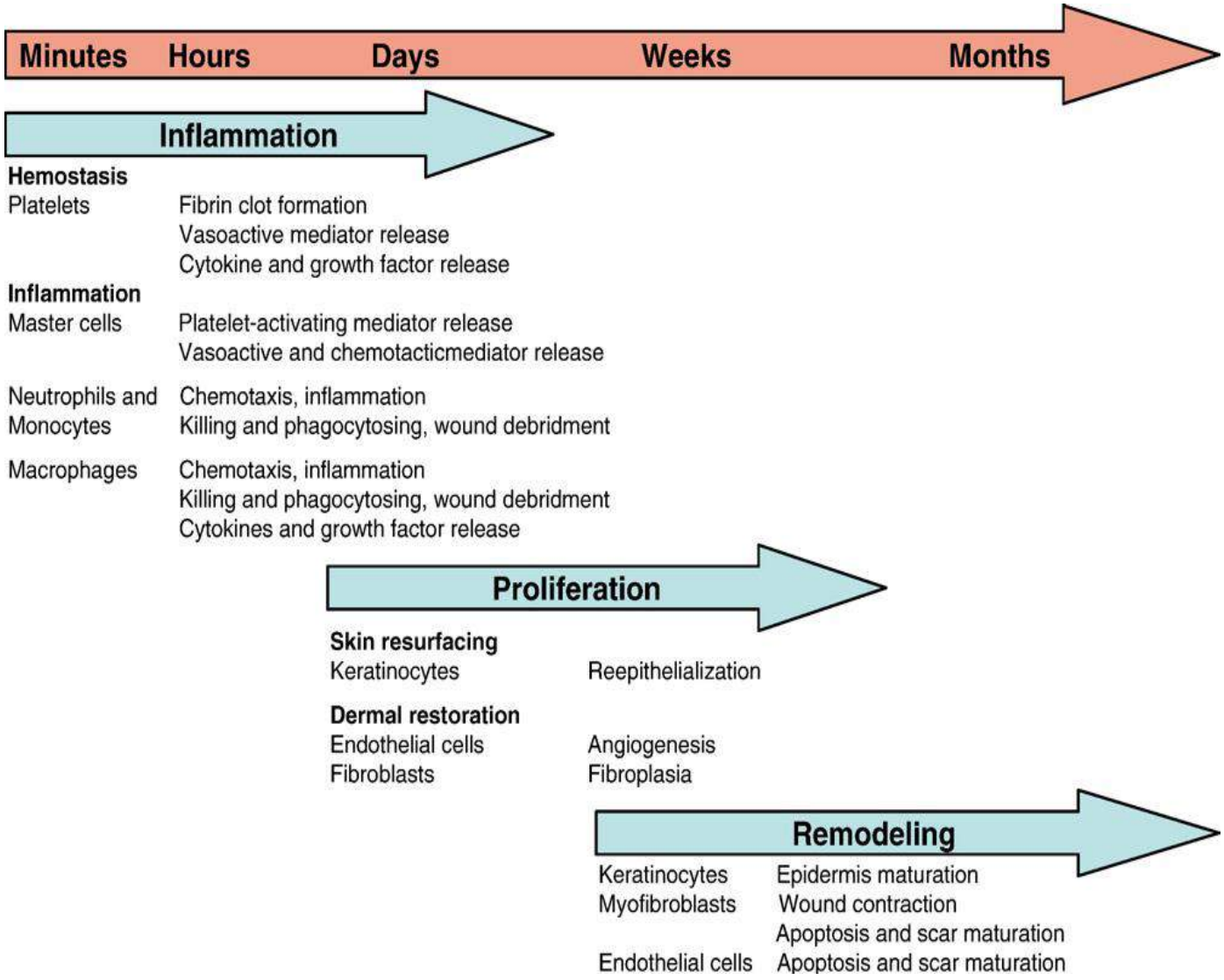
In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exist in a steady-stated equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the normal (physiologic) process of wound healing is immediately set in motion. The classic model of wound healing is divided into three or four sequential, yet overlapping, phases: (1) hemostasis (not considered a phase by some authors), (2) inflammatory, (3) proliferative and (4) remodeling,(Nguyen, et al., 2009).

2-2-1-Pathogenesis of wound healing:

Wound healing is a complex series of reactions and interactions among cells and mediators. Wound healing has traditionally been divided into three distinct phases: inflammation, proliferation, and remodeling.

Within each phase, a myriad of orchestrated reactions and interactions between cells and chemicals are put into action. There is considerable overlap for each phase, and lines separating them are blurred, (Broughton, et al. 2006). The phases are summarized below in table (1) , cited by (Li, et al. 2007).

Table (1): summarizes the three phases of wound repair demonstrable by microscope examination.



Inflammatory phase

Injury to vascular tissue initiates the extrinsic coagulation cascade by releasing intracellular calcium and tissue factor that activate factor VII. The resulting fibrin plug achieves hemostasis aided by reflex vasoconstriction. This plug acts as a lattice for the aggregation of platelets, the most common and "signature" cell type of the early inflammatory phase.

Platelets elaborate a number of pro- inflammatory substances, such as adenosine diphosphate, tissue growth factor beta (TGF-ß), and platelet-derived growth factors (PDGF). These growth factors act on surrounding cells and stimulate chemotaxis of neutrophil, monocytes, and fibroblasts to the area of injury.

Injured tissues, through activated phospholipase A, simultaneously catalyze arachidonic acids to produce vasoactive prostaglandins and thromboxane, collectively known as eicosanoids. Eicosanoids mediate activity influencing platelet plug formation, vascular permeability, and cellular chemotaxis to influence wound healing. For example, thromboxane A2 mediates vasoconstriction and platelet aggregation.

After initial vasoconstriction, the classic signs of inflammation manifest from increased vascular permeability. Rubor results from vasodilation, mediated by prostacyclin (PGI2), prostaglandin A (PGA), prostaglandin D (PGD), and prostaglandin E (PGE). Tumor and calor develop as vascular endothelial gaps enlarge, allowing the egress of plasma protein and fluid into the interstitial space. These changes are potentiated by PGE2 and prostaglandin F2α (PGF2α) and allow the ingress of inflammatory cells into the area of injury, including cells that elaborate. Dolor is sensed as PGI2, PGE, and PGE2 act on peripheral nociceptors.

In the second stage of the inflammatory phase, leukocytes(fig.3) supplant platelets as the dominant cell type, attracted by chemotaxis. White blood cells (WBCs) are the predominant cells for the first three days after wounding; their numbers peak at approximately 48 hours. Polymorphonucleocytes (PMNs) are the first to begin bactericidal activities using inflammatory mediators and oxygen free radical metabolites. However, normal wound healing can occur without PMNs. Another leukocyte, the helper T cell, elaborates interleukin-2 (IL–2). The later promotes further T cell proliferation to augment the immunogenic response to injury.

As PMN leukocytes begin to wane after 24-36 hours, circulating monocytes enter the wound and mature into tissue macrophages. These cells debride the wound on the microscopic level and produce a wide variety of important substances, such as IL-1 and basic fibroblast growth factor (bFGF). IL-1 stimulates the proliferation of inflammatory cells and promotes angiogenesis through endothelial cell replication. bFGF is a chemotactic and mitogenic factor for fibroblasts and endothelial cells. Unlike PMNs, macrophage depletion severely impairs wound healing, as debridement, fibroblast proliferation, and angiogenesis all diminish.

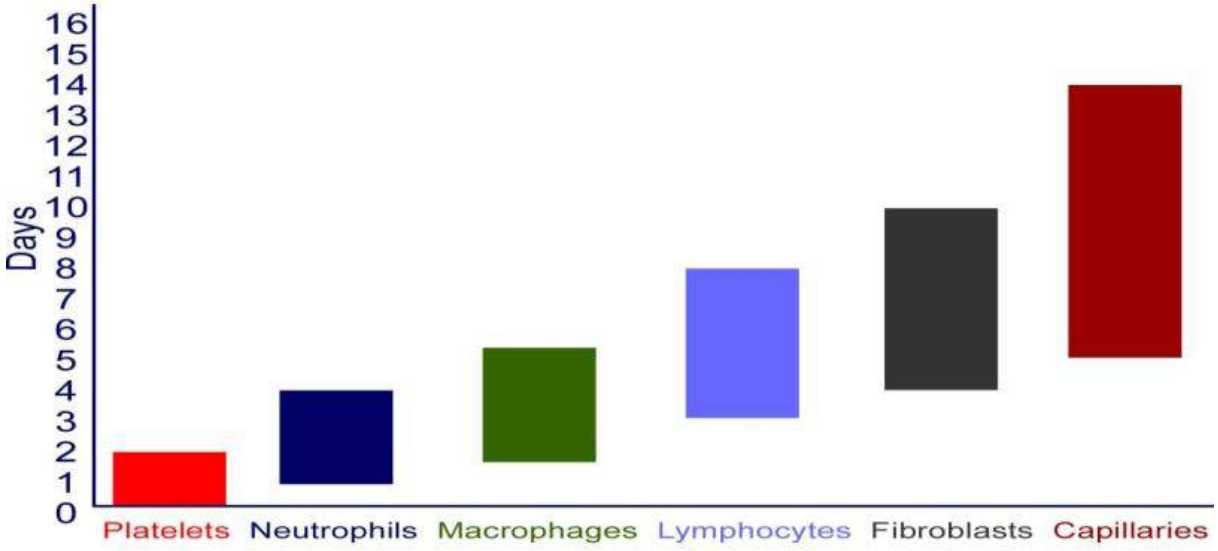
Toward the end of the inflammatory cycle, the level of the eicosanoids in the wound interact with the cell types already present, resulting in synthesis of collagen and ground substance . Additionally, the macrophage - derived growth factors are now at optimal levels, strongly influencing the influx of fibroblasts and then keratinocytes and endothelial cells into the wound. As mononuclear cells

continue to replace WBCs and macrophages, the proliferative phase begins. (Torre and Chambers, 2008)

Proliferative phase

This phase begins approximately on the third day; it overlaps with the inflammatory phase. The most important cell is the fibroblast (Fig.4) cited by ,(Baum, et al.**,** 2006) , fibroblasts peak approximately on the seventh day from injury and are responsible for initiating angiogenesis, epithelialization, and collagen formation.

Epithelialization is from the basement membrane if the basement membrane remains intact but if the basement membrane is not intact, the epithelialization is from the wound edges. Fibroblasts produce mainly type III collagen during this phase. The Granulation tissue formed in this phase , it is very important in wound healing by secondary intention.(Baum,et al.**,** 2006)



Fig(4): Wound healing and growth factors. Cells involved in wound healing. The cells appearing in a wound are depicted in sequence from left to right, and the color bars represent the range of days each cell type has in the wound.

Remodeling Phase:

Increased collagen production and breakdown continue for 6 months to 1 year after injury. The initial type III collagen is replaced by type I collagen until a type I: type II ratio of 4:1 is reached, which is equal to normal skin. Also, fibroblasts differentiate into myofibroblasts, causing tissue contraction during this phase of wound healing. Collagen reorganizes along lines of tension and crosslinks, giving added strength. Strength eventually approaches 80% of the strength of uninjured

tissue. Vascularity decreases, producing a less hyperemic and more cosmetically appealing wound as this phase progresses.

The timetable for wound healing can be quite variable. Chronic wounds can stall in the inflammatory phase because of poor perfusion, poor nutrition, or a myriad of other factors causing excessive buildup of exudates in the wound base. These wounds tend to remain unhealed unless active and aggressive means are undertaken to correct the underlying co-morbidities while providing proper wound care**,**(Gabriel,2009).

2-2-2-Categories of wound healing:

Primary healing, delayed primary healing, and healing by secondary intention are the three main categories of wound healing. Even though different categories exist, the interactions of cellular and extracellular constituents are similar. A fourth category is healing that transpires with wounds that are only partial skin thickness.

1st. category: primary wound healing or healing by first intention occurs within hours of repairing a full-thickness surgical incision, such surgical insult results in mortality of a minimal number of cellular constituents.

2nd.category: if the wound edges don't approximated immediately, delayed primary wound healing transpires. This type of healing may be desired in the case of contaminated wounds. By the fourth day, phagocytosis of contaminated tissues is well underway, and the processes of epithelization, collagen deposition, and maturation are occurring. Foreign materials are walled off by macrophages that may metamorphose into epithelioid cells, which are encircled by mononuclear leukocytes, forming granulomas. Usually the wound is closed surgically at this juncture, and if the "cleansing" of the wound is incomplete, chronic inflammation can ensue, resulting in prominent scarring.

3rd. category: a third type of healing is known as secondary healing or healing by secondary intention. In this type of healing, a full-thickness wound is allowed to close and heal. Secondary healing results in an inflammatory response that is more intense than with primary wound healing. In addition, a larger quantity of granulomatous tissue is fabricated because of the need for wound closure. Secondary healing results in pronounced contraction of wounds.

4th. Category: epithelization is the process by which epithelial cells migrate and replicate via mitosis and traverse the wound. In wounds that are partial thickness, involving only the epidermis and superficial dermis, epithelization is the predominant method by which healing occurs. (Mercandetti and Cohen**,**2008)

2-2-3-Factors Affecting Wound Healing:-

For a wound to heal successfully, all the phases of the healing must occur in a proper sequence and time frame. Many factors can interfere with one or more phases of this process, causing improper or impaired wound healing. This most significant factors that affect cutaneous wound healing and the potential cellular and/or molecular mechanisms involve: (Guo and DiPietro,2010)

1-**Nutrition** : adequate carbohydrate, fat, and protein intake is required for healing to take place, but research in the laboratory has suggested that other specific nutritional interventions can have significant beneficial effects on wound healing.

Experimental evidence for the use of arginine, glutamine, vitamins, and micronutrient supplementation is described.

Summary of clinical implications showed that care must be individualized to optimize the relationship between nutrition and wound healing. (Arnold and Barbul,2006)

2- **Oxygenation:** Oxygen is a prerequisite for successful wound healing due to the increased demand for reparative processes such as cell proliferation, defense against bacteria, angiogenesis and collagen synthesis ,many experimental and clinical observations have shown that wound healing impaired under hypoxia.(Schreml ,et al. 2010)

3- **Age**: There is accumulating evidence that intrinsic aging processes have a detrimental effect on the healing of acute wounds. Recent studies have highlighted the pivotal roles of a prolonged inflammatory response, up-regulated protease activity, and reduced matrix deposition in age-related impaired healing.(Gilliver, et al.,2007)

4- **Sex hormones**: In recent years, the increasing size of the geriatric population and the consequently bigger burden of non-healing or difficult-to-heal wounds associated with this age group has heightened interest in finding novel treatment modalities for wound healing. Sex hormones play a key role in numerous physiologic processes and functions and could potentially impact wound healing in the elderly.(Oh and Phillips, 2006)

5- Stress: stress can significantly slow wound healing. The effects of stress on healing have important implications in the context of surgery and naturally occurring wounds, particularly among at-risk and chronically ill populations. Recent evidence suggests that interventions designed to reduce stress and its concomitants (e.g., exercise, social support) can prevent stress-induced impairments in healing .(Christian, et al., 2006)

6- Diabetes: Diabetes' influence on wound healing is complex, multifactorial and impacts all stages of healing. Hyperglycemia causes tissue damage through the nonenzymatic glycation of proteins. Proteins with a longer half-life, such as collagen, fibrin, albumin, and hemoglobin can cause thickening of the basement membranes in microcirculation, leading to ischemia and impaired wound healing. A lack of insulin in diabetic wounds results in increased protein degradation and decreased collagen formation thus impacting the body's ability to heal the wound.

Because carbohydrate is the primary energy source for cells, including those involved in wound healing, impaired utilization of carbohydrate due to hyperglycemia leads to more proteolysis, glycogenolysis, and lipolysis and subsequent decreased wound healing.

Hyperglycemia also leads to osmotic diuresis and loss of water and electrolytes, which negatively impact wound healing by decreasing tissue oxygenation. Both extracellular and intracellular dehydration occurs, impairing the ability of the skin to heal, it impairs the action of white blood cells, macrophages, and immune functions, leading to an increased risk of infection and decrease in wound healing ability. Defects of fibroblast function, angiogenesis, and collagen production play a role in impaired wound healing found in people with diabetes**.(** Grieger**,** 2009)

7- Medications: Medications can be divided into two groups: inhibitory and stimulatory drugs. Drugs interfere with specific phases of wound healing and will affect cells, pathways, growth factors, cytokines and other important components of the wound healing cascade. Additionally some drugs will, as part of their side effects, reduce blood flow, blood cells and organ functions critical to wound healing. Many systemic medications used regularly by patients –both prescription and over the - counter – may have impact on the healing of a wound. A majority of patients with chronic wounds have co-morbidities often requiring multiple medications. These medications may have a positive or a negative impact on wound healing.(Sussman, 2007)

8- Smoking: Many of the chemicals in cigarette smoke have adverse effects on wound healing. These include nicotine, carbon monoxide and hydrogen cyanide. Nicotine diminishes red blood cells, fibroblasts and macrophages and increases platelet adhesiveness. This produces cutaneous vasoconstriction. Carbon monoxide affects the transport of oxygen via hemoglobin and hydrogen cyanide inhibits enzyme systems necessary for oxygen transport at the cellular level as well as oxidative metabolism. One cigarette reduces the peripheral blood flow by 50% for one hour and reduces oxygen tension for two hours. Smoking can therefore be a major cause for wounds not healing.(Sussman, 2007)

There is another division of the factors that affect the healing of wounds shown in figure 5 cited by , (Nayeem and Karvekar,2010)

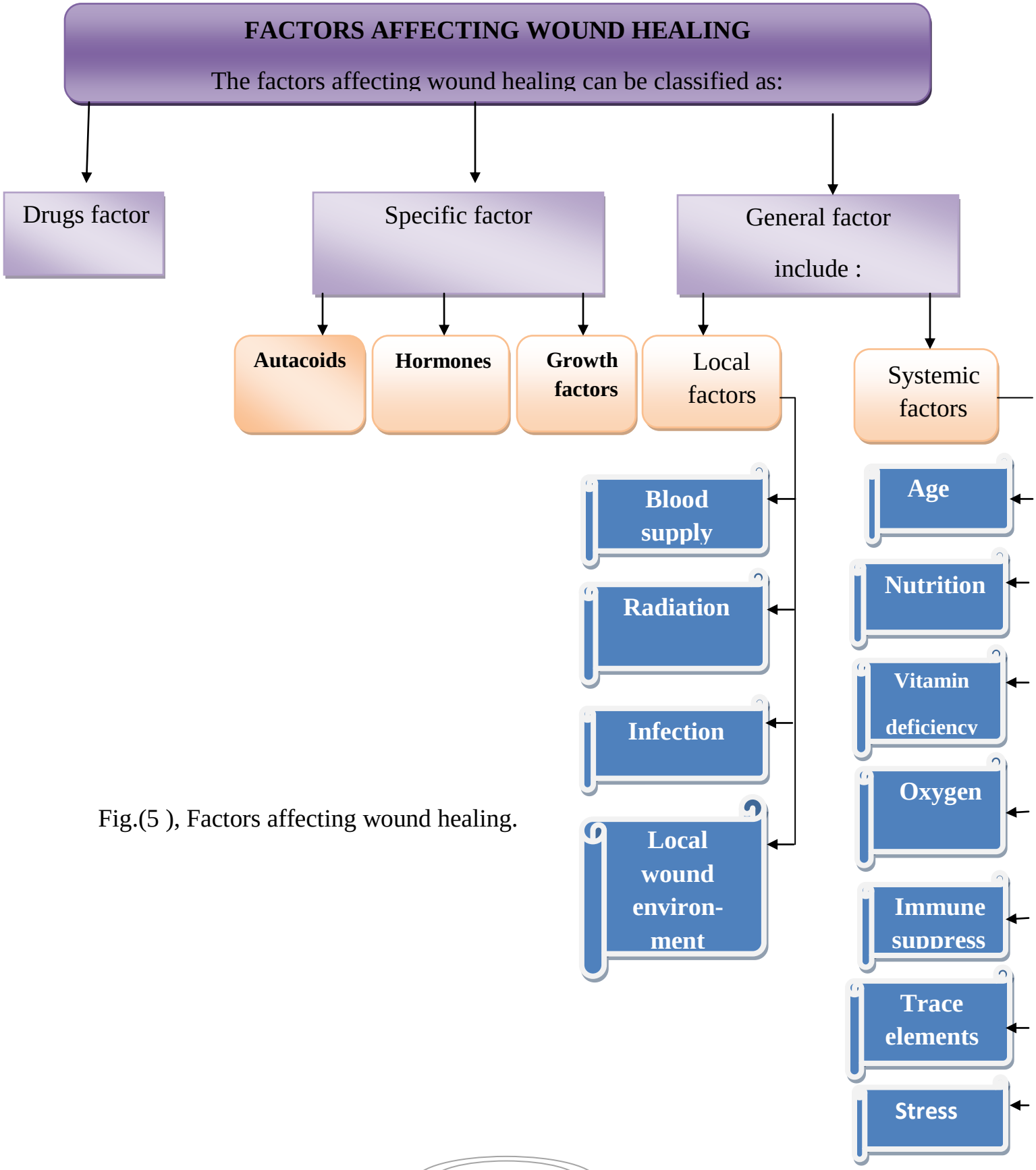


Fig.(5), Factors affecting wound healing.

2-2-4-**Prostaglandins**

Prostaglandins were detected first in seminal fluid of rams, but were thought to be derived from the prostate, hence, the name prostaglandin in1930. Chemically, the primary prostaglandins are unsaturated hydroxy - acids with a five-membered ring in a 20-carbon skeleton.

Trivial names are by letter E, F, A, and B and number 1 and 2. Prostaglandins of the 1 and 2 series have one and two double bonds, respectively. The E-prostaglandins have an oxygen at carbon 9 while the F-prostaglandins have an alpha-hydroxyl , as shown in figure 6 cited by , (Christie, 2010)

The A-prostaglandins are dehydrated derivatives of the E's in which there is a double bond between carbons 10 and 11. There are over a dozen others, with minor variations in double bonds and hydroxyls.

Hundreds of prostaglandin analogs and derivatives have been reported, from which could come "second generation" prostaglandins.

Many of these have interesting biological actions, and promising early clinical studies have been reported for the 15 [SI-methyl analogs of PGE2 and PGF2α. These particular analogs are interesting because they are not substrates for prostaglandin 15-hydroxy dehydrogenase, which is responsible for one of the important routes of metabolism of natural prostaglandins. As oxytocics, these analogs are from 10 to a hundred times more active than the corresponding natural compound.

The word "ubiquitous" has been utilized extensively in papers describing prostaglandins. This adjective seems appropriate since prostaglandins have been detected in, or released from, lung, thymus, brain, spinal cord, kidney, iris, umbilical cord, fat, adrenals, ovaries, stomach, intestines, nerves, skin and other tissues of the body.
(Stratton and Shiwen, 2010)

The E and, to a lesser degree, the F compounds are dilators of the cutaneous vasculature with long-lasting (up to ten hours) responses.

They may play a role in cutaneous inflammation, both by releasing histamine and independent of histamine, and have been isolated from perfusates of contact dermatitis and ultraviolet light-induced inflammation.

Many of their effects seem to be mediated via the adenyl cyclase - cyclic adenosine monophosphate system, cAMP, (Akaneya, 2007)

Biosynthesis of prostaglandins is not stored in tissues in a preformed state but synthesized and released in response to a given stimulus. All types of mammalian tissue that have been examined are capable of synthesizing at least a small quantity of prostaglandins. The biosynthesis of prostaglandins occurs in the cells of different tissues.

Prostaglandins are synthesized from eicosatrienoic (dihomo-y-linolenic), eicosatetraenoic (arachidonic) or eicosapentanoic acids. These compounds are incorporated in phospholipids and are converted to prostaglandins by the action of cyclo-oxygenase. This enzyme, previously known as prostaglandin synthetase, is present in the microsomal fraction of cells and initially brings about cyclization and inclusion of molecular oxygen in the precursor.

The action of cyclo - oxygenase (and therefore synthesis of prostaglandins) is inhibited by aspirin-like drugs. In the formation of bis-unsaturated compounds, for example, when phospholipids have been hydrolysed by phospholipase A, arachidonic acid is acted on by cyclo-oxygenase or by lipoxygenase (which forms HETE but not prostaglandins).

The cyclooxygenase initially stimulates formation of the cyclic-endoperoxides, prostaglandins G2 and H2 (Fig. 6). Prostaglandins G2 and H2 are then converted to either prostaglandin E2, D2, F2. Or thromboxane A2 which is unstable and decays to thromboxane B2; in some tissues, e.g. lung or platelets, the main products of arachidonic acid metabolism are thromboxanes rather than prostaglandins.

Conversion of the endoperoxides to thromboxanes is enzymically controlled by thromboxane synthetase. prostaglandins G2 or H2 act on blood vessel walls prostacyclin (prostaglandin 12) is formed enzymatically.

The stable metabolite of prostacyclin is 6-oxoprostaglandin F1j. The possibility exists for some interconversion of prostaglandins, for example, prostaglandin E2 may be reduced to prostaglandin F2, by the action of 9-oxo-reductase which is present in tissues of anumber of species. E-type prostaglandins are converted to A-type by acid or alkaline conditions (outside the range pH 5-8) and A-type rearranges to B in alkaline solution. An isomerase present in plasma of some species converts prostaglandins .

At first to prostaglandin C and then to B. Prostaglandins of the E and F series have a short half-life in the circulation and lose up to 95%4 of their biological activity on one passage through the pulmonary circulation and are further degraded on passage through other vascular beds.

Initially the prostaglandins are taken up from the vascular space and then metabolized by a series of enzymes to metabolites which usually have less biological activity than the prostaglandins. The enzymes are present in the soluble fraction of cells and are shown in Fig. 3. 15-OH prostaglandin dehydrogenase causes the oxidation of the secondary alcohol group at C15 which is the rate-limiting step in the breakdown of prostaglandins.

Prostaglandin A are not so rapidly metabolized by prostaglandin dehydrogenase and do not lose activity in the pulmonary circulation but in the hepatic portal circulation. Prostaglandin A lose its activity upon conversion to prostaglandin B which is biologically inactive.

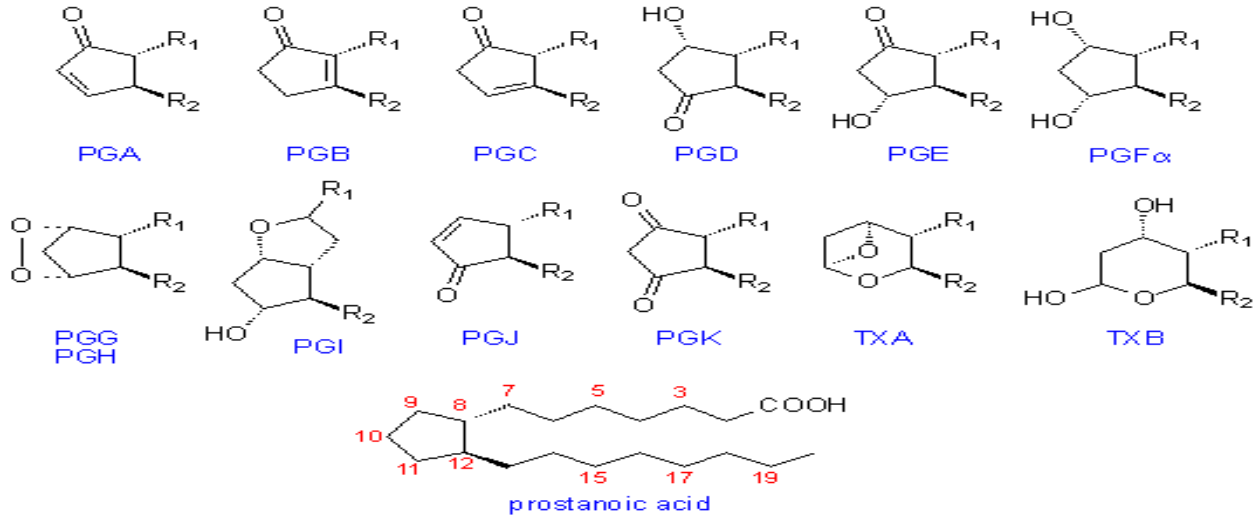


Fig (6): chemical structure of Prostanoids, Prostaglandins, Prostacyclins and Thromboxanes. (Christie, 2010)

All eicosanoids are very potent compounds present in low concentration in cells. They are local mediators, meaning that they perform their function in the environment in which they are synthesized. This distinguishes them from hormones, which are first synthesized and then transported in the bloodstream to their site of action. Eicosanoids are not stored in cells; rather, they are synthesized from arachidonic acid in response to an external stimulus.

The synthesis of prostaglandins, thromboxanes, and prostacyclins being with the oxidation of arachidonic acid with O2 by a cyclooxygenase enzyme, which forms an unstable cyclic inter mediate, PGG2. PGG2 is then converted via different pathways to these three classes of compounds.

Leukotrienes are formed by a different pathway, using an enzyme called a lipoxygenase . Prostacyclin analogues are used in the treatment of intermittent claudication; severe limb ischaemia; prevention of imminent gangrene and to reduce pain They can be used to promote healing in arterial and vasculitic ulcers. (Sussman ,2007)

Prostaglandins cause vasodilation through the activation of the adenylate cyclase pathway via the production of cyclic adenosine monophosphate. Prostaglandins also accumulate at the area of injury through the activation of phospholipases located on injured cell membranes. Phospholipases stimulate the release of arachidonic acid, ultimately leading to the production of prostaglandins, leukotrienes, and other factors. (Romo, 2010)

2-2-5-Growth hormone

Growth hormone, also called somatotropic hormone or somatotropic, is a small protein molecule that contains 191 amino acids in a single chain and has a molecular weight of 22,005. It causes growth of almost all tissues of the body that are capable of growing, promotes increased sizes of the cells and increased mitosis, with development of greater numbers of cells and specific differentiation of certain types of cells such as bone growth cells and early muscle cells.

Aside from it`s general effect in causing growth, growth hormone has multiple specific metabolic effects, including:

a- increased rate of protein synthesis in most cells of the body,

b - increased mobilization of fatty acids from adipose tissue, increased free fatty acids in the blood, and increased use of fatty acids for energy, and

b- decreased rate of glucose utilization throughout the body.

Thus, in effect, growth hormone enhances body protein, uses up fat stores, and conserves carbohydrates. Growth hormone fails to cause growth in an animal that lacks a pancreas; it also fails to cause growth if carbohydrates are excluded from the diet. This shows that adequate insulin activity and adequate availability of carbohydrates are necessary for growth hormone to be effective.

Part of this requirement for carbohydrates and insulin is to provide the energy needed for the metabolism of growth, but there seem to be other effects as well. Especially important is insulin's ability to enhance the transport of some amino acids into cells, in the same way that it stimulates glucose transport. (Guyton and Hall, 2006)

It is secreted by alpha cells situated in the adenohypophysis, part of the pituitary gland, (Standring ,et al., 2008).

Growth hormone - releasing hormone (GHRH) is produced by the hypothalamus and acts on the pituitary, stimulating the production and release of growth hormone (GH), (Dioufaa ,et al., 2010)

There are a number of key hormones involved with energy production, 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.

There are 4 major anabolic hormones that directly or indirectly affect wound healing. They are growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin, and testosterone (and its analogs).

GH is a potent endogenous anabolic hormone produced in daily doses of 0.5 to 0.8 mg in children and young adults. Growth hormone is a large polypeptide that

contains 2 receptor-binding sites. There is a number of growth hormone – binding proteins, and growth factor–binding sites found on a large variety of tissues, especially liver.

GH has a number of metabolic effects, but the most prominent is its anabolic effect. Cell proliferation is accentuated as is overall protein synthesis and new tissue growth. GH also stimulates IGF-1 production by the liver and some of the anabolism seen with HGH is that produced by IGF-1, another anabolic agent.

As to its direct wound healing effects, skin is a target tissue for HGH, both directly through HGH receptors on the surface of epidermal cells and indirectly through the action of IGF - 1.30,34. Exogenously administered HGH has been shown to increase skin thickness in normal humans.

Other effects on the wound include increased rate of re- epithelialization of skin graft donor sites in adults and children with severe burns or trauma. In addition, HGH has been shown to increase wound collagen content, granulation tissue and wound tensile strength, and the local production of IGF-1 by fibroblasts. (Demling ,2005)

GH has been used in patients with severe burn injuries, and in most studies enhanced rates of wound healing and patient survival were observed. In addition, GH was shown to stimulate granulation tissue formation and biomechanical wound strength in animal models of impaired healing.

The role of GH on wound repair and its mechanisms of action at the wound site were determined. Full-thickness incisional and excisional wounds of transgenic animals developed an extensive, highly vascularized granulation tissue. However, wound bursting strength was not increased.

Wound closure was strongly delayed as a result of enhanced granulation tissue formation and impaired wound contraction. The latter effect is most likely due to a significantly reduced number of myofibroblast at the wound site. GH *in vitro* studies with stressed collagen lattices, identified as an inhibitor of transforming growth factor β-induced myofibroblast differentiation, resulting in a reduction in fibroblast contractile activity. These results reveal novel roles of growth hormone in angiogenesis and myofibroblast differentiation, which are most likely not mediated via insulin like growth factors at the wound site. Furthermore, our data suggest that systemic GH treatment is detrimental for wound healing in non-healing impaired individuals.

Generally Growth factors (GFs) are biomolecules that regulate a great variety of key functions in the body, including mitosis, cell differentiation, extracellular matrix synthesis, and metabolism. During the ontogeny, some GFs also display chemotactic activity to direct cell migration. Several families of GFs are expressed in specific tissues where they play a protective role against the natural and pathologic cell death. Generally, the production and the physiological activity of

GFs occur at low concentration (picomolar, pM) in a wide variety of cells.(Fernandez , et al. 2011)

2-2-6-cAMP

It's a monophosphoric diester of adenosine, cyclic Adenosine Monophosphate (cyclic AMP *or* cAMP); adenosine 3′,5′-cyclophosphate; adenosine 3′,5′- (cyclic) phosphate; It is a universally distributed key metabolite, produced by the action of adenylate cyclase on adenosine 5′-triphosphate, ATP.

It is the first compound to be named a second messenger, it mediates many effects in signal transduction pathways. It was first identified as a heat-stable activator of glycogen phosphorylase kinase, and is now known also to activate cyclic-AMP-dependent protein kinase and to regulate numerous other enzymic activities or physiological processes , figure (7) shows the chemical structure of cAMP , cited by ,(Attwood, et al.2006)

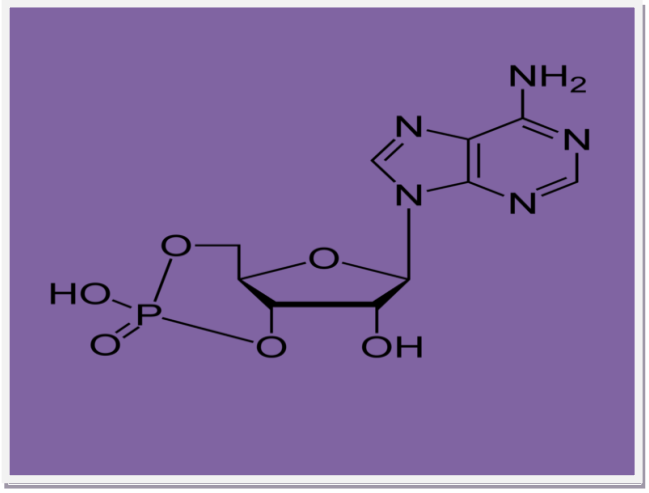


Fig.(7), Chemical structure of cyclic Adenosine Monophosphate.

cAMP is a second messenger mechanism for mediating intracellular hormonal functions . One of the means by which hormone exerts intracellular actions is to stimulate formation of the second messenger cAMP inside the cell membrane. The cAMP then causes subsequent intracellular effects of the hormone. Thus, the only direct effect that the hormone has on the cell is to activate a single type of membrane receptor. The second messenger does the rest.

The Prostaglandins E1, E2 (PGE1, PGE2) use cAMP as second messenger. Prostaglandins increase cAMP in platelets, thyroid, corpus luteum, fetal bone, adenohypophysis, and lung but reduce cAMP in renal tubule cells and adipose tissue,(Granner, ,et al.,2010)

cAMP is the intracellular signal for many responses. cAMP was the first intracellular signal identified in mammalian cells. Several components comprise a system for the generation, degradation, and action of cAMP is formed from ATP by adenylyl cyclase at the inner surface of cell membranes and acts as an intracellular second messenger in response to hormones such as epinephrine, norepinephrine, and glucagon. cAMP is hydrolyzed by phosphodiesterase, so terminating hormone action. In liver, insulin increases the activity of phosphodiesterase.(Granner, ,et al.,2010)

cAMP acts as enzyme activation. Enzymes that are normally inactive can often be activated when needed. An example of this occurs when most of the ATP has been depleted in a cell. In this case, a considerable amount of cyclic adenosine monophosphate (cAMP) begins to be formed as a breakdown product of the ATP; the presence of this cAMP, in turn, immediately activates the glycogen-splitting enzyme phosphorylase, liberating glucose molecules that are rapidly metabolized while their energy is used for replenishment of the ATP stores.

Thus, cAMP acts as an enzyme activator for the enzyme phosphorylase and thereby helps control intracellular ATP concentration.(Guyton and Hall,2006)

2-3-Laser

2-3-1-Definition

The word laser is an acronym for the words; Light Amplification by Stimulated Emission of Radiation, it's one of the outstanding inventions of the second half of

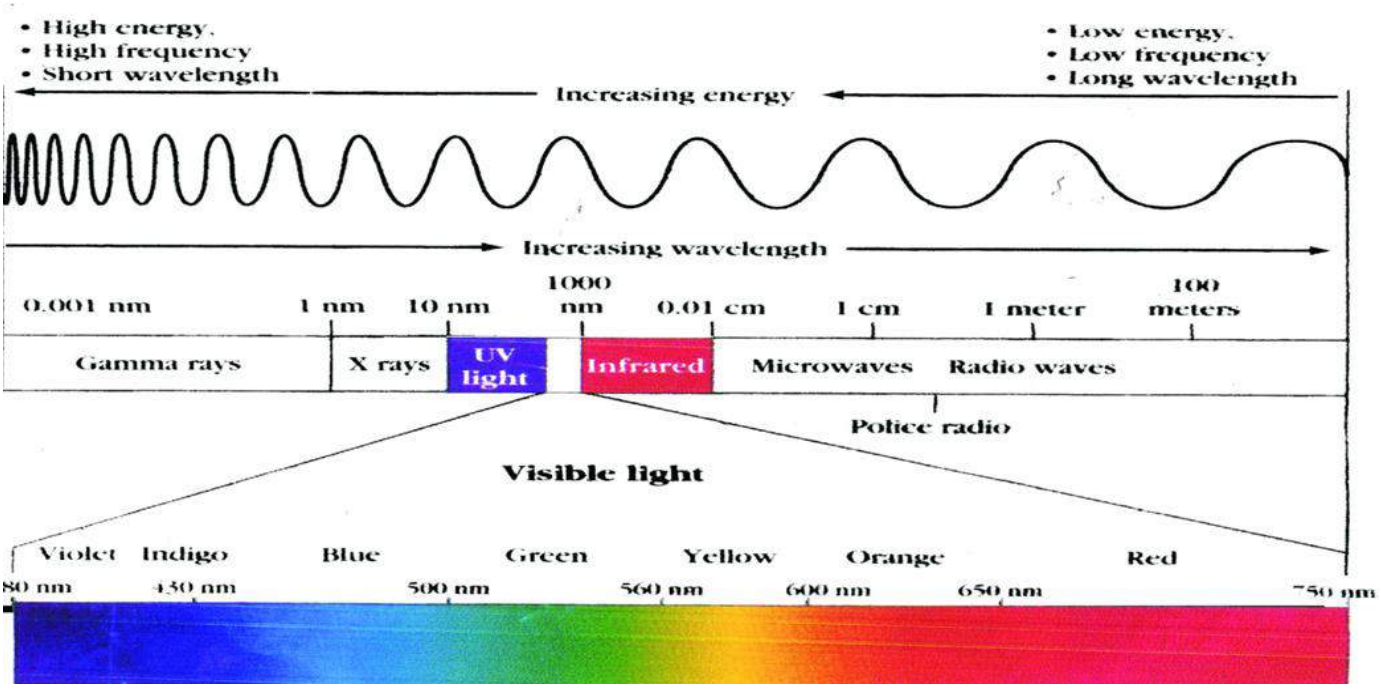


Fig (8): Different electromagnetic wavelengths

ʎ Less than 400 nm = ultraviolet spectrum

ʎ between 400 - 700 nm = Visible light

ʎ 700 - 100 000 nm = infrared spectrum

the last century which has become a valuable tool in a variety of fields starting with medicine to communications. It is a light source but it is very highly different from many traditional light sources.

We can't use the laser for illumination purpose as well as other light sources because it produces a highly directional and high intensity beam with a narrow frequency range than that available from the common type of light sources .

So the lasers are more widely used as a high power electromagnetic beam rather than a light beam , figure (8), shows the different electromagnetic wavelengths , cited by(Zungu,et al. (2008).The beam of laser is used as special type of drill bit to drill holes in hard materials, as a saw to cut thick metal sheets ,as a phonograph needle for compact discs ,as a knife during surgical operations ,as a target designators for military weapons and so on .Thus ,it's a high technology device affecting our lives in many ways, (Avadhanulu, 2009)

2-3-2-Laser components:

Every working laser of the atomic or molecular varieties has certain common elements of structure and function, these common elements are:

1. A material medium having the proper energy levels to produce the desired wavelengths of light.

2. A resonant optical cavity, shaped in the form of a cylinder whose length is much greater than It's diameter, and having coaxial mirrors at opposite ends of it.

3. An external source of energy to provide the excitation of the atoms or molecules of the medium by the process of pumping.

The media available today for lasers include hundreds of different materials: gases, liquids, and solids. The resonant cavity is usually fitted with mirrors that are sectors of spheres having radii much greater than the distance between the mirrors, because flat (plane) mirrors are very difficult to align properly. At one end of the resonator (the cavity and the medium), the mirror must have a reflectance greater than 99.8% at the wavelength of the laser. At the other end, the mirror must have a transmittance between 1% and 20%, depending upon wavelength and other factors. This is necessary to let some of the laser light escape from the resonator for external use.

The energy source is necessary because the medium cannot spontaneously generate energy for its own excitation, except in case of chemical lasers, which consume their active media. The pumping energy is most often electrical (an

electric current flowing through the medium) or radiant (light from a non-coherent source or from another laser). Thermal energy can be used if means are provided to create regions of different temperature within the medium, but a medium heated to a uniform temperature throughout will always have more atoms or molecules in lower energy levels than high, thus making it impossible to produce more individual atoms or molecules in the excited state than in the ground state.(Fisher, 2007)

2-3-3-How a laser work?

Laser is a device that converts electrical or chemical energy into light energy. In contrast to ordinary light that is emitted spontaneously by excited atoms or molecules, the light emitted by laser occurs when an atom or molecule retains excess energy until it is stimulated to emit it, fig ; 9-a , cited by (Jawad, et al.,2011).

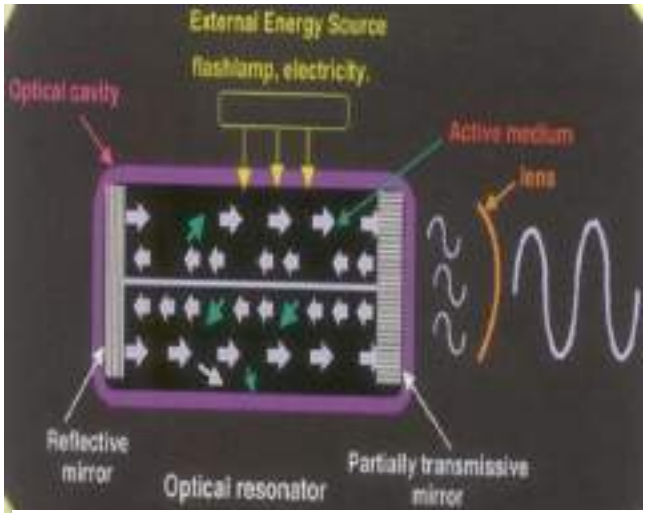


Fig. (9a): Components of the laser

The radiation emitted by lasers including both visible and invisible light is more generally termed as electromagnetic radiation. The concept of stimulated emission of light was first proposed in 1917 by Albert Einstein. He described three processes: Absorption, spontaneous emission and stimulated emission .

Einstein considered the model of a basic atom to describe the production of laser. An atom consists of centrally placed nucleus which contains positively charged particles known as protons, around which the negatively charged particles .

When an atom is struck by a photon, there is an energy transfer causing increase in energy of the atom. This process is termed as absorption. The photon then ceases to exist, and an electron within the atom pumps to a higher energy level. This atom is thus pumped up to an excited state from the ground state .

In the excited state, the atom is unstable and will soon spontaneously return back to the ground state, releasing the stored energy in the form of an emitted photon. This process is called spontaneous emission

If an atom in the excited state is struck by a photon of identical energy as the photon to be emitted, the emission could be stimulated to occur earlier than would occur spontaneously. This stimulated interaction causes two photons that are identical in frequency and wavelength to leave the atom. This is a process of stimulated emission

If a collection of atoms includes, more that are pumped into the excited state that remain in the resting state, a population inversion exists. This is necessary condition for lasing. Now, the spontaneous emission of a photon by one atom will stimulate the release of a second photon in a second atom, and these two photons will trigger the release of two more photons .

These four then yields eight, eight yields sixteen and so on. In a small space at the speed of light, this photon chain reaction produces a brief intense flash of monochromatic and coherent light which is termed as laser,(Jyoti ,et al., 2010).

In order to obtain a laser action, it must be ensured that more atoms in the lasing medium are in an excited state than in the lower - energy state. When this condition is met, it is said that a population inversion takes place in the medium, pumping energy into the lasing medium can create the condition .

Now a stray photon of the correct wavelength, produced by spontaneous emission, is enough to set off a chain of stimulated emissions. The lasing medium lies between two mirrors, one of them is totally reflecting and the other is partially reflecting.

Photons can bounce back and forth, stimulating more and more atoms to emit photons, thus rapidly increasing the intensity , as they leave through the partially reflecting mirror. If the pumping energy is applied continuously, population inversion is maintained and new excited atoms will recoup the exhaustive atoms and give rise to a continuous wave laser. While if the pumping energy is applied

intermittently, as pulsed laser , the stimulated emission die down as the atoms that are in an excited state are developed and lose its excessive energy, destroying the population inversion,(Jawad, et al.,2011).

2-3-4- Properties of Laser Beams:

Laser radiation is characterized by an extremely high degree of Monochromaticity, Coherence, Directionality and Brightness.

1-Monochromaticity: The light from a laser is monochromatic, which means that it is of a particular wavelength, or of a single color. Light from sodium lamp is monochromatic. i.e. of single color or of single wavelength of about 58930A.

The wavelength of sodium light is 5893 ^{0}A, that means simply that intensity is maximum at this value. However, intensity is not zero for wavelengths above and below 5893 ^{0}A up to even 500^{0}A on either side. This spread of wavelength (or frequency) about the wavelength of maximum intensity is called 'band width ' (or range). The band width of a conventional monochromatic light is of the order of 1000^{0}A.

On the other hand, the band width of an ordinary laser is of the order of 10^{0}A and for a high quality laser it is only 10^{-8} at 6000^{0}A. This narrow band width of a laser light is called 'high monochromacity'. Because of this monochromaticity, large energy can be concentrated into an extremely small band width.

2-Coherence: The light from a laser is coherent, which means that each photon is in synchrony with the other photons, or the patterns of their waves are aligned with each other, thus increasing the intensity of the light emitted.

Visible light energy is emitted when the excited electrons in atoms undergo transitions to the ground state. In ordinary light sources, these transitions take place at random in time and the light waves received at a point on a screen bear no definite phase relation among them. But, in a laser source, electronic transitions take place in an 'orderly way' and the light waves emitted have a consistent phase relation which does not change with time , figure 9b shows coherent waves cited by ,(Svelto, et al.2010)

This is called 'temporal coherence' and is the most important characteristic of laser light.

Lack of coherence makes ordinary light an 'optical noise'. But coherence makes a laser light ' optical music '. Because of this coherence, tremendous amount of power of

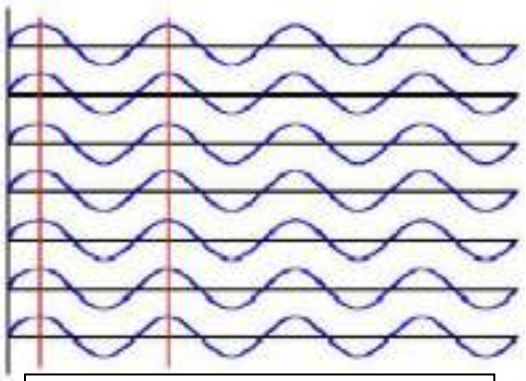


Fig.9 b: coherent waves

the order of 10^{13} Watts can be concentrated in a narrow space of linear dimension of 10^{-6}m.

3- Directionality: The light from a laser is highly directional, which means that the light emitted is very tight, concentrated, and intense. In contrast, the light from a flashlight or a light bulb, for example, is comparatively diffuse and weak, since the light emitted is scattered in many directions.

The conventional sources like lamp, torch light and sodium lamp emit light in all directions. This is called 'divergence'. Laser, on the other hand, emits light only in one direction. This is called 'directionality' of laser light.

An example is the powerful search or guide light. If the beam from it travels a distance of 1km, it spreads to about a kilometer in diameter. If a laser travels a distance of 1km, it spreads to a diameter less than 1 cm. The directionality of laser enables us to focus the light to a point on a target at large distance.

4- High intensity: The intensity (I) of a wave is the energy per unit time flowing through a unit normal area. The light from an ordinary light source spreads out uniformly in all directions and forms spherical wave fronts around it. If you look at a 100 watt lamp filament from a distance of 30cm, the power entering your eye is less than 1/1000 of a watt. In the case of a laser light, energy is emanated in small region of space and in a small wavelength range and hence is said to be of great intensity.

Looking at a beam of a laser directly, allows all the power in the laser to enter the eye. Thus, even a 1 watt laser would appear many thousand times more intense than 100 watt ordinary lamp. For certain lasers, the intensity is so enormous that a power of 10^{15} watt can be concentrated into an area of 1 square centimeter , (Svelto, et al.2010) and (Ganesh,2010) . Fig (10) illustrates the variations between laser and conventional lights , cited by, (Martinm, 2008).

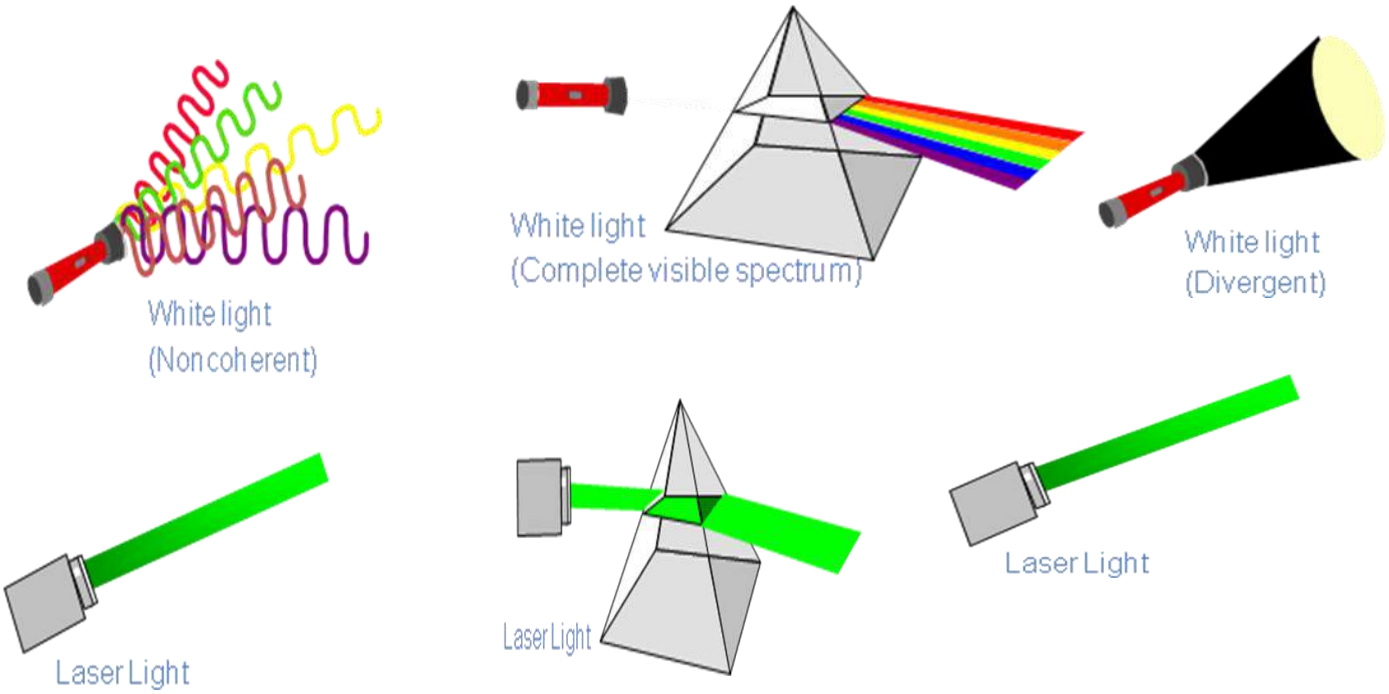


Fig (10): Laser beam characteristics (variations between laser and conventional lights.

2-3-5-Laser Safety

Fig (11) :Laser warning sign

Lasers are divided into five classes depending upon the power or energy of the beam and the wavelength of the emitted radiation. The classification is based on the laser's capability to cause immediate injury to the eye or skin and / or potential for causing fires from direct exposure to the beam or from reflections from diffuse reflective surfaces. Since August 1, 1976, commercially produced lasers have been classified and identified by hazardous labels affixed to the laser.
Class 1: lasers are safe under most circumstances and are incapable of damaging the eye or skin because of either engineered design or inherently low power output. (Whale, 2009)

The most limiting MPE (Maximum Permissible Exposure Level) values cannot be exceeded and no specific safety controls are required for CW (continuous

Wave) visible laser, the maximum limit is 70 microwatts. Thus, therapeutic lasers that fall into class 1 do not harm tissue and do not affect the eyes. (Dais, 2009)

Class 2: lasers emit in the visible wavelength range 400 – 700 nm and have sufficient power output to cause damage to the eyes if viewed continuously. However, their outputs are low enough where eye protection is afforded by the blinking reflex. Additional hazard control measures take the form of cautionary signs or labels. (Whale, 2009)

Class 3R (Previously Class 3A) **:**lasers emit in the wavelength range106–302.5 nm and have the potential to cause damage to the eyes from intra-beam viewing but the risk is lower than for class 3B lasers.
Class 3B lasers. Precautions are required to prevent both direct viewing and viewing with optical instruments, warning signs must be placed on the operation room doors , figure 11 , cited by , (Whale, 2009)
Class 3B: lasers are more hazardous because of either higher output or operation outside visible wavelengths. In addition, specular reflections i.e. non- diffuse surface reflections may also be hazardous. In general, more stringent controls are needed to prevent exposure,(Whale, J., 2009).

Therapeutic lasers that fall into class 3B do not harm tissue, but protective eyewear is necessary for the therapist and the client, (Dais, 2009).

Class 4**:** lasers are high power devices capable of producing eye damage even from diffuse reflection. They may cause skin injuries and could also constitute a fire hazard. Examples of class 4 lasers include surgical lasers and those used in the plastic, wood and metal fabrication industries.(Whale, 2009)

2-3-6-Types of Lasers

There are several ways in which the lasers can be classified. It can be done according to what material or element is used as an active medium. It can be done according to the operation of laser in a pulsed mode or in a continuous wave (CW) mode. The classification may be done basing on other parameters such as gain of the laser medium, power delivered by the laser, efficiency or applications.

According to the active medium; lasers are broadly divided into four categories- solid lasers, gas lasers, liquid lasers and semiconductor lasers, (Avadhanulu , 2009)

- Solid state laser is one in which the active centers are fixed in a crystal or glassy material .Solid state lasers are electrically non-conducting. They are also called doped insulator lasers to avoid connotation of semiconductor. (Avadhanulu , 2009)
- Gas Lasers: Gas lasers are the most widely used lasers and the most varied. They range from the low power Helium-Neon (He-Ne) laser used in college laboratories to very high power carbon dioxide laser used in industrial applications. These lasers operate with rarefied gases as their active media and excited by an electric discharge .There are three different types of gas laser: ion lasers, neutral atom lasers and molecular lasers. In gases , unlike in crystals, the energy levels of atoms involved in the lasing process are well defined and narrow. Broad pump bands do not exist and the pump levels are also narrow. In order to excite atoms, sources with sharp wavelength are required finding an appropriate optical source for pumping poses a problem. Therefore optical pumping is not used in gases. The most common method of exciting gas laser medium is by passing an electric discharge through the gas .Electrons in discharge transfer energy to atoms in the laser gas by collisions. (Avadhanulu.2009.
- liquid lasers or dye lasers belong to the family of liquid lasers. The active material is a dye dissolved in a host medium of a liquid solvent, such as ethylene glycol. The situation is very similar to solid state lasers where Cr^{3+}, Nd^{3+} or Ti^{3+} ions are used in a solid host. The advantage of a liquid host is that the concentration of the active ions can be easily varied. There are over 200 laser dyes, the most important one being rhodamine 6G. Dye lasers operate both in cw and pulsed modes. Pulsed dye lasers are pumped by a flash lamp or other lasers and can produce up to 400J output in a 10 μs – pulsed. Cw dye lasers are pumped by other cw lasers such as argon ion laser and produce powers of about 2W. (Avadhanulu.2009)

2-3-7-Semiconductor Laser Diodes

Also called diode lasers or injection lasers, they were developed in the early 1960s and are now the most widespread lasers in the world, largely because they are so compact and inexpensive .

Semiconductor laser present in the DVD , barcode product, swipes through the checkout , long-distance telephone call by fiber- optic cable , a laser printer. Semiconductor lasers make powerful, precise beams of light (like ordinary lasers), but they are about the same size as simple colored lamps seen on electronic instrument panels,(Chris,2010).

A laser consists of a cavity, with plane or spherical mirrors at the ends, that is filled with lasable material. This material can be excited to a semistable state by light or an electric discharge. The material can be a crystal, glass, liquid, dye, or gas as long as it can be excited in this way.

The simplest cavity has two mirrors, one that totally reflects and one that reflects between 50 and 99%. As the light bounces between these mirrors, the intensity increases. Since the laser light travels as an intense beam, the laser produces very bright light.

The type of mirror determines the type of beam. A very bright, highly monochromatic (one wavelength or one color) and coherent beam is produced when one mirror transmits only 1-2% of the light. If plane mirrors are used, the beam is highly collimated (made parallel).

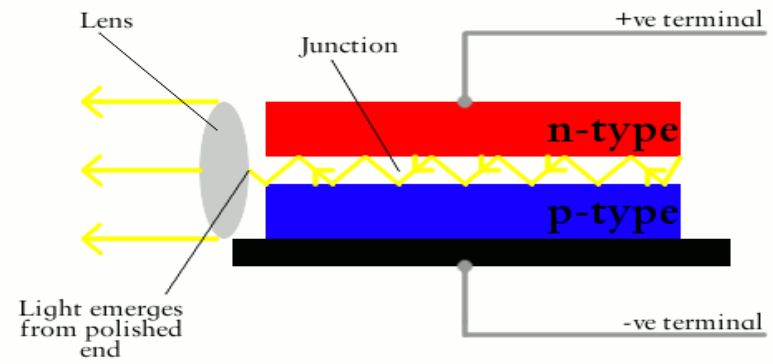


Figure (12) : Schematic diagram of Semiconductor laser diodes

The beam comes out near one end of the cavity when concave mirrors are used.

A semiconductor laser converts electrical energy into light. This is made possible by using a semiconductor material, whose ability to conduct electricity is between that of conductors and insulators. By doping a semiconductor with specific amounts of impurities, the number of negatively charged electrons or positively charged holes can be changed, figure 12 , cited by ,(Chris,2010) .

The conventional semiconductor laser consists of a compound semiconductor, gallium arsenide. This material comes in the form of ingots that are then further processed into substrates to which layers of other materials are added.

The materials used to form these layers are precisely weighed according to a specific formula. Other materials that are used to make this type of laser include certain metals (zinc, gold, and copper) as additives (dopants) or electrodes, and silicon dioxide as an insulator.

The basic design of a semiconductor laser consists of a "double heterostructure." This consists of several layers that have different functions. An active or light amplification layer is sandwiched between two cladding layers. These cladding layers provide injection of electrons into the active layer. Because the active layer has a refractive index larger than those of the cladding layers, light is confined in the active layer.

The performance of the laser can be improved by changing the junction design so that diffraction loss in the optical cavity is reduced. This is made possible by

modifying the laser material to control the index of refraction of the cavity and the width of the junction.

The index of refraction of the material depends on the type and quantity of impurity. For instance, if part of the gallium in the positively-charged layer is replaced by aluminum, the index of refraction is reduced and the laser light is better confined to the optical cavity.

The width of the junction can also affect the performance. A narrow dimension confines the current to a single line along the length of the laser, increasing the current density. Peak power output must be limited to no more than 400 watts per cm (0.4in) length of the junction and current density to less than 6,500 amperes per centimeter squared at the junction to extend the life of the laser.(Goldwasser, 2010)

2-3-8-Low Level Laser Therapy L.L.L.T:

Low Level Laser Therapy is also known as Low Intensity Light Therapy (L.I.L.T.), cold laser, phototherapy, light therapy, low-energy laser therapy, photobiomodulation among other L.L.L.T.

Typically, lasers used for therapeutic purposes fall in the red and near-infrared ranges of electromagnetic radiation and thus in the non-ionizing range. The wavelength range for red light is 630 - 700 nm and the range for near-infrared radiation is 700 nm to 1mm. Therapeutic lasers use these wavelengths because other wavelengths are absorbed by melanin pigment in skin, hemoglobin in blood or water in the tissues and thus do not reach the mitochondria of the targeted tissues. In addition, studies have shown that wavelengths in the red through near-infrared spectrum (630-900 nm) are best absorbed by the iron or copper atoms associated with the cytochrome system in mitochondria for ATP production, (Dais,2009).

With true lasers, the intensity of the light remains consistent even when the source of the beam is moved away from the target; a characteristic called coherence. The wavelengths of light from light emitting diodes, or LED lasers, spread out in all directions when pulled away from the target (non-coherent) and are thus thought not to penetrate tissue as well. However, these types of “cold lasers” do not give off heat and can be held directly against the skin. More powerful lasers can give off heat; even enough to cut tissue and damage the retina.

2-3-9-Laser Tissue Interaction

Different types of lasers react differently with tissue. The wavelength of the laser is of primary importance. However the power density and exposure time also play a critical role in determining tissue interaction. When a beam of laser light strikes the surface of living tissue, one of the four basic physical phenomena may occur:

1-Reflection: The redirection of a ray of light from its impact point on the boundary surface between different media back into the hemisphere of space, centered at the impact point, from which that ray originated, in such a way that the angle of incidence is equal to the angle of reflection (both measured from the perpendicular to the reflecting surface in the plane defined by the incident and reflected rays).

2-Transmission: into, or through, the tissue.

3-Scattering can be defined as a change in direction of a light ray without a change in wavelength.

4-Absorption: of radiant energy occurs at the level of atoms, ions, molecules, and radicals (combinations of atoms that pass unchanged through chemical reactions, but may be incapable of existing alone). It is a process of conversion of the radiant energy into other forms of energy. (Fisher , 2007)

A variety of interaction mechanisms may occur when applying laser light to biological tissue. Specific tissue characteristics as well as laser parameters contribute to this diversity. Most important among optical tissue properties are the coefficients of reflection, absorption, and scattering which, together, they determine the total transmission of the tissue at a certain wavelength.

Although the number of possible combinations for the experimental parameters is unlimited, mainly five categories of interaction types are classified today. These are photochemical interactions, thermal interactions, photo ablation, plasma-induced ablation, and photodisruption.

1- Photochemical Interaction: a group of photochemical interactions stems from empirical observations that light can induce chemical effects and reactions within macromolecules or tissues. One of the most popular examples is the photosynthesis. In the field of medical laser physics, photochemical

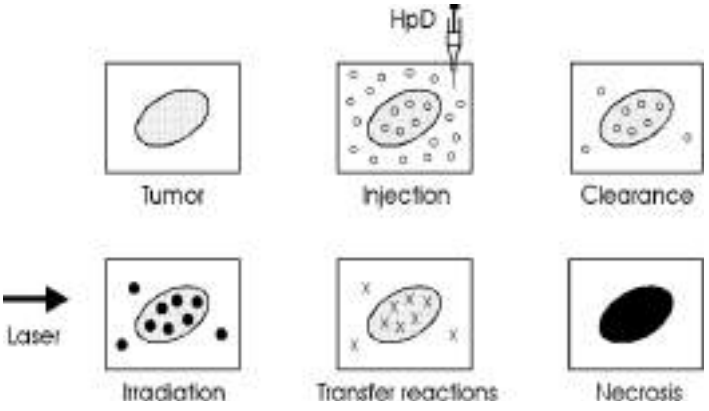


Fig.(13):Scheme of photodynamic therapy

2- interaction mechanisms play a significant role during photodynamic therapy (PDT),

frequently, biostimulation is also attributed to Photodynamic Therapy (PDT): Photodynamic therapy is performed as follows: first, a photosensitizer, e.g. hematoporphyrin derivative (HpD), is injected into a vein of the patient.

Within the next few hours, HpD is distributed among all soft tissues except the brain. The basic characteristic of a photosensitizer is that it remains inactive until irradiated. After 48–72 hours, most of it is cleared from healthy tissue, its concentration in tumor cells has not decreased much even after a period of 7–10 days, as shown in figure 13 , cited by , (Markolf, 2007) .

Thus, HpD does not accumulate in tumor cells immediately after injection, but these cells show a longer storage ability (affinity) for HpD. The initial concentration is the same as in healthy cells, but the
clearance is faster in the latter cells.

After about three days, the concentration of HpD in tumor cells is about thirty times higher than in healthy cells. Laser irradiation usually takes place after the third day and up to the seventh day after injection if several treatments are necessary. Within this period, tumor cells are still very sensitive and selective necrosis of tumor cells is enabled. However, many healthy tissues may retain certain constituents of HpD and are thus photosensitized,

b- Biostimulation occurs at very low irradiances and belong to the group of photochemical interactions. Wound healing and anti-inflammatory properties by red or near infrared light sources such as helium–neon lasers or diode lasers were reported. Local wound healing effects with helium–neon He-Ne or diode lasers may be explained by the action of low-intensity light on cell proliferation. In the area of such injuries, conditions are usually created preventing proliferation such as low oxygen concentration or pH. The exposure to red or near infrared light might thus serve as a stimulus to increase cell proliferation.

2- Thermal Interaction : the term thermal interaction stands for a large group of interaction types, where the increase in local temperature is the significant parameter change. Thermal effects can be induced by either CW or pulsed laser radiation. Depending on the duration and peak value of the tissue temperature achieved, different effects like coagulation, vaporization, carbonization, and melting may be distinguished.

a- Coagulation : during the process of coagulation, temperatures reach at least 60◦C, and coagulated tissue becomes necrotic as it will be discussed in this section.

b- Vaporization: vaporization is also referred to sometimes as a thermo mechanical effect due to the pressure build-up involved. The resulting ablation is called thermal decomposition, when a laser beam strikes a tissue. Water strongly absorbs its wavelength leading to vaporization within these layers.

c- carbonization: In this case, however, too much energy was applied and carbonization occurred. Thus, the local temperature of the exposed tissue had been

increased. At temperatures above approximately 100◦C, the tissue starts to carbonize, i.e. carbon is released, leading to a blackening in color.

d- Melting : Temperature must reach a few hundred degrees to melt for example the tooth substance which mainly consists of hydroxyapatite, a chemical compound of calcium and phosphate, the pulse duration of a few microseconds is still long enough to enable a sufficient increase in temperature, since the applied repetition rate of 1 Hz is extremely low, the different effects of laser irradiation are listed in table 2, cited by , (Markolf, 2007).

Table (2): Thermal effects of laser radiation

Temperature	Biological effect
37◦C	Normal
45◦C	Hyperthermia
50◦C	
60◦C	Denaturation of proteins and collagen, coagulation
80◦C	Permeabilization of membranes
100◦C	Vaporization, thermal decomposition (ablation)
> 100◦C	Carbonization
> 300◦C	Melting

3-Photoablation : photo ablation was first discovered by Srinivasan and Mayne - Banton (1982). They identified it as ablative photodecomposition, meaning that material is decomposed when exposed to high intense laser irradiation. Typical threshold values of this type of interaction are 107–108 W/cm2 at laser pulse durations in the nanosecond range.

The ablation depth, i.e. the depth of tissue removal per pulse, is determined by the pulse energy up to a certain saturation limit. The geometry of the ablation pattern itself is defined by the spatial parameters of the laser beam. The main advantages of this ablation technique lie in the precision of the etching process, its excellent predictability, and the lack of thermal damage to an adjacent tissue.

4-Plasma-Induced Ablation: when obtaining power densities exceeding 10^{11} W/cm² in solids and fluids – or 10^{13} W/cm² in air – a phenomenon called optical breakdown occurs. If several laser pulses are applied, a typical sparking noise at the repetition rate of the pulses is heard. By means of plasma-induced ablation, very clean and well-defined removal of tissue without evidence of thermal or mechanical damage can be achieved when choosing appropriate laser parameters. Sometimes, plasma-induced ablation is also referred to as plasma-mediated ablation.

5-Photodisruption: The physical effects associated with optical breakdown are plasma formation and shock wave generation. If breakdown occurs inside soft tissues or fluids, cavitation and jet formation may additionally take place. At higher pulse energies – and thus higher plasma energies – shock waves and other mechanical side effects become more significant and might even determine the global effect upon the tissue. Primarily, this is due to the fact that mechanical effects scale linearly with the absorbed energy. Then, because of the mechanical impact, the term disruption (from Latin: ruptus = ruptured) is more appropriate, (Markolf, 2007)

2-3-10-Biological effect of laser therapy:-

Clinical studies and trials of laser therapy technology indicate the following beneficial effects of laser light therapy on tissues and cells. (Stein,2009).

1- Accelerates tissue repair and cell growth: Photons of light from lasers penetrate deeply into tissue and accelerate cellular reproduction and growth .

Therapeutic lasers increas the energy available to the cell so that the cell can take on nutrients, and to get rid of waste products faster, (Stein,2009).

As a result of exposure to laser energy, the regenerative cells of tendons, bone, ligaments, and muscles repair faster,(Stein,2009) . This action is done by the activation of enzyme in the target cells:- Enzymes are catalysts of extraordinary efficiency, able to accelerate reactions by manifold. Enzyme laser light activation is currently a fast-growing field and a large number of studies have been produced,(Da Silva, and Potrich,2010) .

2-Faster wound healing, (Stein,2009).

3-Reduces fibrous tissue formation: Low Level Laser Therapy reduces the formation of scar tissue following tissue damage from cuts, scratches, burns or surgery, (Stein,2009).

4-Anti-Inflammation: Laser light therapy has an anti-edema effect as it causes vasodilatation, but also because it activates the lymphatic drainage system (drains swollen areas). As a result, there is a reduction in swelling caused by bruising or inflammation, (Stein,2009).

5-Anti-pain (analgesic): Laser therapy has a high beneficial effect on nerve cells which blocks pain transmitted by these cells to the brain and which decreases nerve sensitivity. Also, due to less inflammation, there is less edema and less pain. Another pain blocking mechanism involves the production of high levels of pain killing chemicals such as endorphins and enkephalins from the brain and adrenal gland,(Stein,2009).This action is done because the near-infrared light stimulation targets cytochromec oxidase, a terminal enzyme whose role is to transfer electrons between complex III and IV within the respiratory chain. It is believed that cytochromec oxidase stimulation accelerates the transfer of electrons and promotes an up-regulation of oxidative phosphorylation, producing more adenosine triphosphate molecules (ATP). This stimulation promotes intracellular signaling as well as extracellular signaling, which it is believed to reduce edema and pain, (Jackson, et al., 2009).

6-Improves vascular activity: Laser light significantly increases the formation of new capillaries in damaged tissue that speeds up the healing process.

Additional benefits include temporary vasodilatation, and increase in the diameter of blood vessels.

7-Increases metabolic activity: Laser therapy creates higher outputs of specific enzymes, greater oxygen and food particle loads for blood cells

8-Improves nerve function: Slow recovery of nerve functions in damaged tissue can result in numbness and impaired limbs

9-Immunoregulation: Laser light has a direct effect on immunity status by stimulation of immunoglobulin and lymphocytes .

10-Trigger point and acupuncture point stimulation : Laser therapy stimulates muscle trigger points and acupuncture points on a noninvasive basis providing musculoskeletal pain relief.

2-3-11-Laser in medicine and surgery:

Laser is used as a very unique tool in all medical fields: diagnosis, treatment and surgical operations. They play nowadays a very important role both in diagnostics and surgery, having become in association with optical fibers a powerful tool.

Interior of the body which was otherwise difficult or impossible to be seen can now be seen very easily with the help of optical fibers.

Fiber - optic sensors are very useful both before and during surgery. The focused laser beam proved to be a new and unique "scalpel" in the hands of surgeons. Surgical operations with laser is a highly sterile process since contact does not occur between the surgical tools and the tissues being cut ; furthermore advantage is that the laser does not only cuts but also "welds "blood vessels being cut.

Operations with laser were done very fast and patients do not feel pain. The first big success of lasers in medicine was in the treatment of eye. Argon laser has been in use for several years to treat the detachment of the retina.

In dermatology, laser is used to cause homeostasis, the cessation of bleeding and removal of warts, freckles, acne and various other growths both malignant and benign. One typical example is birthmarks.

Dark-red birthmarks called "port wine stains "appear on the face or neck due to abnormal blood vessel networks under the surface of the skin. Such marks are treated by illuminating with the blue-green light from an argon laser .The radiation is absorbed by the blood and heats it up.

The area thus receives burns and in the process closes the blood vessels. Gradually the burns heal and the birthmarks are bleached. An important area of application of lasers is angioplasty for clearing the blocked arteries. In balloon angioplasty a thin catheter having a tiny deflated balloon is threaded through an artery to the blocked area.

In the urinary system the lasers are used in destroying kidney stones. An optical fiber is threaded through until it faces the stone directly. Lasers pulses launched through the fiber shatters the stone into small pieces that can pass through the ureter without pain. Tunable dye lasers are used in laser lithotripsy, same technique is used for gallbladder stones.

A leading cause of sterility in women is the blockage of one or both oviducts. Conventional surgery is risky in view of the delicate nature of the organs. However, treatment using laser is much simpler and more successful .The technique is similar to that of laser angioplasty.

At time tumors develop in brain and spinal cord which cannot be operated in conventional way because of their delicate nature, using laser in such surgeries. Surgery in such area is possible and much safer.

Welding of human blood vessels using laser is more advantageous than conventional suturing. Argon laser with 1mm spot size is used for this purpose. Argon pumped dye lasers are used to destroy viruses found in donor blood.

In dentistry, Q-switched ruby , CO2 and Nd- Yag lasers are found to arrest decay in teeth by reducing the rate of demineralization of tooth enamel .They are also used for drilling tooth cavities, figure (14) shows the different lasers wavelengths can be used in medicine and surgery,ciewd by ,(Martinm, 2008).

Laser canes and spectacles assist the blind. The laser cane is equipped with three diode lasers. One aims its beam downward, one upward and one straight ahead. Distance is sensed by vibration of the handle. The obstacle level is indicated by the pitch of vibration. In case of spectacle ,the temple pieces house IR LEDs, (Avadhanulu,2009)

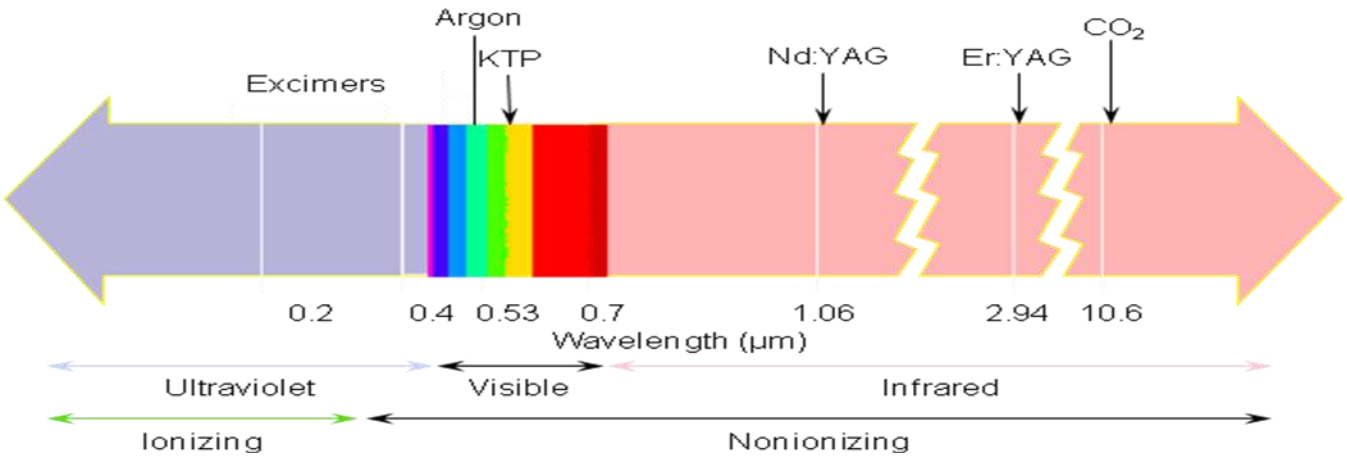


Figure (14): The different laser wavelengths can be used in medicine and surgery.

MATERIALS AND METHODS

3-1-Materials:

3-1-1-Animals: Twenty male adult New Zealand white rabbits, with one to one and half year aged and average weight of (1.5 -2Kg.) were used in this study. They were at least one year old as it is considered as the minimum age required for insuring growth cessation and more adults like metabolism. They were kept in standard separate cages and had free access to tap water and were fed with standard pellets. This model was selected because it provides many desired characteristics to fulfill the requirements of this study. All cages were kept in conditioned room 28-32˚c with controlled lightening .The animals left for one week before the experiment for adaptation.

3-1-2- Solutions and Reagents: All the solutions and reagents including the hormone analysing kits used are summarized in table; 3.

Table(3): Shows all solutions and reagents and their suppliers and sources.

No.	Solutions and Reagents	Sources &suppliers
1	Prostaglandin E2 ELA Kit	Cayaman Chemical Company /Czech
2	Prostaglandin F2α ELA Kit	Cayaman Chemical Company /Czech
3	Cyclic AMP ELI Kit	Cayaman Chemical Company/ Czech
4	Human Growth hormone ELI Kit	Bio Check/Forest City
5	Sterile normal saline solution (0.9 % MIV)	KIMADIA/Iraqi
6	Xylazine 20mg	ADWIA Co. S.A.E. of Ramadan city /Egypt
7	Ketamine Hydrochloride	Panther (London)LTD/10 Westboume Gardens, Billericay , Essex,CM12OUU,United Kingdom.
8	Alcohol 70%	ISO (Registered company) Iraq
9	Antiseptic spray(Chlortetracycline/Orondo spray)	Invesa Esmeralda 19 , E08950 Esplugues de Liobregat , Barcelona/Spain
10	Povidone – Iodine Sol. 10%	Al Ansari for Antiseptic ,ALEPPO-SYRIA
11	Penicillin	
12	Streptomycin	

3-1-3-Equipments & Instruments: Equipments and instruments used in this study are summarized in table; 4 and 5.

Table; (4) Shows the equipments used in the experiment and their suppliers.

No.	Equipments	Supplier
1	The medical laser diode system	Omega /
2	Eliza	BioKit/French
3	Centrifuge	Labtech co./Namyangui-city ,Kyonggi – Do ,Korea
4	Autoclave	Labtech Co.LTD. Namyangni – city, Kyonggi – Do, Korea

Table ;(5) Shows the instruments used in the experiment and their suppliers.

No.	Instruments	Supplier
1	Micropipette 1M , 10µ, 50 µ,500 µ(and Tips (small , large)	**Dragon Med**
2	Ordinary tube	**AFCO-DISPO, Jordan**
3	Scalpel & blades	**Aesculap ,Germany**
4	Scissors	**Aesculap ,Germany**
5	Artery forceps.	**Aesculap ,Germany**
6	Thumb forceps.	**Aesculap ,Germany**
7	Needle holder.	**Aesculap ,Germany**
8	Silk suture 3- 0	**ETHICON.LTD.UK.**
9	Disposable gloves.	**TG Medical /Malaysia**
10	Disposable syringes 2 & 3cc	**Medeco® Inject ,Abu Dhabi Medical Devices Co.L.L.C.**
11	Disposable masks and head caps	**Al Ansary, S.A.R**
12	Disposable surgical gowns.	**Al Ansary, S.A.R**
13	Surgical towels.	**Al Ansary, S.A.R**
14	Surgical Blades	**NFLB,CHINA**

Figures 15 , 16 and 17 show the solutions and reagents including the hormone analysing kits in addition to the instruments and the equipments used in the study.

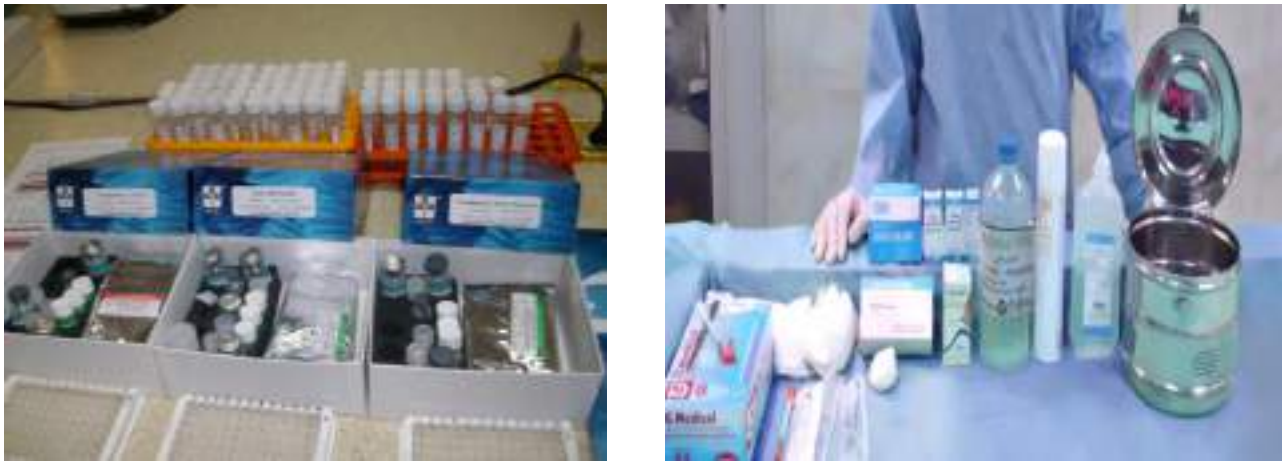


Fig. 15 ; L , The solutions and reagents including the hormone analysing kits , R, the instruments used

Fig. 16; L , Diode laser system (Omega) , R, the centrifuge

Fig. 17; Eliza Bio kit (French).

3-2-Surgical Technique:-

All instruments were sterilized in an autoclave at 121°c, 15 bar/cm, and 220 volt for 30 minutes. The site of the operation which was the lateral aspect of the left thigh clipped, shaved and disinfected using a piece of cotton damped with alcohol 70% for 10 minutes.

Surgery was performed under sterile conditions ,The animal injected first with a premeditation drug Acepromizine malete 10 mg /kg B.W administrated i/m ,after 5 minute the animal given the anaesthesia by injecting a mixture of Xylazine 5 mg /B.W and Ketamine hydrochloride 10 mg /kg B.W injected i/m, the animal reached to the stage of surgical anaesthesia 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 thigh faced to the surgeon, and left for 10 minutes to allow the surgeon to prepare himself while the disinfection and anesthesia reach the peak, then the piece of the cotton was removed and surgical towels were placed around the site of the operation , figure 18 , shows the animal lies anesthetized with the left thigh prepared for the surgical operation , a piece of cotton impregnated with alcohol used to disinfect the area.


Figure 18; L , the animal lies anesthetized with the left thigh prepared for the surgical operation , R, a piece of cotton impregnated with alcohol used to disinfect the area.

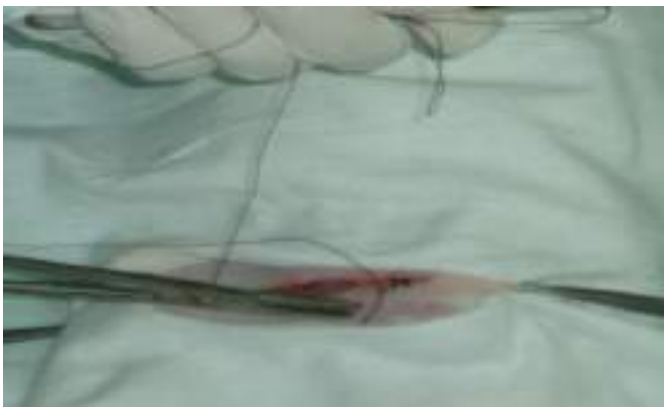



Figure 19; L, an incision with 7cm length was done in the lateral aspect of the left thigh in the animals of group A . R , closing the wound using stitches of simple interrupted 3-0 silk .

In the animals of group A an incision with 7cm length was done in the lateral aspect of the left thigh and involve the whole thickness of the skin , then it was closed using stitches of simple interrupted sututers using 3-0 silk, figure 20 , then the animal injected with systemic antibiotics, penicillin 1000i.u. kg. B.W. and streptomycin 10 mg/kg .B.W. i/m for 3 days post the operation ,while the animals at the group B underwent surgical operations to remove a square peace of a whole thickness of the skin with 1 cm^2 in diameters, figure 20.Then the site of the operation in all the animals was treated with antibiotic spray, figure 21.

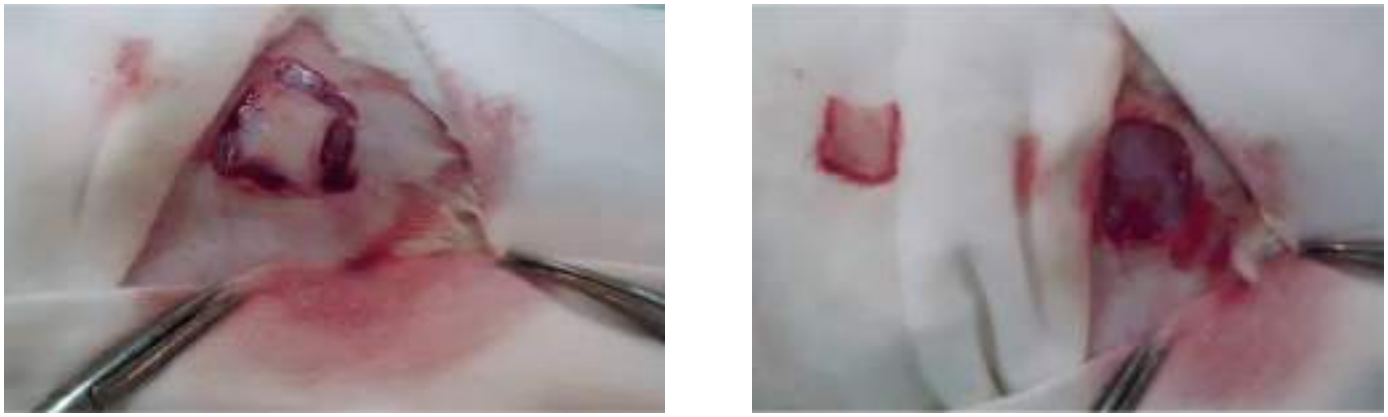


Figure 20; removing a square peace of a whole thickness of the skin with 1 cm^2 in diameters from the animals of group B.

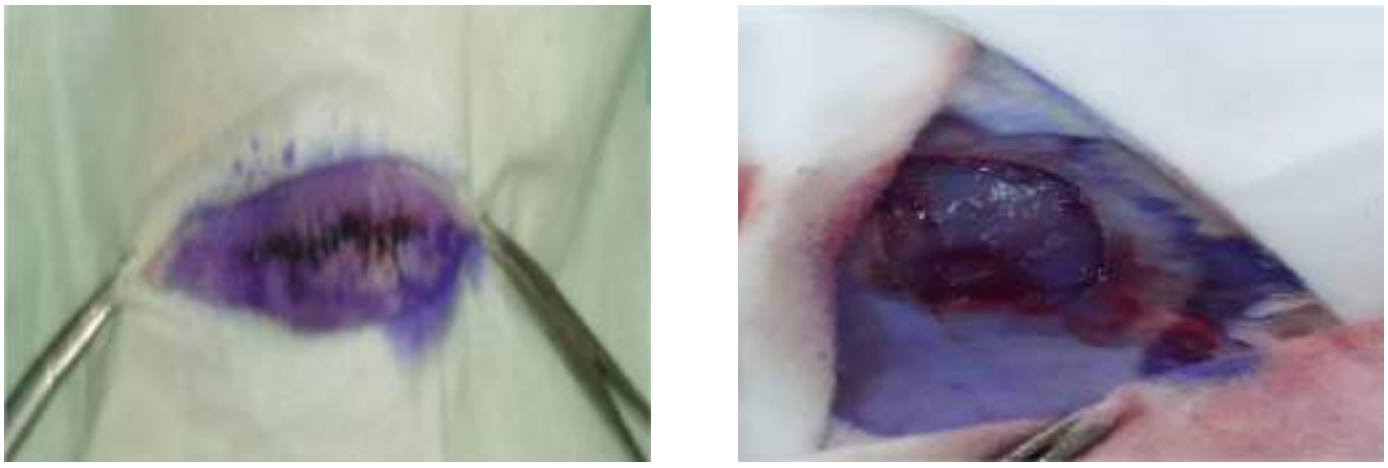


Figure 21; the site of the operation in all the animals was treated with antibiotic spray.

Then the both groups were subdivided in to two subgroups ; control & treated with 5 rabbits each , the difference between the two subgroups that the treated sub groups were irradiated directly after the operation and for 5 subsequent days with laser diode 200 mw for 1.2 min /session .

The experiment carried out in August 2010 and at the surgical theater of the animal`s house of the Faculty of Science.

The animals of the first group (induced wound group) were examined daily observed in 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, if the wound was infected, also they were examined looking for the type of approximation of the wound`s edges and the healing processes.

While the animals of the second group (skin loss group) were examined every 3 days using a very precise caliper to determine the diameter of the skin defects. Also the skin defects were investigated looking for any Infection with a large number of bacteria i.e. colonization which may slow the healing process or contamination with just small number of bacteria which usually does not affect the healing process. The difference between contamination and colonization is the concentration of bacteria.

Signs of infection include red skin around the edges of the wound, discharge containing pus, swelling, warmth, foul odor, and fever.

3-3-laser diode :-

It is a laser where the active medium is semiconductor crystal typically constructed of Ca Al As (gallium aluminum arsenide) its convert an electrical current into light the conversion process in fairly efficient in that is generates little heat compared to incandescent lights .

. The laser used was diode 820nm wave length, a maximum output of 200 mW, density 8J/cm², pulsing frequency 1-10 Hz. Irradiation began after the operation directly and continued for 5 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 (1cm) distance from the wound or around the square area of the lost skin.

3-4-Blood sampling:

Blood samples were collected at regular intervals from the marginal ear vein right after disinfection using 70% alcohol, The amount of blood about 3 cc, blood samples were collected using syringes 2-3 ml , and then serum was separated by centrifuge 2500 cycles / minute for 15 minutes. Serum was used to measure each of: -PGE2, PGF2α, cAMP and GH.

3-5-ELIZA assay:- Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA), is a biochemical method used in the laboratory to aid in the diagnosis of several medical conditions by detecting the presence of antibodies or antigens in the blood or other bodily fluids.

An ELISA or enzyme-linked immunosorbent assay, is a method used in the laboratory to aid in the diagnosis of a wide range of diseases. This test is performed on blood or urine and is used for measuring the amount of a particular protein or substance in these bodily fluids, such as infectious agents, allergens, hormones or drugs.

This test relies on the interaction between components of the immune system called antigens and antibodies. Antibodies are proteins produced by the body to identify and neutralise any foreign substances that may be encountered, such as viruses and bacteria. The substances to which antibodies are produced are known as the antigens as they stimulate an immune response.

3-5-1-Work steps (PGE2,PGF2α and cAMP):-

The methods of each Kit of PGE2,PGF2α,and cAMP were similar, and the difference was in the basic materials and reagents (the labels), Because of this manner remind one working together with the differences among them.

3-5-1-1-Addition of the reagents

1-**ELIBuffer**

Add 100µl ELI Buffer to Non-specific Binding (NSB)wells .Add 50µl ELI Buffer to Maximum Binding (B0) wells .

2-**cAMP ELI Standard**. (PGE2 ELA Standard,PGF2α ELA Standard) Add 50µl from tube #8 to both of the lowest standard wells (s8). Add 50µl from #7 to each of next tow standard wells (s7).Continue with this procedure until all the standards are a liquated . The same pipette tip should be used to aliquot all the standards . Before pipetting each standard , be sure to equilibrate the pipette tip in that standard .

3-**Samples.**

Add 50µl of sample per well .

4-**cAMP AChE Tracer** .(PGE2 AChE Tracer,PGF2α AChE Tracer)

Add 50µl to each well except the Total Activity (TA) And the Blank (Blk)well .

5-**cAMP ELA Antiserum**. (PGE2 Monoclonal Antibody , PGF2α ELA Antiserum)

Add 50µl to each well except the Total Activity (TA) , the Non – specific Binding (NSB) , and the Blank (Blk) wells.

3-5-1-2-Incubation of the plate.

Cover each plate with plastic film (Item No. 400012) and incubate 18 hours at 4ºC.

C-Empty the wells and rinse five times with Wish Buffer .

D-Add 200 µl of Ellman's Reagent to each well .

E-Add 5 µl to the Total Activity wells.

F-Cover the plate with plastic film . Optimum development is obtained by using an orbital shaker equipped with a large , flat cover to allow the plate (s) to develop in the dark . This assay typically develops in 90-120 minutes.

3-5-1-3-Reading the plate .

1-Wipe the bottom of the plate with a clean tissue to remove fingerprints , dirt ,etc.

2-Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover .

3-Read the plate at a wavelength between 405 and 420 nm .

3-5-2-Estimate Growth Hormone:-

The level of hormone GH followers of the way Elisa Kit No. CA 94404, from the factory by a company Bio Check, Inc.

Principle of the test :-

The HGH Quantitative test kit is based on the principle of solid phase enzyme –linked immunosorbent assay (ELISA). The assay system utilizes a sheep anti- HGH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-HGH antibody in the antibody – enzyme (horseradish peroxidise) conjugate solution .

The test sample is allowed to react simultaneously with the antibodies , resulting in HGH molecules being sandwiched between the solid phase and enzyme – linked antibodies . After a 45 – minute incubation at room temperature , the wells are washed with water to remove unbound – labelled antibodies . A solution of TMB reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution and the color is changed to yellow and measured spectrophotometrically at 450 nm .The concentration of HGH is directly proportional to the color intensity of the test sample .

3-5-2-1-Reagents –

1-Sheep Anti-HGH coated microtiter plate with 96 wells.
2-Reference standard set ,containing 0,2.5,5,10,25,and 50 ng/ml HGH (WHO,1st IRP 66/217),lyophilized .
3- Enzyme Conjugate Reagent ,13 ml .
4-TMB Reagent (One – Step),11ml.

5-Stop Solution (1N HCl),11ml.

3-5-2-2-Assay Procedure –

1-Secure the desired number of coated wells in the holder.

2-Dispense 50 µl of standard, specimens, and control into appropriate wells.

3-Dispense 100 µl of Enzyme Conjugate Reagent into each wells.

4-Thoroughly mix for 30 seconds . It is very important to have a complete mixing in this setup .

5- Incubate at Room temperature (18-25ºC) for 45 min.

6-Remove the incubation mixture by flicking plate content into a waste container .

7-Rins and flick the microtiter wells 5 time with distilled or deionized water .

8-Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets .

9-Dispense 100 µl TMB reagent solution into each well. Gently mix for 10 seconds .

10-Incubate at room tem. In the dark for 20 min.

11-Stop the reaction by adding 100 µl of Stop Solution to each well.

12-Gently mix for 30 seconds .

13-Read the optical density at 450 nm with a micro titer plate reader with in 15 min.

3-5-2-3-Calculation of Results

1-Calculate the average absorbance value (A450)for each set of reference standard , control, and samples.

2-Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on liner graph paper ,with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis .

3-Using the mean absorbance value for each sample , determine the corresponding concentration of HGH in ng/ml from the standard curve .

3-6- Statistical Evaluation Methods:

The efficacy of L.L.L.T. has been evaluated by:

a- Minitab using the 2 samples t test, the data was presented as means; the mean values were calculated using Microsoft Excel.

b- SPSS Regression test.

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 superlative exudates investigated.

Two of the animals of the treated subgroup showed clinical wound healing within three days while the remainder showed complete healing and stitches' removal at the fourth postoperative day.

The wound healing of the animals of the control group take approximately eight days, some of the animals showed clinical wound healing at the seventh postoperative days, table (4- 6).

Table (4-6): Clinical wound healing of the both subgroups of the 1st. group (wound healing group).

Subgroups	**Animals**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
Control C	1							√			
	2							√			
	3								√		
	4							√			
	5								√		
Treated T	1				√						
	2				√						
	3				√						
	4			√							
	5			√							

No complications were seen associated with the healing processes in the animals of the both subgroups because they were all injected with a mixture of antibiotic daily after the operation directly and for three days after that.

The skin defects of the animals of the seconds group were measured using a fine measurement caliber looking for the stages of the defect`s contraction and sealing, it was faster in the animals of the treated subgroup taking nine days for complete sealing of the defects, 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 the both subgroups,(table 4-7).

The values of diameter of the skin defect in the animals of the 2nd group for the animals of both subgroups of the 2nd group showed significant variations between the two subgroups P > 0.05, table (4-7) and figures (22).

Table (4- 7): Diameter of the skin defect in the animals of the 2nd group estimated in cm^2.

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 & diameter of the defect), followed by the 1st degree equation (linear), so any additional day passed lead to regression in the diameter of the defect, explained by the R^2 which were 0.94 and 0.99 for both the control and laser treated subgroups respectively.

The regression value in the treated subgroup was 0.11, it estimates a triple when compared with the control subgroup regression value which was just 0.03, fig: 22.

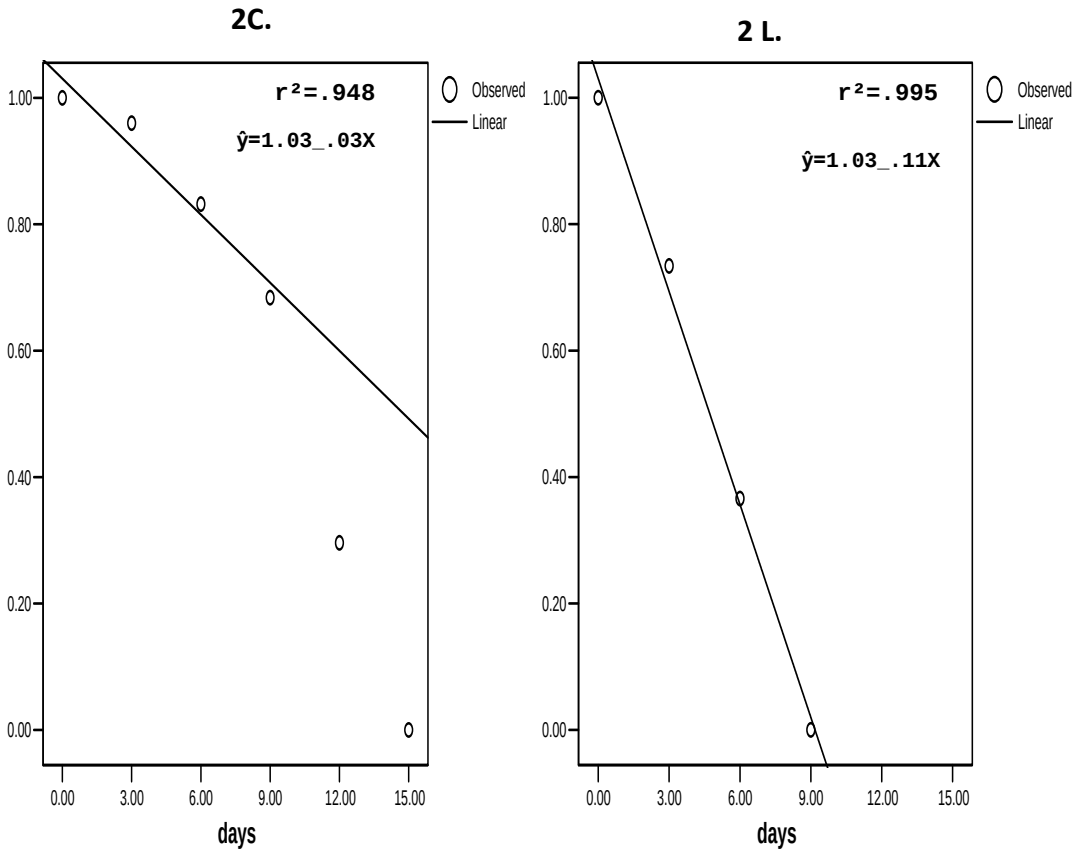


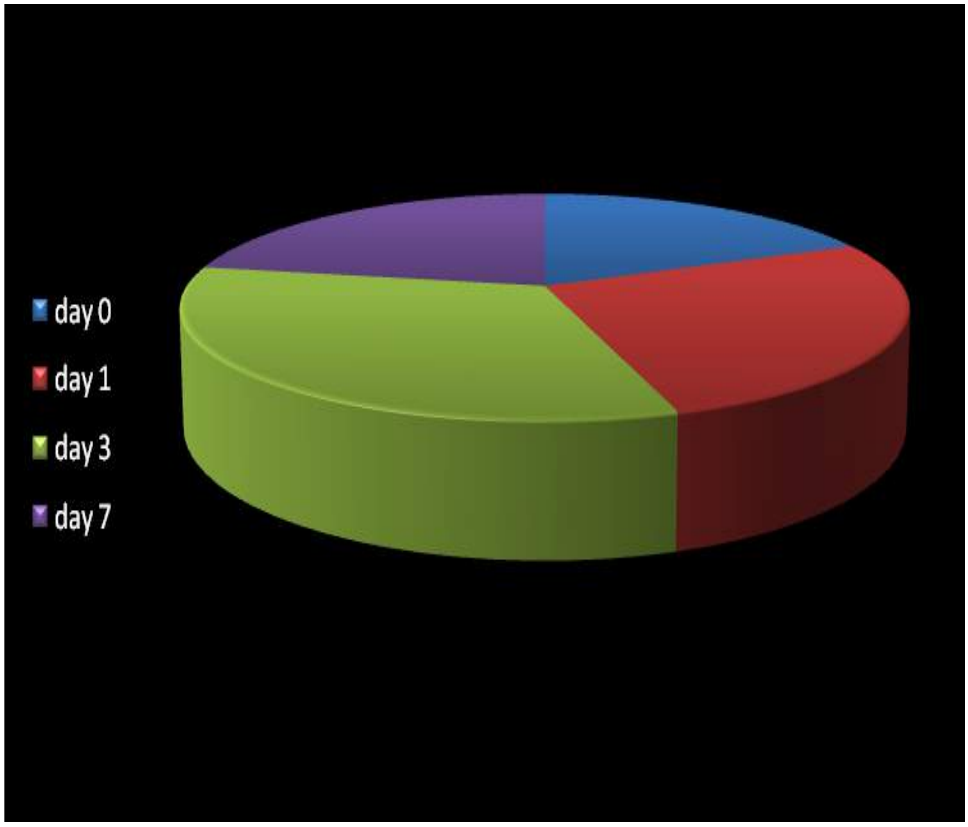
Figure (22): SPSS regression test for the animals of both subgroups for skin loss diameters.

- The vertical axis represent the diameters of skin loss estimated in cm^2.
- The Horizontal axis represent the days .

The results of the Eliza test for the hormones showed significant variations in the values of PGE2, between the two subgroups of the 1st group, P > 0.05, table (4- 8) and figures (23, 24).

Table (4-8): Values of PGE2 in the animals of the 1st group.

Subgroups	**Days**	**Samples**					
Con.of Prostaglandin E2 PGE2(pg/ml)							
Control C		1	2	3	4	5	**Mean**
	0 Day	241.1	227.7	239.4	240.1	230.8	**235.82**
	1st. Day	331.0	335.7	330.5	340.5	341.8	**335.9**
	3rd. Day	400.7	420.0	415.7	418.7	425.9	**416.2**
	7th. Day	279.5	280.5	282.9	281.4	283.5	**281.65**
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**



Figure(23): The values average of PGE2 in the animals of the control subgroup.

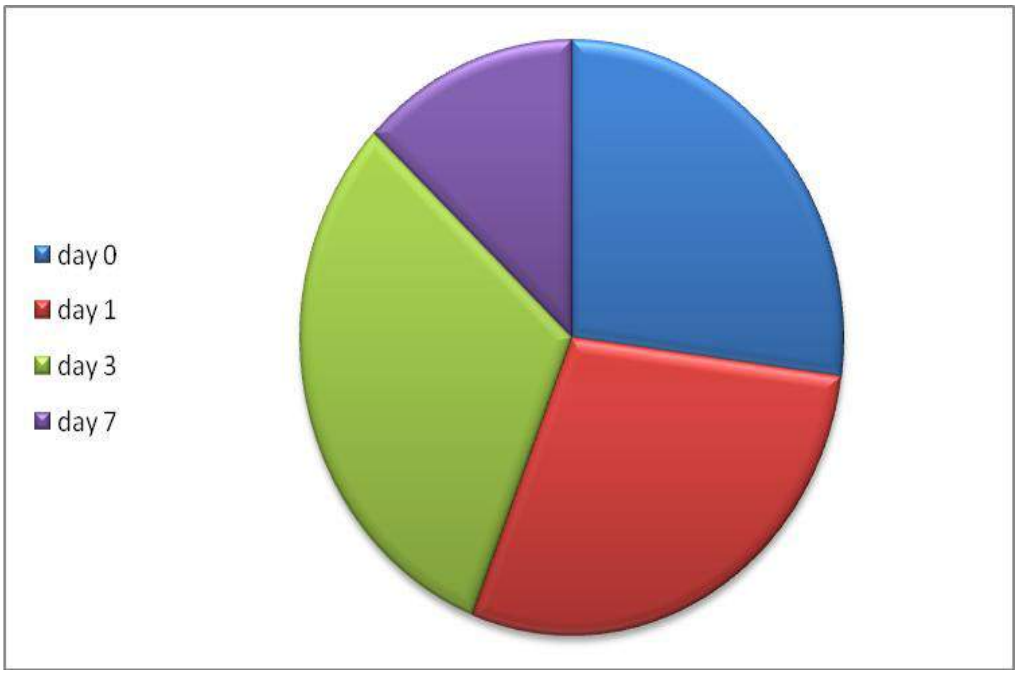


Figure (24): The values average of PGE2 in the animals of the treated subgroup.

Results of regression test showed that there were a relationship between the two variants (time & PGE2 level), following the 2nd degree equation, so any additional day that passed lead to increase in the level of the hormone, but till the 3rd, day then it begin to decrease by time the correlations between the two subgroups was high reaching 0.99.

At the zero time, we can see that the level of the hormone in the treated subgroup was double when compared with the control one, and we can also see that the clearing rate of the hormone in the treated subgroup was higher than that of the control one, fig 25.

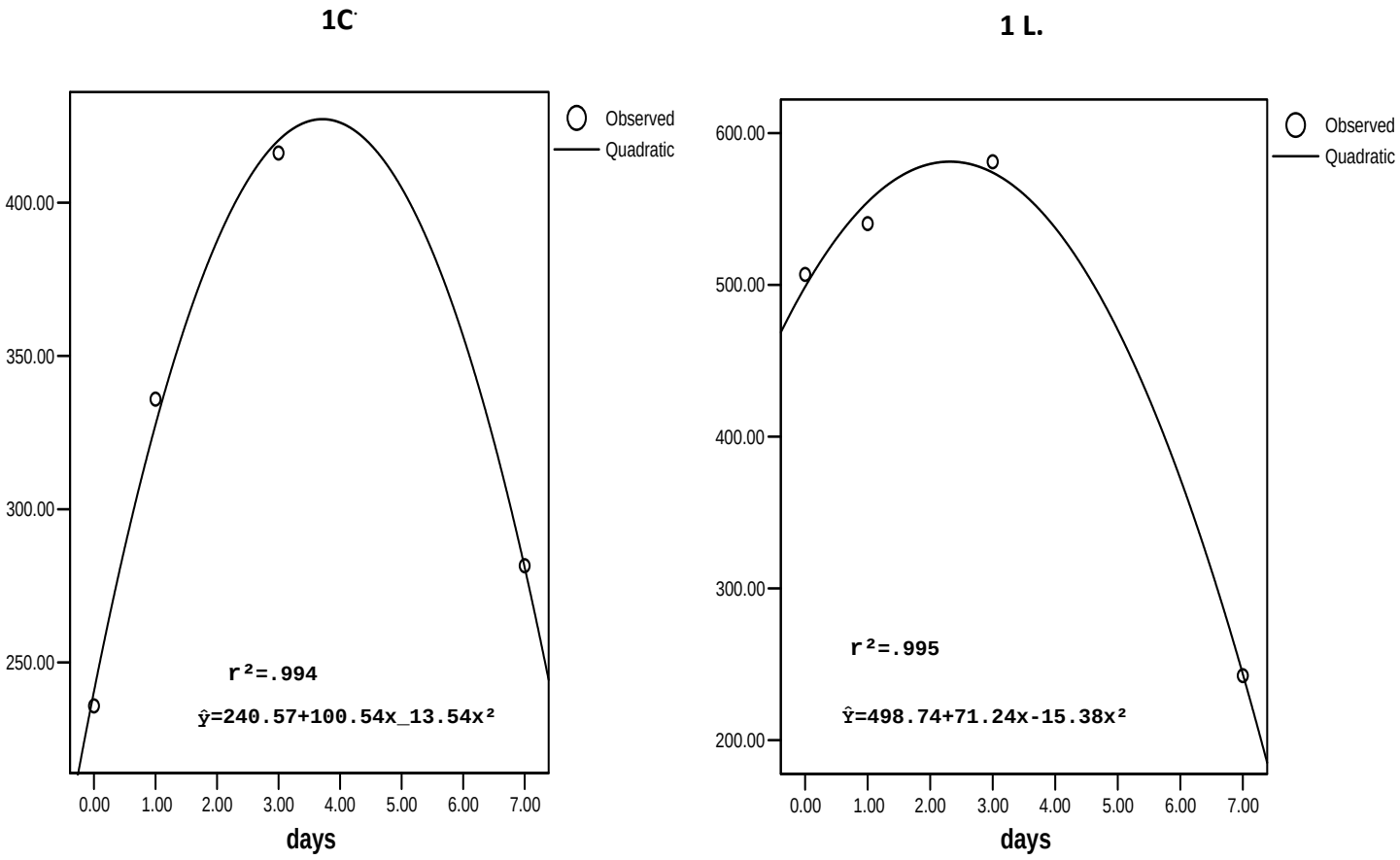


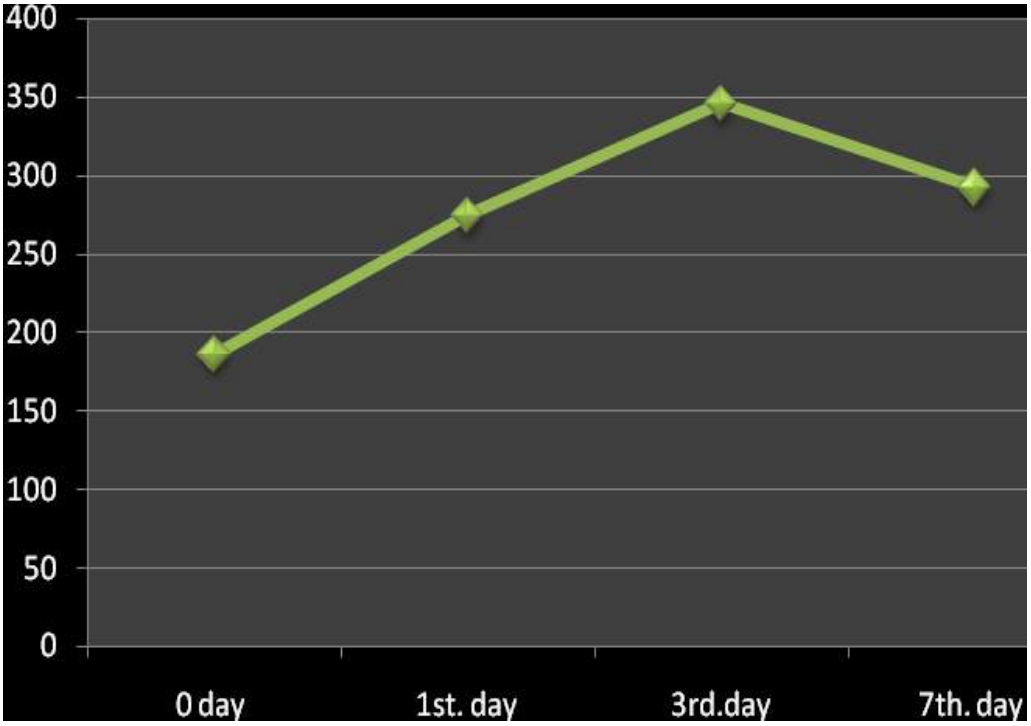
Figure (25): SPSS regression test for the animals of both subgroups for PGE2.

- The Vertical axis represents the concentration of PGE2 estimated in (pg/ml).
- The Horizontal axis represents the days .

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, table (4- 9) and figures (26, 27).

Table (4-9): Values of PGF2α in the animals of the 1st. group.

Subgroups	**Days**	**Samples**					
Con.of Prostaglandin F2α PGF2α (pg/ml)							
Control C		1	2	3	4	5	**Mean**
	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**



Figure(26): The values average of PGF2α in the animals of the control subgroup.

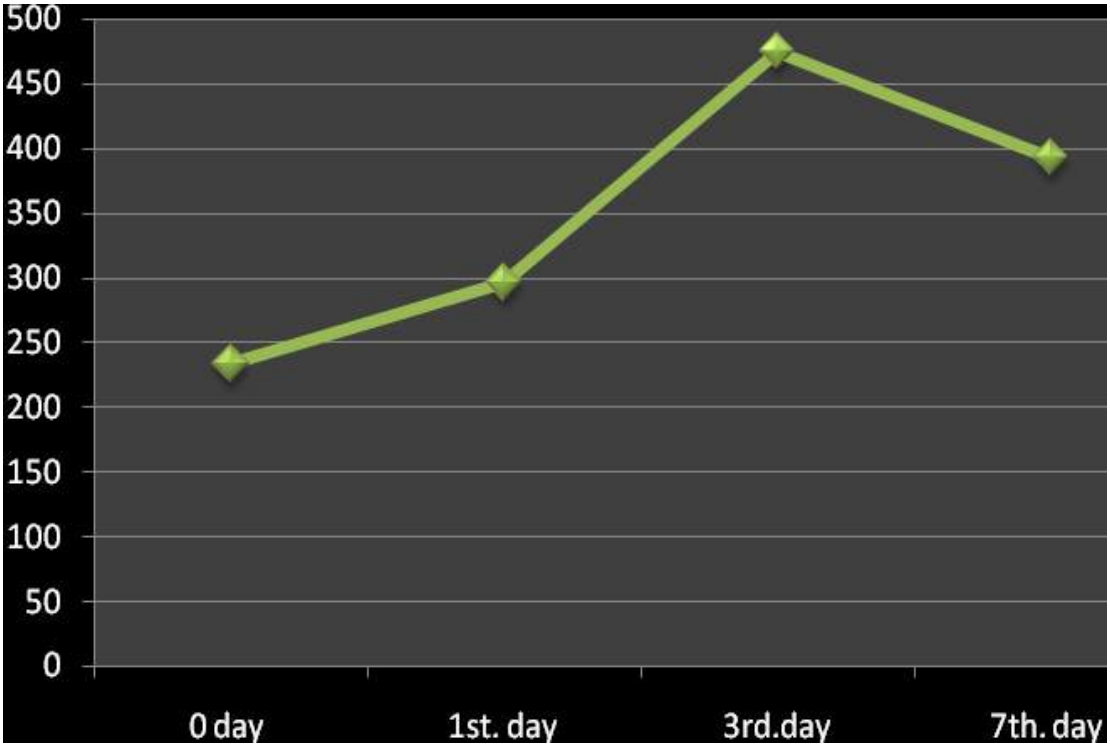


Figure (27): The values average of PGF2α in the animals of the treated subgroup.

Results of regression test showed that there were a relationship between the two variants (time & PGF2α level), followed by the 2nd degree equation, The r^2 was 0.95 and 0.99 for both the control and treated subgroups which means that there was a strong correlation between the two variants.

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, fig. 4-21.

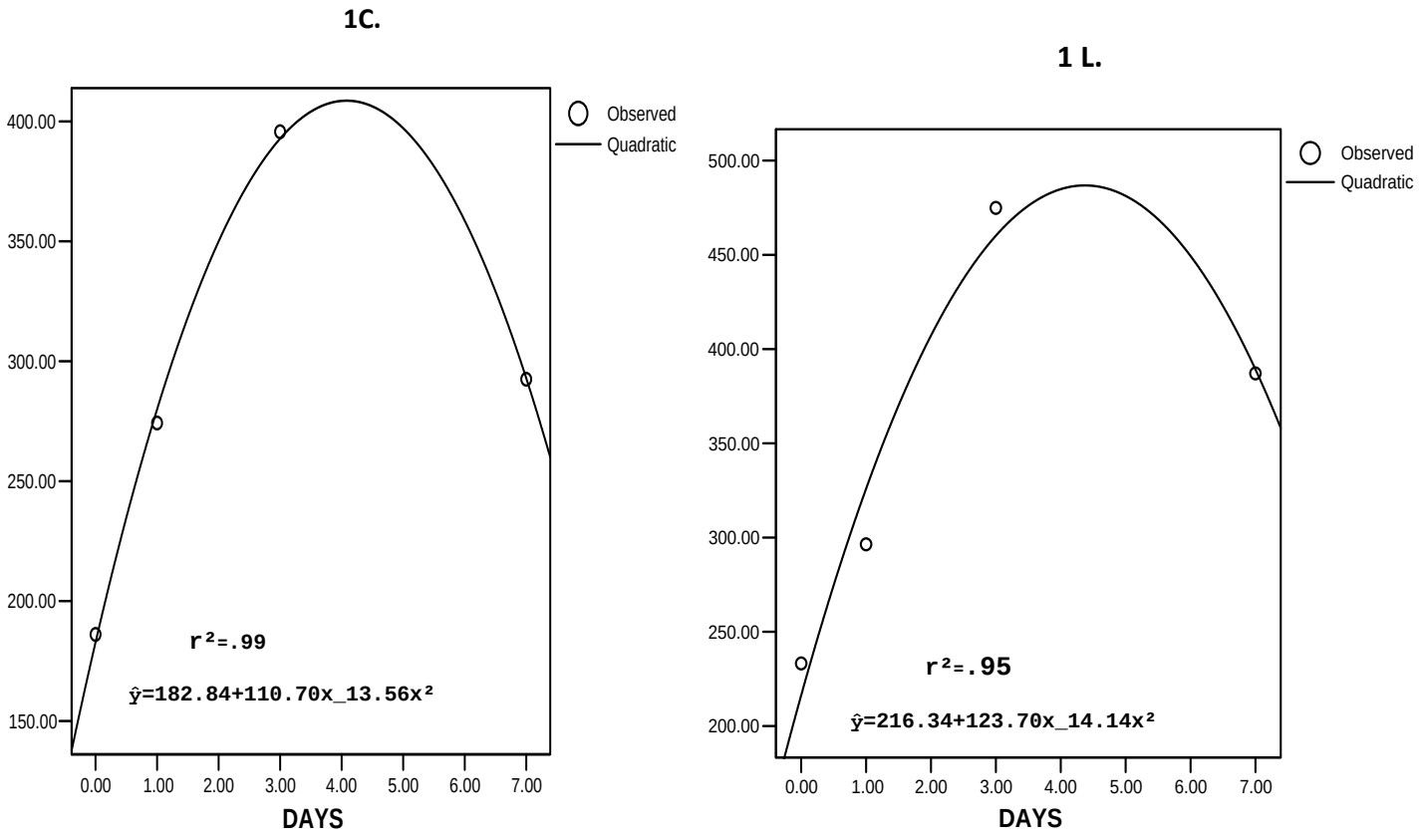


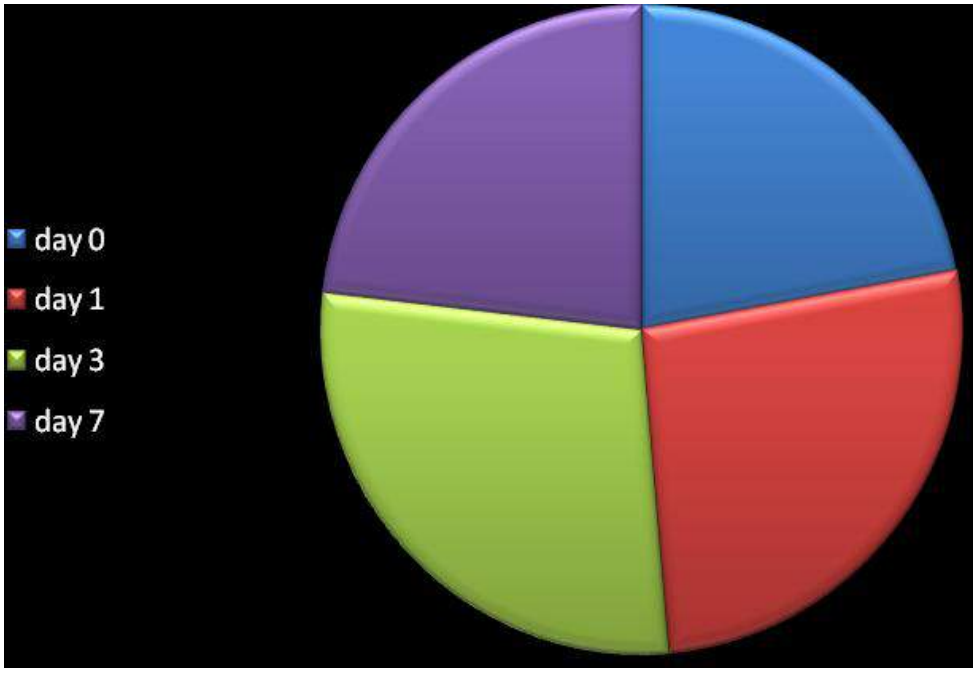
Figure (28): SPSS regression test for the animals of both subgroups for PGF2α.

- The Vertical axis represents the concentration of PGF2α estimated in (pg/ml).
- The Horizontal axis represent the days.

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 (4- 10) and figures (29, 30).

Table (4-10): Values of c AMP in the animals of the 1st group.

Subgroups	**Days**	**Samples**					
Con.of c AMP(pg/ml)							
Control C		1	2	3	4	5	**Mean**
	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**



Figure(29): The values average of cAMP in the animals of the control subgroup.

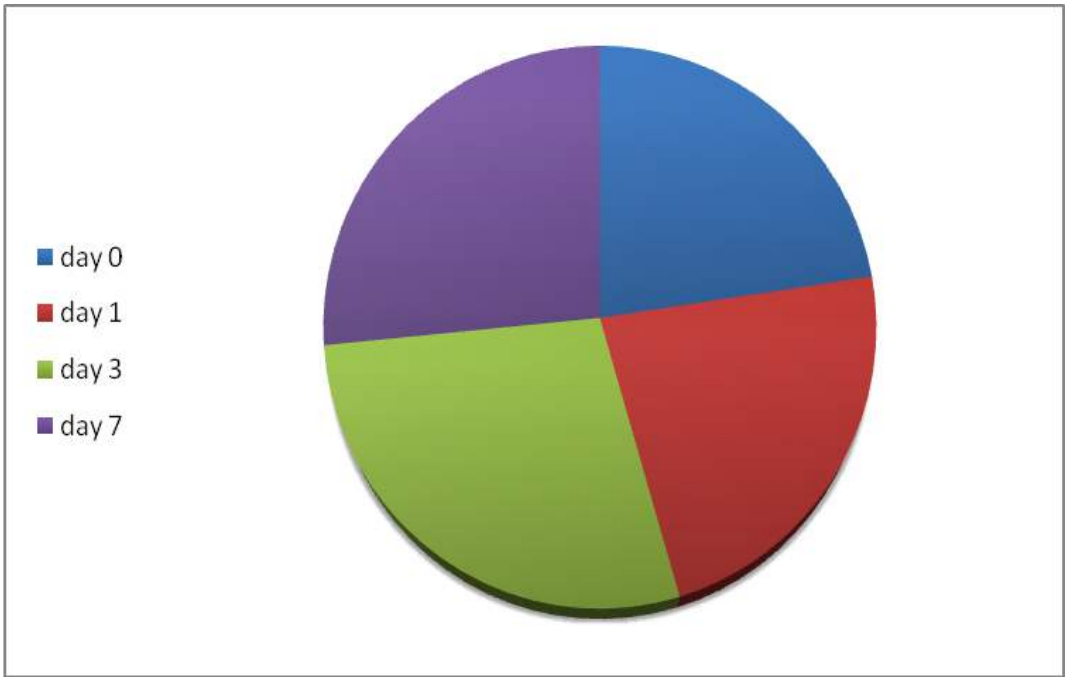


Figure (30): The values average of cAMP in the animals of the treated subgroup.

The results of regression test showed that there were a correlation of the 2nd degree between the two variants (time and cAMP level) . and that there were an increase in the level of the enzyme by time but reaching to a specific value where it began to decrease in both the subgroups.

The degree of r^2 between the two variants was 0.86 and 0.94 in both the control and treated subgroups respectively, the R^2 degree of the level of the enzyme was higher in the treated subgroup and it`s clearing was higher also, fig. (31).

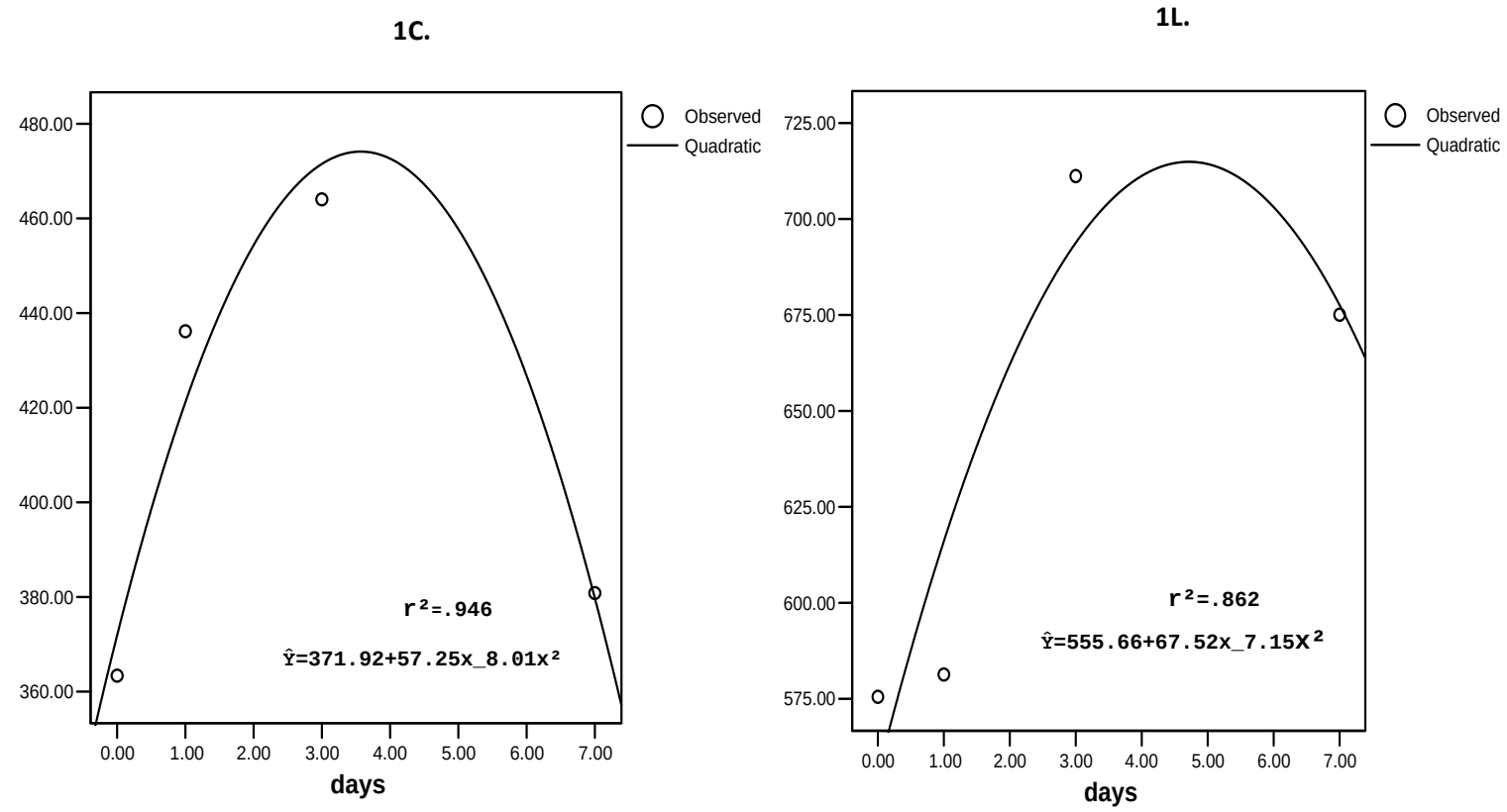


Figure (31): SPSS regression test for the animals of both subgroups for cAMP.

- The Vertical axis represents the concentration of cAMP estimated in (pg/ml).
- The Horizontal axis represents the days.

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 (4- 11) and figures (32, 33).

Table (4-11): Values of GH in the animals of the 1st. group.

Subgroups	**Days**	**Samples**					
Con.of Growth Hormone GH(ng/ml)							
Control C		1	2	3	4	5	**Mean**
	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**

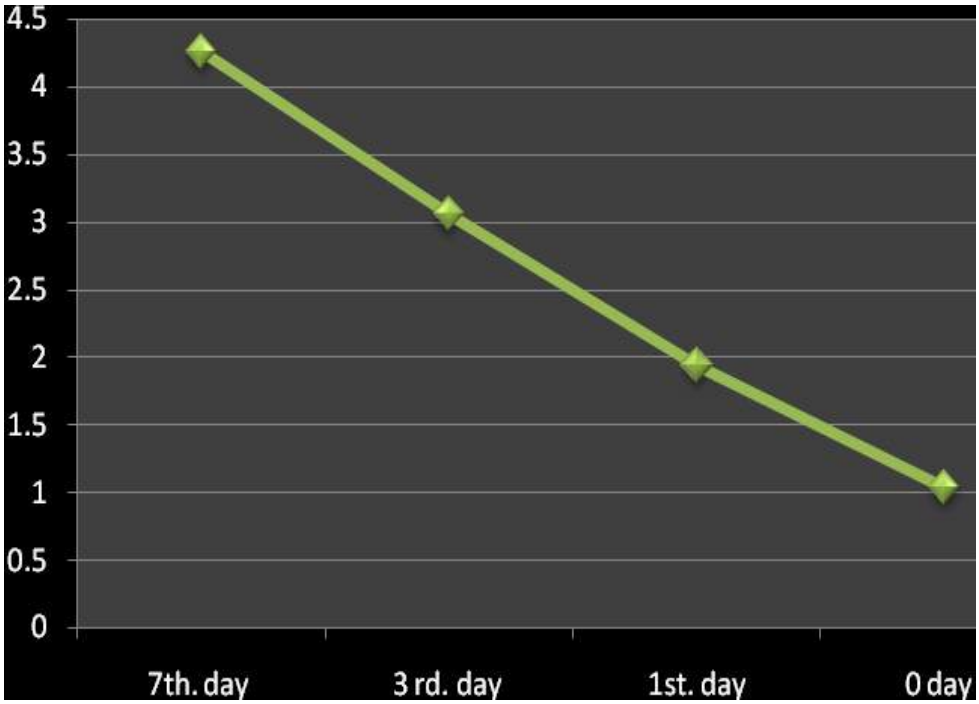


Figure (32): The values average of GH in the animals of the control subgroup.

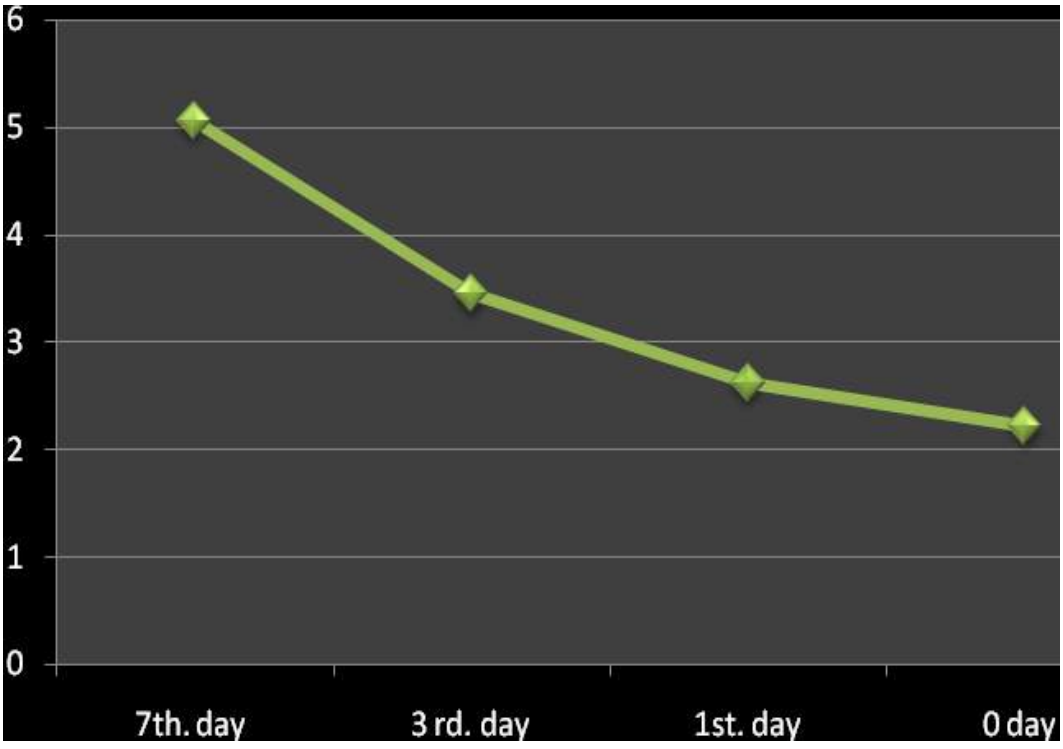


Figure (33): The values average of GH in the animals of the treated subgroup.

The relationship between the two variants (time and GH level) was of the 2nd degree (curved) for the control subgroup and linear for the treated subgroup.

The r^2 was very high reaching 0.99 and 1 in both the control and treated subgroups respectively, which means 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, fig;(34)

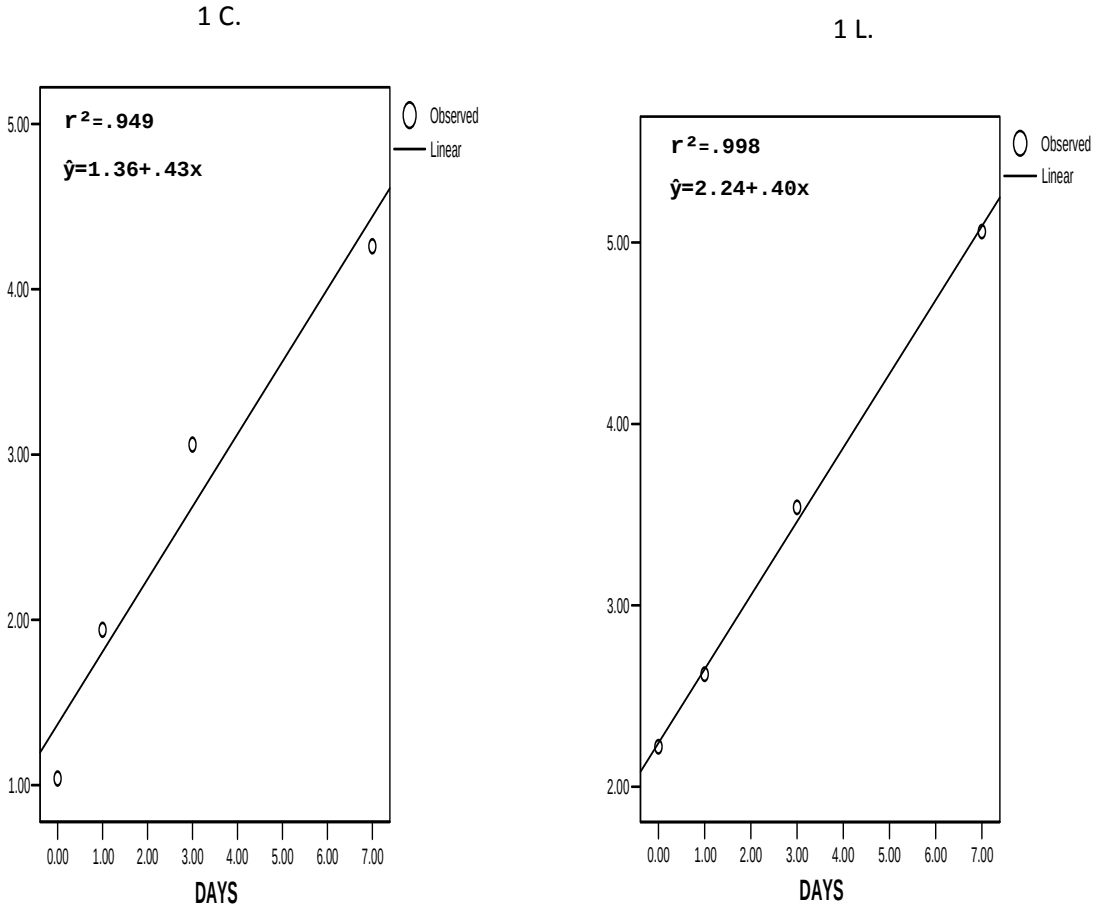


Figure (34): SPSS regression test for the animals of both subgroups for GH.

- The Vertical axis represent the concentration of GH estimated in (ng/ml).
- The Horizontal axis represent the days.

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- 12) and figures (35, 36).

Table(4-12): Values of PGE2 in the animals of the 2nd group.

Subgroups	**Days**	**Samples**					
Con.of Prostaglandin E2 PGE2(pg/ml)							
Control C		1	2	3	4	5	**Mean**
	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**

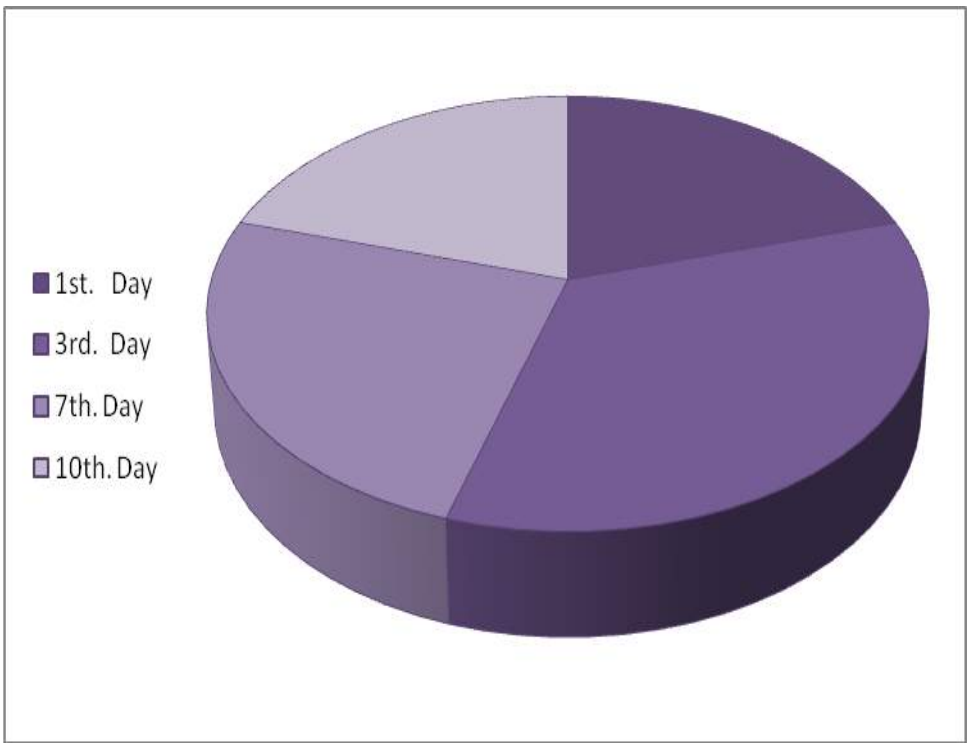


Figure (35): The values average of PGE2 in the animals of the control subgroup.

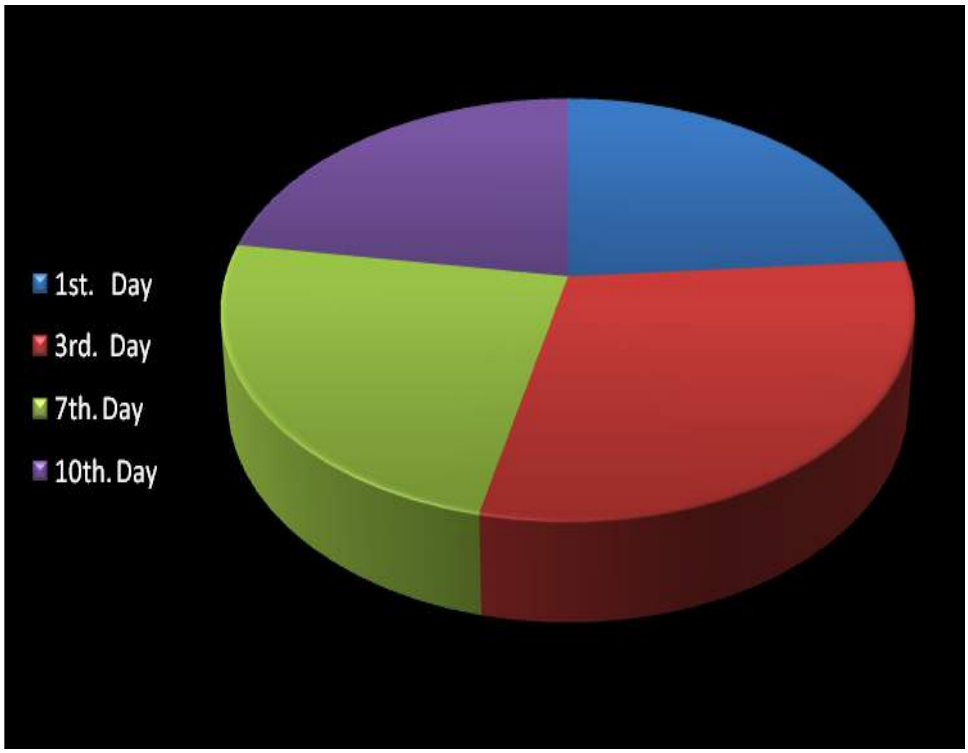


Figure (36): The values average of PGE2 in the animals of the treated subgroup.

The regression test showed that the relationship between the two variants (time and PGE2 level) was linear and curve and that there was an increase in the level of the hormone in both groups reaching to a value then ceased by time , this regression occurred in a slow rate between the two variants which were represented by r^2, it`s value was ٠.5680and ٠.515 in both subgroups respectively, fig. (37) .

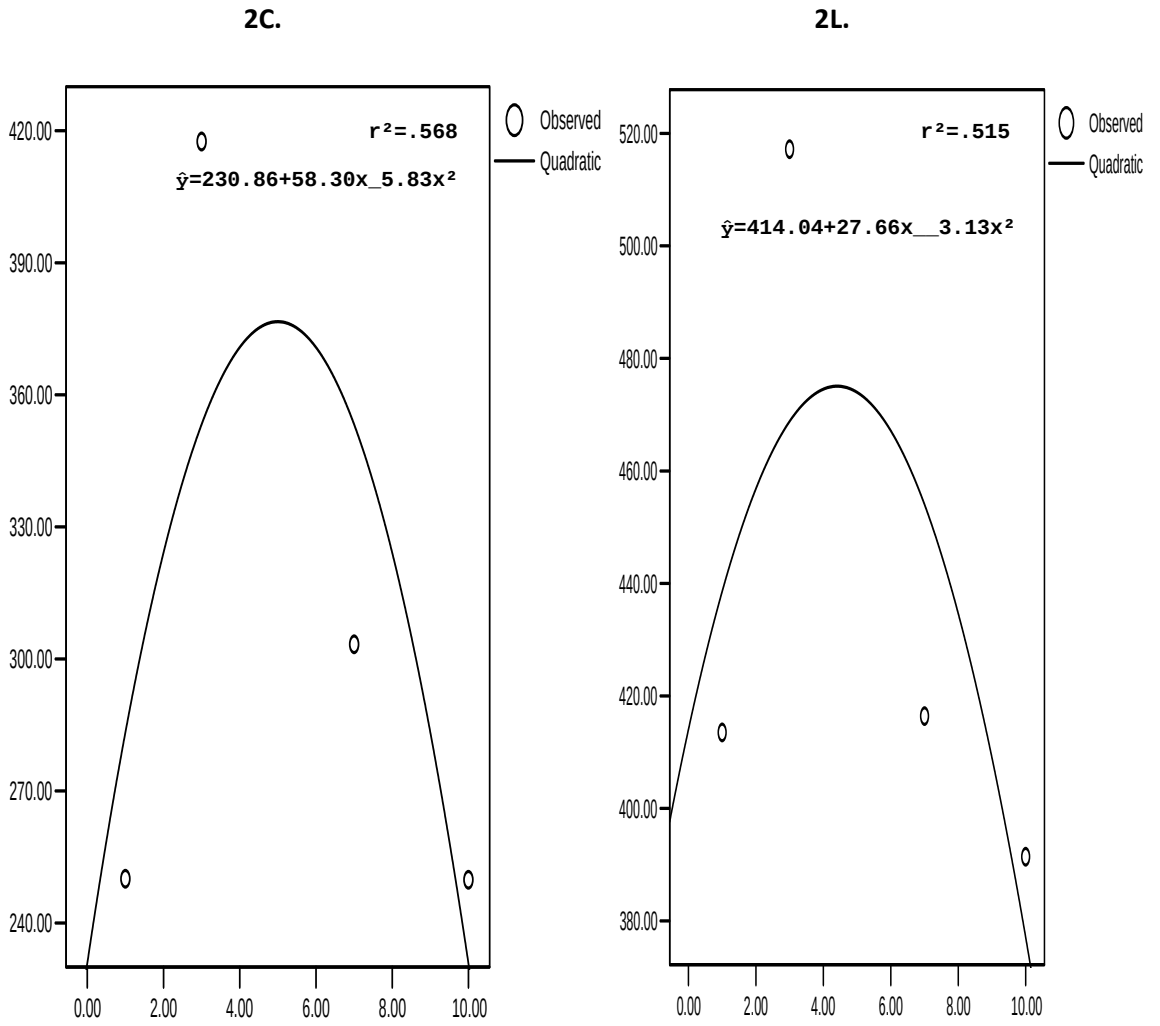
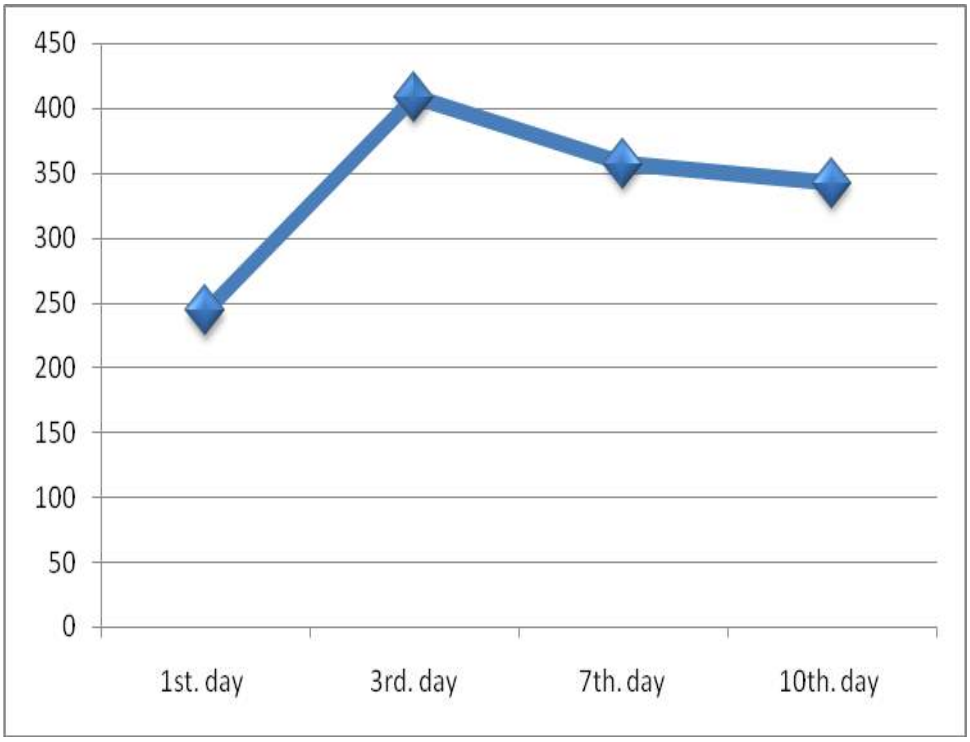


Figure (37): SPSS regression test for the animals of both subgroups of the 2nd group for PGE2.

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- 13) and figures (38, 39).

Table (4-13): Values of PG F2α in the animals of both subgroups of the 2nd. group.

Subgroups	**Days**	**Samples**					
Con.of Prostaglandin F2α PGF2α (pg/ml)							
Control C		1	2	3	4	5	**Mean**
	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**



Figure(38): The values average of PGF2α in the animals of the control subgroup.

Figure (39): The values average of PGF2α in the animals of the treated subgroup.

The relationship between the two variants was of the 2nd degree, the level of the hormone stopped increasing after a several days then begin to decrease.

At the zero time the level of the hormone in the control subgroup was higher than the treated one , also the percentage of the hormonal increase fig. 40.

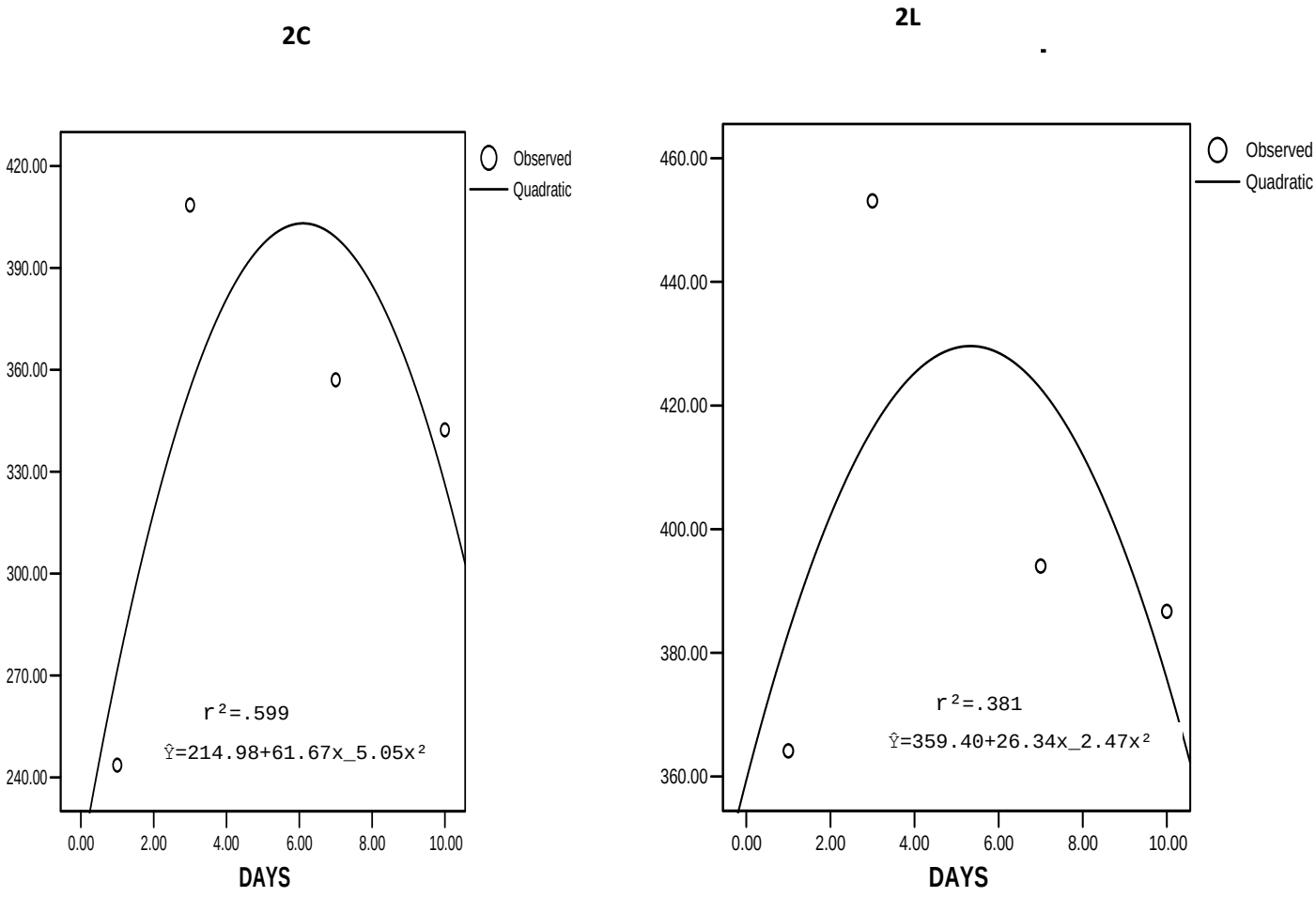


Figure (40): SPSS regression test for the animals of both subgroups of the 2nd group for PGF2α.

- The Vertical axis represents the concentration of PGF2α estimated in (pg/ml).
- The Horizontal axis represent the days .

The values of hormonal assessment of c AMP for the animals of both subgroups of the 2nd group showed significant variations between the two subgroups P > 0.05, table (4- 14) and figures (41, 42).

Table(4-14): Values of c AMP in the animals of the 2nd group.

Subgroups	**Days**	**Samples**					
Con.of c AMP(pg/ml)							
Control C		1	2	3	4	5	**Mean**
	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**

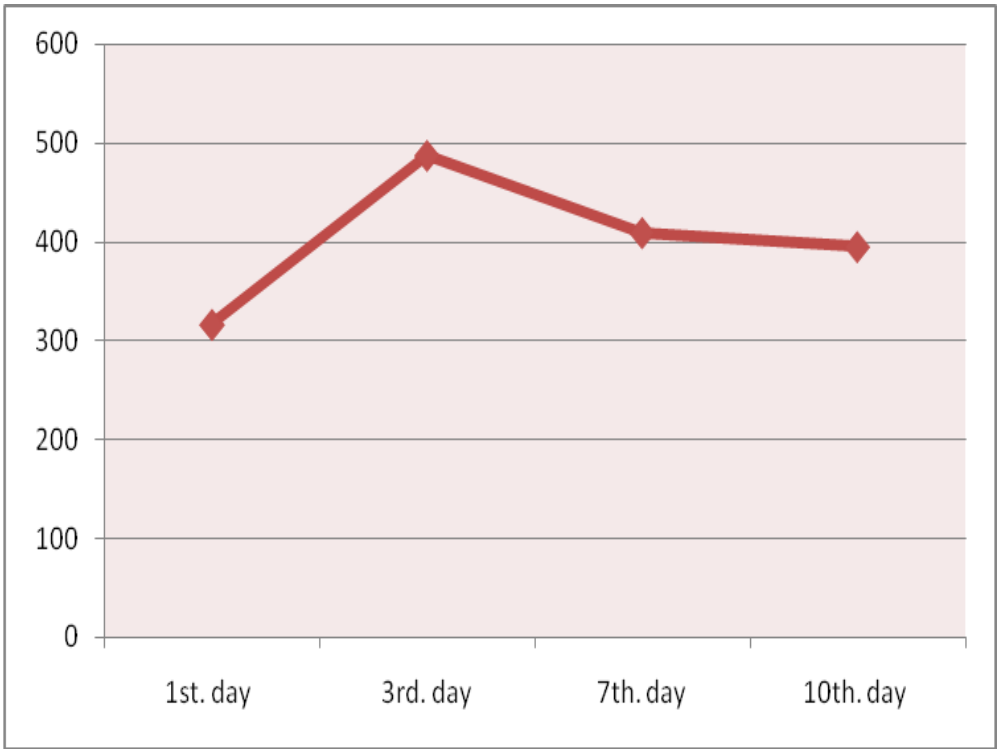


Figure (41): The values average of cAMP in the animals of the control subgroup.

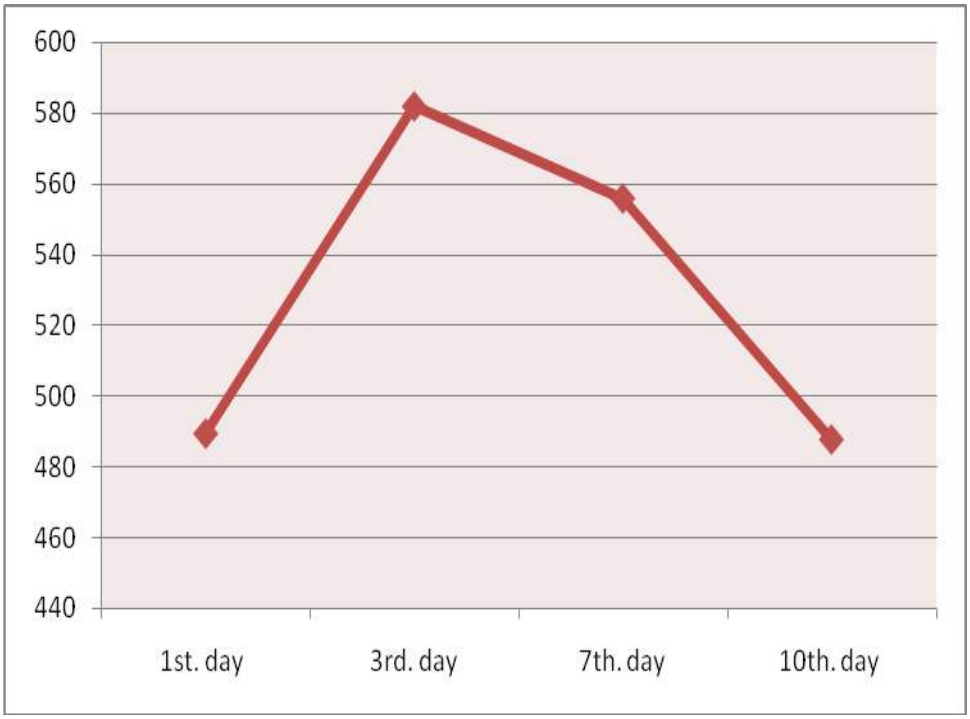


Figure (42): The values average of cAMP in the animals of the treated subgroup.

There was an increase in the level of the cAMP by time but reaching to a limited value when it began to cease, the level of regression was approximately equal in both subgroups, fig. (43).

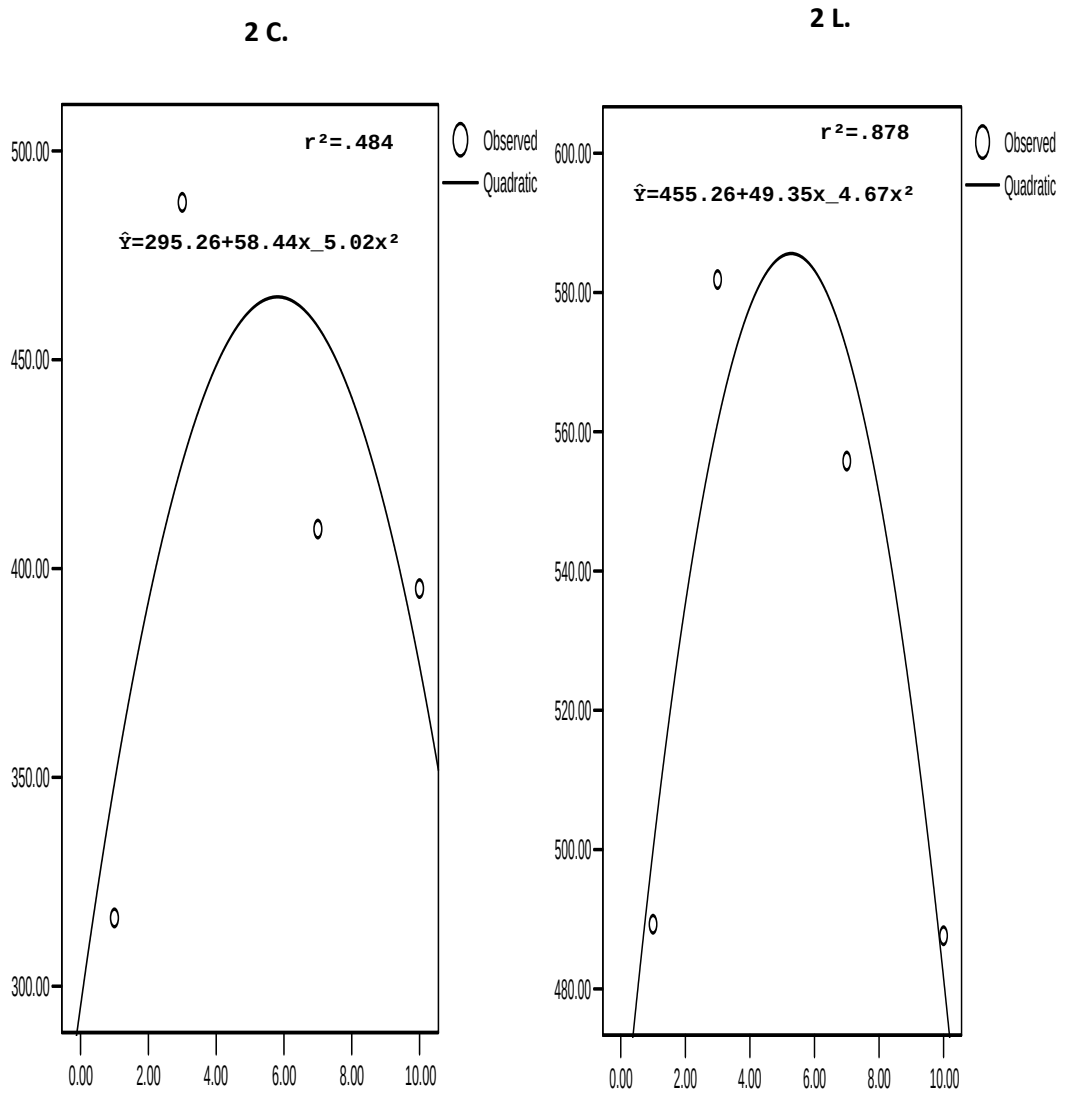


Figure (43): SPSS regression test for the animals of both subgroups of the 2nd group for cAMP .

- The Vertical axis represents the concentration of cAMP estimated in (pg/ml).
- The Horizontal axis represent the days.

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, table (4- 15) and figures (44, 45).

Table (4- 15) : Values of Growth Hormone GH in the animals of the 2nd group.

Subgroups	**Days**	**Samples**					
Con.of Growth Hormone GH(ng/ml)							
Control C		**1**	**2**	**3**	**4**	**5**	**Mean**
	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**

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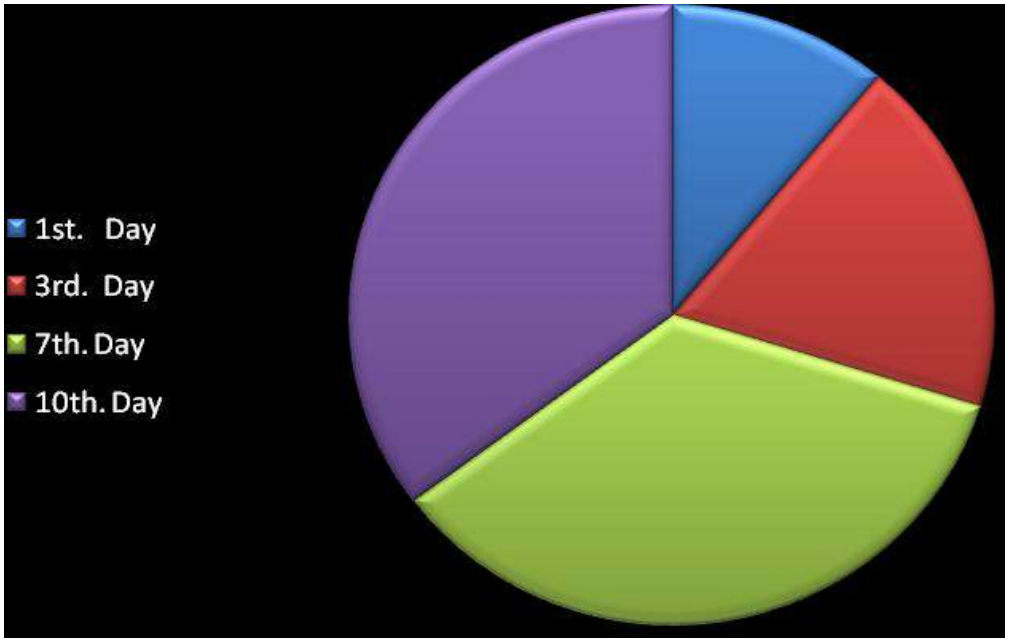

Figure (44): The values average of GH in the animals of the control subgroup.

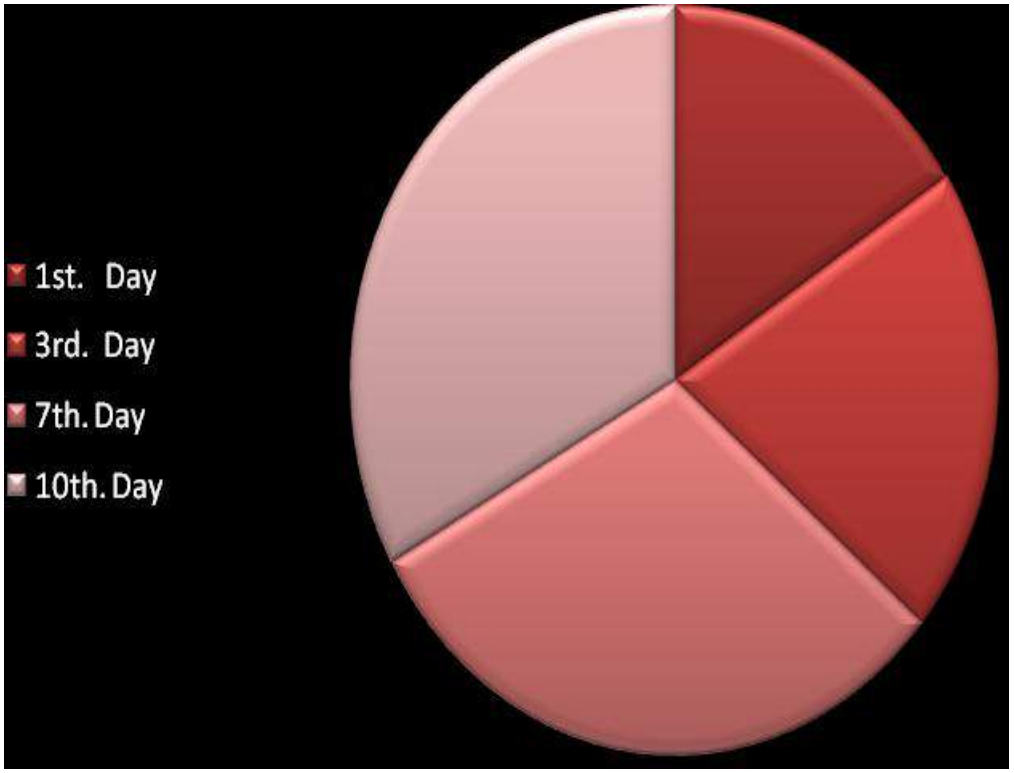

Figure (45): The values average of GH in the animals of the treated subgroup.

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, fig 46.

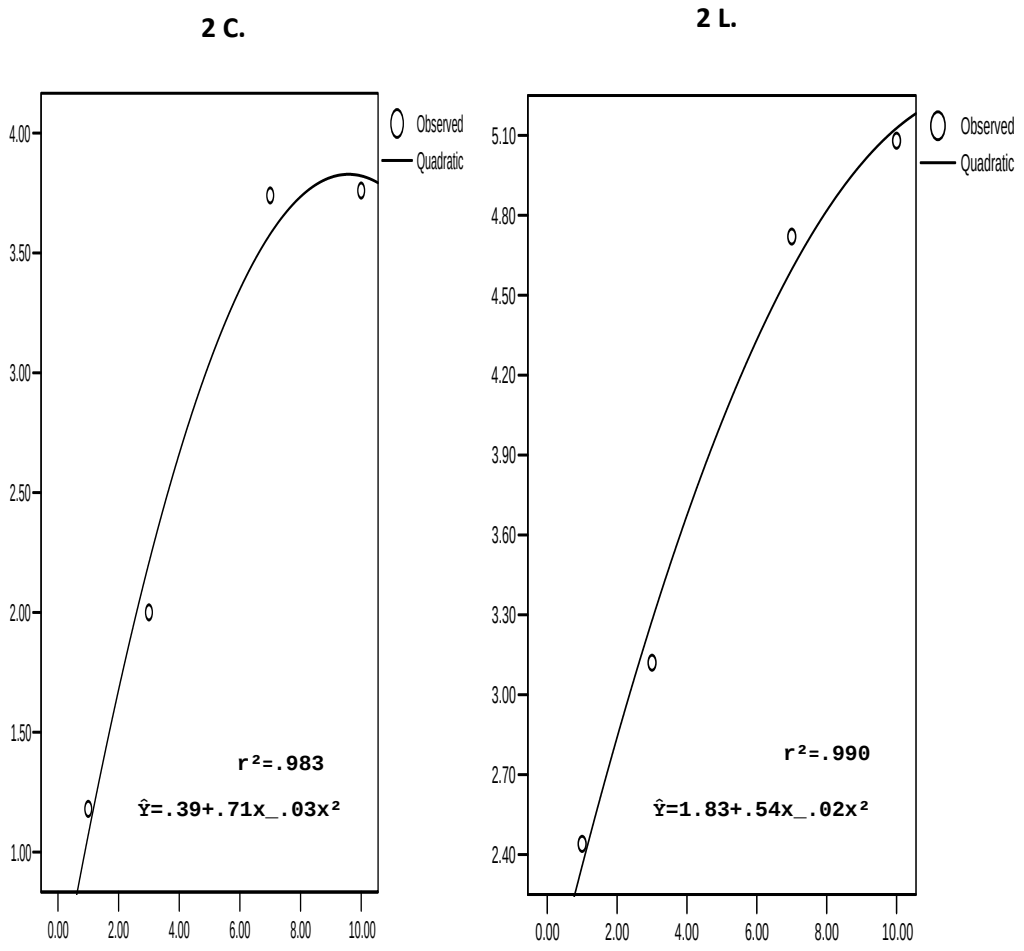

Figure (46): SPSS regression test for the animals of both subgroups of the 2nd group for GH .

- The Vertical axis represent the concentration of GH estimated in (ng/ml).
- The Horizontal axis represent the days.

Discussion:

Following tissue injury via an incision, the initial response is usually bleeding. The cascade of vasoconstriction and coagulation commences with clotted blood immediately impregnating the wound, leading to hemostasis, and with dehydration, a scab forms. An influx of inflammatory cells follows, with the release of cellular substances and mediators. Angiogenesis and re-epithelization occur and the deposition of new cellular and extracellular components ensues . (Mercandetti and Cohen, 2008)

Acute wounds generally proceed through orderly and timely reparative processes that result in a durable restoration of anatomic and functional integrity. However, various physiologic and mechanical factors may impair the healing response, resulting in a chronic wound that fails to proceed through the usual stepwise progression. Local infection, hypoxia, trauma, foreign bodies, or systemic problems such as diabetes mellitus, malnutrition, immunodeficiency, or medications are most frequently responsible . (Torre and Chambers,2008)

Chronic wounds were defined as those expected to take more than 4 to 6 weeks to heal because of 1 or more factors delaying healing, including venous leg ulcers, pressure ulcers, diabetic foot ulcers, extended burns, and amputation wounds. Acute wounds were defined as those expected to heal in the expected time frame, with no local or general factor delaying healing. These included burns, split-skin donor grafts, skin graft donor site, sacrococcygeal cysts, bites, frostbites, deep dermabrasions, and postoperative-guided tissue regeneration.(Barclay,2007)

Non-healing wounds have traditionally been defined as those that fail to progress through an orderly sequence of repair in a timely fashion. Such wounds are sometimes thought of as being caused by neglect, incompetence, misdiagnosis, or inappropriate treatment strategies. However, some wounds are resistant to all efforts of treatment aimed at healing, and alternative end points should be considered; measures aimed at improving the quality of life will be paramount in these instances.

Patients with non-healing wounds have a decreased quality of life. Reasons for this include the frequency and regularity of dressing changes, which affect daily routine; a feeling of continued fatigue due to lack of sleep, restricted mobility, pain, odor, wound infection and the physical and psychological effects of polypharmacy.

The loss of independence associated with functional decline can lead to changes, sometimes subtle, in overall health and wellbeing. These changes include altered eating habits, depression, social isolation, and a gradual reduction in activity levels .(Grey, et al. 2006)

Many alternative treatments are available to help heal wounds that do not respond to the conventional methods described above. These treatments should be undertaken in coordination with your healthcare provider.

Among these treatments are using the hyperbaric oxygen therapy to treat very serious wounds. The patient breathes 100% oxygen in a pressurized chamber for 90-120 minutes. The oxygen dissolves into the blood and is distributed throughout the body, providing extra oxygen to the cells attempting to heal the wound. Ultrasound treatment uses mechanical vibration delivered at a frequency above the range of human hearing also used to accelerate the wound healing.

Electrical stimulation using a current of energy generated between the skin and inner tissues when a break in the skin occurs is believed to accelerate the healing .The use of magnets has been reported to increase blood flow and enhance cell growth by transferring energy.

A large number of remedies used to accelerate the wound healing while the hormones used successfully in experiments and clinically to accelerate wound healing and caretaker in optimizing healing conditions.

Nutritional problems: Protein-calorie malnutrition and deficiencies of vitamins A, C, and zinc impair normal wound-healing mechanisms.(Stillman , 2010)

Laser irradiation was first used in medicine when a pioneer laser apparatus was built by T. Maiman in 1960. The first low-level laser for tissue biostimulation was applied by Mester in 1969.

Despite initial doubts concerning its efficiency, low level laser therapy L.L.L.T. has been used for 30 years, and it occupies a prominent place in contemporary medicine. The range of LLLT's medical applications in tissue stimulation continues to increase as new devices are constructed.

Continued research into tissue biostimulation revealed that L.L.L.T. has a beneficial effect on living organisms. The use of laser irradiation for biostimulation broadens our knowledge of the mechanisms responsible for polarized light effects on living tissue. (Chyczeweski ,et al. ,2010)

Both light absorption and scattering in tissue are wavelength dependent, and the principal tissue chromospheres (hemoglobin and melanin) have high absorption bands at wavelengths shorter than 600 nm. For these reasons, there is a so-called 'optical window' at red and near-IR wavelengths, where the effective tissue penetration of light is maximal. Thus, although blue, green, and yellow light may have significant effects on cells growing in optically transparent culture medium, LLLT use in animals and patients almost exclusively involves red and near-IR light 600–1070 nm; this is the reason why we choose the 820 nm laser diode in our procedure.(Huang, et al.2009)

Each cell contains a number of power plants, called mitochondria. The function of these power plants is to produce adenosine triphosphate ATP, the form of energy, which can be used by the cell to function properly. Low level laser light reaches the mitochondria of low lying cells where the photonic energy is absorbed by the collector surfaces and is converted to chemical energy within the cell in the form of ATP as an additional source of energy. Mitochondria produce more ATP, which leads to normalization of cell function, pain relief, and healing . Sufficiently high supply of cellular energy enables cells to work under optimum conditions and is the essential prerequisite to ensure successful self-healing process.

The complex physiological process of wound healing commences at the time of injury. The immune and circulatory systems are stimulated while cell migration, cell division, and several chemical and cellular responses occur.

The three overlapping phases of healing are the inflammatory phase, followed by the proliferative phase and matrix remodeling. Any device that can accelerate any of these processes could accelerate the healing process of wounds.

Literature indicates that laser photobioactivation accelerates inflammation, modulates the level of prostaglandin, enhances the action of macrophages, promotes fibroblast proliferation, facilitates collagen synthesis, fosters immunity, and even accelerates the healing process.

Fibroblasts are cells of paramount importance in the process of wound healing. At low doses 2 J/cm² phototherapy stimulates fibroblast proliferation while higher doses 16 J/cm² are suppressive, pointing to the dose dependence of biological responses after light exposure.

Low energy laser irradiation alters the cellular function by influencing protein synthesis, cell growth and differentiation, cell motility, membrane potential and binding affinities, neurotransmitter release, ATP synthesis, and prostaglandin synthesis.(Hawkins and Abrahamse, 2006)

The classic model of wound healing is divided into three or four sequential, yet overlapping, phases: hemostasis not considered a phase by some authors, inflammatory, proliferative and remodeling.

Upon injury to the skin, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage. Within minutes post-injury, platelets (thrombocytes) aggregate at the injury site to form a fibrin clot. This clot acts to control active bleeding (hemostasis).

In the inflammatory phase, bacteria and debris are phagocytized and removed, and factors that cause the migration and division of cells involved in the proliferative phase are released.

The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound

contraction. In angiogenesis, new blood vessels are formed by vascular endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin. Concurrently, re-epithelialization of the epidermis occurs, in which epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue.

In contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis.

When the regression test values of the 2st group are analyzed looking for the relationship between the two variants (time and defect contraction) , an estimation can be easily come to mind that every new day brings with it an increase in the contraction of the wound . The laser treated subgroup showed an increase of triple (0.11) as that of the control one 0.3.That mean the laser radiation was unique in increasing the ability of wound defects sealing.

Because inflammation plays roles in fighting infection, clearing debris and inducing the proliferation phase, it is a necessary part of healing. However, inflammation can lead to tissue damage if it lasts too long. Thus the reduction of inflammation is frequently a goal in therapeutic settings. Inflammation lasts as long as there is debris in the wound. Thus the presence of dirt or other objects can extend the inflammatory phase for too long, leading to a chronic wound.

Blood vessels that traversed the wound are severed at the time of injury and it is these cut vessels that allow whole blood into the wound, which then coagulates, seals the injured vessels and lymphatic channels in order to close the wound, and prevents further hemorrhage. The simultaneous release of histamine and other triggers by the injured tissue causes the intact vessels to dilate.

Histamine causes brief vasodilation in neighboring non-injured vessels and it is this combination of whole blood exudates and serous transudate that creates a reddened, hot, swollen and painful environment. Bradykinins, derived from plasma in the area of the injury, contribute to more prolonged vascular permeability.

Prostaglandins are produced by all cell 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.

Mast cells also release hyaluronic acid and other proteoglycans into the cocktail of chemicals accumulating within the wound and these bind with the watery wound fluid to create a non-flowing gel that slows down leakage and fluid loss. The inflammatory edema fills up all the spaces in the wound and surrounds all the damaged or repaired structures and binds them together. (Mallefet&Anthony,2008)

The prostaglandin released as a result of any injury or insults and later initiating the inflammatory reaction can be seen in both subgroups but, within this framework we can see that the laser radiation shots sparks of the PGE2 concentration in the treated subgroups (of both the wound healing or skin loss sealing groups), twice higher than that of the control one.

But on reaching the 3rd. day the concentration of the hormone reached its 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 PGE2was stimulated by the laser irradiation and this stimulation plays a great role in initiating the inflammatory stage and terminating it in the treated subgroups.

Comparing these results with those of the PGF2α, it will be easy to conclude that the level of the hormone reached the peak in the 3rd regardless which subgroup or the group and the level ceased after the 3rd day 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.

Assumptions may not be correct that tissue levels of prostaglandins reflect biologic activity. If tissues were not rapidly removed from the body and either quick frozen or immediately placed into solutions containing inhibitors of prostaglandin synthesis, the prostaglandin levels in excised tissues would not reflect prostaglandin levels in the body, prostaglandins were rapidly synthesized or released by manipulation of tissues.

Release of prostaglandins carried out in association with physiologic function suggests a cause and effect association, but this association may be incidental.

In general, both alteration of smooth muscle contractility and modulation of hormonal activity were detected following prostaglandin administration. Although, prostaglandins are synthesized locally, over 90% of infused prostaglandins have been shown to be inactivated with

one pass through the lungs. These data suggest that prostaglandins act locally as modulators rather than as classical circulating hormones.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have important anti-inflammatory effects. In animal models, expression of both PGD and PGF synthases declines during acute inflammation, only to rise again during the resolution phase, suggesting their possible role in resolving inflammation. Prostaglandin E_2 (PGE_2), the classic model of a proinflammatory 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.(Jose, et al. 2009)

The adenyl cyclase cyclic AMP system appears to be involved in action of prostaglandins as modulators and is receiving much research scrutiny at the present time. PGEl and PGE2 induced bronchodialation, whereas PGF2a induced bronchoconstriction, 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.(Akaneya ,2007)

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 increase because the enzyme acts as the second messenger which triggesr 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.

Prostaglandin E2 PGE2 is a lipid mediator of inflammation involving wound healing. ProstaglandinE2 PGE2, synthesized from arachidonic acid by cyclooxygenases COX and synthases PGES, acts as both an inflammatory mediator and fibroblast modulator. The release of PGE2 from skin tissue after toxic stimuli produces local edema and hyperalgesia. PGE2 is the lipid mediator of inflammation in diseases, such as rheumatoid arthritis and osteoarthritis, and is also involved in skin inflammation .(Su, et al .2010)

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.(Stratton and Shiwen, 2010)

Laser-related research has demonstrated a number of interesting biochemical responses that can have a positive clinical effect. These effects include stabilization of the cell membrane, enhancement of ATP synthesis, stimulated vasodilation along with increased histamine, NO and serotonin. Acceleration of leukocyte activity, increased prostaglandin synthesis, reduction in Interleukin-1 levels and increased angiogenesis

(Kneebone,2010).It is for this reason that we can see the increase in the values of the prostaglandins both E2 and F2α in the inflammatory phase , represented by the readings of the first up to the third days in both the induced wounds and skin loss groups irradiated with laser therapy.

Following laser irradiation, absorption of photons by molecules leads to electronically excited states and consequently can lead to acceleration of electron transfer reactions. More electron transport necessarily leads to increased production of ATP.

Light induced increase in ATP synthesis and increased proton gradient leads to an increasing activity of the Na+/H+ and Ca2+/Na+ antiporters and of all the ATP driven carriers for ions, such as Na+/K+ ATPase and Ca2+ pumps. ATP is the substrate for adenyl cyclase, and therefore the ATP level controls the level of cAMP. Both Ca2+ and cAMP are very important second messengers. Ca2+ especially regulates almost every process in the human body (muscle contraction, blood coagulation, signal transfer in nerves, gene expression, etc.). Researchers in PDT have known for a long time that very low doses of PDT can cause cell proliferation and tissue stimulation instead of the killing observed at high doses.

The beneficial effect of L.L.L.T. on wound healing can be explained by considering several basic biological mechanisms including the induction of expression cytokines and growth factors known to be responsible for the many phases of wound healing.

Firstly it is proved that L.L.L.T. increases both protein and mRNA levels of IL-1α and IL-8 in keratinocytes. These are cytokines responsible for the initial inflammatory phase of wound healing.

Secondly there are reports that L.L.L.T. can upregulate cytokines responsible for fibroblast proliferation and migration such as bFGF, HGF and HGH.

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 followed by a significant increase after that reaching to the 7th day; after that the level of the hormone takes a semi constant level till the end of the healing process in the 1st group and the defects sealing in the 2nd. group.

These results contribute to the anabolic role of this hormone which started from the point of terminating the inflammatory process.

Thirdly it has been reported that L.L.L.T. can increase growth factors such as VEGF responsible for the revascularization necessary for wound healing.

Fourthly TGF-β is a growth factor responsible for inducing collagen synthesis from fibroblasts and has been reported to be upregulated by L.L.L.T. .

Fifthly there are reports that L.L.L.T. can induce fibroblasts to undergo the transformation into myofibroblasts, a cell type that expresses smooth muscle α-actin and desmin and has the phenotype of contractile cells that hasten wound contraction. (Hamblin, et al.2006)

Low-level laser therapy (LLLT), is an alternative for the modulation of inflammatory processes, it has also been recommended for use postoperatively because of its capacity to biomodulate healing.

However, data in the literatures are not in agreement about the anti-inflammatory effect of laser energy on wound healing. Some studies suggest that laser therapy may minimize inflammatory reactions,

whereas others report that LLLT accelerates inflammation in the healing process, and makes it more severe initially, thus decreasing healing time.

Thus the application of laser energy to injured tissues may provide earlier healing with better histologic quality.(Viegas , et al. 2007)

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.(Peplow, 2007)

Injury to the skin initiates a series of events, which finally lead to at least partial reconstruction of the wounded tissue. The important role of locally acting cytokines and growth factors in wound repair is well documented. However, crucial functions of endocrine acting hormones have only recently been recognized. One of the hormones, which are thought to influence the repair process, is growth hormone GH.

This hormone is successfully used for the treatment of growth defects in children. In addition, an increasing number of healthy adult patients receive GH therapy as an anti-aging strategy. However, the side effects of GH treatment have been poorly defined and it is as yet unclear if GH affects the wound healing process in healthy individuals.

Treatment with GH has been used in patients with severe burn injuries, and in most studies enhanced rates of wound healing and patient survival were observed. In addition, GH was shown to stimulate granulation tissue formation and biomechanical wound strength in animal models of impaired healing, although a lack of efficacy of GH has also been reported. Thus, the role of GH in wound healing remains to be determined.

Many effects of GH are mediated via the insulin-like growth factor IGF system, suggesting that this might also be a mechanism of GH action in

the skin. IGF-I is a mitogen for keratinocytes, and it stimulates collagen, glycosaminoglycane and proteoglycane synthesis by dermal fibroblasts.

It exerts its effects in concert with a family of six IGF-binding proteins IGFBPs which control the availability of IGF for signaling through its receptor in a complex manner: While IGFBP-3 and IGFBP-4 have a predominantly negative effect on IGF activity, IGFBP-5 can act to enhance IGF signaling, but only when associated with the surface extracellular matrix of target cells .In addition, IGFBPs have also IGF-independent effects on cell growth.

Local IGF gene therapy or protein treatment improved the wound healing process in different animal models. In contrast, raising the serum levels of IGF-I by systemic application did not improve wound healing, whereas GH was effective in the same experimental set-up, possibly via induction of IGF-I at the wound site.

It is known that the growth hormone-releasing hormone (GHRH) stimulates the production and release of growth hormone (GH). GHRH plays a role in wound healing and tissue repair by acting primarily on wound associated fibroblasts. (Dioufa , et al. 2010)

As to its direct wound healing effects, skin is a target tissue for HGH, both directly through HGH receptors on the surface of epidermal cells and indirectly through the action of IGF-1.

Exogenously administered HGH has been shown to increase skin thickness in normal humans. Other effects on the wound include increased rate of re-epithelialization of skin graft donor sites in adults and children with severe burns or trauma. In addition, HGH has been shown to increase wound collagen content, granulation tissue and wound tensile strength, and the local production of IGF-1 by fibroblasts. These data are mainly derived from animal studies. (Demling ,2005)

The beneficial wound healing effect of the systemic growth hormone (GH) mediated by insulin-like growth factor 1 (IGF-1) has been widely reported. Recent studies have suggested that GH facilitates wound healing not by circulating IGF-1, but by local IGF-1 produced in the wound itself.(Kim, et al.2009)

For all these considerations we saw the increase in the level of the growth hormone in the groups treated with L.L.L.T., it is very important to know that the need for the hormone in the subgroup of sin loss is higher and longer than that in the first group in which we induced the wounds only because the size of insult to the skin in this group is larger which make the need for acceleration and activation of the regenerative processes is much more.

These results come in agree with those got from a team of workers on stem cells and their effect on wound healing . In spite of recent advances

from breakthroughs in recombinant growth factors and bioengineered skin, up to 50% of chronic wounds that have been present for more than a year remain resistant to treatment. Stem cells offer the possibility that some structures within the wound may be reconstituted.

Because of the relative ease with which they are cultured, bone marrow–derived mesenchymal stem cells have been tested in pilot studies, and there are now promising approaches for introducing them into the wound. It might turn out, however, that other types of stem cells will be more effective, including those derived from hair follicles, or perhaps subsets of bone marrow–derived cultured cells. Still, proper wound care and adherence to basic principles cannot be bypassed, even by the most sophisticated approaches .(Cha and Falanga , 2007)

The sutures were removed on the fourth postoperative day from the treated subgroup and the seventh postoperative day in the control subgroup, (the both subgroups are from the wound induced group) and these results can be considered more advanced than those got by Mihaela Antonina Calin et. al. from their work on rabbits, they induced wounds with 8 cm length and removed the sutures from the treated group in the sixth day while the healing process is over in ten days in the control group.(Calin ,et al.2010)

In the second group (the skin lost group) the defect is closed in the treated subgroup after Nine days while they took Fifteenth days to be closed in the control subgroup, these results are very encourage if compared with those got by Suk Hwa Kim et.al. 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 .

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.(Kim, et al.2009)

The results of the current study can be evaluated and assessed, at the same time they may remind us with the relationship between nutrition and wound healing after injury or surgical intervention, we have no doubt that adequate carbohydrate, fat, and protein intake is required for healing to take place, but research in the laboratory has suggested that other specific nutritional interventions can have significant beneficial effects on wound healing.

Malnutrition after injury results from multiple factors, including poor nutritional intake to a host's perturbed metabolic equilibrium. Studies

over the past century have shown that changes in energy, carbohydrate, protein, fat, vitamin, and mineral metabolism affect the healing process. Loss of protein from protein-calorie malnutrition, the most common form of malnutrition in the world, leads to decreased wound tensile strength, decreased T-cell function, decreased phagocytic activity, and decreased complement and antibody levels, ultimately diminishing the body's ability to defend the wound against infection. These immune compromises correlate clinically with increased wound complication rates and increased wound failure after clean surgical procedures.

In elderly nursing home patients, malnutrition is also associated with increased mortality, an increased risk of developing pressure ulcers, and a lower quality of life, in such patients low level laser irradiation has no benefits, because it lacks the basics of it`s work inside the body.(Arnold, & Barbul,2006)

The results of the current study agree with those got by a team of workers who used T3 cream to accelerate wound healing in rats They evaluated mice treated with topical T_3 *vs*. the same mice receiving vehicle alone. They established ten-millimeter diameter (79 mm^2) dorsal skin wounds in all animals, and wounds were remeasured 4 days after injury. All animals were evaluated twice: once with the T_3 treatment and once with the vehicle alone. Daily topical application of 150 ng T_3 resulted in 58% greater wound closure relative to wounds on the same animals receiving vehicle alone . (Safer, et al.2005)

A group of researchers carried out their trail on mice using laser therapy , they found that the wound healing was significantly stimulated. Illuminated wounds started to contract while control wounds initially expanded for the first 24 hours, they found a biphasic dose–response curve for fluence of 635-nm light with a maximum positive effect at 2 J/cm^2 . 820 nm was found to be the best wavelength tested compared to 635, 670, and 720 nm. (Demidova – Rice ,et al.2007)

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 increase the level of cAMP , mitochondrial intracellular calcium ion ca^{2+} , Mitochondrial Membrane Potential MMP and Adenine Triphosphate .(Zungu, et al. 2008)

The conditions were prepared in the lab animal unit so that the temperature didn't raise more than 35º c , because we know that increasing room temperature has a reverse effect on the wound healing .

They induced full thickness skin wounds in hooded Lister rats and kept them at an environmental temperature of 30º C, these wounds closed more rapidly than did those in similarly wounded rats housed at 20° C.

The differences were statistically significant. This response was exhibited by both sexes.

The wounds in male rats were found to close more rapidly than those in female rats housed at the same temperature. The rectal temperature of the animals was not affected by the environmental temperature but the temperature of wounded skin was significantly higher (ca. 0.90 C) in the animals held at 30° C. The epithelium of the wounds advanced at a linear rate in the final stages of closure.

Experimental works matching with those of the current study. They used rats as experimental models which induced 1cm² wounds on the back and treated them with laser irradiation. The laser was able to slightly reduce the intensity of the inflammatory reaction as well as to enhance substantially the epithelization process at both 8th and 14th day. In addition, it also appeared to stimulate the deposition of collagen fibers at the final stages of wound healing. (Ribeiro, et al.,2009)

The results we got from the second group (the group of skin loss) are satisfied with those got by Nayak, B.S. et.al. using excision wounds in Wister rat model. The circular wounds were created on the dorsum of the back of the animals. The animals were divided into two groups. The treated group (n = 12) wound was treated with 632.8 nm He-Ne laser at a dose of 2.1J cm-2 for five days a week until the complete healing. The control group was sham irradiated. 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 15th day, whereas the control group healed, on average by 22nd day.

Wound contraction and experimental observations suggested that low intensity Helium-Neon laser photo stimulation facilitates the tissue repair process by accelerating collagen production in chronic wounds.(Nayak,et al.,2007)

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 (also called galvanotaxis).(Huttenlocher and Horwitz, (2007) or an external herbal formulation. (Honnesh, 2010)

Conclusions

Better understanding of the physiology of wound healing will eventually contribute to progress in the treatment of major acute wounds and excessive skin defects and losses.

1- L.L.L.T. accelerates wound healing and the skin defects 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. There is a complementary relationship or synergy between each of the PGE2, PGF2α, cAMP, and GH with each other during the healing process

3- 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.

Recommendations

Expansion the scientific research base so that we can reach to :

1- Wide band of information about all the hormones and enzymes which interlaced with wound healing and skin defects filling.
2- Make a sound base of the role of each hormone in the specific stage of wound healing and the possibility of enhancing this role by the use of the L.L.L.T.
3- Studying the possibility of healing of the wounds and skin defects with the minimum amounts of scar tissue.
4- Studying the possibility of using the L.L.L.T. in decreasing to the minimum the deterioration in wound healing, through the control of any physiologic or mechanical factor which may impair the healing response and leads to a chronic wound that fails to proceed through the usual stepwise progression.
5- Designing a large number of experimental works and clinical applications of L.L.L.T. in treatment of many chronic cases which failed in healing when using the traditional remedies like; Diabetic ulcers , Pressure ulcer and Venous stasis ulcer.

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Summary:-

The objective of this study is to investigate the effect of Low Level Laser Therapy L.L.L.T. on cell proliferation of epithelial and connective tissue in the healing of incised cutaneous wounds or lost skin flaps and to determine the adaptive response of cells to irradiation with laser from the physiological point of view depending on the laboratory determination using the Eliza for the hormones which greatly interlace with wound healing processes.

The experiment was conducted on twenty adult white New Zealand male rabbits with 1.5 -2 Kg body weight each, 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 first 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 stitches of simple interrupted sutures using surgical silk 3-0. while the 2nd group operation involved removing of a whole thickness skin square graft of (1x1 cm) dimensions.

The site of the operation in all the animals was treated with antibiotic spray then the animals were injected with systemic antibiotics: penicillin (1000 iu/kg. B.W.) and streptomycin (10mg/kg. B.W.) i/m for three subsequent days after the operation.

The animals of each group were divided into two subgroups (control and treated with laser irradiation). The laser used was diode 820nm wave length, with maximum output of 200 mW, density 8J/cm², pulsing frequency 1-10 Hz.

Irradiation began after the operation and continued for 5 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 and 7) from the animals of the first group and (1, 3, 7 and 10) in the animals of the second group. The samples were taken from the marginal ear vein from all the animals and sent for examination with Eliza to determine the levels of Prostaglandin E2

(PGE2), Prostaglandin F2α (PGF2α), Growth hormone (GH) and cyclic Adenosine Monophosphate (c AMP).

Results of the current study revealed a highly significant increase in the levels of PGE2, PGF2α, GH and cAMP in the samples of the treated subgroups as compared with those of the control one; there was rapid stabilization of the hormonal status.

We can conclude that the treating of the surgical wounds and skin disorders with low level laser radiation was useful and efficient because the primary healing was promoted and accelerated. The process ending in the 4^{th}. postoperative day in case of the surgical wound group, and 9^{th}. postoperative day in the skin loss group.

The results obtained from this study should be attributed to the improvement of rheological properties of blood, increase blood capillary, blood flow, reduced vascular resistance and vascular tone which lead to increasing the motion and outflow of fluids from the interstitial spaces into the lymphatic system, Energy is needed to activate certain processes in the cell to trigger the aforementioned sequence of pathways which in turn promote regeneration and accelerate wound healing.

We tested the results statistically using SPSS regression test and found that the results of the Eliza test for the hormones showed significant variations in the values of PGE2,PGF2α,cAMP and GH, between the two subgroups of the $1^{st.}$ group, $P > 0.05$,

It was also found that the values of hormonal assessment of PGE2, PGF2α, cAMP, GH and the diameter of the skin defect for the animals of the 2^{nd}. group showed significant variations between the two subgroups $P > 0.05$.