

## ULTRASONOGRAPHY, COMPUTED TOMOGRAPHY AND HISTOPATHOLOGICAL EVALUATION OF THE USE OF PLATELETS RICHED PLASMA (PRP) AND INSULIN ON LONG BONE FRACTURE HEALING IN RATS

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### ABSTRACT

To evaluate the effect of PRP and insulin on promotion of healing rate in experimental animal's long bone fracture. The current study was conducted with 27 white albino rats weighing between 150 and 200 gm. These animals were divided into three groups (nine rats in each). The experiment consisted of two stages: the first stage, involved the surgical induction of complete tibial fracture under the effect of general anesthesia. The second stages, cast was applied only in G1, while in G2, cast was applied after injection of insulin in the site of fracture, as well as cast was applied after inject PRP at the site of fracture in G3. ultrasonography, computed tomography and histopathology were used to evaluate the rate of bone fracture healing for each group. The result showed that, the echogenicity change at the fracture rapidly in PRP group followed by insulin group and slowly in control group. The bone density was increased rapidly and high in PRP group followed by insulin group and finally the bone density is low in control group and the histopathological evaluation confirms these results. In conclusion, using of PRP has a greater useful effect in promoting bone fracture healing rate and tissue proliferation than insulin.

**Key words:** Bone Fracture, Rats, Platelets Riched Plasma, Insulin, Ultrasound, C.T, Pathology.

### INTRODUCTION

Fracture healing is a compound process that includes different regulators and biochemical, hormonal and growth factors which interact with the healing process (Einhorn, 1995; Wilkens *et al.*, 2003 and Say *et al.*, 2014). The fracture healing process should be passed via three stages, they includes deposition, resorption and remodeling (Kalfas, 2001). Fracture healing is looking as a tardily operation that causing long immovable periods for complete healing, and delayed fibrosis and/ or non-union healings are the most subsequent problems that face the surgeon and the animals (Bostrom and Camacho, 1998 and Bouxsein *et al.*, 2001).

The biology of bone, ligaments, and tendons healing that directed us to investigate numerous products that promote the fracture healing, using of these products is depend on stimulation of growth factors to fast the healing of bone and soft tissues. Insulin

and Platelet rich plasma (PRP) are examples of this product (Foster *et al.*, 2009). PRP is defined as higher plasma concentration of platelet than platelet concentration found in whole blood (Foster *et al.*, 2009; Wroblewski *et al.*, 2010).

In fracture healing, platelets play as source of growth factors that promot bone growth (Bolander, 1992 and Thiede *et al.*, 1993). At the fracture site, platelets release about fifteen growth factors that have the greatest potential for fracture repair because both chondrocytes and osteoblast are enriched with receptors TGF- $\beta$ 1. (Shantaram, 2016).

There is another product that has the ability to accelerate the healing process is insulin which has an anabolic role in bone (Graves, *et al.*, 2011).

Bone is a unique organ in the body because its scarless regenerative capacity (Glowacki, 1998). Repair of fractures by callus production occurs in four overlapping phases (Einhorn, 1995; Einhorn, 1998; Mandracchia *et al.*, 2001; Hadjiargyrou *et al.*, 2002 and Gerstenfeld *et al.*, 2003). Following damage to the musculoskeletal system, disruption of blood vessels leads to activation of the coagulation cascade and formation of a hematoma, which encloses the fracture area. Removal of the hematoma

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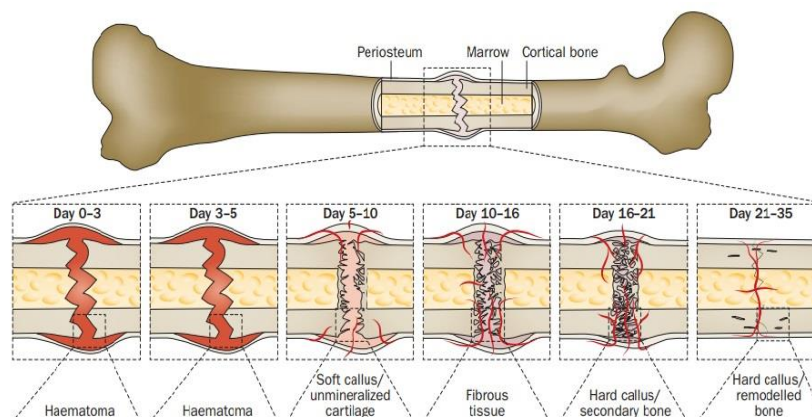
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significantly attenuates repair, and transplantation of the hematoma produces new bone, consistent with the angiogenic activity of the hematoma (Mizuno *et al.* 1990; Grundnes, and Reikeras, 1993; Street *et al.*, 2000 and Street *et al.*, 2001). Inflammatory cells, fibroblasts, and stem cells are recruited to the site, and new blood vessels are formed from pre-existing ones (i.e. angiogenesis). The inflammatory response is associated with pain, heat, swelling, and the release of several growth factors and cytokines that have important roles in repair (Einhorn, 1995; Hauser *et al.*, 1997; Barnes *et al.*, 1999;

Mandracchia *et al.*, 2001 and Gerstenfeld *et al.*, 2003).

Initially, granulation tissue forms at the ends of bones, gradually being replaced by fibrocartilage, in a manner seemingly related to the vascular pattern (Trueta, 1963). Meanwhile, the periosteum undergoes direct bone formation, or intramembranous ossification, to create an external callus. Subsequently, the internal callus becomes mineralized with calcium hydroxyapatite, to form a hard callus. In the final, remodeling phase of bone regeneration.



Fracture healing: mechanisms and interventions  
Einhorn and Gerstenfeld (2015).

The aim of this study is to evaluate the local effect of PRP and insulin on bone healing and to assess the fracture healing process by using ultrasonographic, Computed Tomographic and histopathological techniques to know the effect of these substances on enhancing and/or promotion of bone fracture healing.

## MATERIALS AND METHODS

### Animals

This study was conducted on 27 white albino rats weighing between 150 and 200 gm. These animals were divided into three groups (nine rats in each group).

### The experiment

The animals were anaesthetized by using 0.1 ml of anesthetic mixture (10 mg/kg xylazine hydrochloride and 100 mg/kg ketamine HCl.) intra-peritoneal injection. The experiment was consisted of two steps: the first step includes surgical induction of complete tibial fracture at the distal third (Fig. 1). The second step using external fixation only in control group (GI) (Fig. 2). While in the second group (GII), 0.2ml of freshly prepared platelet Richet plasma (PRP) was injected at the fracture site then a cast was applied (Fig. 3). In group (III), inject 0.2ml of insulin (NovoRapid® Penfill, 100 U/ml, Egyptian Drug Trading Company) at the fracture site

was performed and then a cast was applied (Fig. 4). The next stage of the experiment was performed to evaluate the different changes of bone fracture healing and effect of different treatments in different groups at one, two, and four weeks interval by using of the following techniques:

#### 1-Ultrasonography:

Ultrasonographic examination was done at one, two and four weeks by using Mindray veterinary ultrasound device 5000 connected with linear multi-frequency transducer 5.0-10.0 MHz. The examined area was shaved and ultrasound coupling gel was applied. The prepared areas were scanned in sagittal plane to early and easily detection any changes at the fracture site by 10.0 MHz.

#### 2 - Computed Tomography (CT):

This method was done at one, two and four weeks to evaluate the pattern of bone healing in different groups by determination the bone density at the fracture site by using CT (Go Now, 16 slices, Siemens, Germany).

### 3 - Histopathology

Both gross and microscopic pathological evaluation of experimentally induced simple aseptic tibial fracture of albino rats were done after euthanasia of animals according to the Local Experimental Animal Care Committee and approval of the ethics of the Beni- Suef university committee.

The gross alterations were photographed and recorded by the use of digital cam (Nikon Coolpix L 340, 28x optical zoom wide20.2 Megapixels. NikonCorp, Japan) after collection of legs of each rat groups at time of one week and two weeks post fracture after gross examination for periosteal, endosteal reactivity and nature of callus.

Soft tissues that covering fractured bones were removed for obtaining only tibial bone samples then preserved in 10 % neutral buffered formalin for 3 days. After fixation process, all specimens were washed from formalin by running tap water for about 4 hours, then immersed in a decalcifying agent of 17% EDTA disodium solution (Ethylenediamine tetra-Acetic acid disodium salt B.P.93©: El Nasr

pharmaceutical chemical, Egypt) for one month during which the specimens were inspected weekly for signs of complete decalcification appeared (Shibata *et al.*, 2000).

After complete decalcification, the specimens were washed in running tap water for 6 hours, then dehydrated in ascending grades of ethyl alcohol (70 %, 80 %, 90 % and 96 %) (Absolute I, Absolute II and Absolute III), cleared in xylene (xylene I, xylene II and xylene III) and embedded in soft paraffin (paraffin I, paraffin II and paraffin III) then blocked in hard paraffin wax, sectioned 5-7  $\mu$  and stained with routinely Hematoxylin and Eosin according to (Bancroft and Gamble, 2008 and Fathy *et al.*, 2018). The stained sections were imaged by use Leica Full HD Camera that fixed on Leica DM2500 – Leica DF290 microscope.

### 4 - Image analysis:

This technique was done to compare the diameter of callus formation between different three groups by using image j software (<http://rsb.info.nih.gov/ij/>) (Fig. 5).

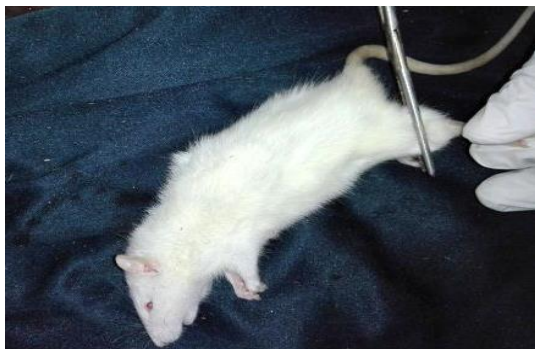


Fig. (1) Experimental induction of tibial fracture



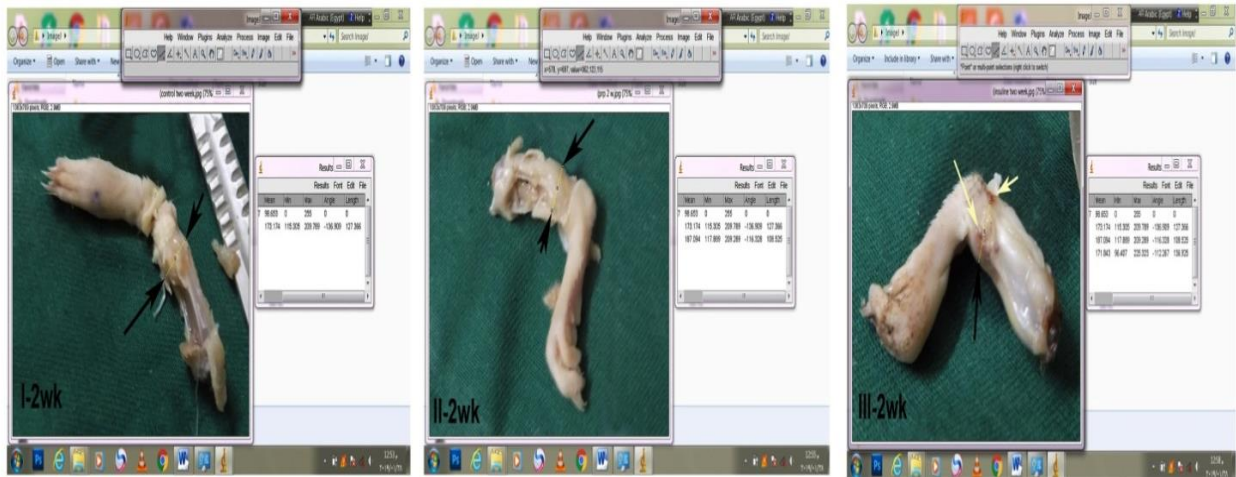
Fig. (2) Application of cast



Fig. (3) Local application of PRP



Fig. (4) Local application of insulin



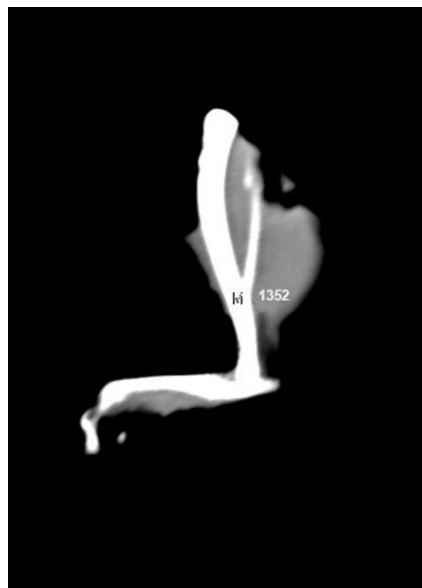
**Fig. (5):** Showing the image analysis, the length between two black arrows in control group is 127 while in PRP injected group is 108 but in insulin injected group is 136

**RESULTS**

No death or complications to the rats were observed during the entire duration of the study. Animals were allowed to walk within their cages until the stage of euthanasia at week 4. Upon resection observation of

the fractured tibia, no evidence of inflammation or infection was present at the fractured site.

The normal appearance of the healthy bone ultrasonography is a hyper-echoic line and the normal bone density by CT measured by 1352. (Fig. 6).



**Fig. (6):** CT showing the normal bone density is 1352.

In group (I), ultrasonographically, at first week, the fractured site appeared as a hypo-echoic area (Fig. 7) while at two weeks, the fractured site has small hypo-echoic area and the fractured end as a hyper-echoic points which are near to each other (Fig. 8) but at four weeks, the fracture gap appeared as hyper-echoic line which connect the part of two ends of fractured ends (Fig. 9).

Computed tomography, at first week, the fractured site appeared as a hypo-dense area and the bone density is 213 (Fig. 10) while at two weeks, increase in dense at the fractured site and the bone density is 353 (Fig. 11). At four weeks, the fracture gap was appeared as hyper-dense area and the bone density is 577 (Fig. 12).

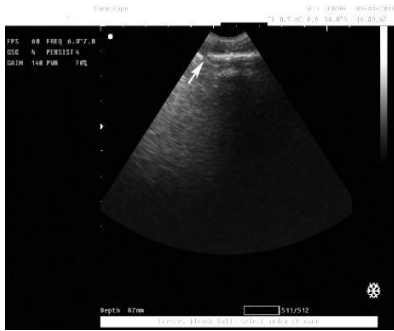


Fig. (7)



Fig. (8)



Fig. (9)

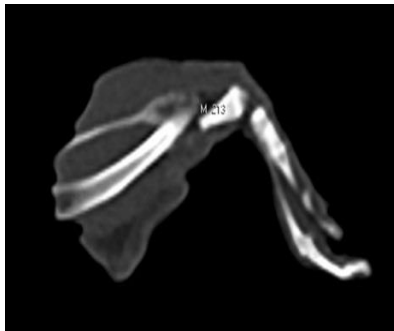


Fig. (10)

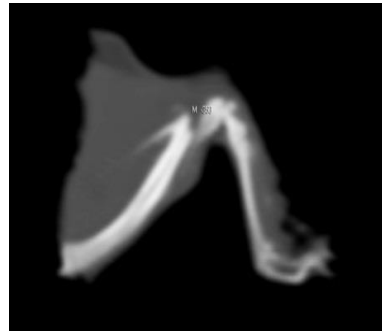


Fig. (11)

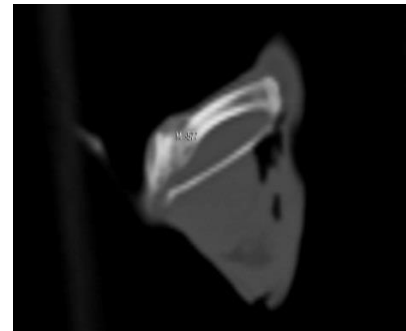


Fig. (12)

**Fig. 7:** Ultrasonogram showing the fractured site appeared as a hypo-echoic area (white arrow)

**Fig. 8:** Ultrasonogram showing the fractured site has small hypo-echoic area and the fractured end as hyper-echoic points which are near to each other (white arrow).

**Fig. 9:** Ultrasonogram showing the fracture gap appeared as hyper-echoic line which connect the two ends of fracture

**Fig. 10:** CT showing the fractured site appeared as a hypo-dense area and the bone density is 213.

**Fig. 11:** CT showing increase in dense at the fractured site and the bone density is 353.

**Fig. 12:** CT showing the fracture gap was appeared as hyper-dense area and the bone density is 577.

In group (II), Ultrasonographically, at first week, the fractured site appeared as a hypo-echoic area due to presence of soft callus (Fig. 13). At two weeks, increase of echogenicity at the fracture site due to presence of hyper-echoic denote in fracture gap due to presence of initial stage of hard callus (Fig. 14). At four weeks, presences of hyper-echoic line fill in the fracture gap and this is attributed to hard callus formation (Fig. 15).

Computed tomography, at first week, the fractured site appeared as a hypo-dense area and the bone density is 779 (Fig. 16) while at two weeks, increase in dense at the fractured gap and the bone density is 1081 (Fig. 17). At four weeks, the fracture gap was appeared as hyper-dense area and the bone density is 1230 (Fig. 18).



Fig. (13)

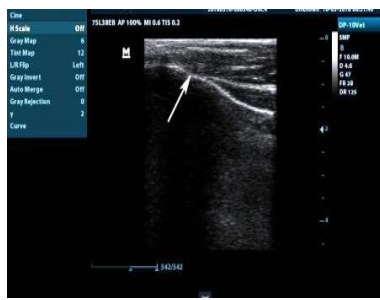


Fig. (14)



Fig. (15)

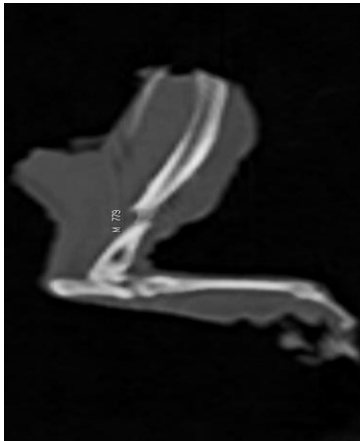


Fig. (16)



Fig. (17)

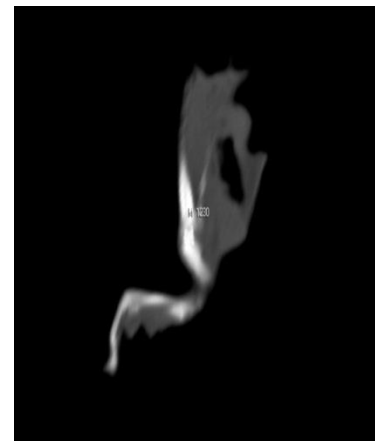


Fig. (18)

**Fig. 13:** Ultrasonogram showing the fractured site appeared as a hypo-echoic area due to presence of soft callus (white arrow)

**Fig. 14:** Ultrasonogram showing increase of echogenicity at the fracture site due to presence of hyper-echoic denote in fracture gap due to presence of initial stage of hard callus (white arrow)

**Fig. 15:** Ultrasonogram showing presences of hyper-echoic line fill in the fracture gap and this is attributed to hard callus formation

**Fig. 16:** CT showing the fractured site appeared as a hypo-dense area and the bone density is 779

**Fig.17:** CT showing increase in dense at the fractured site and the bone density is 1081.

**Fig. 18:** CT showing the fracture gap was appeared as hyper-dense area and the bone density is 1230

In group (III), at first week, the fractured site appeared ultrasonographically as a hypo-echoic area (Fig. 19) while at two weeks, increase of echogenicity at the fracture site due to initial presence of soft callus (Fig. 20). At four weeks, presences of hyper-echoic denote in the fracture gap and this is attributed to initial stage of hard callus (Fig. 21).

Computed tomography, at first week, the fractured site appeared as a hypo-dense area and the bone density is 459 (Fig. 22) while at two weeks, increase in dense at the fractured gap and the bone density is 824 (Fig. 23). At four weeks, the fracture gap was appeared as hyper-dense area and the bone density is 990 (Fig. 24).



Fig. (19)



Fig. (20)



Fig. (21)

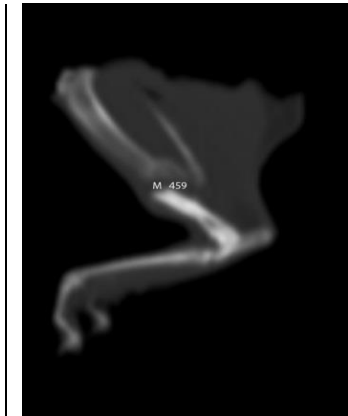


Fig. (22)

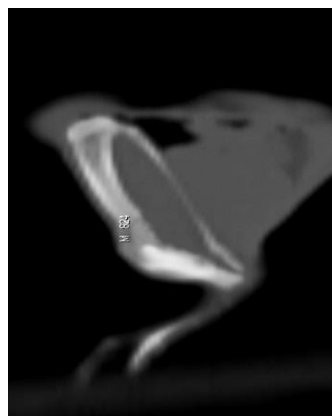


Fig. (23)



Fig. (24)

**Fig. 19:** Ultrasonogram showing the fractured site appeared as a hypo-echoic area (white arrow)

**Fig. 20:** Ultrasonogram showing increase of echogenicity at the fracture site due to presence of soft callus (white arrow)

**Fig. 21:** Ultrasonogram showing presences of hyper-echoic denote in the fracture gap and this is attributed to initial stage of hard callus

**Fig. 22:** CT showing the fractured site appeared as a hypo-dense area and the bone density is 459

**Fig. 23:** CT showing increase in dense at the fractured site and the bone density is 824.

**Fig. 24:** CT showing the fracture gap was appeared as hyper-dense area and the bone density is 990.

The gross pathological alterations of the fracture site were recorded at one week (1wk) and two weeks (2wk) for each group (I-Control group; 0.2 ml normal saline / one dose immediately after fracture induction), (II-Platelet Rich Plasma PRP group; 0.2 ml PRP / one dose immediately after fracture induction), and (III-Insulin group; 0.2 ml insulin / one dose immediately after fracture induction) Plate (1 and 2).

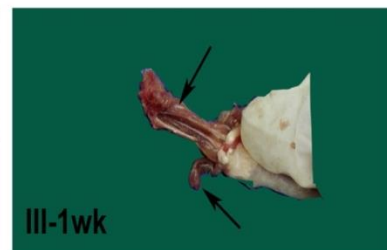


Plate (1)



Plate (2)

(Plate, 1) Fig. (I-1wk): Photograph of rat tibia of control group showing inflammatory red zone at fracture site (black arrow), Fig. (II-1wk): Photograph of rat tibia of PRP group showing fracture site (black arrow) while the surrounding musculature constitute minimal inflammatory reaction, Fig. (III-1wk): Photograph of rat tibia of insulin group showing sever inflammatory reaction in muscles either far from or near to surrounding fracture site (two black arrows).

(Plate, 2) Fig. (I-2wk): Photograph of rat tibia of control group showing beginning of formation of hard callus at fracture site (two black arrows), Fig. (II-2wk): Photograph of rat tibia of PRP group showing hard callus formation at the fracture site (black arrow), Fig. (III-2wk): Photograph of rat tibia of insulin group showing callus formation (black arrow) associated with congested blood capillaries (two yellow arrows) surrounding fracture site.

When the length of hard callous determined by image analyzer software of gross images taken from tibial bone, two weeks post fracture revealed that in control group, the callous length was 127, while in PRP injected group was much more lesser than control one 108 even lesser than insulin injected group which was 136.

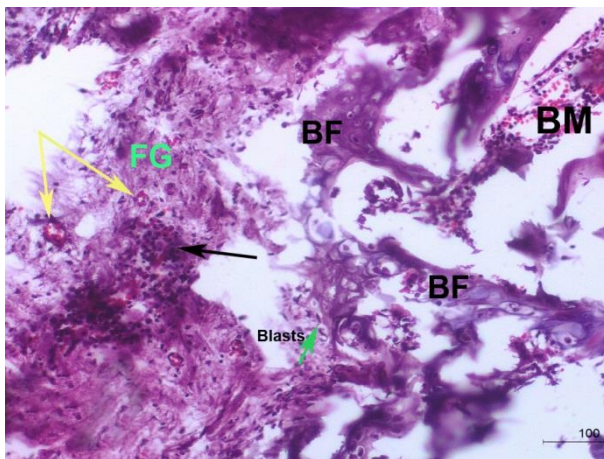
The histopathological examination of the fracture site of different groups revealed that:

In control group, in 1<sup>st</sup> week post fracture, the fracture gap (FG) was filled with granulation tissue

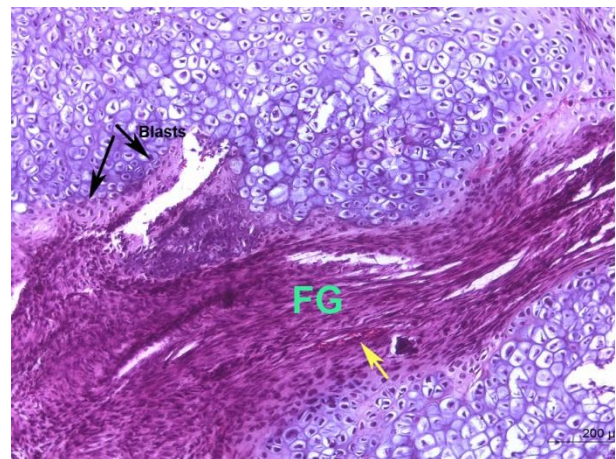
which characterized by loose interlacing fibrous connective tissue which is rich in blood capillaries entangling in it lymphocytes representing the end of the inflammatory phase of the bone fracture healing subsequently after hematoma formation (Fig. 25). While bone fragments (BF) still present near to the fracture gap next to it the bone marrow of the bone (BM). In 2<sup>nd</sup> week post fracture, the blood capillaries were absent, while the fibrous connective tissue become much more abundant and compact filling the fracture gap (FG) as the beginning of the proliferative phase of bone fracture healing (Fig. 26). The neighbors bone beside the fracture gap showing activated osteoblasts penetrates toward the fracture site (Fig. 27).

In PRP injected group, by examination of the fracture site at the 1<sup>st</sup> week, the fracture site showed that an early proliferation of fibrous connective tissue filling the fracture gap with minimal numbers of blood capillaries and leucocytes (Fig. 28). The end of inflammatory phase and the beginning of the proliferative phase of fracture bone healing.

In insulin injected group, the histopathological examination of the fracture site in 2<sup>nd</sup> week post fracture revealed that connective tissue rich in blood capillaries and inflammatory cells, while the bony matrix showing activation of osteoblasts (Fig. 29 and Fig. 30). As an inflammatory phase of bone fracture healing.

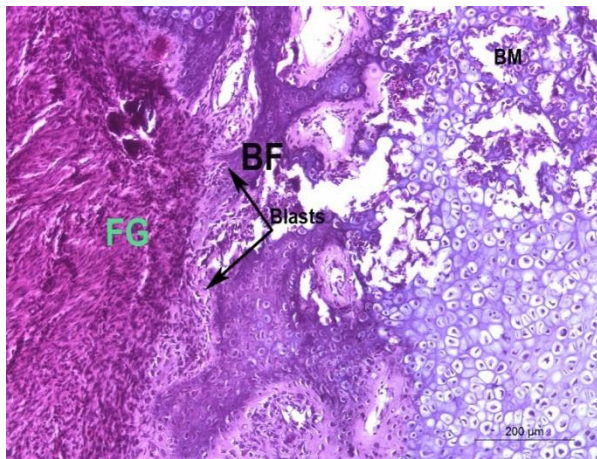


**Fig. (25):** Photomicrograph of rat tibia of control group post fracture by one week showing granulation tissue filling fracture gap (FG), rich by blood capillaries (two yellow arrows), and entangling in it lymphocytes representing the end of the inflammatory phase of the fracture (black arrow), while bone fragment (BF) Bone marrow (BM) (H&E; Bar= 100 μm).

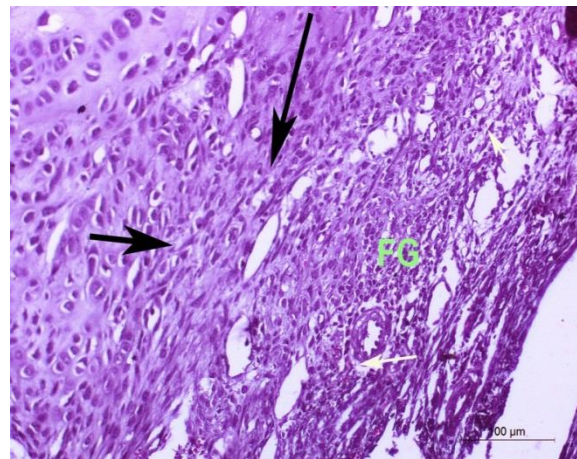


**Fig. (26):** Photomicrograph of rat tibia of control group post fracture by two weeks showing proliferative connective tissue filling fracture gap (FG), with minimal numbers of blood capillaries (yellow arrow), and activated osteoblasts (two black arrows) (H&E; Bar= 200 μm).

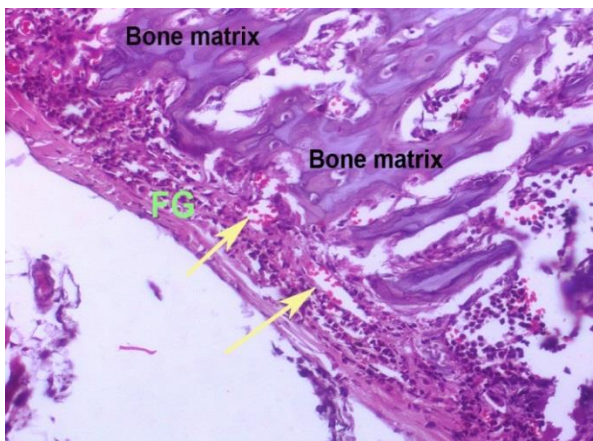




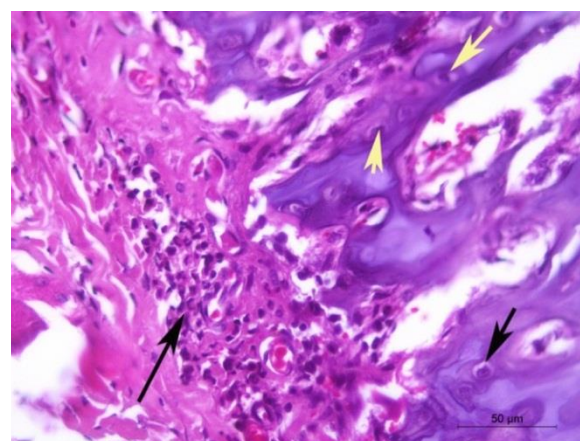
**Fig. (27):** Photomicrograph of a higher magnification of the previous picture showing compact connective tissue filling fracture gap (FG), and activated osteoblasts (two black arrows) (H&E; Bar= 200 µm).



**Fig. (28):** Photomicrograph of rat tibia of PRP group post fracture by one week showing proliferative connective tissue filling fracture gap (FG), with minimal numbers of blood capillaries (yellow arrow), and activated osteoblasts (two black arrows) (H&E; Bar= 100 µm).



**Fig. (29):** Photomicrograph of rat tibia of insulin group post fracture by two weeks showing granulation tissue rich in blood capillaries (two yellow arrows) filling fracture gap (FG), with (H&E; Bar= 200 µm).



**Fig. (30):** Photomicrograph of rat tibia of insulin group post fracture by two weeks showing inflammatory cells (black arrow) filling fracture gap (FG), with high numbers of blood capillaries, and activated osteoblasts (two yellow arrows) (H,E; Bar= 50 µm).

**DISCUSSION**

The strategy of this study was designed to identify and to evaluate the local effect of PRP and insulin in acceleration of bone regeneration and healing at the fractured sites of long bone using an experimentally laboratory animal model.

PRP stimulate and promote the healing rat of bone fractures. This finding was in agreement with that given by Anitua *et al.* (2006) and Castillo-Cardiel *et al.* (2017) who mentioned that the PRP therapeutic is a relatively new biotechnology that used to accelerate the healing of soft and hard tissues which applied in multiple aspects of medicine like (orthopedics and maxillofacial surgery).

In this study, PRP technique is simple low cost technique and does not causes any harmful effect to the animal, reducing the time for fracture healing. These findings are in agreement with that given by (Castillo-Cardiel *et al.*, 2017).

Application of PRP was associated with lower healing period and less postoperative infection and pain. This result was in correlation with (Ghaffarpasand *et al.*, 2016). Because the PRP release interleukin 1 (IL-1) that play an important role in the regulation of immune and inflammatory responses to infections.

This study detects the local application of the autologous PRP which considered as an effective

method for stimulation bone regeneration (BR) and noticeable acceleration at the fracture site without complications. This evidence was in coincide with (Castillo-Cardiel *et al.*, 2017).

By determination of both ultrasonographic echogenicity and computed tomography density and bone density at 1<sup>st</sup>, 2<sup>nd</sup> and 4<sup>th</sup> weeks' time intervals of PRP group and insulin group in comparison to the normal bone density which determined before fracture induction. The PRP group becomes much more similar to the normal bone density at 4<sup>th</sup> week or at the end of the experiment. While insulin group, the density not reach or approximate the normal bone density, (Ultrasonographic echogenicity computed tomography bone showed significant differences favoring the two experimental group than the control group) and these findings agreed with (Castillo-Cardiel *et al.*, 2017).

A good effect of PRP on bone regeneration (BR) and its clinical and surgical importance and it has many properties include autologous product, induces angiogenesis and promotes osteo-integration and cell proliferation. Also it has a hemostatic and lymphatic bioseal which decreasing post-operative drainage and edema (Anitua *et al.*, 2016 and Pocaterra *et al.*, 2016) this way improving BR and shortening the time for recovery.

Subsequent to an injury, capillaries invading a fibrin clot are one of the most important cellular components of early granulation tissue because they deliver nutrients, inflammatory cells, and oxygen to the wound site, on the other hand, the lack of angiogenesis can result in impaired wound healing (Lingen, 2001). Also Battegay, (1995) mentioned that new blood vessel growth is required for the removal of necrotic tissue and considered starting point for the repair process.

It is possible that the local insulin injections promote wound healing by stimulating the expression of vascular endothelial growth factor VEGF, which promotes endothelial cell proliferation, also increases the permeability of capillaries thus promoting the formation of new blood vessels (Puddu *et al.*, 2016).

One popular theory was that the cells were differentiated from the endothelial cells of the nearby blood vessels, whereas others believed that the cells came from the peripheral blood or resting wandering cells.

The PRP have cytokines and growth factors important to improve the vascularization and tissue proliferation (Hee *et al.*, 2003 and Coetzee *et al.*, 2005).

Transforming growth factor- $\beta$  (TGF- $\beta$ ) mainly comes from blood platelets, it can promote the chemotaxis, migration, proliferation, and differentiation of cells and the formation of granulation tissue by extracellular accretion and secretion, essential factors of wound healing. (Kryger *et al.*, 2007 and Koskela *et al.*, 2016). IL-1 makes the generation of cells, neutrophils and lymphocytes possible by chemotaxis. It stimulates the fibroblasts to produce collagen and controls the formation of scarring tissue, playing a crucial role in wound healing.

Platelet-rich plasma contains up to 8 times the concentration of platelets found in whole blood. The fact that PRP contains several different growth factors, present in physiological proportions, is an appealing benefit compared to isolated growth factors (Sánchez *et al.*, 2007; Creaney and Hamilton 2008 and Hammond *et al.*, 2009).

The lower degrees of inflammation and angiogenesis in the study group was caused by the proven effect of PRP on the increased expression of the matrix degrading enzymes in addition to the increased cell proliferation and collagen production at tendon cells, resulting in accelerated remodeling of the injured tendon (Anitua *et al.*, 2005 and De Mos *et al.*, 2008).

Additionally, Hammond *et al.* (2009) reported improvement and recovery from muscle strain with limitation of the inflammation in a rat muscle injury model as early as possible, 3 days after the injection.

Local Insulin application improve chondrogenesis and cellular proliferation within the first 4 weeks. This finding in correlation with (Gandhi *et al.*, 2005; Graves *et al.*, 2011 and Da-Wei Wang *et al.*, 2013) who suggests that insulin treatment following fracture is accompanied with increasing of VEGF biosynthesis in the fractured tissues, which may be responsible for the improved bone healing.

Local application of insulin has the important effect in accelerate early and late stages of fracture healing. This finding is coincide with (Gandhi *et al.*, 2005) who mentioned that the various stages of fracture healing could be accelerated with the effect of expression of three soluble factors (pro-inflammatory cytokines, the TGF-h superfamily and angiogenic factors) insulin that play a critical role in modulating the repair process. Local low-dose insulin acts independently of blood sugar metabolism in promoting wound healing, and it plays an important regulatory role in the course of regeneration of cell factors, growth factors and inflammatory reactions of multiple epithelia. Also, it could markedly promote wound healing and the survival of transplanted flaps after operation for deep burns, and this is probably associated with the

stimulation of higher expression levels of HSP-90, VEGF, TGF- $\beta$  and IL-1, which nevertheless, exert little influence on systemic blood glucose (Ming *et al.*, 2016).

Whether the insulin is injected via subcutaneous, infiltration or intravenous injection, its actions depend on its ability to regulate the blood sugar metabolism. Insulin is a polypeptide with a short half-life *in vivo*. It may be decomposed by proteases in tissue when directly applied to wounds, and therefore reapplications are needed for treatment. The problem of how to continually release insulin in an effective manner to promote the growth of granulation tissue and wound healing is a known concern (Dhall *et al.*, 2015).

This suggests that the insulin effect on wound healing is related to its concentration. When the concentration is increased above a certain threshold, the effect is weakened, probably restraining normal wound healing speed. From this, it seems clear that a low-dose insulin can promote the regeneration of wound tissue (Zhang *et al.*, 2007 and Zhang *et al.*, 2011).

In conclusion, the local application of small dose of PRP in the site of long bone fracture results in higher cure rate, shorter healing duration, and less postoperative pain. Insulin comes in the second place after PRP but it is clear that PRP products are very preferable than insulin product due to easy to have with low costs of production as, all we need autologous blood from the same animal species, aseptic syringe, collecting tube and centrifuge.

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## الموجات فوق صوتية ، الأشعة المقطعية والهستوباثولوجي لتقييم استخدام البلازما الغنية بالصفائح الدموية والانسولين على التئام الكسور في الفئران

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تهدف هذه الدراسة الى تقييم تأثير البلازما الغنية بالصفائح الدموية والانسولين على تعزيز معدل الالتئام في كسر العظام الطويلة في حيوانات التجارب. أجريت الدراسة الحالية مع ٢٧ من الفئران البيضاء والتي يتراوح وزنها بين ١٥٠ و ٢٠٠ جرام. تم تقسيم هذه الحيوانات إلى ثلاث مجموعات (تسعة فئران في كل منها). تألفت التجربة من مرحلتين: المرحلة الأولى ، شملت على الاستحداث الجراحي للكسر الكامل لعظمة الساق تحت تأثير التخدير العام. المراحل الثانية ، تم تطبيق المصبوب فقط في المجموعة الاولى ، بينما في المجموعة الثانية، تم تطبيق المصبوب بعد حقن البلازما الغنية بالصفائح الدموية في موقع الكسر ، وكذلك تم تطبيق المصبوب بعد حقن الأنسولين في موقع الكسر في المجموعة الثالثة. باستخدام الموجات فوق الصوتية والتصوير المقطعي المحوسب والهستوباثولوجي لتقييم معدل التئام كسر العظام لكل مجموعة. أظهرت النتائج أن تغيير الصدى في موقع الكسر كان سريعاً في مجموعة البلازما الغنية بالصفائح الدموية متبوعة بمجموعة الأنسولين وأخيراً كان تغيير الصدى بطيئاً في المجموعة الضابطة. وجد أيضاً زيادة في كثافة العظام بسرعة عالية في مجموعة البلازما الغنية بالصفائح الدموية متبوعة بمجموعة الأنسولين وأخيراً كانت كثافة العظام منخفضة في مجموعة التحكم والتقييم النسيجي يؤكد هذه النتائج. في الختام ، استخدام البلازما الغنية بالصفائح الدموية له تأثير مفيد أكبر في تعزيز معدل التئام كسر العظام وانتشار الأنسجة من الانسولين.