Radiological study of the influence of platelet rich-plasma and low level laser therapy on healing of experimentally fractured proximal sesamoid bone in equine: Part II

Sinan A, MJ Eesa and Raffal A Omar

Abstract
The objective of this study was to evaluate the effects of platelet rich plasma and low level laser therapy (diode laser) on the healing of proximal sesamoid bone fracture. Twelve healthy adult donkeys were used during the period from June 2015 to August 2017. Animals were divided into three equal groups: First group was used as control, second was treated by platelet rich plasma and third group was treated by diode laser. All animals were exposed experimentally to transverse mid body fracture of proximal sesamoid bone, in first group the bone fixed internally by screw and followed by external fixation with Plaster of Paris under general anesthesia, the second and third groups were exposed to similar surgical procedure except in second group injected of autologous platelet rich plasma in the fracture site, and the third group exposed to serial session of low level laser. All experimental animals were in a good health postoperatively, except hyper granulation tissue at the site of surgical incision was observed in one animal of the first group and another in the second group exhibited superficial exudate at the site of operation. At 60 post-operative days, the x-rays of the fracture line show a slight visible fracture line, new bone formation with still visible fracture line and denser fracture line, in first, second and third groups respectively. At 90 post-operative days, in three groups, they showed invisible fracture line, but with superior of third group in compared with first and second groups.

Keywords: Proximal sesamoid bone, Low level laser, Platelet rich plasma, Fracture, Equine.

1. Introduction
The proximal sesamoid bones were paired bones consisting of spongy bone in its structure due to their position in the palmar/planter aspect of the fetlock. They give stability and support to the suspensory apparatus. These two bones act as single bone during the joint movement due to the attachment of inter sesamoid and ligaments on the axial aspect of both bones [1,2]. Platelet rich plasma was prepared by centrifuging the blood with anticoagulant so it can be prepared easily and during the operation [3]. Because platelet rich plasma (PRP) contains different growth factors and cytokines it can enhance the fractured bone and damaged soft tissue healing, for that reason studies recommended to use PRP in inflammation, postoperative blood loss, infection, narcotic requirements, and osteogenesis, in addition to the local hemostasis at the sites of vascular injury [4]. Laser effects on tissues may be attributed to thermal and non-thermal effects; the non-thermal effects of laser beam on tissue include photodynamic therapy and photobio-stimulation therapy. The beneficial biological effects of laser light therapy on tissues can accelerate tissue repairing and cell growth, improve vascular activity and increase metabolic activity [5]. The use of laser could enhance callus development in the early stage of the healing process, with doubtful improvement in biomechanical properties of the healing bone; therefore, laser therapy may be recommended as an additional treatment in non-union fractures in humans [6]. The aim of this study was to evaluate the effects of PRP and low level laser therapy (diode laser) on healing experimental transverse proximal sesamoid bone fracture.

2. Materials and Methods
This study was carried out on twelve apparently healthy donkeys during the period from June 2015 to August 2016. Animals were divided into three equal groups. The first group (control group): In which transverse fracture of a proximal sesamoid bone was made, and internal
fixation (lag full-thread screw) was used to fix the two fragments of the fractured bone. The animals were fasted 12 hrs. of water and 24 hrs. of food before the surgical operation. The surgical area which extended from mid-metacarpal bone to the coronet was prepared aseptically. All operations were achieved under general anesthesia, intravenous administration of Acepromazine 0.05 mg/kg B.W. as a premedication, and after the appearance of action, a mixture of both chloral hydrate 50 mg and magnesium sulphate 25 mg at 7% was injected intravenously. The animal was placed on the right lateral recumency and about 8-10 cm longitudinal skin incision was made in the posterior aspect of the lateral proximal sesamoid bone of the left forelimb (Fig. 1). Blunt dissection of subcutaneous tissue was done to identify the lateral proximal sesamoid bone. Then palpated groove between this bone and sheath of the superficial and deep digital flexor tendons and about 1.5-2 cm longitudinal tendon sheath incision was made to investigate the area between tendons and lateral proximal sesamoid bone (Fig. 2). After these steps, the bone edge was clear and, mid fractured bone was induced by bone chisel (Fig. 3). Electrical drill machine (0.8 Mm auger size) was used to induce longitudinal canal through two fragments of fractured bone, inorder to facilitate insertion of lag full threaded screw (0.8 Mm size) (Fig. 4). Normal saline was used during drilling to minimize heat, and screw driver was used to insert the screw in the canal up to approximate the two fragments of the fractured bone (Fig. 5). This technique is not mentioned in ours avaliable references.

Polyglactine 910 (1 size) suture material was used to close the incision of tendon sheath by simple continuous pattern, also subcutaneous tissue was closed by the same suture material. Skin was sutured by simple interrupted suture pattern. Plaster of Paris cast with window was used to support internal fixation. Penicillin (20,000 I.U) and streptomycin (20 mg/kg. B.w) were injected intramuscular daily for four days postoperative, and dressing of wound was done daily until healing was completed and skin stitches were removed after 12 postoperative days. Clinical examination of experimental animals such as movement, ability to bear body weight on affected limb and posture of animal, were observed. In second group (PRP): similar procedure as in the first group was done, but after internal fixation PRP(1-1.5 ml) was injected via needle G 18, at the fracture site (PRP prepared by collecting 20 ml of jugular blood sample from the same animal (autologous) before surgical interference in tubes with anticoagulant (sodium citrate) and centrifuged at 2000 rpm for 20 min., where the upper layer was collected and centrifuged at 2000 rpm for 10 min., PRP were collected with small amount of plasma. Third group (laser group): Similar procedure as in the first group was done, but the fracture site was exposed to serial session of portable diode laser type K-laser. Laser therapy was given for 3 days then 1 day rest and continued as the same system for three weeks in which 15 min. per day for each session. The dose was measured in joules, and means that a certain amount of energy per cm² (J/cm²) Supplied: Dose= Time x Treatment laser power per cm². Anterio-posterior radiographic view was used immediately after the surgical operation to show the approximated of fracture ends and later to determine the degree of fracture healing along the period of experiment, at 15, 30, 60 and 90 days post operation. This article has been carried out in accordance with the Code of Ethics of the World Medical Association and EU Directive 2010/63/EU for animal experiments.

3. Results and Discussion
One animal in the first group was suffered from hyper granulation tissue formation at surgical site. This hyper granulation may be due to the increase in the skin tension below the carpal joints in equine and the increase in movement in this area; this observation coincided with Jacobs
et al.,\textsuperscript{11} who mentioned that the lower leg skin wounds had slower rates of wound contraction than those on the shoulder, also in agreement with Bader and Eesa\textsuperscript{12}\textsuperscript{12} who reported that hyper granulation tissue was formed in wound healing below the carpal or tarsal joint in equine. In second group one animal was exhibited superficial exudate at surgical site, this may be due to wound infection, in which treated until the wound gap filled with tissue. Clinical observation after one month post-operative shown that animals in third group were able to bear weight on treated limb but lesser degree in other two groups was observed, while at the ends of three months, it revealed that animals in the third and second groups were normal in movement, which is more prominent in third group, but in the first group the animal still lame. These observations may be related to the effect of Laser on bone healing. This fact agrees with other finding reported about laser improved bone healing by an increase of osteoblastic proliferation, collagen deposition, and new bone formation when compared to non-irradiated bone\textsuperscript{13}. In three groups, the x-rays showed a visible fracture line at 15 days postoperative as shown in (Fig. 6, 7, and 8). At 30 days in first and second groups the x-rays showed a slight visible fracture line, and the space between fractured ends was decreased (Fig. 9, and 10). The visible fracture line may be indicated that the two fractured ends were connected by soft callus which made bridge between fractured ends but not seen in radiograph. This phenomenon was agreed with Kealy et al.,\textsuperscript{14} who explained that the repair began by soft callus formation then converted into hard callus. The slight visible fracture line especially in PRP may be indicated that the PRP enhanced fracture healing. This was agreed with Doler et al.,\textsuperscript{15} who reported that PRP has been shares to stimulate cells proliferation of osteoblasts and fibroblast and to regulate osteocalcin in these cells. Also Berner et al.,\textsuperscript{16} reported that platelet play an important role in the initial wound healing, platelets release multiple growth factors and cytokines involved in healing. In third group show new bone formation at the fracture site with narrowing of fracture line (Fig. 11). These observations were coincided with histopathological study at one and three months post operation who revealed that, the fracture gap were infiltrated with collagen and woven bone in addition to thin layer of trabecular bone in control group, while in PRP group, slight granulation tissue, more woven bone, trabecular bone and thin area of lamellar bone were formed and more advanced of lamellar bone formation in laser group. This development may be due to the action of growth factor of PRP which plays an important role in fracture healing to activate osteoblasts and fibroblasts to produce additional growth factors\textsuperscript{18} and in the laser therapy, might be increased osteogeneses, this was coinciding with Tavares et al.,\textsuperscript{19} Also Lopes et al.,\textsuperscript{13} reported that the use of LLLT in early stages of bone repair decrease the total period of osteoid formation and it aids the organization of clot and promotes granulation.

At day 60 post operation, in the first group, the radiographies showed slight visible fracture line, which gradually decreased the space of the fracture line (Fig. 12). In second group, showed new bone formation with still visible fracture line (Fig. 13), while in third group showed denser fracture line (Fig. 14). These results might be explained that the slight radiopaque of fracture line, might be due to the gradual conversion of woven bone into the trabecular and compact bone but no completely filled the fracture so that the fracture line still visible in first group. In second group very slight visible fracture line, that might be due to the most of osteoid tissue converted into compact bone. This observation agreed with Franchini et al.,\textsuperscript{20} who reported that the osteoinduction effect of autologous platelets concentrate used in reconstruction surgery and treatment for impaired fracture repair. In third group, fracture line very slight visible, this might be return to the action of LLT on proliferation of osteoblasts and other body cells. These results were agree with Catao\textsuperscript{21} who described that laser can stimulate cells proliferation at the fracture site that were vital for the progress and resolution of healing progress via tissue bio-stimulation, such as increased mitochondrial ATP production, lymphocyte, mast cell activation, proliferation of fibroblasts and other cells, beside promoting analgesia and anti-inflammatory effects. At 90 days post-operative in three groups, there was invisible fracture line that means the fractured gap was occupied with bony tissues and appearance radiopaque in radiography (Fig. 15, 16 and 17). However the histopathological founding at this period exhibited that the amount of compact bone formation at the fracture gap on laser group more developed than first and second groups. These results indicated that the healing process was more advanced in the laser group compared with other two groups. This might be due to the actions of the LLLT to proliferation of osteoblasts and accelerated fracture healing. These observations were coincided with Orhun et al.,\textsuperscript{22} who reported that alkaline phosphatase was significantly increased six days after irradiation of standardized bone lesions, which reflected an enhanced osteoblasts activity.
4. Conclusion
The present study indicated that the healing process was more advanced in the laser.

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6. References
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