

## **An experimental study of the effect of autologous platelet rich plasma on the healing process of tendon in donkeys**

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### **Abstract**

The aim of this study was to evaluate the regenerative effect of platelets rich plasma PRP on tendon healing in donkeys. Eight adult donkeys from both sexes were used. The age range from 2-4 years and weighting 150-180 kg. The animals divided into two groups control and treatment group each group consists of four animals. The donkeys in both groups were given deep narcosis with 10% chloral hydrate at a dose of 5 g / 50 kg of body weight I.V after the premedication with 2% xylazine at a dose of 1.1mg/kg of body weight I.V. The superficial flexor digital tendon of forelimb was severed transversely and sutured by bunnell-Mayer technique using stainless steel suture and the limb was totally immobilized with plaster of paris. In the second group donkeys underwent the same procedures with topical application of platelets rich plasma on the site of sectioned tendon. At 30 post operative days the results revealed that there was a noticeable improvement of the functional activity of the injured tendon treated with platelets rich plasma PRP. Histologically there was granulation tissue at the site of healing of sectioned tendon. We conclude that platelets rich plasma lead to functional improvement of the injured tendon.

**Keywords:** Tendon, Healing, Platelet rich plasma, Donkey.

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## **دراسة تجريبية لتأثير المزيغ الذاتي للبلازما الغنية بالصفائح الدموية على تجدد الوتر في الحمير**

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### **الخلاصة**

أجريت هذه التجربة لدراسة تأثير البلازما الغنية بالصفائح الدموية على تجدد الوتر المقطوع كلياً في الحمير. إذ تم استخدام ثمانية حيوانات من كلا الجنسين تراوحت أعمارها ما بين ٢-٤ سنة وأوزانها ما بين ١٥٠ - ١٨٠ كغم. قسمت حيوانات التجربة إلى مجموعتين ضمت كل مجموعة أربعة حيوانات. تم تخدير كل حمير التجربة بعد تهدئتها مسبقاً وقبل ٥ دقائق بالزايلازين ٢٪ (١.١ ملغم / كغم بالحقن الوريدي) بالكولورالهايدريت ١٠ ٪ (٥ غم / ٥٠ كغم بالحقن الوريدي) وبعد ذلك تم قطع الوتر الانقباضي السطحي للقائمة الأمامية لحيوانات المجموعة الأولى بصورة كلية بعد ذلك تمت خياطة الوتر المقطوع بطريقة بانيل - ماير وباستخدام خيط جراحي نوع ستانليس ستيل ثم غلق الجلد بواسطة الخياطة المتقطعة البسيطة باستخدام خيط نوع حرير. تم تثبيت القائمة بواسطة التثبيت الخارجي باستخدام الجبس. أما في المجموعة الثانية فقد تم إجراء نفس الخطوات السابقة مع إضافة البلازما الغنية بالصفائح الدموية موضعياً على منطقة قطع الوتر. أظهرت النتائج العيانية والوظيفية حدوث تحسن واضح في وظيفة القائمة المعالجة بالبلازما الغنية بالصفائح الدموية بعد مرور ٣٠ يوم من إجراء العملية في حيوانات المجموعة الثانية بعكس حيوانات المجموعة الأولى التي كان فيها الأداء الوظيفي للقائمة غير جيد. أما نسيجياً فأظهرت النتائج حدوث التئام لمنطقة القطع من خلال تكون نسيج حبيبي جديد في كلتا المجموعتين. نستنتج أن إضافة البلازما الغنية بالصفائح الدموية أدت إلى تحسن الأداء الوظيفي للوتر المصاب فقط.

## Introduction

The process of tendon repair was described with accuracy as far back as the seventeenth century (1). Flexor tendon wounds are serious because of the weight bearing function of this structure, Further more distal limb wounds usually involve additional soft tissue structures such as joint capsules, tendon sheaths and neurovascular bundles which complicates treatment and healing and negatively affect the prognosis (2). Rehabilitation following tendon injury and repair is a long process. In case of flexor tendon injuries, return to normal function is not allowed until at least eight to twelve weeks after repair (3). The platelet rich plasma (PRP) is an autologous product that concentrate a high number of platelets in a small volume of plasma (4). Its biocompatible and biodegradable properties prevent the PRP from inducing foreign body reactions, tissue necrosis, or extensive fibrosis (5). Reports in the literature differ about the intensity and duration of blood centrifugation, the number of platelets present in the PRP, and the use of thrombin or other factors to activate PRP (4-6). PRP can be used as a healing substance for different kinds of tissue injury causing less post-surgical pain (7,8). During platelet degranulation many biologic active substances are released which participate in the primary homeostasis and help the following reparation and regeneration of the soft and hard tissues. The most important substances are: serotonin, catecholamine, fibrinogen, factor V, factor VIII, thromboxan A<sub>2</sub>, calcium etc. Many growth factor are also very important: Platelet-derived growth factor (PDGF); Transforming growth factor-b (TGF-b); Vascular endothelial growth factor (VEGF); Interleucin-1(IL-1); Basic Fibroblast Growth Factor (BFGF); Insulin like Growth Factor (IGF). In the platelet rich plasma there are also small quantities of immune cells as well as plasma (9-11). The aim of present study was to evaluate the effect of platelet rich plasma on the healing process of the injured tendon.

## Materials and methods

### Animals

Eight adult donkeys weighing between 150 and 180 kg and aging between 2 and 4 years were used in the current study. The animals were kept in the farm of the College of Veterinary Medicine, University of Mosul. The animals divided into two groups of four animals in each group the control group underwent surgical procedure in which the superficial flexor digital tendon of the forelimb was sectioned and repaired surgically by bunnell-mayer technique using stainless suture material and was totally immobilized with plaster of Paris. In the second group the same procedure performed with local application of platelet

rich plasma on the cut surface of the tendon. Postoperative observations were daily performed during the first week and weekly thereafter.platelets rich Plasma preparation.

Four blood samples from four animals were collected. A 100 ml of blood was drawn under strict aseptic condition from the jugular vein the blood was aspirated with a 21 G needle. A 50 ml sized syringe preloaded with 7 ml of anticoagulant citrate dextrose solution ACD was used to avoid coagulation. Each blood sample was centrifuged 2400 prm for 10 minute at 5 °C by cooled centrifuge resulting in three following layers. The inferior layer composed red blood cells, the intermediate layer composed of white blood cells and the superior layer made of plasma, the plasma layer was centrifuged for another 15 minute at 3400 prm in order to obtain a two part: the upper part which is platelet poor plasma PPP and the lower part was the platelet rich plasma PRP. Then the PRP was aspirated with another pipette and placed in a sterile tube. The PRP was thus prepared for activation by 10% calcium chloride CaCl<sub>2</sub> which inhibit the blood – thinning effect of ACD. After activation, PRP turned into a gel-like solution that is ready for use (12).

### Anesthesia

Animals were premedicated with 2% Xylazine at a dose of 1.1mg /kg of body weight intravenously then 10% chloral hydrate at a dose of 5 g /50 kg of body weight intravenously was given as deep narcosis (13).

### Surgical procedure

Under aseptic condition a longitudinal skin incision was made directly over the superficial flexor digital tendon and then complete transverse incision was made with surgical blade through the midway of the tendon. This incision was immediately sutured with bunnell-Mayer technique using stainless steel suture (figure1) then the sectioned tendon was allocated to receive either platelet rich plasma or control treatment. Total immobilization of the affected limb was made by plaster of paris which extended from carpal joint to the coffin joint for two weeks.

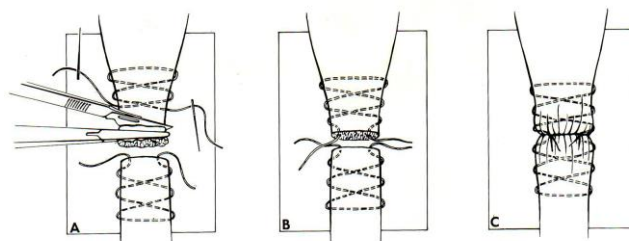


Figure 1: Drawing showing bunnell-Mayer technique utilized for end to end anastomosis of tendon (14).

### Gross pathological study and biopsy collection

Histopathological examination of the specimens were done 30 days after surgery. Histological specimens were stained with hematoxylin and eosin.

### Results

#### Clinical observation

The ability of the animals in the treatment group to use the operated leg is better than the animals in the control group in which the animal exert great efforts in order to stand. The animals in the control group tend to remain recumbent most of the time and standing occurred after several trials as compared to those of the treatment group. The gait of the animals of the both group was observed clinically two weeks after operation. The treatment groups showed significant improvement in comparison with control group animals. And the treatment with PRP was considered successful when the animals return to the same pre-injury movement without relapse of the injury in the follow-time. While the control animals suffered from lameness for longer period when compared with treatment animals.

**Gross finding:** There were macroscopic differences between the groups of animals at four weeks after operation the skin repair site was showed increased granulation tissue especially in the treatment group. The repair site was hypertrophic and semi translucent. The cross sectioned area of tendon was increased in diameter in both groups.

Histopathological results of the control group revealed skin wound healing through fibroplasia and epithelization at 30 postoperative days. Healing of sectioned tendon was occurred by proliferation of collagen fibers and fibroblasts "fibroplasia", associated with angioplasia in intertendineous gap which was filled with mature granulation tissue that arranged in a parallel fashions to long axis of the tendon. This granulation tissue can recognized from original tendon tissue and there is severe infiltration of mononuclear inflammatory cells in the healing tendon tissue as well as edema (figure 2 and 3). In the treatment group healing of tendon occurred in similar pattern that mentioned above histopathologically only there was evidence for polymorphonuclear inflammatory cells (inflammatory nodules) in the healing tissue within the intertendineous gap (figure 4 and 5).

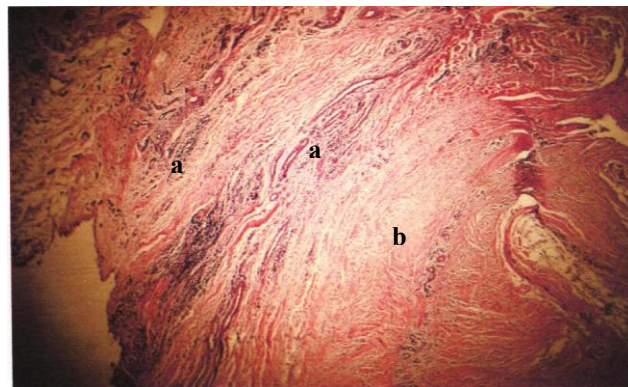


Figure 2: Photomicrograph of healing tendon at 30 days after tenotomy in group 1, showed presence of granulation tissue consisting from newly capillaries (a) and collagen fiber (b). H&E at 40 X.

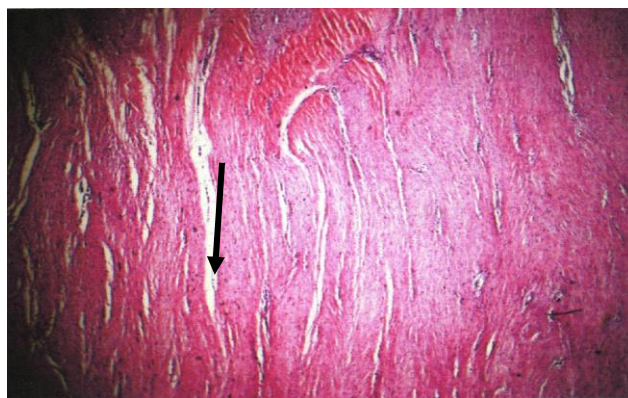


Figure 3: Photomicrograph of healing tendon at 30 days after tenotomy in group 1, showed mature granulation tissue within intertendineous gap (arrow). (H & E 40 X).

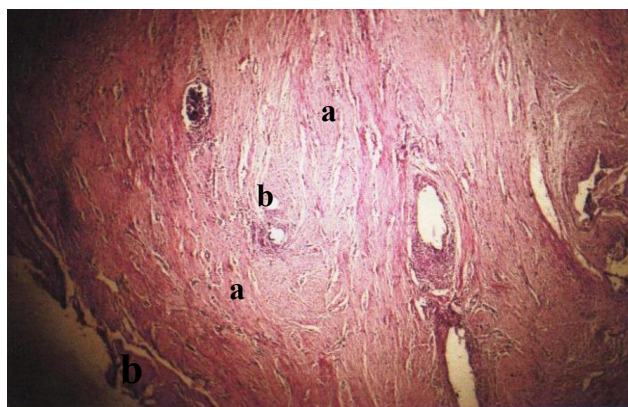


Figure 4: Photomicrograph of healing tendon at 30 days after tenotomy in group 2 treated within PRP after tenotomy, showed the presence of collagen fiber (a), newly capillaries (b), (H & E 40 X).



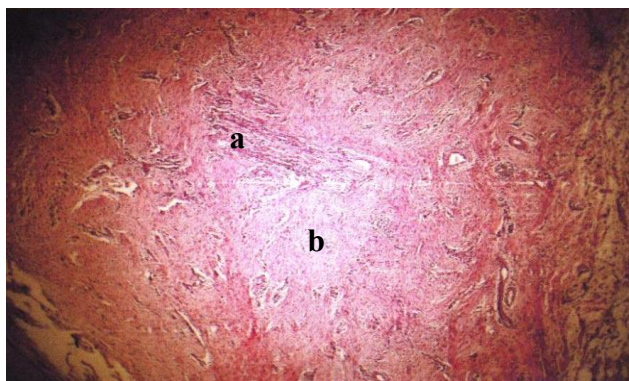


Figure 5: Photomicrograph of healing tendon at 30 days after tenotomy in group 2 treated within PRP after tenotomy, showed the presence of mature granulation tissue (a) with inflammatory nodules (b), (H & E 40 X).

## Discussion

A chemical substance 10% calcium chloride  $\text{CaCl}_2$  was used for the activation of the PRP and this could avoid the use of heterologous blood components such as bovine thrombin as activator which can cause immune reactions and associated with development of antibodies which had occasionally lead to life-threatening coagulopathies, and in turn, lead to negative results that could be falsely ascribed to PRP (14). PRP is identical in substrate to the blood clot that form in every wound and therefore it cannot support bacterial growth in addition to that PRP has a pH of 6.5 to 6.7 where as mature blood clot has a pH of 7.0 to 7.2 and therefore may even inhibit bacterial growth as other acidic solutions do (15). The sectioned tendon was sutured with stainless steel suture material because it is biologically inert to decrease tissue reaction. The injured limb was totally immobilized postoperatively in both groups to prevent further extension which may negatively affect healing process. The clinical improvement of the gait of the animals in the treatment group during the first two weeks after operation may related to the powerful and long-term analgesic and anti-inflammatory effect of the PRP due to presence of important amount of transforming growth factor TGF- $\beta$  and angiogenic VEGF and proliferative and anabolic PDGF, IGF growth factors which have a significant importance in the wound repair process (16). Histologically there was proliferation of collagen fibers and fibroplasia, angioplasia with mature granulation tissue due to the effect of the platelet derived growth factors upon the proliferation phase and also these factors enhance angiogenesis, fibroblast proliferation via activation of their mitotic activity (17,18). Histopathologically there were no differences at repair site in both groups because of short period of the study. The presence of polymorphonuclear

cells in the treatment group was due to PDGF which released as result of platelets degranulation and this factor amplified the inflammatory responses with increased wound influx neutrophils (18). In both groups there was infiltration of inflammatory cells because it is thought that the inflammatory responses were markedly diminished at six weeks and were minimal at twelve weeks (19). In conclusion the healing of tendon seem to be a complicated process and the PRP had a positive effect on functional improvement of injured tendon. It is recommended that the duration of the studies in the future should be extended for more than twelve weeks.

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## References

1. The History of Tendons Suture In Historical Medicine. Med J Ree. Cited by Wrenn RN, Goldner JL, Markee JL. An experimental study of the effect of cortisone on the healing process and tensile strength of tendon. J Bone Joint Surg. Am. 1954;36:588-601.
2. Auer JA, Stick JA, Equine surgery. 2 nd ed. W.B Saunders Company, Philadelphia 1999. 707 p.
3. Boyer MI, Strickland JW, Engles D, Sachar K, Leversedge FJ. Flexor tendon repair and rehabilitation : state of the art in 2002. Instr course lec. 2003;52:137-161.
4. Marx RE. Platelet-Rich Plasma: evidence to support its use. J Oral Maxillofac Surg. 2004;62:489-496.
5. Dijkstra-Tiekstra MJ, Van der Schoot CE, Pietersz RN, Reesink HW. White blood cell fragments in platelet concentrates prepared by the platelet-rich plasma or buffy-coat methods. Vox Sang. 2005;88:275-277.
6. Efeoglu C, Akcay YD, Erturk S. A modified method for preparing platelet-rich plasma: an experimental study. J Oral Maxillofac Surg. 2004;62:1403-1407.
7. Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A. Role of platelet-derived growth factor in wound healing. J Cell Biochem. 1991;45:319-326.
8. Simon D, Maneul S, Geetha V, Naik BR. Potential for osseous regeneration of platelet-rich plasma – a comparative study in mandibular third molar sockets. Indian J Dent Res. 2004; 15:133-136
9. Cochran DL, Rouse CA, Lynch SE, Graves DR. Effect of platelet-derived growth factor isoform on release from neonatal mouse calvarine Bone 1993;14(1):53-58.
10. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 2001;10(4):225-228.
11. Petrungaro PS. Using platelet-rich plasma to accelerate soft tissue maturation in esthetic periodontal surgery. Compend Contin Educ Dent. 2001;22(9):729-732,734, 736.
12. Gimeno FL, Gatto S, Ferro J, Croxatto JO, Gallo JE. Preparation of platelet-rich plasma as a tissue adhesive for experimental transplantation in rabbits Thromb J. 2006;4:18.

13. Hall L W, Clarke K W. Veterinary anesthesia. 8<sup>th</sup> ed. London : W.B saunders company ; 1983. 87-220 p.
14. Slatter DH, Small Animal Surgery. 1<sup>st</sup> ed. Philadelphia: S.B Saunder Company ; 1985, Vol 2: 2344 P.
15. Marx RE, Garg AK, Dental and craniofacial application of platelet-rich plasma. Chicago: Quintessence Publishing Co; 1995, 43 P.
16. Anitua E, Andia I, Arpanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration Thromb haemost2004;91:4-15.
17. Dennison DK, Vallone DR, Pinerro GJ, Rittman B, Caffesse RG. Differential effect of TGF- $\beta$  and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts J. Periodontology 1994; 65(7): 641-648.
18. Wiltfang J, Schlegel KA, Schultze-Mosgau S, Nkenke E, Zimmermann R, Kessler P. Sinus floor augmentation with betatricalciumphosphate(beta-TCP): does platelet-rich plasma promote its osseous integration and degradation? Clin Oral Implants Res. 2003; 14(2): 213-218.
19. Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A. Role of platelet-derived growth factor in wound healing. J Cell Biochem. 1991; 45: 319-326.
20. Chong AKS, Ang AD, Goh JCH, Hui JHP, Lim AYT. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit achilles tendon model. J Bone Joint Surg Am. 2007; 89: 74-81.