Effect of Freshly Prepared Autologeous Platelets Rich Plasma on Limb Gait in induced complete Femoral transverse fractures in Dogs

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Abstract:

The presents study was designed to evaluate the effect of freshly prepared autologeous platelets rich plasma (A-PRP) on limb gait , and body weight bearing of induced femoral mid shift fracture in 18 adult stray male dogs .The fractures created under general anesthesia with highly aseptic technique .The operated animals divided to tow equal group each (n = 9) , group A control group which followed for normal healing processing without additive ,while group B treatment group which injected 3ml of the A-PRP locally at the site of fracture after the end of surgical operation .The evaluation done according to the limb gait score , which consist of 5 different degree and level ,the results revealed ,there is gradual improvement in limb gait in treatment group compare to the control group ,in which the animals use the affected limb normally in walking and running with complete body weight support at the end of 28th day p. o. with score (1.83±0.16 d), while the control group persist until the end of 35th day p. o. which take the score (1.00±0.00 e) .The conclusion was, injected single dose of A- PRP for treatment of induced femoral mid shift fractures that fixed by intramedullary pining ,rapidly promote and enhance used the limb normally in walking running with complete support body weight better than control group.

Key words: autologeous platelets rich plasma (A-PRP), body weight bearing, femoral mid shift fracture, limb gait score.

Introduction:

A-PRP is an autogenously concentrate fluid of platelets and growth factors, Local applications of Activated PRP releases cytokines, chemokines, histamine and other bioactive compounds to mediate and promote wound and bone healing (1and 2),and play important roles in enhances local vascular permeability, and promotes local aggregation of immune cells (neutrophils), macrophages, and monocytes, to promotes immune cells to engulf necrotic and pathogens tissue **(2-5).**

PRP application has rare side effects, like risk of morbidity, infection, injury to the blood vessels or nerves, scar tissue formation, and calcification at the injection site of (6 and 7), can be used successfully for the management of osteoarthritis (8-11).

The mechanisms of PRP in wounds and bones healing are due to the action of platelet-derived growth factors and cytokines, which regulate the immune system and trigger cellular regenerative processes,

and the plasma offers a fibrin scaffold during wound healing (12). (2 and 13) declare that activation of platelets, release growth factors and cytokines to regulate the inflammatory phase of bone healing and subsequently modulate soft and hard callus formation and bone remodeling.

Application of PRP on bone graft in non-union long bone after open reduction and internal fixation results in higher cure rate, shorter healing duration, lower limb shortening, less postoperative pain. Higher infection rate is a complication of PRP application(14).Activated PRP released growth factors promote muscle recovery reduces pain, swelling,and time for return normal (15).

The aim of present study:

To evaluate the effect of freshly prepared autologuos (A-PRP) on limb gait ,and body weight bearing in the experimental femoral fractures in dogs.

Methodology:

Experimental animals:

18 adult healthy local breed dogs, employed to create femoral mid shift, transverse fracture ,and fixed by intramedullar pining ,the animals divided to 2 equal groups (N=9) rabbits, A. control group followed for normal healing ,while B. treatment group , exposed locally to single dose A- PRP.

Preparation of platelet rich plasma:

10 ml of blood were collected from the cephalic vein by sterile tube coated with EDTA (Ethylene Diamine Tetra Acetic Acid), 0.5-1 hours prior to operation, by double centrifugation (8), first done at (1800 rpm for 15 min) to induced three layers: The lower layer contains red blood cells, the middle layer contain a buffy coat of leukocyte and platelets, the upper layer contain plasma(poor platelets plasma (PPP) and PRP), the second centrifugation (3500 rpm for 10 min) for pellet formation of platelets that accumulate in the bottom , the PPP in the upper that drawn off. 1ml PRP mixed with 2ml of 10% calcium chloride (activator) to form 3ml of A-PRP as mentioned by (16) (Fig.1).

Anesthetic protocol and surgical procedure:

Induction of general anesthesia done by i/m injection 5mg/ Kg. B.W. of 2% xylazine, after10 minutes 15mg /Kg. B.W. of 10% ketamine hydrochloride was injected (17).4-6 cm skin incision at the lateral aspect of thigh region ,the fascia lata was sharply incised ,the underlying muscle vastus laterials and biceps femoralies bluntly incised ,exposed femur and separate the periostium , Transverse mid shift fracture was done by gaggle wire saw, with dropping sterile warm isotonic normal saline to prevent thermal necrosis ,the two femoral fragments fixed internally using medical intramedullay pins ,the muscles and fascia lata sutured by simple continuous suture patterns using no.0 absorbable sutures materials ,the skin close by simple interrupted suture patterns using no.0 of nonabsorbable suture materials .The treatment group injected locally

3ml of freshly prepared A-PRP immediately after the end of the surgical operation at the fracture gap. Both group followed to end 12th wk p. o. (Fig. 2).

Post operative examination and care:

1. Systemic antibiotics 3-5 days p. o.,10000 IU/ Kg.B.W penicillin hydrochloride and 5mg/ Kg. B.W. streptomycin hydrochloride .
2. Daily observation normal limb gait, body weight bearing at 3rd,10th,21th.28th,35th,and 42th day p. o., to the end of 60th day of experimental periods according to (18) , (Table 1)..
3. Remove the skin suture materials after 7days p. o.

Table 1. Limb gait scoring system cited by (18).

Degree	Description
0	Normal
1	Normal walking, sporadic lameness at trot and gallop without limb elevation.
2	Gait showing lameness in the operated limb at trot and gallop. Total weight limb bearing.
3	Gait showing lameness in the operated limb at walking and trot. Partial weight bearing with elevation of the limb at gallop.
4	Infrequent and intermittent support of the limb. No weight bearing.
5	No limb use

Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of A-PPP limb gait in the current study. Least significant difference –LSD test (Analysis of Variation-ANOVA) was use to significant compare between mean in this study (19).

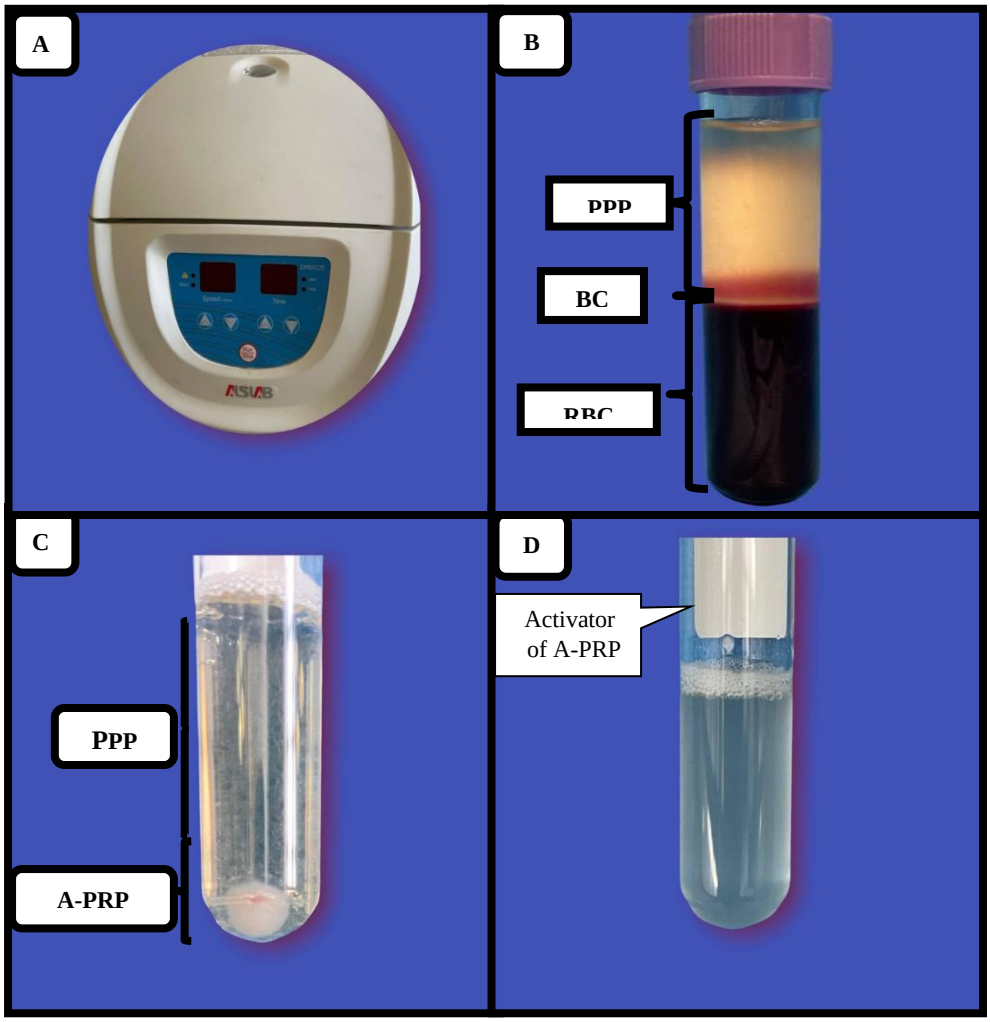

Figure .1. Steps of A-PRP preparations , A- Digital centrifuge devise, B- Blood sample after the first spin, C- Plasma after the second spin, D- activator of (A-PRP).

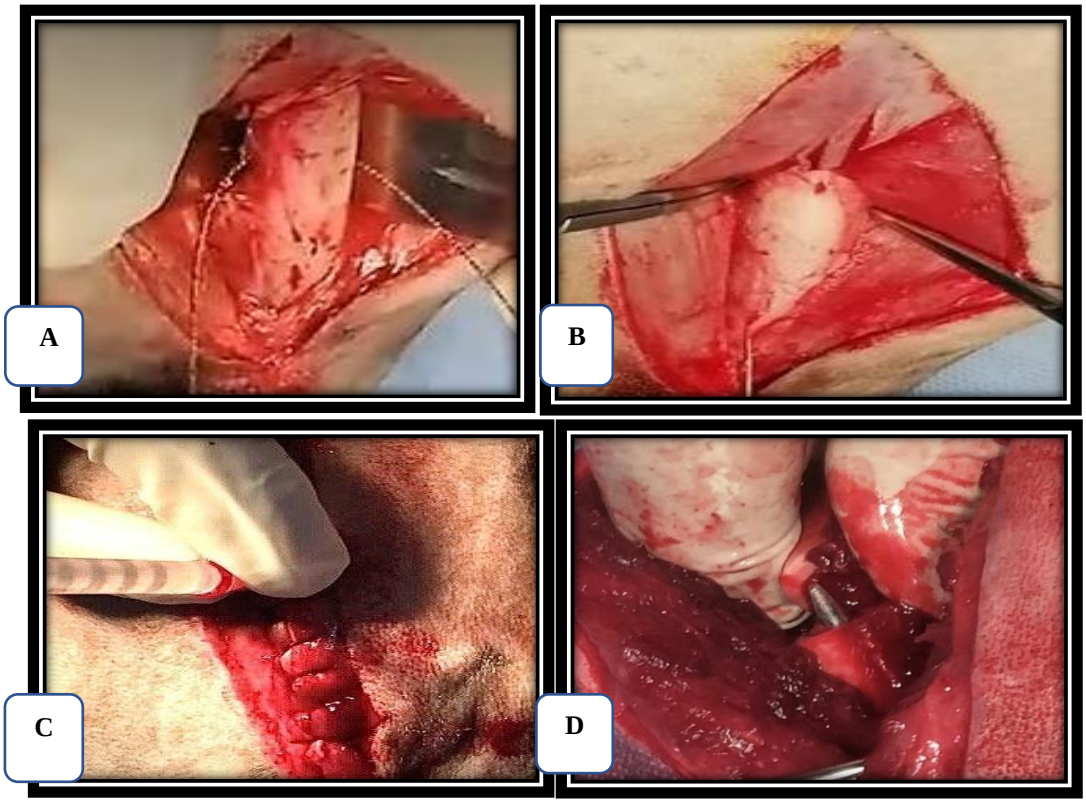

Figure 2. Surgical procedure **A-**Skin incision **B-** Induced complete fracture by Giglie wire saw **C-** Inserted the needle at fracture site to injection of A-PRP. **D-** the two femoral fragments were fixed by intramedullary pinning.

The results of Limb Gait, Lameness and Support Body Weight (Table 2) and (Fig.3):

Within 24 hours P.O. all the operated animals refuse to move, in lying or standing position, at 3rd day P. O. All the animals stand all the time holding the affected limb without supporting body weight, at 10th day P. O. the treatment group can bear the weight on the affected limb in a standing position but hold the limb when walking which takes score (3.78 ±0.14 b), while the control group was (4.00 ±0.00 b),at 21th day p. o. show significant between treatment group and control group with mean value(2.50 ±0.22 c and3.00 ±0.00 c) respectively, at 28th day P. O. the treatment group take score (1.83 ±0.16 d) can used the limb normally,the control group take score(2.00 ±0.00 d) ,at 35 th day P. O. the treatment group take score (0.33 ±0.17 e) while the control group take score (1.00 ±0.00 e) the animals bear body weight and normally walking ,at 42thday p. o. the control group normally use limb .

Table 2 . Different mean value of limb gait in both groups, show of the transverse row (same group), and in vertical column between tow group ,with significant changes in treatment group at the 21th day p. o.

Group	Mean ± SE						LSD value
	Day 3	Day 10	Day 21	Day 28	Day 35	Day 42	
Control	5.00 ±0.00 a	4.00 ±0.00 b	3.00 ±0.00 c	2.00 ±0.00 d	1.00 ±0.00 e	0.00 ±0.00 f	0.522 *
Treatment	5.00 ±0.00 a	3.78 ±0.14 b	2.50 ±0.22 c	1.83 ±0.16 d	0.33 ±0.17 e	0.00 ±0.00 e	0.337 *
LSD value	0.020 NS	0.311 NS	0.498 *	0.371 NS	0.334 NS	0.00 NS	---
Means having with the different letters in row column differed significantly. * (P≤0.05).							

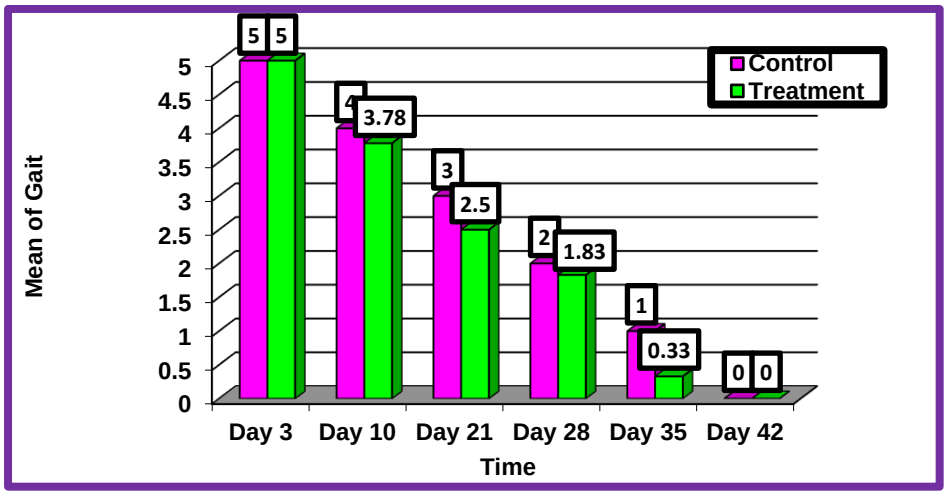

Figure 3. Graphic illustrated the improvement limb gait scores of treatment group compare with the control group , during the period of the study

Discussion:

From the results above, no significant changes recorded between two groups from 24th hour- 3rd day p. o. Which takes grad 5 in which the animals hold the affected limb without supporting body weight ,either in lying or standing position .The data recorded gradually improvement in limb gait and body weight bearing in treatment group compare to the control group during the experimental periods ,these note agree with **(20)** in treatment animals that treated with PRP .

At 10 day p. o. slight improvement in limb gait and body weight support in treatment group which take 3.78, than the control group that take grade 4

At 21th day p. o. limb gait score showed significant different between both groups .that information conducted with (21) they refer that at the 2nd and 3rd week after orthopedic surgery lead to development callus formation and enhance of bone repair and lead to more obvious and active of limb function .

At 28th day p. o. the lameness scale showed improvement in normal weight bearing in treatment group in which the animals can walk running with bear body weight than control group agree with (21), also these note conducted with (22) that the treated animals with PRP can use the affected limb normally with complete body weight support .At 35th day in control group normal walking until the 42th day in which the animals used the limb normally in walking, running and jumping which take grade 0 and remain until the end of experimental periods. These finding of limb gait observation agreed with (23-25). they reported that beginning weight bearing from 2nd day and continues until 60 days, also, these results come in a partial agreement with (26)who revealed that dogs started to support whole body weight on 7th- 10thday P. O. and walked normally without any signs of pain at 15th to 20th days p. o. and these observation of limb gait of the current subject were due to the effect of PRP on fracture healing processing and lameness improvements as mentioned by (13and 15),they concluded that slight improvement in the degree of lameness was observed in PRP group but with no significant difference with dogs of the control group on the 6th and 8th weeks postoperatively**.**

The continuous improvement of limb gait in treatment group than the control group ,due to effect of applying PRP to increase fracture healing and relieve pain, this observation has been conducted with **(** 27) that PRP decreases the postoperative discomfort and enhances healing processes. The body bearing weight score was better in treated dogs with PRP as mentioned by **(28).**

In the present study local applications of PRP promote wound healing , reduce swelling area and promote fracture healing in the treatment group better than control group due to the high concentration of platelets within PRP that releases many active tissue factors and provides supra-physiologic concentrations of growth factors to an injury site, possibly accelerating or otherwise improving connective tissue regeneration as mentioned by (5 ,15,and 29).other researchers refer to the transient pain and inflammation at the injection site after PRP application **(7).**

Application of A-PRP release numerous growth factors within platelets and plasma , effects on tissue repair but at concentrations much different than those found in PRP(30).Local application of A-PRP lead to rapidly repair of bone defect in long bones in rabbits (31).The results of the control group conducted with (32) .

Conclusion:

Local application of freshly prepared autologus derived platelets rich plasma (A-PRP) enhance and improve the limb gait and rapidly use the affected limb normally in walking running and jumping with complete support body weight compare with control group in induced femoral fracture in dogs that fixed internally by intramedullary pins.

Recommendation:

- Study the effect of A-PRP on blood vessels angiogensis of fracture case in dogs.
- Study the effect of A-PRP on osteoblast cells by electron microscope during fractures healing
- Study the effect of A-PRP on the remodeling phase of fractures healing processing in dogs.
- Histomorphometric study of the effect of A-PRP on fracture healing processing.
- Study the effect of the combination of PRP with stem cells on long bones fractures healing

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