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Evaluation of Locomotor and Morpho-histological Effect for Platelet Rich Plasma and Silver Nanoparticles on Healing Process of Achilles Tendon (Comparative Experimental Study in Rabbits)

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Abstract

Tissue engineering is a new treatment option provided encouraging results in orthopedic surgery. In the present study, we compare between platelet rich plasma (PRP) and silver nanoparticles (AgNPs) in the healing process of achilles tendon. This comparison depending upon the evaluation of locomotor and morpho-histological parameters for each treatment. The current study was carried out on 45 apparently healthy adult female rabbits. Locomotor evaluation was done through numerical score at intervals of 2, 4, 6 and 8 weeks postoperatively. Morpho-histological evaluation was performed through numerical score at intervals of 2, 3 and 6 weeks postoperatively. Results of the current study revealed that PRP and AgNPs were better than control group regarding to lameness, pain on complete flexion of tarsal joint, adhesion of tendon to the skin and signs of inflammation. However, PRP is better than both control and AgNPs groups in decreasing lameness and adhesion of tendon to the skin. Morpho-histological examination revealed that PRP is better than both control and AgNPs in improving color of the tendon. PRP and AgNPs were better than control group concerning collagen maturation, fibroblast number, angiogenesis, and surgical gap reduction. However, PRP is better than both control and AgNPs in decreasing signs of inflammation and increasing collagen maturation. We concluded that using PRP for treatment of ruptured achilles tendon provides better prognosis than AgNPs treatment.

KEYWORDS
Healing, Plasma rich platelets, Silver nanoparticles, Tissue engineering, Rabbits

INTRODUCTION

Platelet rich plasma (PRP) contains several growth factors within their alpha granules that are released upon activation. These growth factors act together to improve access of healthy inflammatory cells, angiogenesis, fibroplasias, and re-epithelization (Werner and Grose, 2003), consequently PRP has been previously used to enhance bone, tendon, meniscal, cartilage and wound healing (Murray *et al*., 2006; Ishida *et al*., 2007; Nazhvani *et al*., 2013).

Silver nanoparticles (AgNPs) play an important role in tissue healing as it has antimicrobial effect, accelerating burn wound healing, minimizing wound inflammation, and modulating collagen deposition (Liu *et al*., 2010; Kwan *et al*., 2014). AgNPs has a pro-angiogenic properties due to its role as gene delivery vectors that altering intracellular gene expression and protein synthesis related to the wound healing process, and it modulates cytokines production (Nethi *et al*., 2014; Charafeddine *et al*., 2015).

The Achilles tendon is one of the most injured tendons in the hind limb of animals (Avella *et al*., 2009; Isaka *et al*., 2014). Healing of tendons is low due to its hypo-cellularity, hypo-vascularity, and a low metabolic rate. The healing process normally results only in tissue repair. However, regeneration of the original tensile strength and elasticity are usually not regained (Sharma and Maffulli, 2005a). Therefore, tendon healing has been a challenging issue for orthopedic surgeons in treatment and prognosis (James *et al*., 2008; Rajabi *et al*., 2015). The present study aimed to evaluate the effectiveness of platelet rich plasma and silver nanoparticles on the healing process of achilles tendon. As well as to compare between the effect of PRP and AgNPs on improving tendon healing.

MATERIALS AND METHODS

Animals and study design

The present study was approved by ethical committee of animal use in Sohag university and adhered to national and international animal ethics and welfare laws and guidelines, including those of ARRIVE. This study was carried out on a total 45 apparently healthy adult female rabbits, age ranged 3-6 months and weight ranged 1.5-3 kg. All rabbits were examined clinically before the experimental study to assure that there are no signs of lameness, normal tendon mobility and no pain on manipulation. Rabbits were equally and randomly allocated into 3 groups according to the type of treatment used (control, PRP and Ag-

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NPs groups). Each group had 15 rabbits. All rabbits subjected to unilateral transverse severing of the right pelvic limb's gastrocnemius tendon. Rabbits were anaesthetized by intramuscular injection of a mixture of xylazine - ketamine hydrochloride {Xylazine: 5mg/kg (Xyla-ject® 2%; ADWIA Co., Egypt) and Ketamine: 35 mg/kg (Ketamine® 5%; Sigma-Tec Co., Egypt)}. The right pelvic limb was prepared for aseptic surgery. Then after the site of incision was locally anesthetized by 1.5 ml lidocaine 1% (Debocain®2%, Al-Debeiky pharma., Egypt).

Surgical and treatment procedures

A 3-cm medial skin incision was made in the right hind limb just above the achilles tendon and the gastrocnemius tendon was separated from the plantaris and flexor digitorum superficialis tendons. A complete transverse cutting was applied to the gastrocnemius tendon 1.5 cm above the calcaneus. Then the two tendon stumps were sutured by Becker suture using 4/0 polyglycolic acid thread. Saline (control group) or plasma rich platelets (PRP group) or silver nanoparticles (AgNPs group) were applied directly on the severed tendon. Then the subcutaneous tissue and skin were closed routinely. Following skin closure, the operated limb was immobilized by splint cast.

Rabbits supposed to treatment using PRP gel (400µl of PRP and 200 µl of autologous thrombin). Platelet rich plasma was prepared in two main steps; preparation of autologous thrombin and preparation of autologous PRP according to Yamada *et al.* (2004). Rabbits supposed to treatment using 1ml of AgNPs was injected between the achilles tendon and suture site. Silver nitrate ($AgNO_3$) crystal (ACS $AgNO_3$ F.W. 169.87 Gamma laboratory chemicals, assay: 99%), sodium citrate ($C_6H_5Na_3O_7 \times 2H_2O$, purity 99.0%, Sigma-Aldrich) were used for preparation of silver nanoparticles. Stable Ag-NPs <100 nm were synthesized by using sodium citrate, the molar ratio of silver nitrate to sodium citrate was 1:7 (Ranoszek- Soliwoda *et al.*, 2017).

Study evaluation

All groups were evaluated at intervals of 2, 4, 6 and 8 weeks postoperatively for locomotor evaluation while they evaluated at intervals of 2, 3 and 6 weeks postoperatively for morpho-histological valuables.

Locomotor evaluation

All groups were subjected to locomotor examination based on the blind observer's reports. Evaluations were assessed through numerical scale in which zero score is the worse and most abnormal condition (table 1). This numerical scale was modified from those scales previously mention by Conzemius *et al.* (2002); Kim *et al.* (2010); Gibson and Donnelly (2011) and Stoll *et al.* (2011).

Morpho-histological evaluation

The harvested tendon and neighboring structures were examined grossly at intervals of 2, 3 and 6 weeks postoperatively for the tendon surface at the area of defect, level of tendon defect as well as the color of tendon through numerical scale in which zero score is the worse (Table 2). This numerical scale was modified from those scales previously mention by Stoll *et al.* (2011). Tendon tissue samples were excised and fixed in neutral buffered formalin solution (10 %) and transported to the pathology laboratory for processing. For histo-pathological evaluation, tissue samples were processed, paraffin embedded and sectioned at 5 µm thickness, stained with Harris hematoxylin and Eosin H&E and Masson's Trichrome stains (specific stain for collagen content). The assigned brief score for each animal based on the tissue histopathological examination. Samples were scored qualitatively and semi-quantitatively based on modified scoring systems (Rosenbaum *et al.*, 2010; Oryan *et al.*, 2011; Bigham-Sadegh *et al.*, 2016). Grading was performed through evaluating the following components: Surgical gap widening, inflammation degree, fibroblast maturation, collagen maturation and regular orientation, and angiogenesis (Table 2).

Statistical analysis

The obtained data were statistically analyzed with statistical

Table 1. Showing locomotor evaluation score.

Category	Item description	Score
Connection of tendon to skin	Not conjoined (slidable)	1
	Adhesion	0
Signs of inflammation	No signs of inflammation	2
	Moderate inflammation (only 2 signs exist)	1
	Edema, tenderness, skin color changes, hotness and redness	0
Neighboring structures	Unchanged	1
	Changed (color, surface, thickness)	0
Lameness	Hind limb fully loading	3
	Stiffness of hock joint	2
	Dragging of hind limb	1
	Elevated hind limb	0
Pain on manipulation	No pain	2
	Mild pain	1
	Severe pain	0
Pain in complete flexion of the tarsal joint	No pain	3
	Pain only at full flexion	2
	Pain at less than flexion	1
	Pain at any attempt	0

Table 2. Showing morphohistological evaluation score.

Parameters	Item description	score
Tendon surface at the area of defect	Intact and smooth	1
	Uneven and harsh	0
Level of tendon defect	At the same level of tendon surface	1
	Above the level of tendon surface	0
Color of tendon	Bright white	1
	Translucent, dull white, rose	0
Angiogenesis (semi-quantitative)	Marked infiltration of tissue with arterioles	3
Average number of vascular sections in 5 microscopic fields	Moderate infiltration of tissue with arterioles	2
	Presence of capillaries	1
	No vasculature infiltration	0
Fibroblast maturation (qualitative)	Normal tendocyte (fibrocyte and fibroblast) (full mature)	2
	Marked fibroblast and fibrocyte maturation	1
	Moderate fibroblast and fibrocyte maturation	0
Collagen maturation & orientation (qualitative)	Normal collagen oriented tangentially	3
	Mild changes with collagen fibers less than 25% disorganized	2
	Moderate changes with collagen fibers between 25% and 50% disorganized	1
	Marked changes with collagen more than 50% disorganized	0
Surgical gap widening (qualitative)	No gab (normal)	2
	Marked closed surgical gab	1
	Moderate closed surgical gab	0

software program (IBM SPSS version 21). All data were analysed for normality using Kolmogorov-Smirnov test and then we used nonparametric (kruskal wallis) test. All data were expressed as median (minimum-maximum) values. P < 0.05 was considered statistically significant.

RESULTS

Locomotor evaluation (Table 3)

Regarding to connection of tendon to the skin, PRP group showed a significant less adhesion at the 4th, 6th and 8th weeks than the 2nd week (P = 0.002, 0.001and 0.001 respectively). While in AgNPs group a significant less adhesion was detected at the 6th and 8th week than other evaluation times (P < 0.001). But in control group, a significant less adhesion was notices at the 8th than the 2nd week postoperatively (P < 0.001). However, there were no significant differences among the treated and control groups or between AgNPs and PRP treated groups.

Inflammation score results revealed that the inflammation score significantly decreased at the 4th, 6th and 8th weeks postoperatively than the 2nd week in the control group (P = 0.003, 0.001 and p < 0.001 respectively). On comparing between groups, only at the 2nd week, there were less inflammatory signs in AgNPs and PRP treated groups than in the control group (P < 0.001). However, there were no significant differences between groups at the 4th, 6th and 8th weeks postoperatively.

All groups showed changes in neighboring structures (color, surface, and thickness) at the 2nd week. Medians showed no significant differences between different evaluation times and there were no significant differences between different treated groups.

Lameness was observed in all groups at the 2nd week post operatively. With respect to the 2nd week postoperatively, PRP and AgNPs treated groups had significant improvement in lameness score at the 4th, 6th and 8th week (P < 0.001 in all groups). While lameness in control group improved significantly at the 6th and 8th weeks than the 2nd week (P= 0.048 and 0.001 respectively). However, no significant differences were noticed between the treated groups.

All operated rabbits in three groups showed signs of pain during manipulation of the operated limb at the 2nd week. By the time, pain decreased gradually till disappeared at the 6th week postoperatively. However, there were no significant statistical differences between different times of evaluation or among different groups.

Pain on complete flexion of tarsal joint of operated limbs was detected in all animals at the 2nd week postoperatively. Regarding to the timeline differences, both PRP (P = 0.007) and control (P = 0.038 and 0.011 respectively) groups showed significant less pain score at the 6th and 8th weeks than the 2nd week. While AgNPs group had significant less pain score at the 4th, 6th and 8th week than the 2nd week (P = 0.033, P < 0.001 and P < 0.001 respectively). However, there were no significant differences between all three groups.

Morpho-histological evaluation

Regarding to tendon surface at the area of defect, both treated and control groups showed uneven harsh tendon surface at the area of defect at the 2nd week, and then it decreased gradually till disappeared at the 6th week postoperatively. In comparing between weeks, the changes in the tendon surface at the area of defect in PRP and AgNPs groups showed significantly better enhancement with intact and smooth tendon surface at the 6th week (P = 0.002) than at the 2nd and 3rd weeks. Moreover, there were no significant differences between groups (Table 4).

All operated tendons had healed tissue above the level of tendon surface at the 2nd week, and then it gradually decreased till disappeared at the 6th week postoperatively. On comparing within the timeline, in the 6th week, level of tendon defect in

PRP and AgNPs groups showed significantly better healing and the level of tendon defect became at the same level of tendon surface (P = 0.002) than in the 2nd and 3rd weeks. Regarding to differences between groups there were no significant differences between treated and control groups or among the different treated groups.

Table 3. Results of locomotor score in control, PRP (plasma rich platellets) and AgNPs (silver nanoparticles) groups.

Connection of tendon to skin			
Weeks	Control group	PRP group	AgNPs group
2 (n=15)	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}
4 (n=9)	0 (0 - 0) ^{Aa}	0.5 (0 - 1) ^{Ab}	0 (0 - 1) ^{Aa}
6 (n=9)	0.5 (0 - 1) ^{Aa}	1 (1 - 1) ^{Ab}	1 (1 - 1) ^{Ab}
8 (n=6)	1 (1 - 1) ^{Ab}	1 (1 - 1) ^{Ab}	1 (1 - 1) ^{Ab}
Signs of inflammation			
2 (n=15)	1 (0 - 1) ^{Aa}	1.5 (1 - 2) ^{Ba}	1.5 (1 - 2) ^{Ba}
4 (n=9)	2 (2 - 2) ^{Ab}	2 (2 - 2) ^{Aa}	2 (2 - 2) ^{Aa}
6 (n=9)	2 (2 - 2) ^{Ab}	2 (2 - 2) ^{Aa}	2 (2 - 2) ^{Aa}
8 (n=6)	2 (2 - 2) ^{Ab}	2 (2 - 2) ^{Aa}	2 (2 - 2) ^{Aa}
Neighboring structures			
2 (n=15)	0.5 (0 - 1) ^{Aa}	1 (0 - 1) ^{Aa}	1 (0 - 1) ^{Aa}
4 (n=9)	1 (1 - 1) ^{Aa}	1 (1 - 1) ^{Aa}	1 (1 - 1) ^{Aa}
6 (n=9)	1 (1 - 1) ^{Aa}	1 (1 - 1) ^{Aa}	1 (1 - 1) ^{Aa}
8 (n=6)	1 (1 - 1) ^{Aa}	1 (1 - 1) ^{Aa}	1 (1 - 1) ^{Aa}
Lamness			
2 (n=15)	1.5 (2 - 1) ^{Aa}	2 (2 - 2) ^{Aa}	2 (2 - 1) ^{Aa}
4 (n=9)	2.5 (3 - 2) ^{Aab}	3 (3 - 3) ^{Ab}	3 (3 - 3) ^{Ab}
6 (n=9)	3 (3 - 3) ^{Ab}	3 (3 - 3) ^{Ab}	3 (3 - 3) ^{Ab}
8 (n=6)	3 (3 - 3) ^{Ab}	3 (3 - 3) ^{Ab}	3 (3 - 3) ^{Ab}
Pain on manipulation			
2 (n=15)	1 (0 - 2) ^{Aa}	1.5 (1 - 2) ^{Aa}	1 (0 - 2) ^{Aa}
4 (n=9)	1 (1 - 2) ^{Aa}	2 (1 – 2) ^{Aa}	1.5 (1 - 2) ^{Aa}
6 (n=9)	2 (2 - 2) ^{Aa}	2 (2 - 2) ^{Aa}	2 (2 - 2) ^{Aa}
8 (n=6)	2 (2 - 2) ^{Aa}	2 (2 - 2) ^{Aa}	2 (2 - 2) ^{Aa}
Pain on complete flexion			
2 (n=15)	1.5 (1 - 2) ^{Aa}	2 (1 - 2) ^{Aa}	1.5 (1 - 2) ^{Aa}
4 (n=9)	2 (3 - 2) ^{Aab}	2.5 (2 - 3) ^{Aab}	2.5 (2 - 3) ^{Ab}
6 (n=9)	3 (3 - 2) ^{Ab}	3 (3 - 3) ^{Ab}	3 (3 - 3) ^{Ab}
8 (n=6)	3 (- 3) ^{Ab}	3 (3 - 3) ^{Ab}	3 (3 - 3) ^{Ab}

A, B median (minimum-maximum) values with different letters in the same column are significantly different at P<0.05. a, b median (minimum-maximum) values with different letters in the same raw are significantly different at P<0.05.

All operated rabbits showed changes in the color of the tendon in the form of translucent, dull white or rose color at the 2nd week postoperatively, but it decreased gradually till reaching the normal tendon color. On comparing between different weeks postoperatively, the changes in the color of tendon score in PRP and AgNPs treated groups showed a significantly bright white color at the 6th week than the 2nd and 3rd week postoperatively (P = 0.002). In comparison with the control tendons, there were no significant differences and there were no significant differences also among the two treated groups.

Score for angiogenesis in PRP treated group showed significant lower angiogenesis at the 6th week than the 2nd and 3rd weeks (P < 0.001). While comparing between groups revealed significant lower angiogenesis in the tendons treated with PRP at the 6th week than control (P = 0.003) and AgNPs groups (P < 0.001).

Table 4. Results of morphohistological score in control, PRP (plasma rich platellets) and AgNPs (silver nanoparticles) groups.

Tendon surface at area of defect			
Weeks	(Control group)	PRP group	AgNPs group
2 (n=3)	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}
3 (n=3)	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}
6 (n=3)	0.5 (0 - 1) ^{Aa}	1 (1 - 1) ^{Ab}	1 (1 - 1) ^{Ab}
Level of tendon defect			
2 (n=3)	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}
3 (n=3)	0 (0 - 0) ^{Aa}	0 (0 - 1) ^{Aa}	0 (0 - 1) ^{Aa}
6 (n=3)	0.5 (0 - 1) ^{Aa}	1 (1 - 1) ^{Ab}	1 (1 - 1) ^{Ab}
Color of tendon			
2 (n=3)	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}	0 (0 - 0) ^{Aa}
3 (n=3)	0 (0 - 0) ^{Aa}	0 (0 - 1) ^{Aa}	0 (0 - 1) ^{Aa}
6 (n=3)	0.5 (0 - 1) ^{Aa}	1 (1 - 1) ^{Ab}	1 (1 - 1) ^{Ab}
Angiogenesis			
2 (n=3)	3 (2 - 3) ^{Aa}	3(3 - 3) ^{Aa}	3 (2 - 3) ^{Aa}
3 (n=3)	3(2 - 3) ^{Aa}	3(2 - 3) ^{Aa}	3 (1 - 3) ^{Aa}
6 (n=3)	2(1 - 3) ^{Aa}	1(0 - 2) ^{Bb}	2 (0 - 3) ^{Aa}
Fibroblast maturation			
2 (n=3)	0 (0 - 0) ^{Aa}	0 (0 - 1) ^{Aab}	0 (0 - 1) ^{Ba}
3 (n=3)	0(0 - 1) ^{Aab}	1 (0 - 2) ^{Bb}	1 (0 - 2) ^{Ba}
6 (n=3)	1(0 - 1) ^{Ab}	1 (1 - 2) ^{Bb}	1 (0 - 2) ^{ABab}
Collagen maturation			
2 (n=3)	0 (0 - 0) ^{Aa}	1(0 - 2) ^{Ba}	1 (0 - 1) ^{Ba}
3 (n=3)	0 (0 - 1) ^{Aa}	1(0 - 2) ^{Ba}	1 (0 - 1) ^{Aa}
6 (n=3)	1 (0 - 1) ^{Ab}	2(0 - 2) ^{Aa}	1 (1 - 1) ^{Aa}
Surgical gab			
2 (n=3)	0 (0 - 1) ^{Aa}	1 (0 - 1) ^{Ba}	0 (0 - 1) ^{ABa}
3 (n=3)	0 (0 - 1) ^{Aa}	1(0 - 1) ^{Aa}	0 (0 - 1) ^{Aab}
6 (n=3)	1(0 - 2) ^{Aab}	1(0 - 2) ^{Bab}	1 (0 - 2) ^{ABbc}

A, B median (minimum-maximum) values with different letters in the same column are significantly different at P < 0.05. a, b median (minimum-maximum) values with different letters in the same raw are significantly different at P < 0.05.

The fibroblast maturation score in PRP group showed significant greater value at the 3rd and 6th weeks than in the 2nd week (P = 0.045 and 0.005 respectively) (Fig. 1). Moreover, the control group showed significant high fibroblast maturation at the 6th week than the 2nd week (P = 0.021). In comparison with the control group, there were significant more fibroblast maturation in the tendons treated with AgNPs at the 2nd and 3rd weeks (P = 0.001 and 0.002 respectively) in addition in PRP group at the 3rd and 6th weeks (P < 0.001). There were no significant differences between PRP and AgNPs groups.

Score for the collagen maturation in control group showed significant more collagen maturation at the 6th week than both 2nd (P = 0.005) and 3rd (P = 0.020) weeks. In comparison with the control group, there were significant increase of collagen maturation in AgNPs treated group at the 2nd and 3rd weeks (P = 0.003 and 0.005 respectively), and at the 3rd week (P < 0.001) in PRP treated group (Fig. 2). Moreover, there were significant less collagen maturation in AgNPs treated group than PRP treated group at the 3rd week postoperatively (P = 0.001).

AgNPs group showed narrower surgical gab at the 6th week (Fig. 3) than the 2nd week (P = 0.022). In comparison with the control group, there were significant narrower surgical gap in the tendons treated with PRP at the 2nd and 6th weeks (P < 0.001). There were no significant differences between the different treated groups.

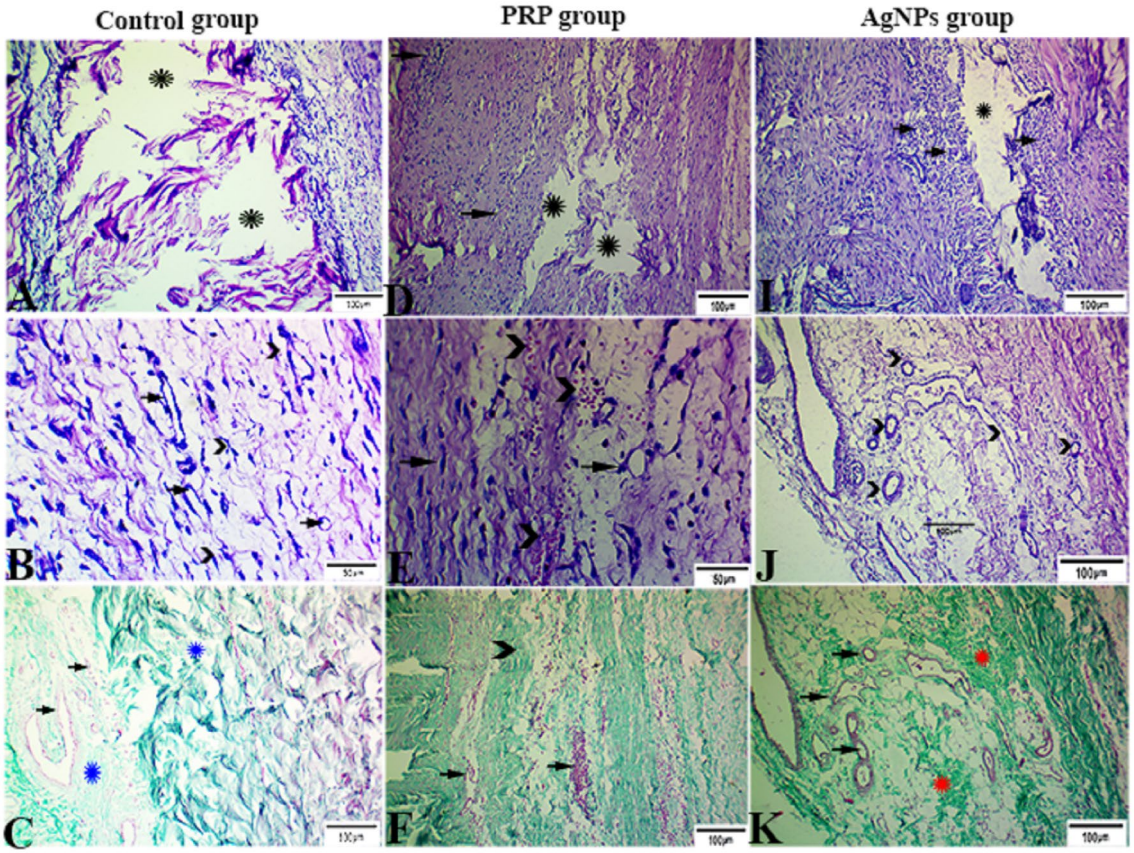

Figure 1. Histopathological section in the tendon from the experimental groups at 2nd week postoperatively, (A-C): control group shows, (A): wide incision space (stars). (B): Angiogenesis (newly formed blood vessels, arrows), fibrogenesis (proliferated fibroblast cells, arrowheads), Hx&E stain. (C): immature collagen fiber formation in the incision space (stars), newly formed blood vessels (angiogenesis) (arrows), (Green Masson's Trichrome staining). (D-F): Plasma rich platelets treated group at 2rd week post-operation shows, (D): incisional space (stars), inflammatory cellular reaction (arrows), Hx&E stain. (E): fibrogenesis (arrows) and hemorrhage (arrowheads), Hx&E stain. (F): immature poorly oriented collagen fibers (arrowheads), and hemorrhage (arrows), (Green Masson's Trichrome stain). (I-K): Silver Nanoparticles treated group (I): wide incision space (star), inflammatory cellular infiltration (arrows), Hx&E stain. (J): angiogenesis (arrowheads), Hx&E stain. (K): Collagen fiber formation in the incision space (stars), angiogenesis (arrows), (Green Masson's Trichrome stain). The bar size was indicated under picture.

DISCUSSION

The current study assessed the role of both of platelet rich plasma (PRP) and silver nanoparticles (AgNPs) for improving healing of severed achilles tendon (complete transverse cutting of the gastrocnemius tendon) in rabbits. However, the present study was conducted on achilles tendon of rabbits as they were the most frequently used model in experimental studies. Moreover, achilles tendon of rabbits is located superficially and somewhat large (Hast *et al*., 2014).

By the 2nd week postoperatively, all the operated rabbits had lameness, pain on manipulation of the operated hind limb, and pain on complete flexion of tarsal joint, and this may be due to the surgical intervention including skin incision and cutting of the tendon, as well as the relatively long period of immobilization of the operated limb and restriction of exercise (Ouyang *et al*., 2003; Stoll *et al*., 2011). However, these signs disappeared earlier in AgNPs group (4th week) than both of the PRP treated group (6th week) and the control group (8th week) and this can be explained by the anti-inflammatory and antimicrobial properties of AgNPs (Tian *et al*., 2007; Wong *et al*., 2009; Kwan *et al*., 2011). Loss of lameness and decrease signs of pain in PRP treated group earlier than control group can be attributed to the regenerative effect of PRP on the tendon as the PRP had anti-inflammatory effect that reduced pain, and it had modulatory effects on inflammation and angiogenesis through secreting signaling proteins which dilute and replace pro-inflammatory cytokines with anabolic growth factors (Ishida *et al*., 2007; Andia *et al*., 2010; Atchia *et al*., 2011; Lippross *et al*., 2011; Sánchez *et al*., 2012).

During tendon healing, adhesion may occur due to invasion of the repair site by the granulation tissue and the tenocytes from the surrounding tissue, after disruption of synovial sheath at the time of injury or surgery, and as these exogenous cells are predominant over endogenous tenocytes, the surrounding tissues attach to the repair area and form adhesions (Sharma and Maffulli, 2006). Furthermore, this scar-like fibrous tissue leads to poor mechanical properties, and lowers strength and elastic modulus, respectively. Fortunately, in the current study, tendons in the treated groups (PRP and AgNPs) improved from adhesion faster than control ones. The different treated groups showed adhesion of the tendon to the skin at the 2nd week post operatively, which decreased gradually and disappeared at the 6th week in AgNPs and PRP treated groups, while in control group it continued till the 8th week postoperatively. Using of AgNPs and PRP possibly relieved acute inflammation and post-surgical edema, and additionally they inhibited peritendinous adhesion formation and resulted in painless movement (Hu *et al*., 2009; Kwan *et al*., 2011).

In the present study, the morphological evaluation of the tendon healing revealed that all rabbits in the different groups had marked site of healing tissue. The semi-quantitative analysis of the morphological evaluation of color, level of tendon defect and surface of tendon at the area of defect showed that in both AgNPs and the PRP groups, there were considerably better results at the tenotomy site by the 6th week postoperatively while in control group prognosis was delayed. In AgNPs and PRP treated groups, the tendon area was filled with a bright white and smooth tissue at the same level of tendon surface and these findings were correlated with the histo-pathological results. These findings in the PRP group agree with Young *et al*. (1998). Moreover, in AgNPs group, our results can be explained by the ability of AgNPs to stimulate and modulate the deposition and alignment of collagen, produce proteoglycans, and to encourage the differentiation of fibroblasts (Tian *et al*. 2007; Wong *et al*., 2009; Liu *et al*. 2010; Kwan *et al*. 2011; Kwan *et al*. 2014).

In this experimental study, the histopathological examinations revealed that the tendon tissue specimens of the treated

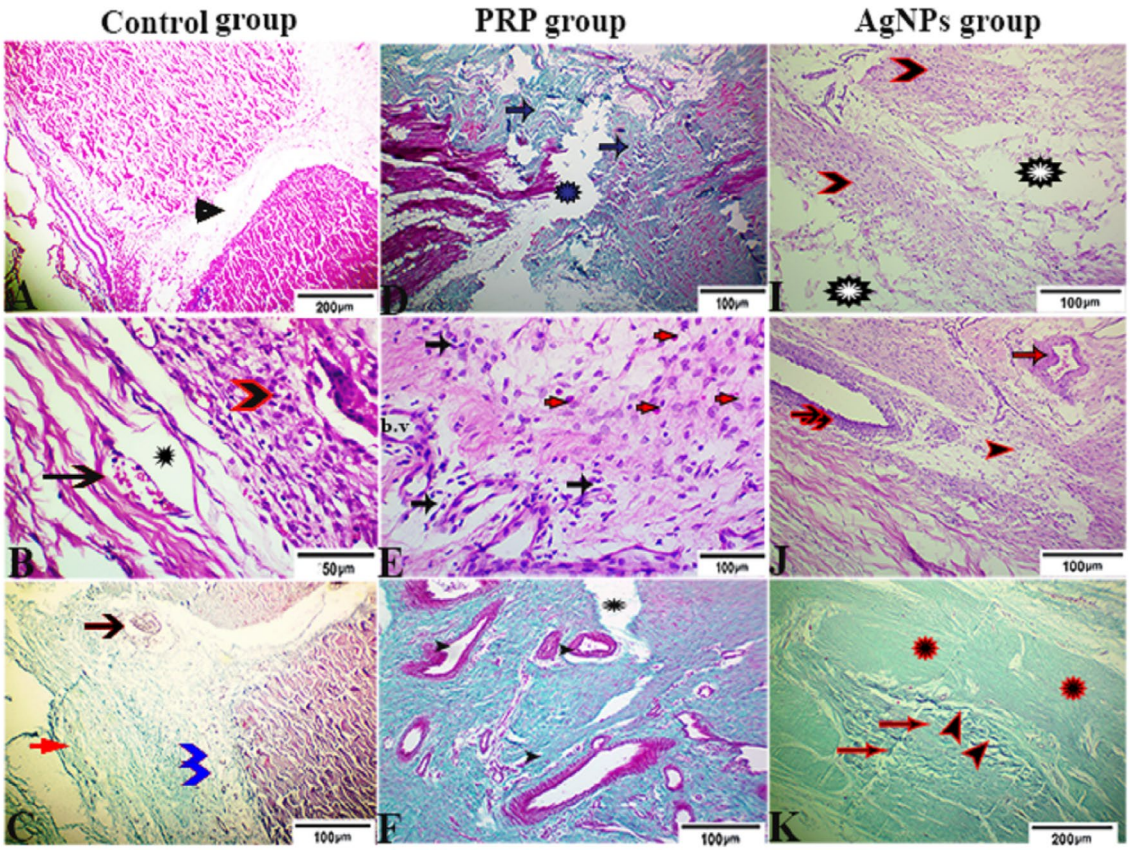

Figure 2. Histopathological section in the tendon from the experimental groups at 3rd week postoperatively, control group (A-C) shows (A): wide incision space with little strands of connective tissue (arrowhead).Hx&E (B): dilated and congested blood vessels (arrow), marked inflammatory cell infiltration (arro head), wide incisional gab (star) Hx&E. (C): immature collagen fiber formation in the incision space (red arrow), inflammatory cellular infiltration around congested blood vessels (black arrow). Note, newly formed blood vessels (angiogenesis) (blue double arrowheads) (Green Masson's Trichrome staining). (D-F): Plasma rich platelets treated group shows (D): immature poorly oriented collagen fibers (arrows) in the incision space (star), (Green Masson's Trichrome stain). (E): newly formed blood vessels (b.v) with intense perivascular inflammatory mononuclear cellular infiltration (black arrows) mixed with eosinophil cells (red arrows), Hx&E stain. (F): dilated and congested blood vessels with thick proliferated wall (arrowheads), wide incision space (star) still present, (Green Masson's Trichrome stain). (I-K): silver Nanoparticles treated group shows (I): wide incision space (stars), dispersed bundles of immature collagen fiber formation (black arrow), Hx&E stain. (J): wide congested blood vessels (arrows), intense perivascular inflammatory cellular infiltration (arrowhead), Hx&E stain. (K): Collagen fiber formation in the incision space (arrows), with fibroblasts (arrowheads), bundles of collagen fibers (stars), (Green Masson's Trichrome stain). The bar size was indicated under pictures.

groups had positive and significant results compared with the control group; the tendons had reparation tissue at the cutting site; had vascularization and improvement of fibroblast maturation; collagen fibers deposition and orientation; as well as decreased gap width overtime. Fibroblasts were the main cell types responsible for collagen production while the endothelial cells were responsible for angiogenesis and vessel production (Sharma and Maffulli, 2005a; Barbosa *et al*., 2013; Kwan *et al*., 2014).

In the current study, there were insignificant increases in the angiogenesis in PRP treated group by the 2nd and 3rd weeks, and a significant decrease by the 6th week after surgery, and this is in agreement with Lyras *et al.* (2010) and Lyras *et al.* (2011) who reported that growth factors accelerated angiogenesis and production of the extracellular matrix during the early phase of achilles tendon healing in rabbit after PRP administration. Moreover, angiogenesis increased insignificantly by the 2nd and 3rd weeks after operation in AgNPs treated group than the control group, and this finding agrees with Tian *et al.* (2007) who reported that the accelerated healing process in tendon may ensue due to increased myofibroblasts, collagen remodeling, and blood vessel neoformation.

In a rabbit achilles tendon transection model, our results proved that the growth factors hasten angiogenesis and production of the extracellular matrix during the early phase of tendon healing and enhanced collagen synthesis by the 4th week and later after PRP administration (Lyras *et al*., 2010 and Lyras *et al*., 2011). Fibroblasts are the main cell type responsible for collagen production. However, endothelial cells are cell type responsible for angiogenesis and vessel production (Sharma and Maffulli, 2005b). However, in the control group, neovascularization and fibroblast proliferation persisted up to 6 weeks after surgery. We proposed that, the absence of exogenous growth factors keep fibroblasts in a proliferative state, even though some remodeling process may have been initiated at this time. Type I collagen expression was lower in the control rabbits too than PRP group, that indicating slower tendon maturation. These results are coincided with a previous study (Sharma and Maffulli, 2005a) that found the stage of remodeling was initiated and cellularity was decreased approximately 6 weeks after injury of the achilles tendon.

There were significant increases in fibroblast maturation in AgNPs group by the 2nd and 3rd weeks as well as by the 3rd and 6th weeks in PRP group than control group. AgNPs and growth factors released from PRP, encouraged fibroblast relocation and proliferation, increased myofibroblasts, and enhanced collagen remodeling. The large number of migrating fibroblasts cells early initiate the proliferative phase and increased the production of collagen fibers. Consequently, the remodeling phase progresses earlier than that in the untreated rabbits. Tension aligns fibroblasts parallel to the line of force (Yasuda *et al*., 2000; Tian *et al*., 2007; Kwan *et al*., 2014). This status increases fibroblast proliferation, collagen synthesis, vascularity, intracellular matrix formation, thickness, and tissue healing (Coussens and Werb, 2002).

With respect to collagen maturation, there were significant increases in the AgNPs group by the 2nd and 3rd weeks than control group. These finding may be attributed to the stimulation of cell proliferation; therefore, the remodeling phase is initiated earlier than that in the untreated rabbits and this in agreement with Tian *et al.* (2007); Wong *et al.* (2009); Liu *et al.* (2010); Kwan *et al.* (2011) and Kwan *et al.* (2014) who reported that AgNPs stimulates and modulates the deposition and alignment of collagen and the production of proteoglycans, and encourages the differentiation of fibroblasts. At the same time, the PRP group showed

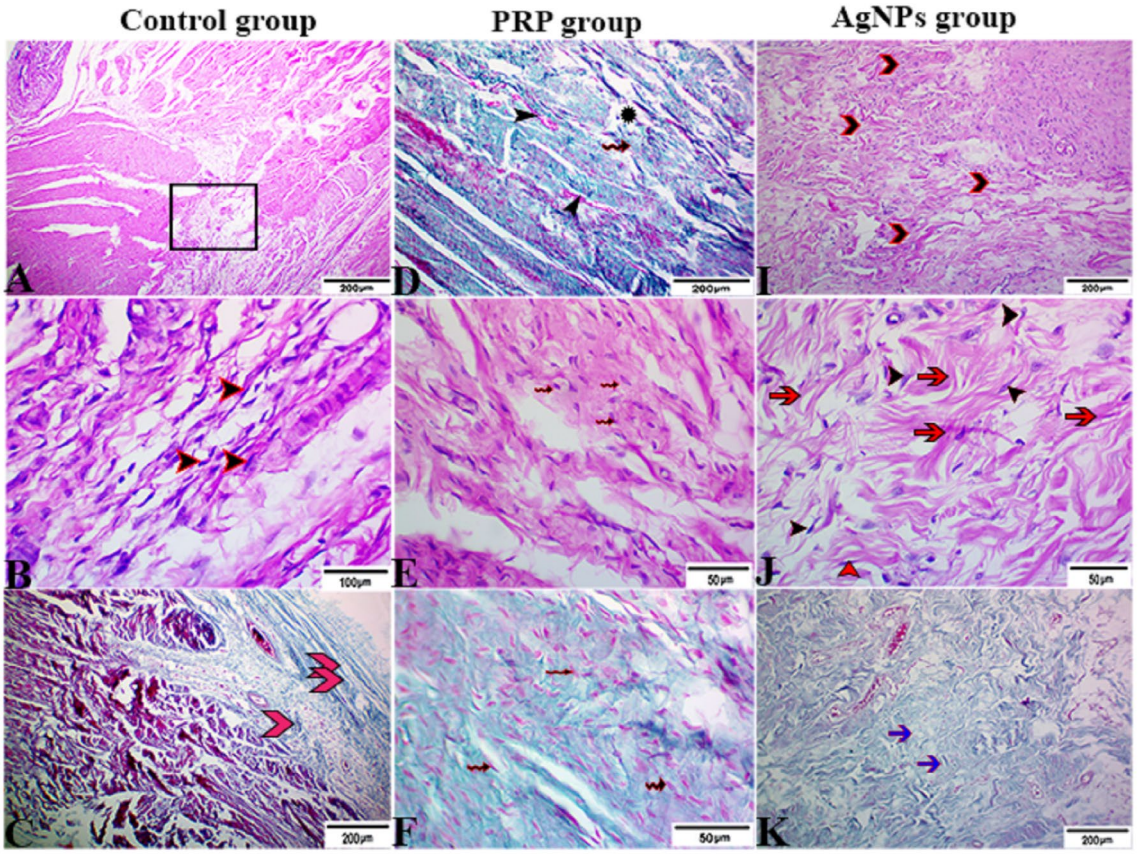

Figure-3. Histopathological section in the tendon from experimental groups at 6th week's postoperatively, (A-C): control group shows (a): Incision space filled by strands of connective tissue (selected square), Hx&E stain. (B): proliferated fibroblast cells (arrowheads), Hx&E stain. (C): collagen fiber formation in the incision space (red arrowheads) (Green Masson's Trichrome stain). (D-F): Plasma rich platelets treated group, shows, (A): incision space filled by strands of connective tissue (stars), connective bundles (arrows) and congested blood vessels (arrow heads). Green Masson's Trichrome stain). (E&F): aggregation of collagens connective fibers filled the incisional space with increased number of fibrocytes (arrows) between collagenous fiber, (E, Hx&E stain-F, Green Masson's Trichrome stain). (I-K): silver Nanoparticles group at 6th week's post-operation, shows (I): Incision space filled by strands of connective tissue (arrowheads), Green Masson's Trichrome stain. (J): aggregation of connective fibers (red arrows), with increased number of fibrocytes (black arrowheads) to fibroblasts, Hx&E. (K): aggregation of collagens connective fibers filled the incisional space (blue arrows), Green Masson's Trichrome stain. The bar size was indicated under pictures.

significant increases in collagen deposition and orientation by the 3rd week post operation than control group, and it may be attributed to the effect of growth factors in collagen synthesis (Molloy *et al*., 2003; Mehta and Watson, 2008 and Bir *et al*., 2009).

Histopathological evaluation in our study revealed significant narrowing of surgical gab in treated groups than control group in all harvested tissue samples, and it is concluded that silver nanoparticles and PRP have a useful effect on provoking fibroblast proliferation, collagen synthesis, vascularity, intracellular matrix formation, thickness, and tissue healing leading to better filling of the surgical gab (Coussens and Werb, 2002; Molloy *et al*., 2003; Kwan *et al*., 2014).

CONCLUSION

Using of PRP and AgNPs for treatment of the severed achilles tendon, in rabbit model improve healing. This study suggests the use of PRP as an original technique for treatment of tendon wounds is better than AgNPs treatment. Moreover, PRP is an autogenic preparation with minimal complications, cheap and easily prepared and applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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