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CLINICAL AND HISTOLOGICAL STUDY OF THE EFFECT PLATELET-RICH AND POOR-PLASMA THERAPEUTIC MODEL ON REGENERATION OF THE SCIATIC NERVE IN RABBITS

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ABSTRACT : There are very similarity in the types of plasma-based treatments: the platelet rich-plasma (PRP) and platelet poor-plasma (PPP). The both types have been established as an autologous source for therapeutic angiogenesis. and the both have two main ingredient: platelets and plasma, fifteen healthy mature male rabbits were divided randomly into three experimental groups: The first group which treated with PRP, the second group, which treated with PPP and the third was control group, the sciatic nerve in all treated animals were transected and served in treatment protocols through surgical procedure after the nerve suture. Then all animals were followed up clinically for fourteen days, and histologically for sixty days after the operations. The results either clinically or histologically indicated that the treatment with PRP has remarkable beneficial effects on nerve healing represented acceleration and improvement of the nerve through reduce adhesion, increase nerve regenerations time and improve nerve function. However, the PRP treated groups were progressed than the PPP and control groups, by findings, demonstrated early weight bearing with fastest healing process.

Key words : Platelet-rich plasma, platelet-poor plasma, regeneration, sciatic nerve, rabbits, histology.

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INTRODUCTION

The Platelets Rich-Plasma (PRP) is one of blood derivatives contains a large amounts of endogenous platelets in a certain volume of plasma more than the basic level found in total natural blood, because of it composed of a high platelet concentrations, it contains a high concentration of growth factors that are produced by these platelets, as well as they, contain large amounts of white blood cells, immune cell activation proteins, anti-bacterial and fungi proteins and other growth factors (Kuffer, 2019; Cai *et al*, 2019).

PRP is a blood-derived fractions that contain a high concentration of leukocytes, high level of platelets and growth factors (Uthappa *et al*, 2017). The improvement effect of PRP is composed a large numbers of platelet in PRP that release significant amounts of the growth factors may aid in healing process (Messora *et al*, 2011; Shen *et al*, 1995; Farrag *et al*, 2007).

The plasma poor-platelet PPP have not many platelets, because of that, the physicians for years regarded it as a by-product of PRP and simply discarded

it. Besides, PPP has it unique property in healing, for instance, PPP can be used for nerve hydro dissection, which is a proceses used to relieve a trapped nerve (Messora *et al*, 2011). PPP used in healing of damaged muscles, which the physician once believed, may not be healed at all. PPP can also be used in the ulnar-neuropathy and many type of carpal tunnel syndrome can thus finds PPP therapy helpful, a substance that used to be considered useless, now healing tissues physicians thought could not be healed. PRP therapy has been used as a viable treatment alternative for several clinical applications and has a potential benefit for use in wound healing (Cristina *et al*, 2016). First uses of PRP in 1950 in the upper jaw, face and mouth surgery, 1987 injections in open-heart surgery (Mlynarek *et al*, 2016; Jalowiec *et al*, 2016). The use of PRP on the humans as a treatment tools for multiple type of treatment like nerve damage, plastic surgery, osteoarthritis, tendinitis, repair and reconstruction of bones and oral surgery. PRP also used in the field of sports injuries treatment (Mishra *et al*, 2009; Andia *et al*, 2012).

The sciatic nerve is the longest in body as well it arise from lumbo-sacral plexus. The sciatic nerve supply the caudal thigh muscles and the caudal hips muscles except for the external obturator (Shively, 1984). In the present study used the PRP and PPP as a model for the treatments of sciatic nerve transaction in rabbits and comparison between two methodologies of treatments.

This study aimed to evaluate PRP effects compared to PPP as the healing process of experimental entire injury of the sciatic nerve and selected the best method for treating through comparing several methods of treatment.

MATERIALS AND METHODS

Preparation of PRP and PPP

The both PRP and PPP are made in the same way, in which the blood samples were taken from the animals and then placed in the centrifuge spins, the sample separate onto it parts and then used this separated samples to make the solutions of PRP and PPP. Before anesthesia was initiated, the blood samples (10 ml) were collected from heart in grouped animals in which PRP and PPP were used, the blood samples were placed in two (5 ml) tubes contained sodium citrate and analyzed by using centrifugation for (10 minutes) at a speed of (1500 cycles/minute) therefore, the blood was initially separated onto

three upper layers representing, the PPP and the middle layer represents the buffy coat and the lower layer represents the red blood cells. The plasma layer with the blood platelets and the insulated coated layer and a section of the top of the red blood cells layer were removed from both tubes and placed in an empty tube and then placed in the face The centrifugation lasts (15 minutes), at a speed of 1500 cycles/minute, as the contents are separated into the upper two parts which are PPP and lower PRP. (10 minutes) before plasma placement on the soil, added (2-4 drops) of calcium chloride at a concentration of (10%) to activate it (Suvarna *et al*, 2013).

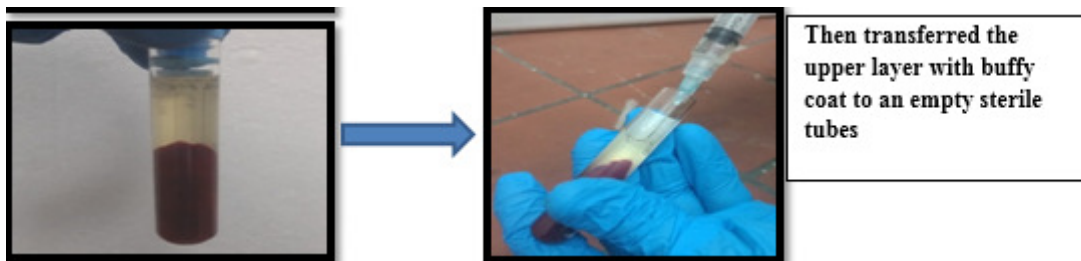
Animals : A fifteen adult healthy males rabbits were study, weight range (1.5-2 kg) and the rabbits were given a mixture of Ketamine and Xylazine (15mg-5mg)/ kg B.W IM, they were randomly divided onto three equal groups.

Surgical procedures

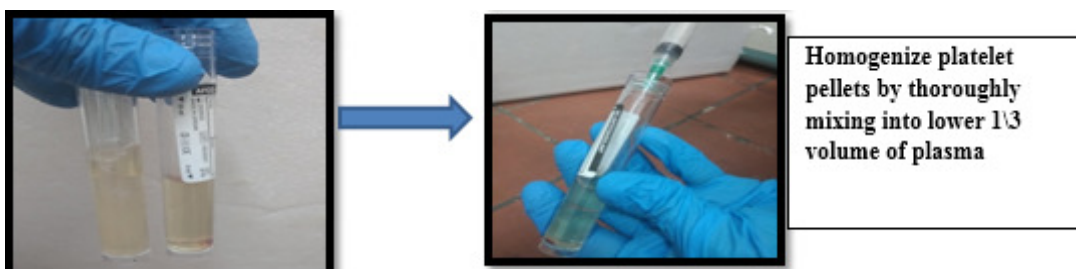
The animals preparing to surgery after anesthesia, incision the skin about (2.5cm) in left thigh under aseptic technique, the sciatic nerves were exteriorized to the wound surfaces as in Fig. 7, the nerves were transected by using a surgical blades, then the two ends of nerve sutures using 5-0 nylon suture placed equidistantly around the nerve by the using epineurium simple interrupted



Figs. 1 and 2 : Blood collection.



Figs. 3 and 4 : First spin.



Figs. 5 and 6 : Second spin.

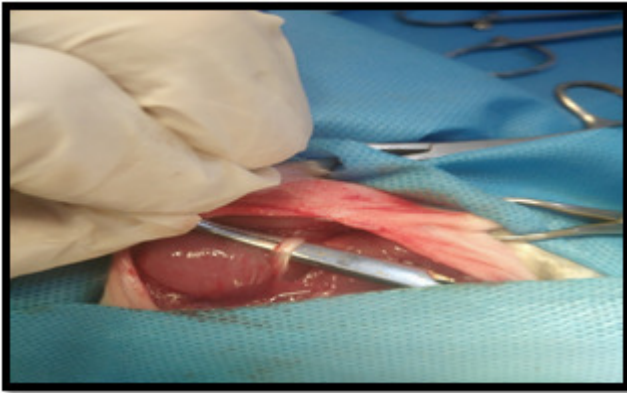


Fig. 7 : Gross section of sciatic nerve.

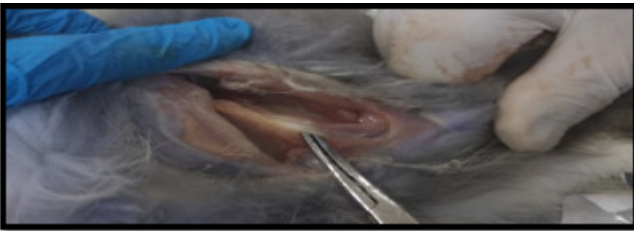


Fig. 8 : show no adhesion in the animal which treated with Platelet-rich plasma.

suture (Fig. 8), then applied PRP in the first group and PPP in the second group. The muscles and subcutaneous fascia closed with 3-0 cut gut suture (China) simple continuous methods, and then closed the skin with 3-0 silk suture. Then the animals gave systemic antibiotics intramuscular daily for 7 days postoperatively by using an intramuscular procaine penicillin 800.000 IU/kg injection, remove the silk suture after 7 days.

Postoperative care and clinical examination

The animals were followed up in all groups clinically for 15 days after the operation and the external wound was cleansed daily with OTC wound spray until the wound healed. Surgical suture removal after 10 days.

Experiment animals were followed in all groups clinically to know the health status of the animals for 15 days after the surgical procedure. The movement of the animal was observed during walking and jogging to see whether there is lameness and the extent of the animal's ability to use the treated party in the event of standing and walking, the examination included the activity of the animal and its appetite for eating. The location of the operation was examined, noting the changes that occurred in the place of the operation note swelling of the wound, and place and infection occurred or not.

Macroscopic examination

The macroscopic examination of the location of the transected nerve which includes adhesion between the treated nerve and adjacent tissue, the type of healing between the two parts of the severed and treatment nerve

and comparison of groups were documented.

Histopathological examination

After the operation of about sixty days, the histopathological examination was taken from animals. The specimens were taken from target tissue, which included the part of Partial lump nerve at the end of all period and fixed directly with 10% neutral buffered formaldehyde then routinely embedded and processed in paraffin. Then were cut into 5 microns (Castillo *et al*,2019) and stained by hematoxyline and eosin stains, finally examined under the light microscope.

RESULTS

The results of this study are shown this technique is completely safe and without any side effects at all in the long term because it depends on the injection of subjective materials from the same animal. The results have shown that the preparation of the PRP and PPP was an easy and fast way without any stress on the animal and the surgeon and its cost is simple without any significant obstacles. The study showed that PRP is an anti-inflammatory and reduces pain and edema.

The results of the clinical examination of the experiment animals showed that all the animals returned to their full activity on the second day of the operation except for the control groups Inactivity was purged during the first two days after the operation. The animals showed severe lameness after the operation in all study animals, and the lameness was evident when walking and jogging, and its duration continued for a day (7.3 ± 0.2) in treated animals with PRP, (7.6 ± 0.2) in animals treated with PPP, while an average duration of (14 ± 0.5) in control animals continued (Table 1), after this period the animals showed an improvement in walking until the movement became normal and relief pain after treatment and the animals returned their affected legs to the right position after operation in the seven days, the tropic ulcers of feet occurs in four animals in the control group and the animals which treated in PPP group in the other animal doesn't occur.

The paralysis of left limbs that were clear, the early developments of mild sores in the hock joints in all animals. The sores it started to reduce in their sizes to be a moderate and then mild in sizes to form scabies like at the end of experimental. Presence of clear swelling at the site of the operation, especially on the second day of the operation, which lasted for a rate of 3.4 ± 0.2 days in the animals treated with PRP, (4.5 ± 0.1) days in animals treated with PPP, (6.5 ± 0.2) in the control animals, which was observed to have a significant duration shortest comparison with other groups (Table 2).

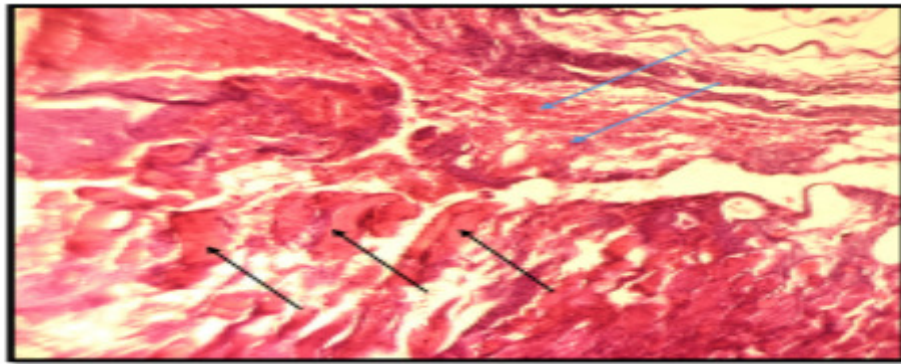


Fig. 9 : Histological section of sciatic nerve of control group showed disorganization of necrotic nerve fibers (black arrows), also present of hemorrhage and cellular infiltration distal to end of the injured nerve (blue arrows). H&E stain. 10X.

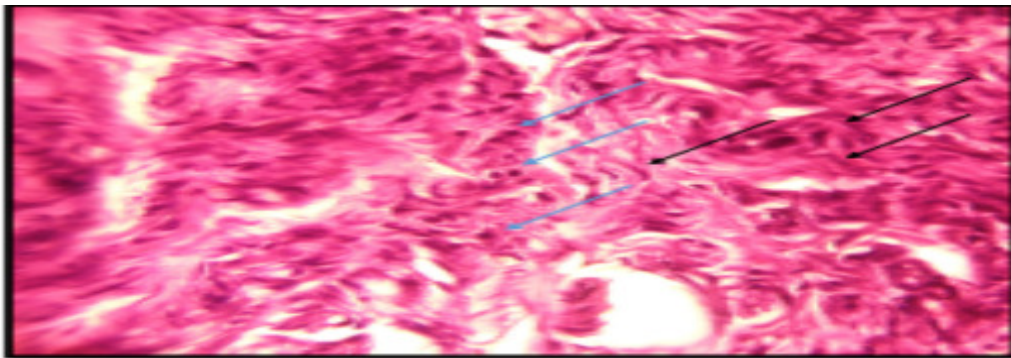


Fig. 10 : Histological section of sciatic nerve of control group showed fibrous tissue disorganization in the area distal to the injured nerve ending (black arrows), also there is an infiltration of inflammatory cells in the injured nerve ending (blue arrows). H&E stain. 40X.

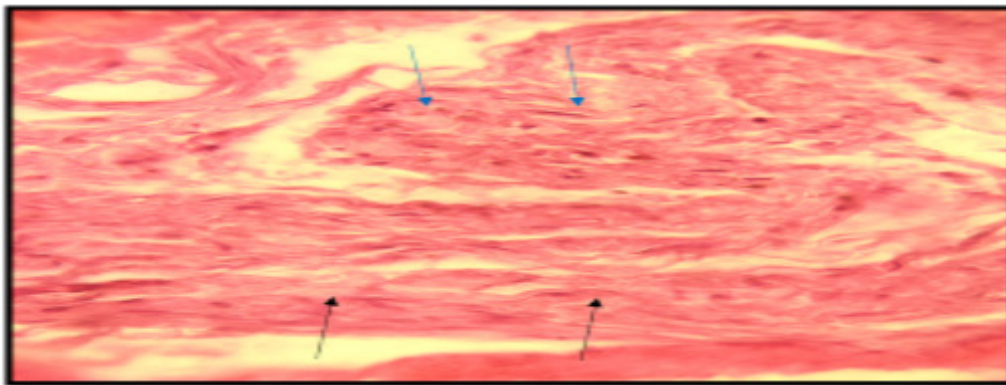


Fig. 11 : Histological section of sciatic nerve of PPP treated group showed a mild area of fibroblastic proliferation in the regenerated nerve fibers (black arrows). H&E stain. 40X.

Sacrifice the animals after 12 weeks and the surgical opening the muscles done to take biopsies of the sciatic nerve. The neuron histopathological and macroscopic examination appears adhesions around the nerve, which is one of the complications during the process of nerve healing and limits its movement and hinder its mechanical function, the current study showed that the beneficial results of the PRP group compared to the PPP group in nerve healing, in which the PPP examination appears increased the angiogenesis and granulation tissue formation significantly.

The current results of histological features of control group showed disorganization of necrotic nerve fibers

with present of hemorrhage and cellular infiltration distal to end of the injured nerve (Fig. 9), in another section it showed a fibrous tissue disorganization in the area distal to the injured nerve ending, also there is an infiltration of inflammatory cells in the injured nerve ending (Fig. 10).

The histological findings of PPP group showed a degree of re-organization of regenerated nerve fibers, also present of marked degree of neuroma like aggregation along of nerve continuity (Fig. 11), in another section of PPP group showed a mild area of fibroblastic proliferation in the regenerated nerve fibers (Fig. 12).

The histological findings of PRP group showed marked degree of organization of regenerated nerve

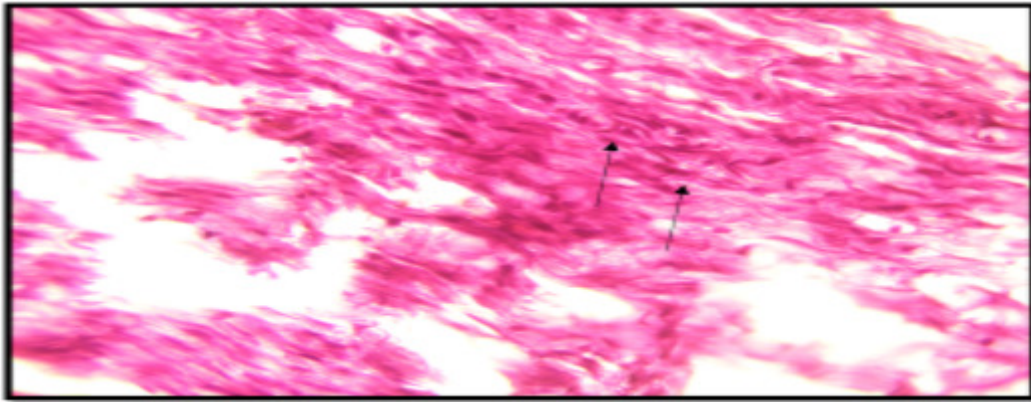


Fig. 12 : Histological section of sciatic nerve of PPP treated group showed a mild area of fibroblastic proliferation in the regenerated nerve fibers (black arrows). H&E stain. 40X.

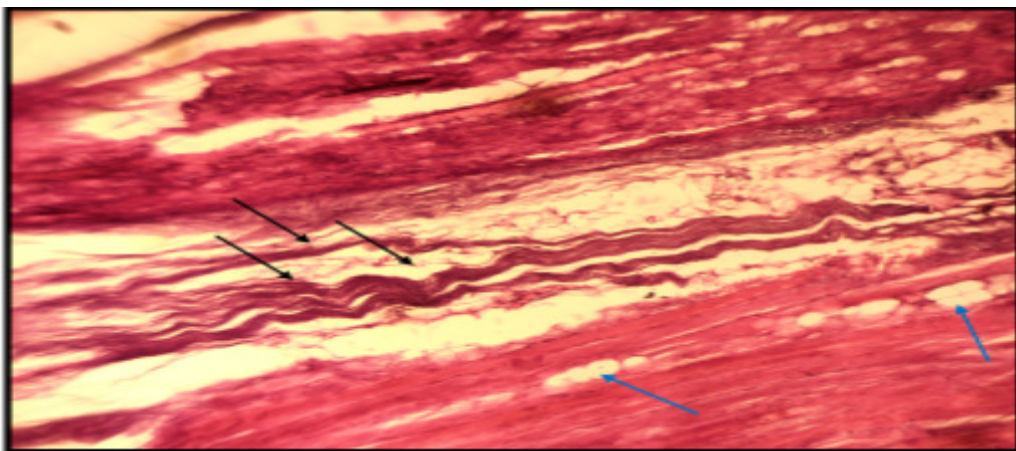


Fig. 13 : Histological section of sciatic nerve of PRP treated group showed marked degree of organization of regenerated nerve fibers (black arrows), also present of marked degree of re-myelination of the previously injured nerve sheath (blue arrows). H&E stain. 10X.

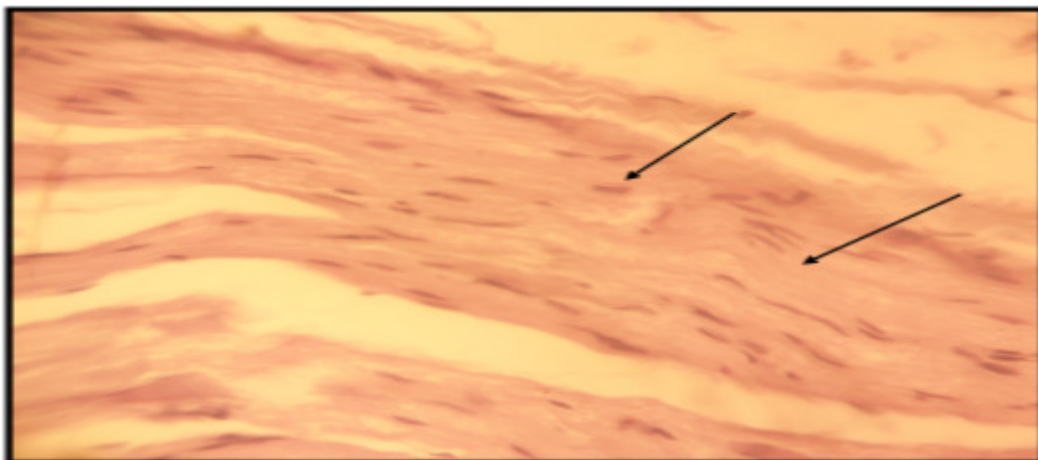


Fig. 14 : Histological section of sciatic nerve of PRP treated group showed marked degree of organization of regenerated nerve fibers (black arrows), also present of marked degree of re-myelination of the previously injured nerve sheath (blue arrows). H&E stain. 10X.

Table 1 : The average time (days) of lameness in all trial groups is sufficient.

Sign	Control group	PRP group	PPP group
Lameness(time)	14 ± 0.5 a	7.3±0.2 c	7.6±0.2 b

There are a significant differences among groups at a level P<0.05.

Table 2 : The average time (days) of swelling in all trial groups is sufficient

Sign	Control group	PRP group	PPP group
Swelling (time)	6.5±0.2	3.4±0.2	4.5±0.1

There are significant differences between groups at a level P<0.05.

fibers, also present of marked degree of re-myelination of the previously injured nerve sheath (Fig. 13), in another section showed organization of regenerated previously injured nerve fibers (Fig. 14).

The histological findings suggest that the PRP group showed an excellent regeneration processes among other PPP and control groups.

DISCUSSION

This study aimed to use PRP and PPP is taken from the animal itself and it can be obtained at the desired time for animal treatment thus this technique is completely safe and without any side effects at all in the long term and therefore there are no concerns about the body rejecting the injected substance or from the transmission of any bacterial infection. preparation of the PRP and PPP was an easy and fast way without any stress on the animal and the surgeon and its cost are simple without any significant obstacles and this is consistent with Sharifi *et al* (2007) and another study, the PRP is a mixtures of both the platelets and plasma that contained a high concentrations of platelets, also released a chemical signals in order to alerts the body in presence of an injuries to spur healing, therefore, the current study showed that PRP is an anti-inflammatory and reduces pain and edema, and that the reason for reducing inflammation is due to the PRP containing growth factors and cellular movements that have a role in controlling inflammation as indicated by Flanagan (2000). This technique is based on stimulating the division of stem cells, which are primitive cells that can divide and multiply, to give different types of specialized cells, such as skin cells, cartilage cells, and others. The stem cell is responsible to regeneration of damaged cell, which leads to the production of new cells by the body and the regeneration of tissues. Promote the growth of new blood vessels and stimulate the process of healing. The pain, which is due to inflammation because of the injury to the animal during the process. The hind limbs paralysis after the operation followed by the dysfunctions of peripheral nerves resulted by the damage to the neuronal schwann cells, or the myelin sheath, the damaged nerves cannot transmitted impulses in the normal fashion this results in agreement with Moshiri and Oryan (2013). The swelling is a natural response to cutting and repairing the nerve through inflammation, which is characterized by the occurrence of pain and edema resulting from increased blood flow to the region and an increase in the maturation of the capillary membranes, this is consistent with Cristina *et al* (2016). The adhesions around the nerve, which is one of the complications during the process of nerve healing and limits its movement and hinder its mechanical function,

this agrees with Duran *et al* (2018). The little adhesions we offered to use PRP was due to the many growth factors presented in the PRP that had a roles in lowering adhesions, including the fibroblasts growth factor FGF-b and TGF-B growth factors (Zheng *et al*, 2013). The superior results in the PRP group in the comparable to the PPP group in nerve healings processes due to quantities of growth factors that may involved in PRP and PPP, The rates of FGF2, VEGF and PDFG measured were 10 times more in PRP than in PPP, this idea may in agrees with Song *et al* (2019). In PPP examination appeared increased the angiogenesis and granulation tissues formations and significantly this agree with Song *et al* (2019).

Besides, the histological findings of the current study indicated that the PRP treated sciatic nerve showed a degree of organization of regenerated nerve fibers, also present of marked degree of re-myelination of the previously injured nerve sheath, these findings agreed with Song *et al* (2019), who mentioned that the PRP of the beneficial effects to stimulates the cellular propagation by inducing the synthesis of neurotrophic factors, and have a significant roles in the increasing the migration of schwann cells, that may playing a beneficial roles to indicate PRP and may providing a beneficial therapeutic effects to the peripheral nerves regenerations post nerve injuries by supplying growth factors. In addition, our histological results agreed with Song *et al* (2019). Who they reported that the PRP led to minimized the healing time significantly by it beneficial effects on the diameters of myelinated nerve fibers in the peri implanted bones. The PRP exerted significant effects on the diameters of the myelinated nerves fibers when compared to PPP results.

CONCLUSION

The PRP represents a biological therapy that accelerates healing process of sciatic nerves comparative to other treatments, in addition to provide an improvement of animal quality of life and have an advantages of biocompatible safety, low cost, simple preparation.

The PRP and PPP was an easy and fast way without any stress on the animal and the surgeon, while the adhesion in the animal, which treated in PRP, was less than in animals treated with PPP.

Histologically, the PRP group showed an excellent regeneration processes among other groups in the current study.

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