Combination local and intraperitoneal injection of platelet-rich plasma (PRP) increased S100B protein expression in sciatic nerve injury rat model

By Meutia Maulina

WORD COUNT 3802
TIME SUBMITTED 15-DEC-2024 10:13PM
PAPER ID 113539540

AMPH
AMALTEA MEDICAL PUBLISHING HOUSE

https://medscience.center/journals/

Words count – 3,799

TYPE OF ARTICLE: Original Article

Combination local and intraperitoneal injection of platelet-rich plasma (PRP) increased S100B protein expression in sciatic nerve injury rat model

Meutia Maulina[1], Yuziani Yuziani[2], Rizka Sofia[3], Ferisha Aulia Balqis[4], Putri Nabilah Lubis[4]

[1]Departement of Histology, Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia
[2]Departement of Pharmacology, Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia
[3]Departement of Tropical Medicine, Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia
[4]Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia

Corresponding author:

Meutia Maulina,

Email: meutia.maulina@unimal.ac.id

ABSTRACT

Background and objectives. Peripheral nerve injuries present a wide array of symptoms based on the severity and the affected nerves. Despite an extensive understanding of injury mechanisms and regeneration, treatments that ensure complete functional recovery are limited. The use of biological therapies such as platelet-rich plasma (PRP) has increased in the treatment of neurological disorders. This study aimed to analyze S100B protein expression as a biomarker of nerve regeneration following post-axonotmesis injury after PRP administration and to compare the efficacy of PRP with that of three different methods of administration.

Materials and methods. Describe briefly the main methods applied. In this post-test-only design, twenty-five Wistar rats from the sciatic nerve injury model were randomly allocated into five groups (n = 5): K1 (sham operation, negative control), K2 (nerve injury with saline injection, positive control), and three nerve injury treatment groups that received PRP injections: local (P1), intraperitoneal (P2), and a combination of local and intraperitoneal (P3). Axonotmesis nerve injury was induced by clamping the sciatic nerve for 60 seconds. S100B expression was evaluated by immunohistochemical examination using monoclonal antibodies anti-S100B and the percentage of expression was calculated.

Results. The PRP-treated group with nerve injury exhibited a greater increase in expression than the K1 and K2 control groups. The group receiving PRP via the combination route showed higher S100B expression than those administered PRP locally or intraperitoneally ($p = 0.000$).

Conclusions. S100B expression was significantly increased in the group receiving combined local and peritoneal PRP compared with those receiving either local or intraperitoneal PRP alone.

Keywords: peripheral nerve injury; platelet-rich plasma; S100B Protein; rats; immunohistochemistry Abbreviations: List all abbreviations & full terms

INTRODUCTION

Injuries to peripheral nerves are the most common and often result in long-lasting disabilities. These disabilities are typically characterized by discomfort, muscle weakness, and diminished sensory function of nerve receptors located in the skin, joints, and target muscular organs [1]. Among traumatic peripheral nerve injuries, motor vehicle accidents were the leading cause (42.1%), with injuries from sharp or penetrating objects ranking second (17.2%) and gunshot wounds being the third most common (15.9%) [2–4].

Peripheral nerve injuries can lead to axonal demyelination, degeneration, or both. These processes typically result in impaired sensory and/or motor functions of the affected nerves. Restoration of nerve function involves myelin regeneration, axonal regrowth, and reinnervation of sensory receptors [5]. Axonotmesis, a type of nerve injury, occurs when axons are damaged, resulting in disruption of axon continuity, demyelination, and impairment of both the endoneurium and the perineurium. This condition typically arises from compression or stretching injuries and generally has an unfavorable prognosis [6,7].

In the last ten years, the use of biological therapies for the treatment of neurological conditions has increased significantly. Platelet-rich plasma (PRP) is a therapeutic method that involves concentrating platelets from the patient's blood through centrifugation. Upon activation, PRP releases various growth factors. These factors can stimulate cell multiplication, differentiation, and blood vessel formation, thereby supporting the natural tissue regeneration process in the body [8,9]. It has been postulated that the ability of PRP to facilitate axon regeneration can be attributed to various factors, including neurotrophic substances released by platelets [5,10].

The restoration and regeneration of nerve tissue, cells, or cellular products, known as neuroregeneration, plays a vital role in addressing neuropathic pain. This process is critical for therapeutic interventions. Among the key components for nerve cell survival are neurotrophic factors; S100B is one such example [10,11]. Neural networks can overcome these neurotrophic

factors; however, they may not be produced at sufficient speed or in adequate quantity [12]. S100B is a crucial protein that mobilizes macrophages and subsequently induces the production of pro-inflammatory cytokines. Inflammation is a fundamental process in degeneration and regeneration. During the inflammatory process, several proinflammatory cytokines exhibit increased expression in various cells, including Schwann cells [13–15].

Histological and immunohistochemical methods can be used to assess peripheral nerve regeneration efficacy. To evaluate axon regrowth using immunohistochemistry, investigators can examine the expression levels of S100B, a protein that exhibits neurotrophic properties, in Schwann cells. This protein shows increased expression when Schwann cells are subjected to injury, rendering it a valuable marker for nerve regeneration studies. This study aimed to analyze S100B protein expression as a biomarker of nerve regeneration following post-axonotmesis injury after PRP administration and to compare the efficacy of PRP with that of three different methods of administration.

MATERIALS AND METHODS

Animals

A group of 25 male Wistar rats (*Rattus norvegicus*), 3-4 months old and weighing–250-300 grams, was obtained from the Laboratory of Experimental Animals at the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. Rats were maintained in controlled environments with unrestricted access to standard food and water. Using a post-test control-only group design, the rats were randomly assigned to five groups (n = 5): K1 (negative control, involving a sham operation without nerve injury or PRP injection), K2 (positive control, with nerve injury and saline injection but no PRP injection), and three treatment groups that received nerve injury and PRP injections: P1 (local PRP), P2 (intraperitoneal PRP), and P3 (a combination of local and intraperitoneal PRP).

Preparation and collection of PRP

Following a seven-day adaptation period, ether was used to anesthetize ten rats. From each rat, 5 ml of whole blood was extracted via intracardiac puncture into a tube containing 0.3 ml of anticoagulant. The blood was centrifuged at 1600 rpm for 10 min, followed by centrifugation at 2000 rpm for an additional 10 min. This process yielded three distinct layers: cell-free plasma at the top, erythrocytes at the bottom, and platelet-rich plasma (PRP) in between. The upper two-thirds of the plasma was extracted, leaving approximately 3 ml of PRP, which was then

resuspended carefully. Before administration, PRP is activated by exposure to light and vibrations [16].

Induction of peripheral injury by axonotmesis of the sciatic nerve

To establish rat sciatic nerve injury models, this study followed previous surgical protocols [17–19]. Rats were administered intraperitoneal anesthesia using a combination of ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight). The gluteal area was shaved and sanitized before making an incision. This incision exposed the sciatic nerve, extending from the sciatic notch to the popliteal branch, and facilitated identification of the right biceps femoris muscle. The injured nerve was marked at the epineurium using a 0.8 nylon suture. An arterial clamp was applied to the nerve for 60 s, approximately 1.5 cm from the sciatic foramen, and the axonotmesis site was marked in the muscle using a 0.4 silk suture.

Experimental design

In the saline control group, axonotmesis injury was induced in the sciatic nerve, whereas in the sham-operated group, it remained uninjured. The axonotmesis site in the saline control group was treated with a gelatin sponge saturated with 0.2 ml of 0.9% saline solution. In the treatment group, the sciatic nerve was enveloped in gelatin sponge moistened with 0.2 ml of PRP. The other group received PRP via intraperitoneal injection without a sponge, administered five minutes after axonotmesis. The sham control group was subjected to sciatic nerve injury, without injury. The wounds were closed using sutures. A single operator performed the entire procedure using the microsurgical tools in a sterile environment. After treatment, the animals were returned to their cages for 21 days [20,21]. On day 22, the rats were euthanized by cervical dislocation and the sciatic nerve was surgically removed. The nerves were divided into two segments: the first starting 5 mm before and extending 10 mm beyond the axonotmesis site, and the second beginning 5 mm beyond the first section. For histological examination, nerve segments measuring 5 mm in thickness (segment 2) were immersed in a 10% BNF solution. Sciatic nerve tissue sections, cut to 5 μm thickness, were embedded in paraffin.

Immunohistochemistry

Paraffin-embedded tissue sections were subjected to immunohistochemical analysis. An anti-S100B antibody was used to assess S100B expression. S100B expression was examined under a light microscope at 400×magnification. ImageJ software was used to manually quantify the percentage of S100B expression.

Statistical analysis

Statistical analysis was conducted using ANOVA to assess the significant effect of different PRP injection methods on S100B protein expression, followed by Tukey's post-hoc test for further evaluation. The S100B expression results are presented as mean ± standard deviation (SD). SPSS software (version 22.0; IBM Corp., Armonk, New York, USA) was used for all the statistical analyses. Statistical significance was set at $p < 0.05$.

RESULTS

S100B expression in each treatment group

S100B expression in each treatment group is shown in Figure 1**.** S100B immunoreactivity was clearly visible under the microscope as fine brownish granules in the cytoplasm. A sporadic expression was observed in the control group. The results showed that the PRP-treated group had higher expression than the K1 and K2 control groups (Figure 1A-B**).** The group that received PRP injections via the combined route (Figure 1E) exhibited enhanced S100B expression in comparison to the groups that received PRP alone through local or intraperitoneal administration (Figure 1C-D)**.**

Percentage of S100B protein expression

The distribution of S100B protein expression across the experimental groups is shown in Table 1. Statistical analysis using one-way ANOVA revealed significant variations among the treatment groups ($p = 0.000$). Notably, the group that received a combination of local and peritoneal PRP exhibited a markedly higher percentage of S100B expression than the groups treated with either local or intraperitoneal PRP alone.

Figure 2 shows that the groups receiving intraperitoneal and combined PRP treatments exhibited significant differences in S100B protein expression compared with the K1 sham operation control group ($p = 0.007$ and $p = 0.000$, respectively). However, the group treated with local PRP did not demonstrate significant expression levels compared to the K1 ($p = 0.082$) or K2 ($p = 0.619$) control groups. When examining the differences between the treatment groups, it was found that the combined local and intraperitoneal PRP administration resulted in significantly different expression levels compared to the P1 (intraperitoneal) treatment group ($p = 0.010$).

DISCUSSION

Treatment efficacy varies significantly depending on how it is administered and its duration. Numerous studies have documented the effectiveness of locally administered PRP for the treatment and regeneration of peripheral nerves, attributing its success to its potential as a neuroprotective, neurogenic, and neuroinflammatory agent. However, studies on the effect of different PRP administration methods on axonal regeneration following peripheral nerve injury are limited. This study aimed to evaluate the effect of local, intraperitoneal, and combined administration routes on S100B protein expression in sciatic nerve injury rat models.

PRP has the potential to deliver external neurotrophic factors [22]. The high concentration of platelets in PRP contains many neurotrophic factors that play important roles in peripheral nerve regeneration after nerve injury [23]. To survive, nerve cells require neurotrophic factors, including S100B [24,25]. Neurotrophic factors can regulate the increase in S100B expression [26].

The S100 protein, encoded by the S100 gene family, contains two calcium-binding sites and is involved in various intracellular and extracellular functions.[27] In studies focusing on nerve regeneration, the S100 protein serves as an indicator of Schwann cell proliferation [28]. S100B expression in Schwann cells is indicative of the degree of myelination, with S100 immunoreactivity correlating with myelin quantity in these cells [12]. In the second week, peak S100 immunoreactivity coincided with the maximum number of Schwann cells, myelin production, and nerve thickness. After 4 weeks, myelin degeneration occurred and S100 expression decreased [29].

The present study revealed that administration of PRP to the site of nerve damage enhanced S100B expression. When acute peripheral nerve injury occurs, Schwann cells in the affected area release S100B, which triggers Receptor for Advanced Glycation End (RAGE) on both infiltrating macrophages and Schwann cells. Furthermore, infiltrating macrophages have favorable effects such as clearance of cell debris and dead neutrophils. Macrophages release cytokines and neurotrophic factors. Activated Schwann cells also produce these substances in the area of nerve damage, which play a crucial role in facilitating nerve repair following injury [30,31].

Compared with the K2 (saline) group, the K1 (sham operation) group, which underwent nerve injury without PRP injection, exhibited low S100B expression. In addition, the combined PRP treatment control group had a 2.07 times higher S100B expression value than the K1 control group, which also received nerve injury treatment and 1.61 times higher S100B expression than the K2 (saline) control group. Under normal physiological conditions, S100B levels remain within the standard range. However, when nerve damage occurs, there is a localized increase in S100B concentration, which triggers various trophic effects in the peripheral nervous system [30]. The effects of S100B are strongly influenced by its concentration. Studies have shown that S100B exhibits neurotrophic properties at low (nanomolar) concentrations, which are believed to be

physiologically relevant. A range of effects has been observed, including stimulation of neurite growth, alteration of long-term potentiation, improvement of neuronal viability, reduction of neurotoxicant-induced harm, and enhanced elimination of reactive oxygen species [12].

The protein S100 is exclusively found in glial cells of the central nervous system and Schwann cells of the peripheral nervous system [26]. The observation of S100 expression by immunohistochemical staining of injured nerves indicates Schwann cell proliferation. In the PRP-treated groups, PRP administration via the combined route increased S100B protein expression compared with that in the local and intraperitoneal groups. Compared with the control group K1 (sham operation), all PRP-treated groups exhibited elevated S100 expression, indicating that PRP administration enhanced Schwann cell proliferation. The PRP delivery method was based on the findings of Ma et al. (2013), who demonstrated that direct application of growth factors to the injured site protects nerve cells from death and substantially accelerates nerve regeneration [32].

Using the Extensor Postural Thrust test, Emril et al. (2021) showed that local and intraperitoneal administration of PRP after sciatic nerve injury effectively inhibited neuropathic pain behavior and improved motor function in rats using the Extensor Postural Thrust (EPT) test [18]. A recent study reported that local and intraperitoneal administration of PRP improved rat motor function in the Toe Out Angel (TOA) test after sciatic nerve injury [21]. Recovery from peripheral nerve injury can be enhanced by the application of PRP, both locally and intraperitoneally. This treatment improves various nerve characteristics, including increased fiber and axon diameters, a higher number of axons, and thicker myelin sheaths [33].

As a results, PRP administration through the combined local and intraperitoneal routes showed the highest nerve regeneration results compared to the local and intraperitoneal routes alone. The accumulation of S100B indicates Schwann cell proliferation in the sciatic nerve, which supports sustained regeneration and functional recovery [34,35].

CONCLUSION

The PRP treatment group exhibited a higher percentage of S100B protein expression than that in the control group. The group administered PRP via the combination route (local and intraperitoneal) showed increased S100B expression both in percentage and by immunohistochemical observation. Further investigations, such as mRNA expression analysis of S100B by RT-PCR, are warranted.

Ethics Committee Approval

The Ethics Research Commission of the Faculty of Medicine at Universitas Malikussaleh granted ethical approval for this study (No. 24/KEPK/FKUNIMAL-RSUCM/2024; May 20, 2024).

Conflict of interest:

All the authors declare that there are no conflicts of interest

Author's contributions:

Conception, Design, Supervision, Fundings, Writing, Critical Review – M.M.; Materials, Data Collection and/or Processing, Analysis and/or Interpretation, Critical Review – Y.Y.; Supervision, Data Collection and/or Processing, Literature Review, Writing – R.S.; Data Collection and/or Processing, Analysis and/or Interpretation – F.A.B; Data Collection and/or Processing, Analysis and/or Interpretation – P.N.L. All authors have read and agreed to the published version of the manuscript."

Acknowledgements:

The authors thank all staff researchers for their support

Funding support:

Universitas Malikussaleh Non-Tax State Revenue Research Grant (PNBP) for providing financial support (072/UN45.3.1/AL.04/2024).

REFERENCES

1. Hatzenbuehler J. Peripheral nerve injury. Curr Sports Med Rep. 2015;14(5):356–7.
2. Mukhtar NB, Umar YI, Mayana KI, Sumaila FG. Prevalence and pattern of peripheral nerve injuries in Kano Metropolis, Nigeria. *African Journal of Physiotherapy and Rehabilitation Sciences*. 2017;9(1–2):22–6.
3. Zavala A, Martinez PC, Gutierrez GG, Vara MD, De Pawlikowski W. The Combined Use of Curcumin and Platelet-Rich Plasma Enhances Axonal Regeneration in Acute Nerve Injuries: An Experimental Study in a Rat Model. *J Hand Microsurg*. 2023;15(01):31–6.
4. Lopes B, Sousa P, Alvites R, Branquinho M, Sousa AC, Mendonça C, et al. Peripheral nerve injury treatments and advances: one health perspective. *Int J Mol Sci*. 2022;23(2):918.
5. Kuffler DP, Foy C. Restoration of Neurological Function Following Peripheral Nerve Trauma. *Int J Mol Sci*. 2020 Mar 6;21(5).
6. Caillaud M, Richard L, Vallat JM, Desmoulière A, Billet F. Peripheral nerve regeneration and intraneural revascularization. *Neural Regen Res*. 2019;14(1):24–33.
7. Rayilla RSR, Naidu MUR, Babu PP. Surgically Induced Demyelination in Rat Sciatic Nerve. *Brain Sci*. 2023 May 1;13(5).
8. Wang S, Liu X, Wang Y. Evaluation of platelet-rich plasma therapy for peripheral nerve regeneration: a critical review of literature. *Front Bioeng Biotechnol*. 2022;10:808248.
9. Ding Z, Jiang M, Qian J, Gu D, Bai H, Cai M, et al. Role of transforming growth factor-β in peripheral nerve regeneration. *Neural Regen Res*. 2023;
10. Kuffler DP. Platelet-rich plasma promotes axon regeneration, wound healing, and pain reduction: fact or fiction. *Mol Neurobiol*. 2015;52:990–1014.
11. Trnka Š, Stejskal P, Jablonský J, Krahulík D, Pohlodek D, Hrabálek L. S100B Protein as a Biomarker and Predictor in Traumatic Brain Injury. *Biomedical Papers*. 2023;167(XX).
12. Michetti F, D'Ambrosi N, Toesca A, Puglisi MA, Serrano A, Marchese E, et al. The S100B story: from biomarker to active factor in neural injury. *J Neurochem*. 2019 Jan 1;148(2):168–87.
13. Radithia D, Soebadi B, Parmadiati AE, Winias S. Nerve growth factor and S100B: Molecular marker of neuroregeneration after injection of freeze-Dried platelet rich plasma. *J Oral Biol Craniofac Res*. 2022;12(5):570–4.
14. Du W, Li H, Sun J, Xia Y, Zhu R, Zhang X, et al. The Prognostic Value of Serum Neuron Specific Enolase (NSE) and S100B Level in Patients of Acute Spinal Cord Injury. *Medical Science Monitor*. 2018;24:4510–5.
15. Rahman BA, Airlangga PS, Saputra AN, Kriswidyatomo P, Salinding A, Hamzah N, et al. Correlation of S100B Level and Postoperative Cognitive Dysfunction (POCD) Events Among Patients With Ear, Nose and Throat (ENT) Surgeries With Controlled Hypotension. *Bali Medical Journal*. 2022;11(3):1860–4.
16. Wu CC, Wu YN, Ho HO, Chen KC, Sheu MT, Chiang HS. The neuroprotective effect of platelet-rich plasma on erectile function in bilateral cavernous nerve injury rat model. *J Sex Med*. 2012;9(11):2838–48.
17. Cámara-Lemarroy CR, Guzmán-de la Garza FJ, Barrera-Oranday EA, Cabello-García AJ, García-Tamez A, Fernández-Garza NE. Celecoxib accelerates functional recovery after sciatic nerve crush in the rat. *J Brachial Plex Peripher Nerve Inj*. 2008;3(01):e128–31.

18. Emril DR, Wibowo S, Meliala L, Susilowati R. Cytidine 5′-diphosphocholine administration prevents peripheral neuropathic pain after sciatic nerve crush injury in rats. *J Pain Res*. 2016;287–91.

19. Jaggi AS, Jain V, Singh N. Animal models of neuropathic pain. *Fundam Clin Pharmacol*. 2011 Feb;25(1):1–28.

20. Emril DR, Hajar S, Ismy. Efikasi platelet rich plasma (PRP) terhadap optimalisasi pengobatan nyeri neuropatik dan perbaikan fungsi neurologi paska cedera saraf. Banda Aceh; 2021.

21. Muttaqien A. Peran Platelet Rich Plasma Terhadap Perbaikan Fungsi Motorik Pada Tikus Putih (Rattus Novergicus) Pasca Cedera Saraf Tepi. [Banda Aceh]: Faculty of Medicine, Syiah Kuala University; 2022.

22. Firat C, Aytekin AH, Durak MA, Geyik Y, Erbatur S, Dogan M, et al. Comparison of the effects of PRP and hyaluronic acid in promoting peripheral nerve regeneration. *Ann Ital Chir*. 2016;87(4):362–74.

23. Lana JF, Huber SC, Purita J, Tambeli CH, Santos GS, Paulus C, et al. Leukocyte-rich PRP versus leukocyte-poor PRP-The role of monocyte/macrophage function in the healing cascade. J *Clin Orthop Trauma*. 2019;10:S7–12.

24. Jesús AR. Plasma growth factors in neuronal regeneration. Austin Clin Neurol. 2017;4(3):1111.

25. Thelin EP, Nelson DW, Bellander BM. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. *Acta Neurochir (Wien)*. 2017 Feb 1;159(2):209–25.

26. Santos G, Barateiro A, Gomes CM, Brites D, Fernandes A. Impaired oligodendrogenesis and myelination by elevated S100B levels during neurodevelopment. *Neuropharmacology*. 2018 Feb 1;129:69–83.

27. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol*. 2001;33:637–68.

28. Jiang X, Ma J, Wei Q, Feng X, Qiao L, Liu L, et al. Effect of frankincense extract on nerve recovery in the rat sciatic nerve damage model. eCAM. 2016;2016.

29. Liu GM, Xu K, Li J, Luo YG. Curcumin upregulates S100 expression and improves regeneration of the sciatic nerve following its complete amputation in mice. *Neural Regen Res*. 2016 Aug 1;11(8):1304–11.

30. Sorci G. S100B protein in tissue development, repair and regeneration. *World J Biol Chem*. 2013;4(1):1.

31. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, et al. S100B's double life: Intracellular regulator and extracellular signal. *Biochim Biophys Acta Mol Cell Res*. 2009 Jun;1793(6):1008–22.

32. Ma SZ, Peng CL, Wu SQ, Wu DJ, Gao CZ. Sciatic nerve regeneration using a nerve growth factor-containing fibrin glue membrane. *Neural Regen Res*. 2013 Dec 25;8(36):3416–22.

33. Maulina M, Emril DR, Mutiawati E, Etriwati E, Hastuti S, Rahman S, et al. Effects of different platelet-rich plasma administration methods on peripheral nerve regeneration: A histomorphometric study. *J Exp Clin Med*. 2024 Sep;(3):557–62.

34. Nagappan PG, Chen H, Wang DY. Neuroregeneration and plasticity: a review of the physiological mechanisms for achieving functional recovery postinjury. Mil Med Res. 2020;7:1–16.

35. Zheng C, Zhu Q, Liu X, Huang X, He C, Jiang L, et al. Effect of platelet-rich plasma (PRP) concentration on proliferation, neurotrophic function and migration of Schwann cells in vitro. *J Tissue Eng Regen Med*. 2016;10(5):428–36.

FIGURES AND TABLES

TABLES

TABLE 1. *Percentage of S100B protein expression between groups*

Group	Mean ± S.D	*p*-value
K1 (Sham operation)	7.75 ± 1.14	0.000[a]
K2 (Saline)	9.94 ± 1.31	
P1 (Local PRP)	11.27 ± 2.18	
P2 (Intraperitoneal PRP)	12.79 ± 2.10	
P3 (Combined PRP)	16.09 ± 2.88	

[a]Analyzed using One-way ANOVA
[a]Statistically significant at p=0.05
PRP:Platelet-Rich Plasma; S.D: Standard Deviation

FIGURES

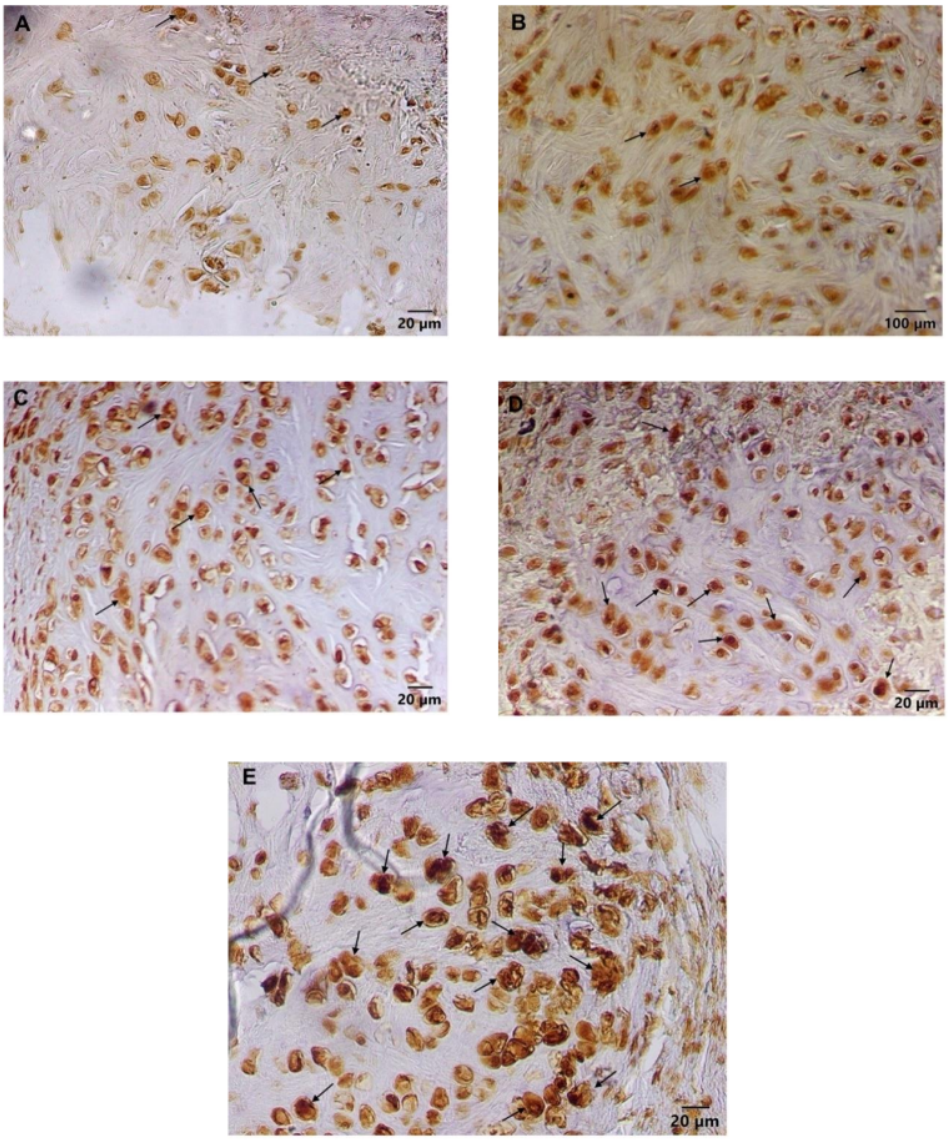

Figure 1. Immunohistochemical staining for S100B showed positive immunoreactivity, as indicated by the black arrows pointing to fine brown granules in the cytoplasm. (A) K1, sham operation; (B) K2, saline; (C) P1, local PRP; (D) P2, intraperitoneal PRP; (E) P3, combined PRP) (400x magnification). PRP:Platelet-Rich Plasma

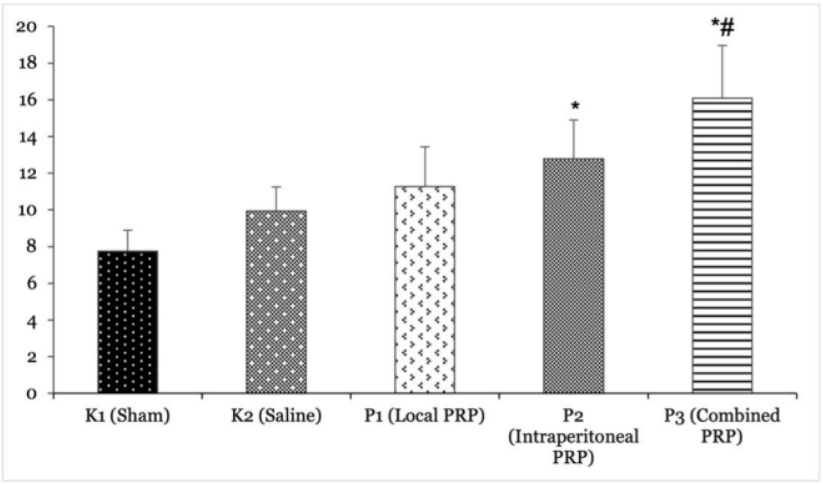

Figure 2. Percentage of S100B protein expression between groups. *Significantly different compared to the control group, K1 ($p = 0.000$); #significantly different between PRP treatment groups ($p = 0.010$). PRP:Platelet-Rich Plasma