Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

Check for updates

# Modified method of producing sciatic nerve crush injury model in Wistar rats: A pilot study

Bamidele Thomas Akingbade [1,*], Simon Irikefe Ovuakporaye [1], Christopher Eseoghene Omajuwa [2], Odomero Thomas Olomo [2], Bartholomew Chukwuebuka Nwogueze [2], Udoka Shalom Nwabuoku [2] and Chukwuemeka Peter Aloamaka [2]

[1] *Department of Physiology, Delta State University, Abraka, Nigeria.*
[2] *Department of Civil Engineering, Delta State University, Oleh, Nigeria.*

Magna Scientia Advanced Biology and Pharmacy, 2024, 12(01), 075–083

Publication history: Received on 29 April 2024; revised on 16 June 2024; accepted on 19 June 2024

Article DOI: https://doi.org/10.30574/msabp.2024.12.1.0038

## Abstract

The production of a standard and reproducible experimental mode of peripheral nerve injury, has been a long-time quest in nerve regeneration research. The aim of this pilot study was to reproduce sciatic nerve crush injury model in Wistar rats and also use the established method for broader and future research.

**Materials and Methods**: twenty (20) rats grouped into five (5) of five (5) rats each. Normal control (G1), the sciatic was not exposed nor crushed; Sham (G2), the sciatic nerve were surgically exposed but not crushed; Crushed (G3), the sciatic nerve were surgically exposed and uniformly crushed, using a non-serrated heamostatic forceps that exerted a compressive force of 33N for a duration of 30 seconds and Transected (G4), the sciatic nerve were exposed and completely transected. Sciatic nerves were harvested at seven (7) days (post-injury) for histological evaluation and the groups were compared for histopathological changes.

**Results:** Transverse sections of toluidine blue (TB) stained sciatic nerve micrograph, showed degenerative changes in the axons, myelin ballooning and surveiling white blood cells, all of which were consistent with Wallerian degeneration in G3, when compared with groups G1 and G2. The G4 group showed more severe degenerative changes when compared with group G3.

**Conclusion**: The histopathological analysis suggest that the modified induction method used in this study, caused Axonotmetic lesion in the crushed sciatic nerves of the Wistar rats.

**Keywords:** Peripheral Nerve Injury; Sciatic Nerve Crush Injury; Universal Testing Machine; Central Nervous System; Peripheral Nervous System

## 1. Introduction

Injury to peripheral nerves happen frequently in humans, leading to severe and long-term physiological and functional disabilities [1, 2]. Given that peripheral nerve injury (PNI) occurs so frequently, it has a high clinical relevance [3,4]. Peripheral neural damage has several and different underlying causes, some of which can be traumatic events or iatrogenic damage that most often result from medical or surgical procedures [5,6]. The primary associative complications of nerve injury may either be loss of motor, sensory or autonomic function in the denervated body segments, which typically causes a mild, moderate or severe functional impairment [7,8]. The peripheral nervous

* Corresponding author: Thomas Bamidele Akingbade

system (PNS), going by its cellular characteristics, has been found to show a better self-reparative and regenerative capacity, when compared with the central nervous system (CNS).

In the quest to optimize functional recovery in victims of PNIs, areas of interest include the investigation factors that directly influence and improve the success of nerve regeneration and reinnervation of end organ. Though invasive experimental studies designed to explore and understand the mechanism of PNI are difficult and unethical in human subjects, the use of animal experimental models of peripheral nerve damage, has since remain the preferred research option [9]. Because of the availability and ease to procure rats and also due to the distribution of nerve trunks of rats, which is similar to that of humans, rats are mostly used in experimental studies of peripheral nerve regeneration, using the sciatic nerve crush injury model [10,11]. The peripheral nerve injury seen in humans, can be modelled in rats with the characteristic features of transient or permanent injury to the neural functions. This is possible because the of the lengthy structure of the nerve trunk, situated within a spacious mid-thigh that allows for surgical procedure that involves the application of standard direct mechanical trauma [12].

The induction methods of nerve crush injury model used in studies, have unique advantages and disadvantages that are associated with them. The use of non-serrated haemostatic forceps in most studies, as a mechanical method of inducing crush injury model, has the advantage of reducing the surgical procedure time [13]. Micro-forceps are used in experimental crush injury procedure sand is usually done by application of effective mechanical pressure that compresses the nerve tissue [14, 15, 16, 17], some studies use clamps [18, 19, 20], tourniquets [21], or other devices and tools [22, 23, 24]. Attempts have been made in published studies to produce crush injury that is reproducible, the efforts notwithstanding [25], standardized method for inducing crush nerve injury model has remain difficult to establish [26, 3]. Studies have reported methods of induction that differ widely as regards the instrument used, the duration of compressive force applied, the injury size, and reproducibility of the resulting nerve injury [27, 28]. Because of these varying methods that is devoid of defined standard of induction, attempt at making comparisons between different experimental studies are often difficult.

Due to lack of standardized method for inducing crush injury model, this pilot study was designed with the aims of producing sciatic nerve crush injury model in rats, using a standardized and reproducible method in terms of quantified compressive force exerted and the duration of the compression, and also, to use the model developed in broader and future research in peripheral nerve injury. Using our proposed modified method, qualitative histological evaluation was used to describe and suggest structural changes that confirmed the degree of induced sciatic nerve injury.

## 2. Materials and methods

### 2.1. Experimental Animals

A total of 20 adult male Wistar rats (weighing 150-200g) were procured from the Animal Laboratory (Faculty of Basic Medical Sciences, Department of Physiology, Delta State University, Abraka, Nigeria). The animals were housed in large cages in a temperature and humidity controlled room, with 12:12 hour light/dark cycles. The animals were acclimatized in the environment 7 days prior to the experiment, during which they had unrestricted access to food and water.

### 2.2. Quantification of Heamostatic-Forceps

Though the use of rat models in studies of PNI by crushing has been done by researchers, there still exist the challenge of establishing a perfect and standardized method of inducing crush model of nerve injury, with respect to duration of force applied, device used, injury size, magnitude and reproducibility [3]. This difficulty therefore, necessitated a pilot study on establishment of nerve crush injury model, as a precursor to PNI study.

The sciatic nerve crush injury model was done using the non-serrated haemostatic forceps (Pakistan TZ). At the Concrete and Material Testing Laboratory, Civil Engineering Department, Faculty of Engineering, Delta State University, Oleh, Nigeria, Universal Testing Machine (UTM) (model: UTM-8001 WINCOM COMPANY LTD CHINA) was used to quantify the compressive force of the forceps. The forceps was mounted on the UTM and gradually operated to the point where the locking sound of the third teeth in the rachet in the handles of the forceps was heard and the operation was stopped. The compressive force value on the computer screen was immediately noted and recorded. Three separate values were obtained (32N, 33N and 34N) and average value of 33N was recorded as the compressive force of the haemostatic forceps used for this experiment (Figure 1. A, B and C).

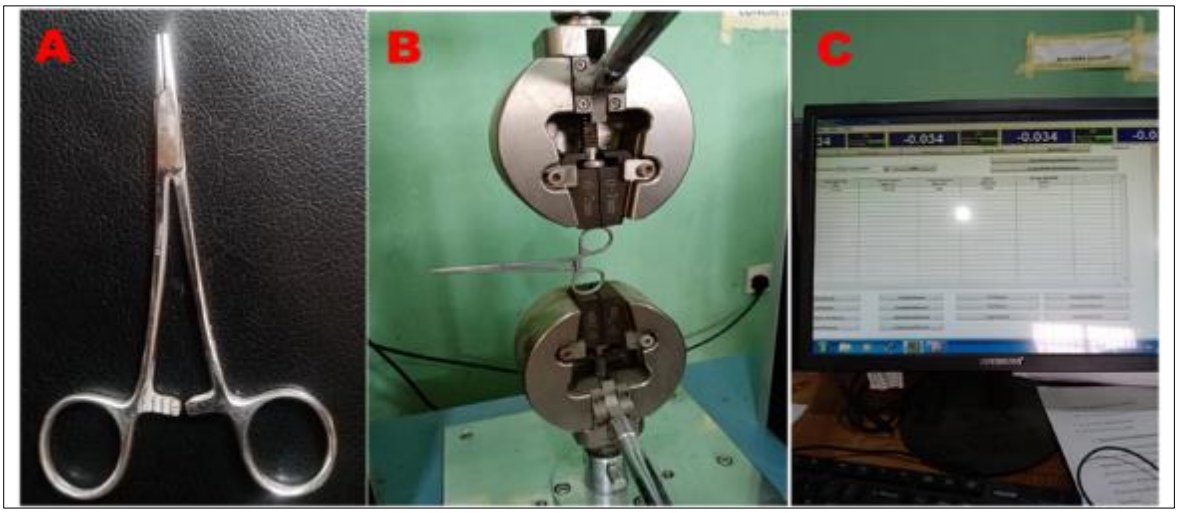


**Figure 1** (A) haemostatic forceps, (B) determination of compressive force of forceps using Universal Testing Machine and (C) digital recording of the compressive force

### 2.3. Surgical Procedures

Rats were randomly divided into four equal groups of five: group G1 (normal control), the sciatic was not exposed nor crushed, group G2 (sham), the sciatic nerve were surgically exposed but not crushed, group G3 (transected), the sciatic nerve were exposed and completely transected and group G4 (crushed), the sciatic nerve were surgically exposed and uniformly crushed (Figure 2). The surgical crush procedure was a modified methods from previous studies [29, 30]. The rats were put under general anaesthesia with intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg), and after routine preparation of the operative field (hair trimming, 20% iodine ethylic alcohol solution), the left sciatic nerve was bluntly exposed

**Figure 2** Isolation of the sciatic nerve, induction of crush injury and anatomical flattening of the sciatic nerve after injury

through a 2 cm long posterolateral longitudinal straight incision on the lateral aspect of the left thigh, following which a straight and non-serrated haemostatic forceps with 3mm width was used to uniformly clamp a 3mm long sciatic nerve by exerting a constant pressure of 33N for 30s at a point perpendicular to the sciatic notch and approximately 10 mm above the sciatic nerve bifurcation, done to obtain good reproducibility of the crush injury model. Distal to the crushed part, the crushed nerve was marked with loosely (non-constrictive) tied 2.0 suture and the incision was thereafter sutured back. Following induction, each rat was watched to ensure that the nerve had been completely crushed, which was demonstrated by the paralysis of the toe muscles and the absence of toes spreading. Rats with toe movement, which is a sign of an incomplete nerve crush was not used for the study.

### 2.4. Histological examination

Seven (7) days (post-injury) after, the rats were sacrificed, sciatic nerves were harvested and fixed with 4% paraformaldehyde. Subsequently, the tissues were dehydrated and embedded in paraffin and sliced into 5 µm sections. The Paraffin embedded tissues were then dewaxed with xylene and alcohol and stained with Toluidine Blue (TB). Finally, any histopathological changes were observed under an optical microscope.

## 3. Results

### 3.1. Histological Evaluation

Seven days after crush, sciatic nerves were removed from the G1, G2, G3 and G4 groups, were TB stained and the microscopic images of transverse-sections were examined (Fig. 3). Transverse sections of the sciatic nerve showed different sizes of spherically-shaped nerve fibres structures and axons appearing blue were arranged closely in the centre of myelinated nerve fibre bundles. The low stained areas that surround the axons, represents myelin structure. While the deeply stained areas depicted the nuclei of the Schwann cells, situated between the myelinated fibre bundles. Wrapped around the surface of each nerve fibre as a thin layer of connective tissue is the Endoneurium, while the perineurium encircled the fibre bundles.

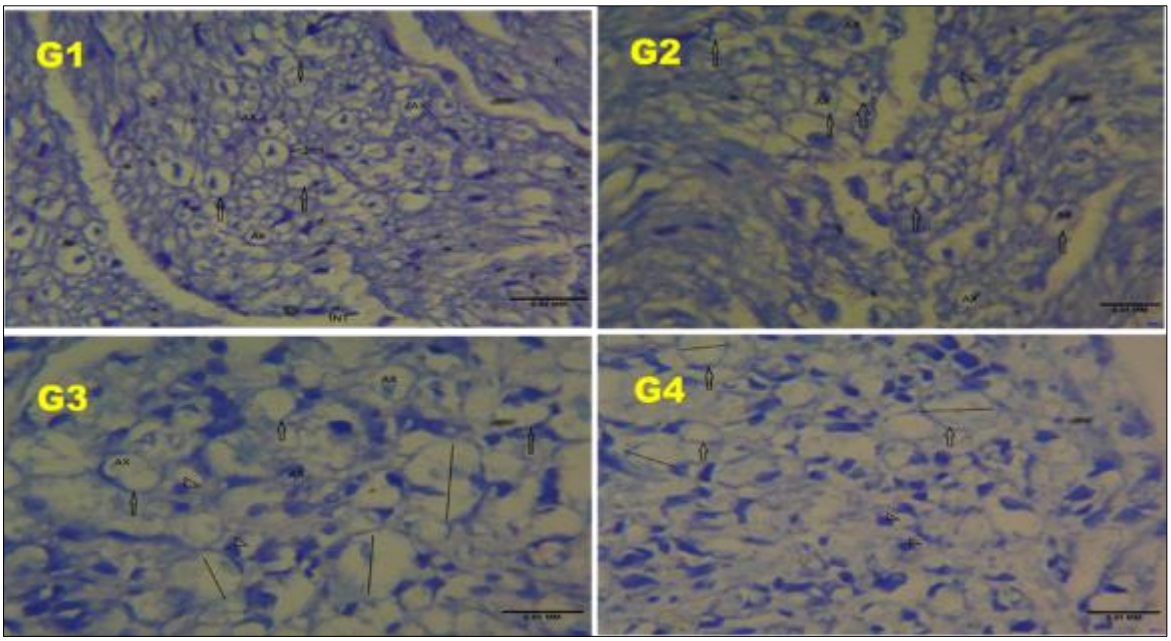


**Figure 3** Photomicrograph of transverse sections of TB stained sciatic nerves of normal control (G1), Sham (G2), Crushed (G3) and Transected (G4) Rats. In G1 and G2 normal nerve histomorphology was observed, as the axons (AX), endoneurium (arrow) and blood capillaries (arrow head) all appeared normal. G3 and G4 showed degenerating and degenerated axon (AX), myelin ballooning (line), degenerating and degenerated myelin sheath (arrow) and surveiling white blood cells (arrow head), which are features consistent with Wallerian degeneration, which more severe in G4. Scale bars = 0.01mm

The histopathologic changes or lack of it in each control and experimental group was observed in cross section. It was found that the sciatic nerve of G1 group, showed nerve fibre consisting of axons (AX) organized in bundles. Each fibre is lined by a thin endoneurium (arrow), separated by a loose connective tissue and blood capillaries (arrow head). All fibres are encapsulated in a thick perineurium containing the vasa vasorum nervorum. Section is free from inflammatory cells infiltration and features consistent with a normal nerve histomorphology. The G2 group, when observed, revealed features closely similar to that of G1 group. A degenerating and degenerated axon (AX), myelin ballooning (line), degenerating and degenerated myelin sheath (arrow) and surveiling white blood cells (arrow head), which are features consistent with inflammatory response, were seen in the section of G3 group. The G4 group, showed features consistent with severe inflammatory response, as evidenced by the level of degenerating axons (AX), myelin ballooning (line), myelin sheath degeneration (arrow) and surveiling white blood cells (arrow head), when compared with the G3 group.

## 4. Discussion

The research interest in understanding the mechanism of peripheral nerve degeneration and regeneration following injury, can be investigated using different experimental models. The axonotmetic class of PNI can be modelled using induced crush injury [31, 32]. the crush injury model has the advantage of not requiring microsurgical suture, which makes it the most commonly used experimental models for PNI by researchers [33]. In the controlled crush injury, and remarkably, some of the axonal protective and connective structures are preserved, which creates enabling environment for regeneration to occur, and these can be achieved with basic skill in surgical techniques, using inexpensive instruments and materials [34].

It is known fact that crush injury to the peripheral nerve leads to complications that may include derangement of the nerve structures and loss of motorsensory functions, the severity of which will depend on the strength of the compressional force applied [35, 36]. Studies have shown that the structural changes and functional loss associated with peripheral nerve compression, may have resulted from non-perfusion of intraneural vessels (vasa nervorum) following application of strong compressive pressures, that ultimately lead to focal ischemia. The nature of the structural derangement that occur in nervous fibres, is characterized by disorganization of the connective tissues that envelopes the axons, which includes the myelin sheath, all of which lead to impairment of motorsensory functions [37, 38].

Studies have abundantly been published about the use of crush nerve injury model to experimentally explore, the different facets of neuro-regeneration and treatment of peripheral nerve injuries [39, 40, 41]. Notwithstanding, the challenge of non-existence of universal standard mechanism of producing crush model of nerve injury, makes the reproduction and comparison of the different methods established in studies difficult [42]. Many mechanisms have been suggested, but none has prevailed over the others. The different crush injury induction methods used by different authors involve the use of different types of machines, adjustable weights, crush duration and compressive force. however, there exist limitations and difficulties with some of the methods. Some of the machines are considerably cumbersome and make ease of use highly challenging. Also, some of the methods are lacking in quantification and standardization of compressive force applied on the nerve [43].

Yun et al [44] in their induction method, used toothless forceps which was qualitatively described, but the compressive force applied was not quantified. The observed suggestions and conclusions from this study and others, informed the idea of using a Universal Testing Machine to quantify the compressive force of the haemostatic forceps, used in crushing the sciatic nerve of the rats used in this present study [45, 46, 47]. The experimental nerve crush injury in rats, models the axonotmetic class of PNI, and to assess the success of this experimental model, previous studies have used electrophysiological, behavioural and histomorphometric assessment as evaluation methods. Research findings that have established the success of crush injury models, showed consistent alignment between the electrophysiological and behavioural results, and that of histomorphometric observations [48, 49].

In this present study, evaluation of the histopathologic micrographs of the rats that had their sciatic nerves crushed (G3 group), showed structural changes that were indicative of Wallerian degeneration, when compared with the nerve tissue micrographs of normal control (G1) and sham (G2) groups, while severe Wallerian degeneration was observed in the transected nerves of rats in G4 group. These observations were made at 7 days after sciatic nerve injury was induced. In the crush injury model, the injured nerves have been reported to have faster rate of regeneration, when compared with transection model that presents an extremely slower rate [50, 51, 52].

In our present study, the Histo-micrographs of the G3 group, showed axons, endoneurium and perineurium that are mildly or moderately damaged, while the epineurium is preserved. This degree of nerve injury presented in G3 group, is similar to PNI classification termed as Sunderland Grade IV nerve injury [53, 25, 54, 55]. Therefore, the crush injury model established in this study, is suitable for basic research in peripheral nerve degeneration, regeneration and recovery management. Our conclusion aligns with findings of studies in animal experiments that modelled Crush injury, where the authors abundantly established and confirmed that the preservation of the epineurium, is a significant factor that enhances spontaneous nerve axon regeneration [56, 57, 58, 59].

### *List of Abbreviations*

- PNI - Peripheral Nerve Injury
- TB - Toluidine Blue
- PNS - Peripheral Nervous System
- CNS - Central Nervous System

- UTM - Universal Testing Machine

## 5. Conclusions

Transverse sections of TB stained sciatic nerve micrograph, showed degenerative changes in the axons, myelin ballooning and surveiling white blood cells, all of which were consistent with Wallerian degeneration in G3 when compared with G1 and G2, while G4 showed severe degenerative changes when compared with G3. Therefore, the histopathological analysis suggest that the modified induction method used in this study, caused Axonotmetic lesion in the crushed sciatic nerves of the Wistar rats. This is a part report of a standardized and reproducible crush injury model in rat, as ongoing work that encompasses functional and biochemical studies of a larger group of rats will be available for publication when completed.

## Compliance with ethical standards

### *Acknowledgements*

The authors are grateful to members of staff of Concrete and Material Testing Laboratory, Civil Engineering Department, Faculty of Engineering, Delta State University, Oleh, Nigeria, for their support and contribution.

### *Disclosure of conflict of interest*

All the authors have approved that this manuscript be published

### *Statement of ethical approval*

Ethical approval (RBC/FBMC/DELSU/23/200) for this study was obtained from Research and Bio-Ethics Committee of the College of Health Sciences, Delta State University, Abraka, and was carried out at the animal facility of Faculty of Basic Medical Sciences, Department of Physiology, Delta State University, Abraka, Nigeria.

### *Availability of data and material*

Data for the Study will be provided by the corresponding author on request.

### *Author's contributions*

- ATN conceptualized and conducted the study and prepared the manuscript
- NBC drafted the manuscript
- OCE carried out the measurement on Universal Testing Machine
- OSI supervised the study
- SLM conducted literature search and prepared the methodology
- OLO assisted in the surgical procedure
- ACP supervised the study.

All authors approved the submission of this manuscript.

## References

[1] Wojtkiewicz DM, Saunders J, Domeshek L, Novak CB, Kaskutas V, Mackinnon SE. Social impact of peripheral nerve injuries. Hand (N Y). 2015; 10(2), 161–167. doi:10.1007/s11552-014-9692-0.

[2] Slavin BR, Sarhane KA, von Guionneau N, Hanwright PJ, Qiu C, Mao HQ, Hoke A, Tuffaha SH. Insulin-Like Growth Factor-1: A Promising Therapeutic Target for Peripheral Nerve Injury. *Front.Bioeng. Biotechnol*. 2021; 9, 695850. [CrossRef].

[3] Rochkind S. Phototherapy in peripheral nerve regeneration: From basic science to clinical study. *Neurosurgical Focus.* 2009; 26(2), E8. doi:10.3171/ FOC.2009.26.2.E8

[4] Rayner MLD, Day AGE, Bhangra KS, Sinden J, Phillips JB. Engineered neural tissue made using clinical-grade human neural stem cells supports regeneration in a long gap peripheral nerve injury model. *Acta Biomater*. 2021; 135, 203–213. [CrossRef] [PubMed].

[5] Antoniadi G, Kretschmer T, Pedro MT, Konig RW, Heinen CP, Richter HP. Iatrogenic nerve injuries: Prevalence, diagnosis and treatment. *DeutschesÄrzteblatt International*. 2014; 111(16), 273–279. doi:10.3238/arztebl.2014.0273

[6] Kerns JM, Walter JS, Patetta MJ, Sood A, Hussain AK, Chung JJ. Histological assessment of Wallerian degeneration of the rat tibial nerve following crush and transection injuries. *J. Reconst. Microsurg*. 2020; 37, 1–14. doi: 10.1055/s-0040-1716870

[7] Navarro, X. Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: a critical overview. *Eur. J. Neurosci.* 2016; 43, 271–286. doi: 10.1111/ejn.13033

[8] Heinzel JC, Oberhauser V, Keibl C, Swiadek N, Längle G, Frick H. Evaluation of Functional Recovery in Rats After Median Nerve Resection and Autograft Repair Using Computerized Gait Analysis. *Frontiers in Neuroscience*. 2021; 14: 593545.

[9] Aksu U, Atukeren P, Terzioğlu D, Belce A, Demirci-Tansel C. Effect of dexamethasone in mediating oxidative stress induced by sodium nitroprusside on frog sciatic nerve action potentials. *Bezmialem Sci*. 2013; 1:21-27.

[10] Andreollo NA, Santos EF, Araújo MR, Lopes LR. Rat's age versus human's age: What is the relationship? *Arq. Bras Cir. Dig*. 2012; 25:49–51. doi: 10.1590/S0102-67202012000100011. [PubMed] [CrossRef] [Google Scholar]

[11] Caillaud M, Chantemargue B, Richard L. Local low dose curcumin treatment improves functional recovery and remyelination in a rat model of sciatic nerve crush through inhibition of oxidative stress. *Neuropharmacology*. 2018; 139:98-116. 10.1016/j.neuropharm.2018.07.001

[12] Chen P, Piao X, Bonaldo P. Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. *Acta Neuropathologica*. 2015; Vol. 130: 605–18.

[13] Gonzalez D, Allende ML. Current Advances in Comprehending Dynamics of Regenerating Axons and Axon-Glia Interactions after Peripheral Nerve Injury in Zebrafish. *Int. J. Mol. Sci*. 2021; 22, 2484. [CrossRef] [PubMed]

[14] Kurtoglu Z, Ozturk AH, Bagdatoglu C, Polat G, Aktekin M, Uzmansel D, Sargon M. Effects of trapidil after crush injury in peripheral nerve. *Acta Medica Okayama.* 2005; 59(2), 37–44. doi:10.18926/AMO/31967.

[15] Fan LY, Wang ZC, Wang P, Lan YY, Tu L. Exogenous nerve growth factor protects the hypoglossal nerve against crush injury. *Neural Regen. Res*. 2015; 10, 1982–1988. doi: 10.4103/1673-5374.172316

[16] Ni XJ, Wang XD, Zhao YH, Sun HL, Hu YM, Yao J. The effect of low-intensity ultrasound on brain-derived neurotropic factor expression in a rat sciatic nerve crushed injury model. *Ultrasound Med. Biol*. 2017; 43:461–468. doi: 10.1016/j.ultrasmedbio.

[17] Suzuki K, Tanaka H, Ebara M, Uto K, Matsuoka H, Nishimoto S. Electrospun nanofiber sheets incorporating methylcobalamin promote nerve regeneration and functional recovery in a rat sciatic nerve crush injury model. *Acta Biomater*. 2017; 53:250–259. doi: 10.1016/j.actbio.

[18] Zhang X, Xin N, Tong L, Tong XJ. Electrical stimulation enhances peripheral nerve regeneration after crush injury in rats. *Molecular Medicine Reports*. 2013; 7(5), 1523–1527. doi:10.3892/mmr.2013.1395

[19] Yüce S, Cemal Gökçe E, Işkdemir A. An experimental comparison of the effects of propolis, curcumin, and methylprednisolone on crush injuries of the sciatic nerve. *Ann Plast Surg*. 2015; 74(6): 684–692, doi: 10.1097/SAP.0000000000000026, indexed in Pubmed: 24317243.

[20] Korkmaz MF, Parlakpinar H, Ceylan MF, Ediz L, Samdanci E, Kekilli E. The effect of sildenafil on recuperation from sciatic nerve injury in rats. *Balkan Med. J*. 2016; 33, 204–211. doi: 10.5152/balkanmedj.2016.14701

[21] Chen LE, Seaber AV, Glisson RR, Davies H, Murrell GA, Anthony DC, Urbaniak JR. The functional recovery of peripheral nerves following defined acute crush injuries. *Journal of Orthopaedic Research*. 1992; 10(5), 657–664. doi:10.1002/jor.1100100508

[22] Sarikcioglu L, Yaba A, Tanriover G, Demirtop A, Demir N, Ozkan O. Effect of severe crush injury on axonal regeneration: A functional and ultrastructural study. *Journal of Reconstructive Microsurgery*. 2007; 23(3), 143–149. doi:10.1055/s-2007-974649

[23] Feng X and Yuan W. Dexamethasone enhanced functional recovery after sciatic nerve crush injury in rats. *Biomed. Res. Int*. 2015; 627923. doi: 10.1155/2015/627923

[24] Hei WH, Byun SH, Kim JS, Kim S, Seo YK, Park JC. Effects of electromagnetic field (PEMF) exposure at different frequency and duration on the peripheral nerve regeneration: in vitro and in vivo study. *Int. J. Neurosci*. 2016; 126, 739–748. doi: 10.3109/00207454.2015.1054032

[25] Beer GM, Steurer J, Meyer VE. Standardizing nerve crushes with a non-serrated clamp. *J Reconstr Microsurg*. 2001; 17, 531–4.

[26] Varejao AS, Cabrita AM, Meek MF, Bulas-Cruz J, Melo-Pinto P, Raimondo S. Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. *J. Neurotrauma*. 2004; 21:1652–1670. doi: 10.1089/neu.2004.21.1652

[27] Tos P, Ronchi G, Papalia I, Sallen V, Legagneux J, Geuna S, Giacobini-Robecchi MG. Chapter 4: Methods and protocols in peripheral nerve regeneration experimental research: Part I-experimental models*. International Review of Neurobiology*. 2009; 87, 47–79. doi:10.1016/S0074-7742(09)87004-9

[28] Alvites R., Rita Caseiro A, Santos Pedrosa S, Vieira Branquinho M, Ronchi G, Geuna S, Varejão ASP, Colette Maurício A, Spurkland A. Peripheral nerve injury and axonotmesis: State of the art and recent advances. *Cogent. Med*. 2018; 5:1466404. doi: 10.1080/2331205X.2018.1466404. [CrossRef] [Google Scholar]

[29] Kalender AM, Dogan A, Bakan V, Yildiz H, Gokalp MA, Kalender M. Effect of Zofenopril on regeneration of sciatic nerve crush injury in a rat model. *Journal of Brachial Plexus and Peripheral Nerve Injury*. 2009; 4(1), 6. doi:10.1186/1749-7221-4-6.

[30] Bobinski F, Martins DF, Bratti T, Mazzardo-Martins L, Winkelmann-Duarte EC, Guglielmo LGA, Santos ARS. Neuroprotective And Neuroregenerative Effects of Low intensity Aerobic Exercise on Sciatic Nerve Crush Injury in Mice. Neuroscience. 2011; 194; 337–348.

[31] Isaacs J. Major peripheral nerve injuries. *Hand Clinics*. 2013; 29:371–82.

[32] Jahromi M, Razavi S, Bakhtiari A. The advances in nerve tissue engineering: From fabrication of nerve conduit to in vivo nerve regeneration assays. *J. Tissue Eng. Regen. Med*. 2019; 13:2077–2100. doi: 10.1002/term.2945. [PubMed] [CrossRef] [Google Scholar]

[33] Huff TC, Sant DW, Camarena V, Van Booven D, Andrade NS, Mustafi S, Monje PV, Wang G (2021). Vitamin C regulates Schwann cell myelination by promoting DNA demethylation of pro-myelinating genes. *J. Neurochem*. 2021; 157, 1759–1773. [CrossRef][PubMed].

[34] Huebner EA, Strittmatter SM. Axon regeneration in the peripheral and central nervous systems. Results *Probl. Cell Differ.* 2009; 48:339–351. doi: 10.1007/400_2009_19

[35] Deumens R, Jaken RJP, Marcus MAE. The CatWalk gait analysis in assessment of both dynamic and static gait changes after adult rat sciatic nerve resection. *J Neurosci Methods*.2007; 164, 120–30

[36] Griffin JW, Hogan MCV, Chhabra AB. Peripheral nerve repair and reconstruction. *Journal of Bone and Joint Surgery - Series A*. 2013; Vol. 95:2144–2151.

[37] Nishimoto S, Tanaka H, Okamoto M. Methylcobalamin promotes the differentiation of Schwann cells and remyelination in lysophosphatidylcholine-induced demyelination of the rat sciatic nerve. *Front Cell Neurosci*. 2015; 9, 298.

[38] Dun XP, Parkinson DB. Transection and crush models of nerve injury to measure repair and remyelination in peripheral nerve. *Methods Mol Biol*. 2018; 251–62.

[39] Campbell WW. Evaluation and management of peripheral nerve injury. *Clin Neurophysiol*. 2008; 119, 1951–65.

[40] Caillaud M, Richard L, Vallat JM. Peripheral nerve regeneration and intraneural revascularization. *Neural Regen Res*. 2019; 14, 24–33.

[41] Byun SH, Ahn KM. Functional and electron-microscopic changes after differential traction injury in the sciatic nerve of a rat. Maxillofac Plast Reconstr Surg. 2021; 43, 12.

[42] Yeoh S, Warner WS, Eli I. Rapid-stretch injury to peripheral nerves: comparison of injury models. J Neurosurg. 2020; 135, 893–903.

[43] Li H, Jia JP, Xu M. Changes in the blood-nerve barrier after sciatic nerve cold injury: indications supporting early treatment. Neural Regen Res. 2015; 10, 419–24.

[44] Yun An, Hui-Xin Yan, Jiang-Na Zhao1, Xu-Ming Yang, Jun-Tao Yan1 J. Evaluation methods of a rat sciatic nerve crush injury model. Integr. Neurosci. 2022; 21(3): 91. https://doi.org/10.31083/j.jin2103091

[45] Dahlin LB. Techniques of peripheral nerve repair. Scandinavian Journal of Surgery. 2008; 97(4), 310–316. doi:10.1177/145749690809700407

[46] de Ruiter GC, Spinner RJ, Verhaagen J, Malessy MJ. Misdirection and guidance of regenerating axons after experimental nerve injury and repair: A review. Journal of Neurosurgery. 2014; 120 (2), 493–501. https://doi.org/10.3171/2013.8.JNS122300

[47] Chen P, Piao X, Bonaldo P. Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. Acta Neuropathologica. 2015; Vol. 130: 605–18.

[48] Grinsell D, Keating CP. Peripheral Nerve Reconstruction after Injury: A Review of Clinical and Experimental Therapies. BioMed Research International. 2014: 698256.

[49] Yue L, Talukder MAH, Gurjar A, Lee JI, Noble M, Dirksen RT, Chakkalakal J, Elfar JC. 4-Aminopyridine attenuates muscle atrophy after sciatic nerve crush injury in mice. Muscle Nerve. 2019; 60(2): 192-201.

[50] Podhajsky RJ, Myers RR. The vascular response to nerve crush: Relationship to Wallerian degeneration and regeneration. Brain Res. 1993; 623, 117–23.

[51] Doron-Mandel E, Fainzilber M, Terenzio M. Growth control mechanisms in neuronal regeneration. FEBS Letters. 2015; 589:1669–1677.

[52] Fissel JA, Farah MH. The influence of BACE1 on macrophage recruitment and activity in the injured peripheral nerve. J. Neuroinflamm. 2021; 18, 71. [CrossRef]

[53] Kingery WS, Lu JD, Roffers JA. The resolution of neuropathic hyperalgesia following motor and sensory functional recovery in sciatic axonotmetic mononeuropathies. Pain. 1994; 58, 157–68.

[54] Karsidag S, Akcal A, Sahin S. Neurophysiological and morphological responses to treatment with acetyl-L-carnitine in a sciatic nerve injury model: preliminary data. J Hand Surg Eur. 20212; ol, 37, 529–36.

[55] Savastano LE, Laurito SR, Fitt MR. Sciatic nerve injury: a simple and subtle model for investigating many aspects of nervous system damage and recovery. J Neurosci Methods. 2014; 227, 166–80.

[56] Sunderland S. A Classification of Peripheral Nerve Injuries Producing Loss of Function. Brain. 1951; 74(4):491–516. Available from: http://dx.doi.org/10.1093/ brain/74.4.491

[57] Moimas S, Novati F, Ronchi G. Effect of vascular endothelial growth factor gene therapy on post-traumatic peripheral nerve regeneration and denervation-related muscle atrophy. *Gene Ther*. 2013; 20, 1014–21.

[58] Blom CL, Mårtensson LB, Dahlin LB. Nerve injury-induced c-Jun activation in Schwann cells is JNK independent. *Biomed Res Int*, 2014; 392971.

[59] Gordon T, Borschel GH. The use of the rat as a model for studying peripheral nerve regeneration and sprouting after complete and partial nerve injuries. *Exp Neurol.* 2017; 287, 331–47.