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RESEARCH ARTICLE
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DEVELOPING SCIATIC NERVE COMPRESSION MODEL IN RATS USING ANEURYSM CLIP AND VALIDATING IT BY HISTOLOGICAL AND BEHAVIOR STUDIES

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ABSTRACT

Aim: The aim of the study was to develop sciatic nerve compression model in rats using aneurysm clip And to validate compression injury by histological examination and behavior testing

Study design and duration: experimental animal model

Methodology: The study included 16 healthy Sprague Dawley rats weighing 250 to 300 gm. The rats were divided into two groups. Each group comprised of 4 male and 4 female rats. Sham group animals were given only skin incision while in control group rats' sciatic nerve injury was induced in right limb under isoflurane anesthesia with an aneurysm clip having a force of 0.6 N. Animals were observed on day 0, 1 and 7 for behavior changes

Results: On histological and behavior examination the animals showed marked changes in crush injury group. On microscopy of tissue after H & E staining clearly evident injury was seen with discontinuity in architecture of nerve and macrophage infiltration at the site of application of aneurysm clamp. Animals in Injury group showed marked mechanical and thermal hyperalgesia and cold allodynia as compared to sham group.

Conclusion: The evident histological alterations supported by the behavior changes in the injury group confirm that the aneurysm clip model is a valid, self-effacing and easily reproducible method to induce sciatic nerve injury in rats with uniform force application for experimental studies.

Keywords: Aneurysm Clip Compression, Sciatic Nerve Injury,

INTRODUCTION

Peripheral Nerve injury remains one of the leading causes of disability resulting in difficulty to carry out routine activities due to pain and functional deficit(DeLeonibus et al., 2021). This debilitation not only leads to financial burden but can cause psychological issues in the patients. It is therefore indispensable to develop different treatment plans to provide best possible therapy and thus improve quality of life(Geuna, 2015). Animal nerve injury models are preeminent opportunity to study nerve regeneration and to evaluate functional and sensory recovery due to therapeutic interventions(Sasso et al., 2020). Male and female rats respond differently to nerve injuries and sexual dimorphism exists for neuropathic pain established by different studies conducted previously(Rosen, Ham, & Mogil, 2017). Rat Sciatic nerve injury model is most preferred due to similarity in morphology and arrangements of nerve fibers between two species(DeLeonibus et al., 2021). Several sciatic nerve injury models have been developed mimicking crush injury or transection injury according to the research needs(Gurkan, Erdogan, Yigitturk, & Erbas, 2021; Zhang et al., 2022). In this regard different methods also have been employed to induce the nerve injury like the use of jewelers forceps(Sharp, Tyreman, Jones, & Gordon, 2018), the non-serrated clamp(An, Yan, Zhao, Yang, & Yan, 2022), the injury induced by hemostatic clamp(Câmara et al., 2011). In all these methods the main aim is that the continuity of epineurium is maintained while the axon continuity is disrupted. The most important aspect while inducing a crush injury is the uniformity of the force that is to be applied. Yasargil-Phynox clamp helped in inflicting a crush injury in sciatic nerve with uniform force but the duration for which the pressure was applied varied between 5 to 20(Sarikcioglu & Ozkan, 2003) minutes which can lead to undue stress to animal. Later different aneurysm clip models were introduced for research purposes with different forces and application time(de Almeida Melo Maciel Mangueira et al., 2022; Omura, Sano, Omura, Hasegawa, & Nagano, 2004). The model used in this study was adapted following the alpha type aneurysm clip model(Sasidharan, Sastri, & Pandey, 2015) to apply a uniform force to crush sciatic nerve, time duration was also kept for 60 seconds so as to minimize the exposure time to anesthesia keeping in mind the principle of Replacement, Reduction, and Refinement (the Three Rs) of animal research. The crush injury was furthermore validated by histological and behavior changes in rats.

MATERIAL AND METHODS

This experimental study was conducted in the "Department of Anatomy, Institute of Basic Medical Sciences of Khyber Medical University Peshawar, Pakistan" from March 2021 to July 2022. Approval from institution ethical review board was taken. Sample size calculation was done using the resource equation formula keeping in mind the 3 Rs of animal research. According to formula 8 animals were taken in Sham group and 8 in Control group. Both male and female rats were included in the study. All animals were allowed 1-week acclimatization to local conditions and had free access to untreated tap water and standard rat chow.

1. **CONTROL GROUP**: - aneurysm clip having a force of 0.6 N was applied for 60 seconds for inducing crush injury and rats were examined for behavioral and microscopic changes after injury.

2. **SHAM GROUP: -** only skin incision was given.

Surgical Preparation

Surgery was performed in surgical room of Khyber Medical University. All instruments were sterilized and surgical field was cleaned with alcohol before start of every procedure. The animal was placed in sterilized induction chamber with a flow of 5% isoflurane. After it was completely anesthetized rat was shifted to surgery table and kept ventrally, the temperature was maintained at 37C by a heating pad. A constant flow of 2.5% isoflurane was ensured throughout the procedure by keeping head inside anesthesia mask. Right limb of rat was shaved above the knee joint and cleaned. To prevent corneal dehydration, the eyes were covered with eye ointment. The right limb of animal

was exposed above knee joint and incision site was cleaned by applying 70% isopropyl alcohol spray and then by 10% iodine solution.

Skin Incision in Sham Group

The animals were tested for achievement of complete anesthesia by pinching their toe and tail. Before Incision was given the sciatic notch was palpated as a reference point for determining the exact location of sciatic nerve. Approximately 2.5 cm above the knee joint near the sciatic notch a single longitudinal incision was given parallel to the direction of thigh. Retractors were applied to hold skin and fascia. The bicep femoris muscle was split bluntly to expose sciatic nerve. No procedure was performed on nerve and the wound was sutured in layers. Muscle was stitched by catgut while silk was used to suture the skin.

Sciatic nerve Compression in control group

The nerve was exposed by following the same steps as in sham group. The nerve once exposed was held gently by forceps. To inflict compression injury aneurysm, clip of force having 0.6 N was used. Aneurysm clip was applied for 60 seconds and then removed. Muscle and skin was sutured in layers as in sham group.

Rat was observed closely during recovery from anesthesia during which time rat was kept placed on the heating pad. After recovery the rat was shifted to cage having clean bedding and observed for sign of nerve compression.

All rats were provided free access to standard food and fresh tap water.

Behavior Testing

Animals were assessed for behavior changes before surgery and then after recovery and again on day 7. Behavior testing was done to validate injury by three different tests

Mechanical hyperalgesia by Von Frey filaments: Von Frey test was done using von Frey filaments. Animals were kept in a cage with a wire mesh floor. The von Frey filament was applied perpendicularly to the right hind paw at 5 different locations following the up down technique. A positive response was recorded when application of filament resulted in lifting, shaking or licking of hind paw(Bonin, Bories, & De Koninck, 2014).

Thermal hyperalgesia by hot plate: To check thermal hyperalgesia animal was placed on hot plate having a constant temperature of 55 c. and with a cutoff time of 15 sec. The time taken by animal to withdraw shake or lick the affected paw after placing it on hot plate was recorded in seconds(Baliki, Calvo, Chialvo, & Apkarian, 2005). **Cold allodynia:** To assess cold chemical thermal sensitivity acetone drop method was used. The rats after being placed in wire mesh cage were given 15 minutes to acclimatize to new environment. After that 0.01 ml acetone was taken in syringe and applied to the right hind paw. The cold chemical sensitivity reaction was considered positive if paw licking, shaking or rubbing the hind paw and brisk foot withdrawal (nociceptive pain response) were seen in first 5 seconds after application of acetone. Absence or delay of these responses was considered as anti-nociceptive effect. The responses were measured for 60 seconds. The mean of three responses was calculated. The interval between each application of acetone was approximately 5 minutes(Yoon, Wook, Sik, Ho, & Mo, 1994).

At the end of day 7 after performing behavior testing half of the animals from each group were sacrificed by cervical dislocation. Sciatic nerve from right hind limb of each animal was removed and was preserved in formalin before preparing it for tissue processing.

Staining: 5µm thick longitudinal section of sciatic nerve was stained with Hematoxylin and Eosin for routine microscopy.

Mounting of sections: Sections after staining were mounted with DPX solution and were carefully coated with glass cover slips for easier handling, storage and preservation.

RESULTS:

After recovery from anesthesia right foot drop was evident in all animals of control group wherein nerve injury was induced. Behavior changes showed significant difference between sham and control group. Thermal as well as mechanical hyperalgesia was clearly evident in control group while no significant changes were apparent in the sham group. Cold allodynia was experienced in all animals of control group as can be observed in the graph while sham group animals did not develop cold allodynia. Light microscopy by H & E staining showed no change in architecture of nerve taken from Sham group. The nerve showed no nerve damage or infiltration of inflammatory cells. In control group on the other hand a break in normal architecture was found with discontinuous myelin sheath clearly visible. The microscopic examination of injury site revealed inflammatory changes. There is an evident break down of nerve fibers with swollen axons. the surrounding myelin appears disintegrated with infiltration of inflammatory cells. In case of the connective tissues, the endoneurium appears broken, perineurium displays partial impairment, but epineurium looks intact. The histological changes corresponded well with the behavior changes.

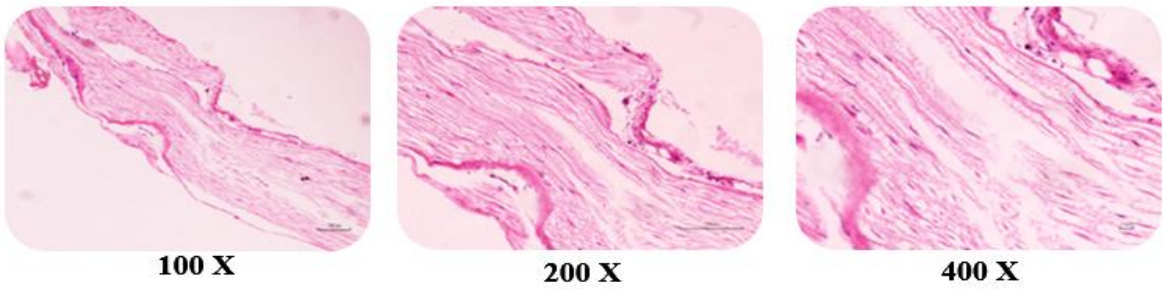


Fig. 1: Photomicrographs of 5µm thick longitudinal section of sciatic injury of control group stained with H&E showing prominent injury

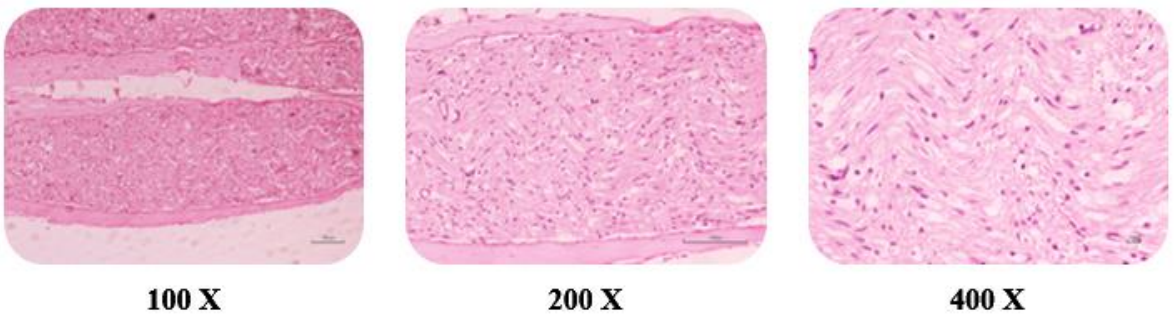
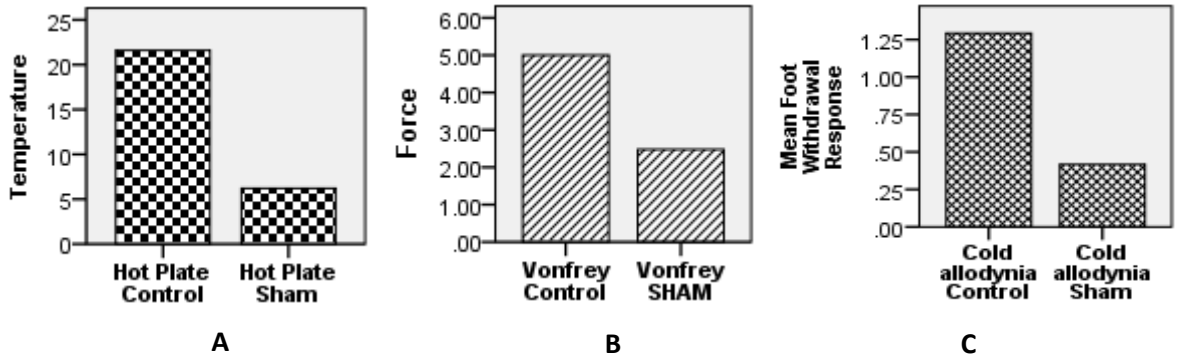


Fig.2: Photomicrographs of 5µm thick longitudinal section of sciatic nerve of sham group stained with H&E showing no disruption in architecture of nerve.

Statistical comparison between the sham and control group shows highly significant difference with a p value of .000. This clearly authenticates the nerve injury in control group resulting in behavior changes as can be seen in Graph 1.



Graph. 1: Highly significant difference of Behavior testing in between control and sham groups as seen in Graph 1 A, B and C.

DISCUSSION:

Nerve injury and repair still remains the most focused issue in research on peripheral nervous system. Nerve injuries can be due to direct trauma or due to compression. In all cases this leads to dysfunction of tissue innervated by the affected nerve(An et al., 2022; Omura et al., 2004). Animal model are widely used in nerve injury evaluation and hence developing new treatment modalities. Sciatic nerve of rodents provides the most suitable model to study crush injuries. Development of a crush injury model needs precision and reproducibility. However, there appears to be no proven pattern regarding the method of production and intensity of injury, which results in great difficulty in reproducing and comparing the proposed methods(Siwei et al., 2022). Result of our studies showed that aneurysm clamp model caused uniform injury to the axon fibers disrupting the myelin sheath. This was the most important step in achieving axontemesis. Whereas in a previous study where serrated clamp was used complete nerve transaction injury(Bridge et al., 1994) was more easily achieved as compared to axontemesis. Additionally, our study contrasted with previous research using a dead weight machine, where smaller fibers were preferentially preserved. In our model, we observed uniform injury affecting all fibers, which was further supported by behavioral studies demonstrating the initial loss of sensory details. Keeping in mind the principles of Replacement, Reduction, and Refinement (the Three Rs) in animal research while using dead weight machine more nerve exposure was required as compared to our study where a small incision was enough to produce a uniform injury in sciatic nerve(Mazzer, Barbieri, Mazzer, & Fazan, 2008). Also, in a previous study aneurysm clamp was used for a longer period of time ranging from 5 to 15 minutes for producing crush injury(Yucel et al., 2023) but it was observed in our study that applying the aneurysm clamp for 60 seconds with a force of 0.6 N was enough to produce axontemesis thus reducing time period of exposing animals to stress. In a similar study conducted by An, Yun, et al, a non-serrated clamp was used for 60 seconds and developed axontemesis. However due to application of different forces a spectrum of damage was observed for each applied force accordingly(An et al., 2022). While in our study a uniform force produced same results in all animals within 60 seconds time period evident by behavior as well as histological changes.

CONCLUSION:

Use of aneurysm clip for inducing sciatic nerve compression is established as a reliable and safe technique. In comparison to other methods it demonstrates cost effectiveness and the precise application of force applied results in yielding a reproducible model of sciatic nerve injury. The force exerted by the aneurysm clip reliably aligns with the histological features corresponding to grade IV Seddon classification of nerve injuries denoted as axontemesis. Consequently, for experimental investigations aimed at elucidating the effects of different pharmaceutical agents in translational research this model can be the best choice. It has induced injury of uniform pattern across all experimental animals and in a shorter time frame as compared to alternative methods, which appears noteworthy for its future use. Furthermore, the diminutive size of aneurysm clip facilitates a more

sparing exposure of nerve and thus effectively diminishing the physiological and psychological stress experienced by experimental animals associated with surgical intervention and anesthesia administration.

Authors Contribution Statement

The authors confirm their contribution to the paper as follows:

- Study conception and design: 1st Author.
- Data collection: 1st & 2nd Author;
- Analysis and interpretation of results: 1st & 3rd Author
- Draft manuscript preparation: 4th Author.
- Supervision: 5th Author
- Final approval: 6th Author

All authors reviewed the results and approved the final version of the manuscript.

Author contribution

All the authors contributed equally.

Conflict of interest

The authors declare no competing interest.

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