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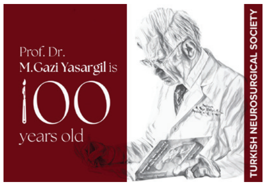

Original Investigation

Spine and Peripheral Nerves

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Pregabalin and Gabapentin's Roles in Nerve Regeneration: Multifaceted Analysis in an Experimental Model

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ABSTRACT

AIM: To compare the effects of pregabalin and gabapentin on the regeneration of rat nerves exposed to experimental axonotmesis by means of histomorphological, immunohistochemical, biochemical, electrophysiological, and clinical evaluations.

MATERIAL and METHODS: Thirty-two female Sprague-Dawley rats were divided into four groups of eight animals each; Group 1 (control), Group 2 (axonotmesis + normal saline), Group 3 (axonotmesis + pregabalin 30 mg/kg), and Group 4 (axonotmesis + gabapentin 30 mg/kg). Medical treatment was given for the first seven days to groups 3 and 4. Functional recovery was assessed using electromyography (EMG) and the sciatic functional index (SFI). Sciatic nerves were excised for histomorphological, immunofluorescence, and immunohistochemical examinations. Biomarkers were measured using the enzyme-linked immunosorbent assay method.

RESULTS: Significant improvement in SFI scores and EMG measurements were observed on the 28th day in groups 3 and 4 (the treated groups), while Group 2 (untreated) exhibited inadequate recovery. Histomorphological and immunohistochemical analyses revealed notable decreases in Wallerian degeneration, necrosis, inflammation, and nuclear factor-kappa B (NF-κB) expression levels in the treated groups compared to Group 2. The groups receiving medical treatment exhibited increased staining areas for nerve growth factor (NGF). Biochemical assessment indicated elevated levels of NGF, ciliary neurotrophic factor, transforming growth factor beta, and myelin basic protein in the treated groups compared to Group 2.

CONCLUSION: Overall, the study findings suggest that both pregabalin and gabapentin exert neuroprotective effects and can contribute to the regeneration process, with no apparent superiority of one over the other.

KEYWORDS: Peripheral nerve injury, Trauma, Pregabalin, Gabapentin, Nerve repair

ABBREVIATIONS: EMG: Electromyography, **SFI:** Sciatic functional index, **NF-κB:** Nuclear factor-kappa B, **NGF:** Nerve growth factor, **CNTF:** Ciliary neurotrophic factor, **TGF-β:** Transforming growth factor beta, **MBP:** Myelin basic protein, **PNI:** Peripheral nerve injury, **HE:** Hematoxylin-eosin, **WD:** Wallerian degeneration

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INTRODUCTION

Peripheral nerve injury (PNI) is a highly prevalent clinical condition deriving from traumatic incidents such as crushing, dissection, and tension. Approximately 2% of patients presenting to trauma centers suffer from PNI, and this rate is believed to be even higher when injuries to the plexus and radix are involved. PNI can lead to complete or partial loss of sensory, motor, and autonomic functions due to axonal discontinuity and nerve degeneration distal to the site of the injury. These functional deficits can result in a diminished quality of life, neuropathic pain, and prolonged healthcare requirements (23,28).

Despite the accumulated body of knowledge concerning nerve injury and regeneration mechanisms, limited effective treatments resulting in complete functional recovery are available. Although recovery rates for PNI have recently improved, the outcomes frequently fall short of entirely obviating the adverse socioeconomic impact on patients and their employment prospects (25,35).

Various surgical repair techniques have been investigated for the reacquisition of damaged sciatic nerve functions and for the improvement of patients' quality of life (34). A wide range of strategies have been developed for PNI treatment, repair, and regeneration, including the systemic use of neuroprotective drugs (2,5). Gabapentin and pregabalin exhibit a broad therapeutic range and are associated with inhibition of the neurotransmitter gamma-aminobutyric acid. These act on voltage-gated Ca++ channels, suppressing the secretion of excitatory neurotransmitters (13). The neuroprotective effects of pregabalin and gabapentin are due to various biological actions in the central nervous system (e.g., changes in glutamate metabolism). Gabapentin was introduced into clinical practice in 1993 as an adjuvant anticonvulsant medication and is of known benefit in managing neuropathic pain, migraines, and spasticity. Pregabalin interacts with the α2δ subgroup of voltage-gated Ca++ channels, thus modulating calcium release in nerve endings. Both are neurological agents with anti-inflammatory properties employed for treating epilepsy, neuropathic pain, fibromyalgia, and anxiety disorders (12,15,19,27,31,36).

Several studies in the literature have investigated the effects of pregabalin and gabapentin on neuropathic pain occurring after nerve damage. However, there has been little scientific research into the effectiveness of gabapentin and pregabalin on nerve regeneration after PNI (4,7,10,17,18). In these studies, gabapentin or pregabalin alone were administered for the treatment of peripheral nerve damage. However, the present study differs from the previous literature since it evaluated and compared the effects of both drugs, and involved multifaceted analyses.

The aim of the study was to investigate the roles of pregabalin and gabapentin in nerve regeneration an experimental axonotmesis model.

MATERIAL and METHODS

This study was performed in accordance with National Institute of Health-approved guidelines. The procedures were approved by the Animal Research Ethics Committee of Ataturk University under protocol number E-42190979-000-2100131573. The research was also supported by the Ataturk University Scientific Research Fund. The research involved 32 female Sprague-Dawley rats weighing 200-250 g and aged 2-3 months, sourced from university laboratories. These were fasted for 12 hours before the surgical procedure, and were housed under stable conditions at 22°C and subjected to a 12 / 12-h light / dark cycle in the treatment center. The animals were subsequently weighed daily throughout the 28-day experiment, and weight-dependent drug dose adjustments were made using a precision scale. Anesthesia was induced intraperitoneally using 40 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis Eczacıbası, Turkey, Ketalar® flacon) and 10 mg/kg xylazine (Rhompun® injectable flacon, Bayer) via insulin injectors. The rats were immobilized by tying all four extremities in such a manner as not to disrupt blood circulation. The right gluteal and lateral femoral regions were first shaved. An incision approximately 3 cm in length was then made parallel to the right femoral region with the rats in the ventral decubitus position. Following a previously described method, the subcutaneous tissue was incised, and the sciatic nerve was exposed after gently moving aside the biceps femoris muscle. In order to create the axonotmesis-type injury model, a Dietrich bulldog clamp (Tekno-Cer® AC-123-20) with a head length of 13 mm and a total length of 5 cm was applied to the sciatic nerve, exerting a force of 180 g [equivalent to 1.76 Newton (N)] for one minute before being removed (6) (Figure 1A). The incision site was then closed using a 4-0 polypropylene suture (Prolene, Ethicon R Ltd, Somerville NJ, USA). Electromyography (EMG) was applied to confirm the successful creation of the nerve injury (Figure 1B). In the postoperative period, the rats were kept in their cages at normal room temperature. Daily povidone-iodine dressings were applied under the supervision of a veterinarian. Amoxicillin-clavulanic acid was given as a prophylactic antibiotic. At the end of the 28th day, the injured right sciatic nerves were removed, including the proximal and distal areas of the injury site. Pregabalin (Alyse®, 75 mg, 14 tablets, oral) and gabapentin (Neurontin®, 300 mg, 30 tablets, oral) were procured from Abdi İbrahim Ilaç Tic. A.Ş. (Istanbul, Türkiye). The drug doses used in this study were based on the findings of previous research.

Thirty-two rats were randomly assigned into four groups of eight animals each.

Group 1 (control group): The sciatic nerves in this group were surgically exposed under general anesthesia without inducing axonotmesis, and the surgical site was subsequently sutured. The structural integrity of the sciatic nerve samples was evaluated at the conclusion of the experiment (n=8).

Group 2 (sham operation): The sciatic nerves in this group were subjected to axonotmesis by clamping, and the animals received a single daily dose of 1 mL normal saline solution via gavage (n = 8).

Group 3: Following axonotmesis, the animals in this group were administered 30 mg/kg pregabalin (n=8).

Group 4: The animals in this group underwent axonotmesis followed by administration of 30 mg/kg of gabapentin (n=8).

Groups 3 and 4 (the treatment groups) received a daily dose of either pregabalin or gabapentin dissolved in 1 mL of distilled water via gavage at the same hour for seven days, commencing from the day of surgery. Specifically, pregabalin and gabapentin were administered at a dose of 30 mg/kg, in line with previous studies (12,16,20).

On the 28th day of the experiment, an anesthetic agent was administered via intraperitoneal injection. Intracardiac blood samples were collected for biochemical analysis, and the sciatic nerves were excised. The animals were subsequently euthanized in accordance with established ethical protocols.

Footprint Analysis and Functional Evaluation

Sciatic functional index (SFI) evaluation was performed on the day preceding the surgery, as well as on the seventh and 28th days postoperatively. This involved the creation of a closed racetrack measuring 8.2 X 42 X 12 cm, along which the animals walked, the soles of their hind feet being marked with blue ink (Figure 1C).

Upon completion of the walking test, precise measurements were taken using millimetric scales from the footprints left behind. Various parameters were assessed from the footprints, including:

1. Heel to third phalanx distance (print length, PL)
2. The distance between the first and fifth phalanxes (toe spread, TS) and
3. The distance between the second and fourth phalanxes (intermediate toe spread, IT)

Measurements were collected for both the experimental limb (E) and the normal limb (N).

The SFI was calculated using the following formula:

SFI = -38.3 (EPL - NPL) / NPL + 109.5 (ETS - NTS) / NTS + 13.3 (EIT - NIT) / NIT - 8.8 (E: experimental side, N: normal side)

The evaluations were assigned values ranging from 0 to-100. An SFI of-100 indicates a complete loss of nerve function, while a value of approximately 0 is considered indicative of normal nerve function (11).

Electrophysiological Evaluation

Electromyography was performed in order to assess nerve conduction following axonotmesis-type nerve injury. The EMG evaluations were performed on the first day after surgery, and at the end of the first and fourth weeks. In order to ensure minimal disruption to the EMG recordings, ketamine and xylazine were administered via the intraperitoneal route to all rats before each EMG procedure (Figure 1B).

A Cadwell Cascade Elite electroneuromonitorization device was employed for electrophysical assessments. The postoperative neuro-monitoring parameters were a filtering range of 1 Hz to 3 kHz, a sweeping time of 10 ms/div, stimulus time of 0.3 ms, and a stimulus frequency of 2.71/s. Twisted needle electrodes were carefully inserted into the gastrocnemius muscle, while reference electrodes were attached to the piriformis muscle. These twisted electrodes consisted of stainless steel with 13 mm-long copper wires. Motor impulses were captured by inserting electrodes into muscles innervated by the corresponding nerves.

The researchers conducting the measurements were blinded to the group assignments. Various parameters were calculated, including compound muscle action potentials resulting from sciatic nerve stimulation, the speed of action potential transmission between successive impulses, and changes in the compound action potential action and area. The experimental limb/reference limb ratio was determined using impulse data from the experimental right lower extremity and the reference left lower extremity. Subsequent statistical comparisons were made using these data.

Histopathological Examination

Tissue specimens collected at the end of the evaluation were fixed for 48 hours in 10% formaldehyde solution, subjected to routine fixation procedures, and embedded in paraffin blocks. Sections 4 mm in thickness were then taken from each block. The preparates made ready for histopathological

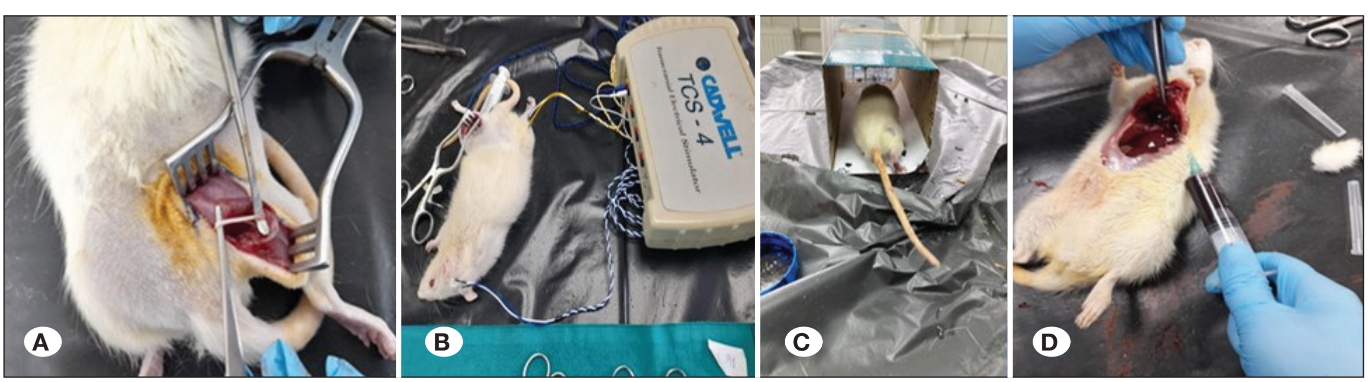

Figure 1: A) The sciatic nerve was exposed after gently moving aside the biceps femoris muscle in order to create the axonotmesis-type injury model. **B)** Electromyography after axonotmesis-type injury. **C)** Footprint analysis after axonotmesis-type injury. **D)** Intracardiac blood collection for biochemical evaluation.

analysis were then stained with hematoxylin-eosin (HE) and examined under a light microscope (Olympus BX 51, Japan). Depending on their histopathological features, the sections were classified as none (-), very mild (+), mild (+ +), moderate (+ + +), or severe (+ + + +).

Immunohistochemical Evaluation

Prior to being mounted onto poly-l-lysine adhesive slides for immunoperoxidase analysis, tissue sections underwent deparaffinization and drying. Next, endogenous peroxidase was inactivated by immersing it in 3% H_2O_2 for 10 minutes. The tissues were then allowed to cool at room temperature after being cooked in a 1% antigen retrieval (citrate buffer (pH + 6.1) 100 X) solution. In order to prevent non-specific background staining, the preparations were incubated with protein block for five minutes. Following the application of primary antibodies (nucleus kappa B (NF-κB) cat. no. sc - 8414, dilution ratio 1 / 100, USA), the tissue was incubated according to the manufacturer's guidelines. The chromogen used in the tissues was 3- 3-' diaminobenzidine. The stained slices were examined using a Zeiss Axio light microscope (Germany).

Immunofluorescence Examination

Tissue sections were dried and deparaffinized before being placed on to poly-l-lysine adhesive slides for immunofluorescence analysis. The slices were subsequently left in 3% H_2O_2 for 10 minutes to inactivate the endogenous peroxidase. After boiling in 1% antigen retrieval (pH+6.1, 100X) citrate buffer solution, the tissues were allowed to cool at room temperature. In order to avoid non-specific background staining, the sections were also allowed to incubate with protein block for five minutes. They were next covered with the primary antibody (NGF cat. no. BS23061941, dilution ratio: 1/100, USA), which was then incubated according to the instructions provided. Secondary antibodies (FITC cat. no. ab6785, dilution ratio: 1/1000) were used as secondary markers for immunofluorescence for 45 minutes and were kept in the dark for five minutes. The sections were then enclosed under with glass covers. Mounting medium containing DAPI (cat. no. D1306, dilution ratio: 1/200 UK) was then applied to the sections. Finally, a microscope equipped with a fluorescent attachment (Zeiss AXIO, Germany) was used to view the stained tissues.

Biochemical Evaluation

A total of 5 mL of intracardiac blood was collected into blood biochemistry tubes (Figure 1D). These were then subjected to centrifugation at 4500 rpm for seven minutes to isolate the sera, which were subsequently stored at -80°C until the study date. Serum NGF, ciliary neurotrophic factor (CNTF), myelin basic protein (MBP) and transforming growth factor beta (TGF-β) values were determined using the ELISA method with a Rat NGF ELISA kit (BTLAB, cat no. E0539Ra, China), a Rat CNTF ELISA kit (BTLAB, cat no. E0358Ra, China), a Rat MBP ELISA kit (BTLAB, cat no. E0576Ra, China) and a Rat TGF-β ELISA kit (BTLAB, cat no. E0778Ra, China), respectively, in accordance with the manufacturer's recommendations.

Statistical Analysis

Statistical Package for the Social Sciences version 25 and GraphPad Prism 8.0.2 software were employed for data analysis. The data were presented as mean and standard deviation values. One-way ANOVA was used for variance analysis, followed by the Duncan test as post hoc analysis. p values < 0.05 were considered statistically significant. Additionally, intergroup analysis of the SFI and EMG results was performed using the Kruskal-Wallis and Tamhane T2 non-parametric tests.

RESULTS

All 32 rats were subjected to comprehensive evaluations throughout the 28-day experiment.

Footprint Analysis and Functional Evaluation

The effectiveness of pregabalin and gabapentin on functional recovery were compared in rats exposed to PNI. The animals traversed the designated walking track on days 1, 7, and 28, and SFI values were subsequently computed using the established formula.

Significant decreases in SFI values were observed in the treated and untreated groups compared to the control group on the seventh day after PNI ($p<0.001$). On the 28th day after injury, the SFI values of the groups treated with pregabalin and gabapentin exhibited significant increases compared to the untreated injury group ($p<0.001$ for both). More specifically, as shown in Figure 2, SFI values were significantly higher in the group treated with gabapentin and pregabalin than in the untreated injury group. Interestingly, no statistically significant difference emerged between the groups treated with pregabalin and gabapentin on the seventh or 28th days post-injury ($p=0.663$ and $p=0.403$, respectively).

Electrophysiological Evaluation

EMG evaluations were carried out on the first, seventh, and 28th days post-injury. Statistical analysis of the EMG data revealed a significant difference between the control group and the other groups on all three days ($p<0.05$ for all). In contrast, no significant change was observed between the treated and untreated groups on days 1 and 7 following PNI ($p>0.05$ for all). Notably, significant improvement was observed on day 28 in groups 3 and 4 compared to the untreated group ($p=0.013$ and $p=0.003$, respectively). However, no significant difference was determined in improvement levels between the treated groups ($p=0.951$) (Figure 3).

Histopathological Findings

Group 1 (control): Histopathological examination of transversal sciatic nerve tissue sections revealed a normal histological appearance (Figures 4 and 5).

Group 2 (sham operation): Histopathological examination of transversal sciatic nerve tissue sections from this group revealed severe Wallerian degeneration (WD) together with necrosis and inflammation in interstitial spaces (Figures 4 and 5).

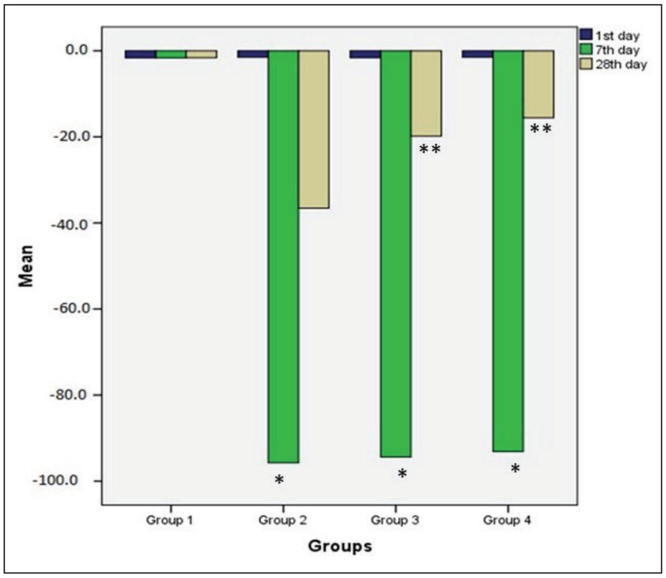

Figure 2: A comparison of the study groups' SFI values on the first day of the experiment and on days 7 and 28 post-injury * between the control group and all other groups on the seventh day post-injury, **between the sham group and treated groups on the 28th day post-injury.

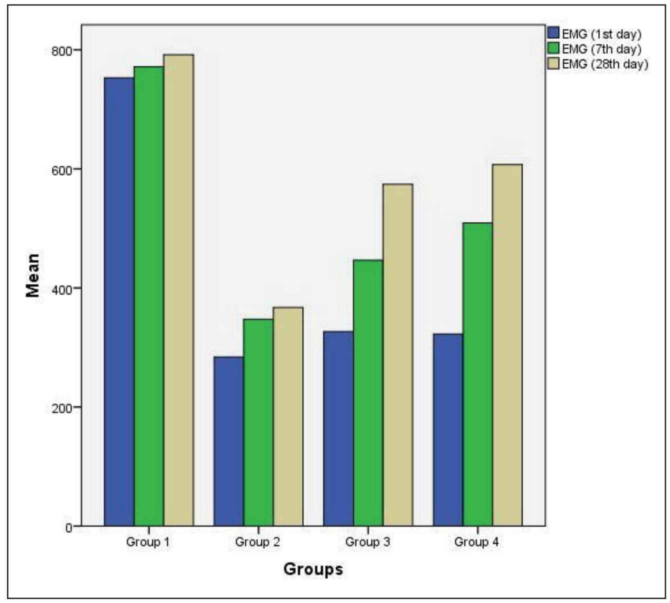

Figure 3: The muscle action potential amplitude of the gastrocnemius muscle on days 1, 7, and 28 post-injury.

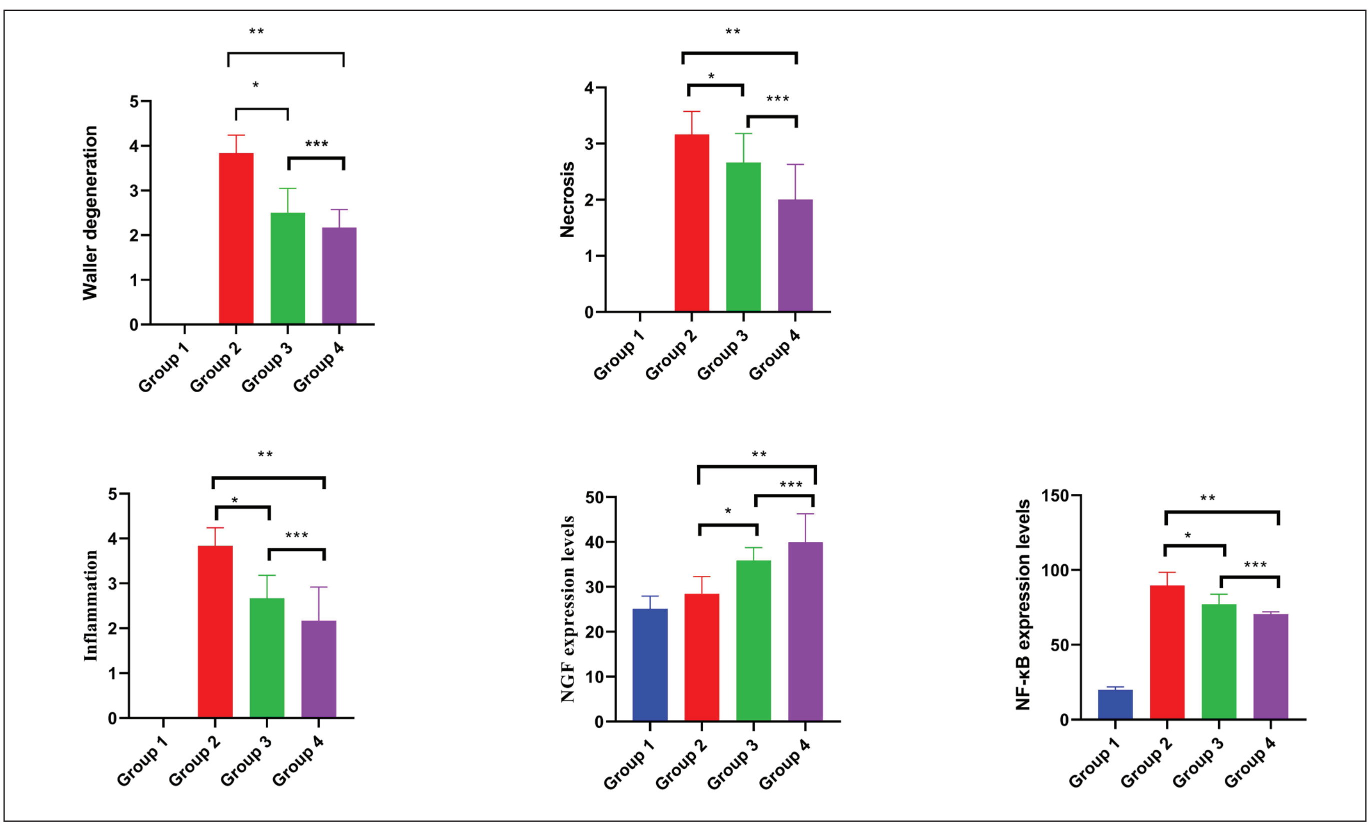

Figure 4: Statistical and analysis data for histopathological and immunohistochemical findings in sciatic nerve tissues. Wallerian degeneration (*p<0.001, **p = 0.021, ***p=0.577); Necrosis (*p=0.015, **p = 0.001, ***p=0.075); Inflammation (*p=0.003, **p=0.012 *** p=0.729); NGF expression level (*p=0.031, **p=0.001, *** p=0.358); NF-κB expression level (*p=0.005, **p<0.001, ***p=0.217).

Group 3 (Axonotmesis + Pregabalin 30 mg/kg): Histopathological examination of transversal sciatic nerve tissue sections from this group revealed moderate WD and necrosis (Figures 4 and 5).

Group 4 (Axonotmesis + Gabapentin 30 mg/kg): Histopathological examination of transversal sciatic nerve tissue sections also revealed moderate WD and necrosis (Figures 4 and 5).

Immunohistochemical Findings

Group 1 (control): Nuclear factor-kappaB (NF-κB) expression was evaluated as negative when the sciatic nerve tissues were examined using the immunohistochemical staining method (Figures 4 and 5).

Group 2 (Sham): Severe NF-κB expression was observed in the interstitial spaces around the vessels when the sciatic nerves were examined using immunohistochemical staining (Figures 4 and 5).

Group 3 (Axonotmesis + Pregabalin 30 mg/kg): Immunohistochemical staining revealed moderate NF-κB expression in the interstitial spaces around the vessels (Figures 4 and 5).

Group 4 (Axonotmesis + Gabapentin 30 mg/kg): Immunohistochemical staining revealed moderate NF-κB expression in the interstitial spaces around the vessels (Figures 4 and 5).

Immunofluorescence Findings

Group 1 (control): NGF expression was evaluated as negative when the sciatic nerve tissues were examined using the immunofluorescence staining method (Figure 5).

Group 2 (Sham): Very mild NGF expression was observed when the sciatic nerve tissues were examined using the immunofluorescence staining method (Figure 5).

Group 3 (Axonotmesis + Pregabalin 30 mg/kg): Moderate NGF expression as observed in nerve cells when the sciatic nerve tissues were examined using immunofluorescence staining (Figure 5).

Group 4 (Axonotmesis+Gabapentin 30 mg/kg): Moderate NGF expression as observed in nerve cells when the sciatic nerve tissues were examined using immunofluorescence staining (Figure 5).

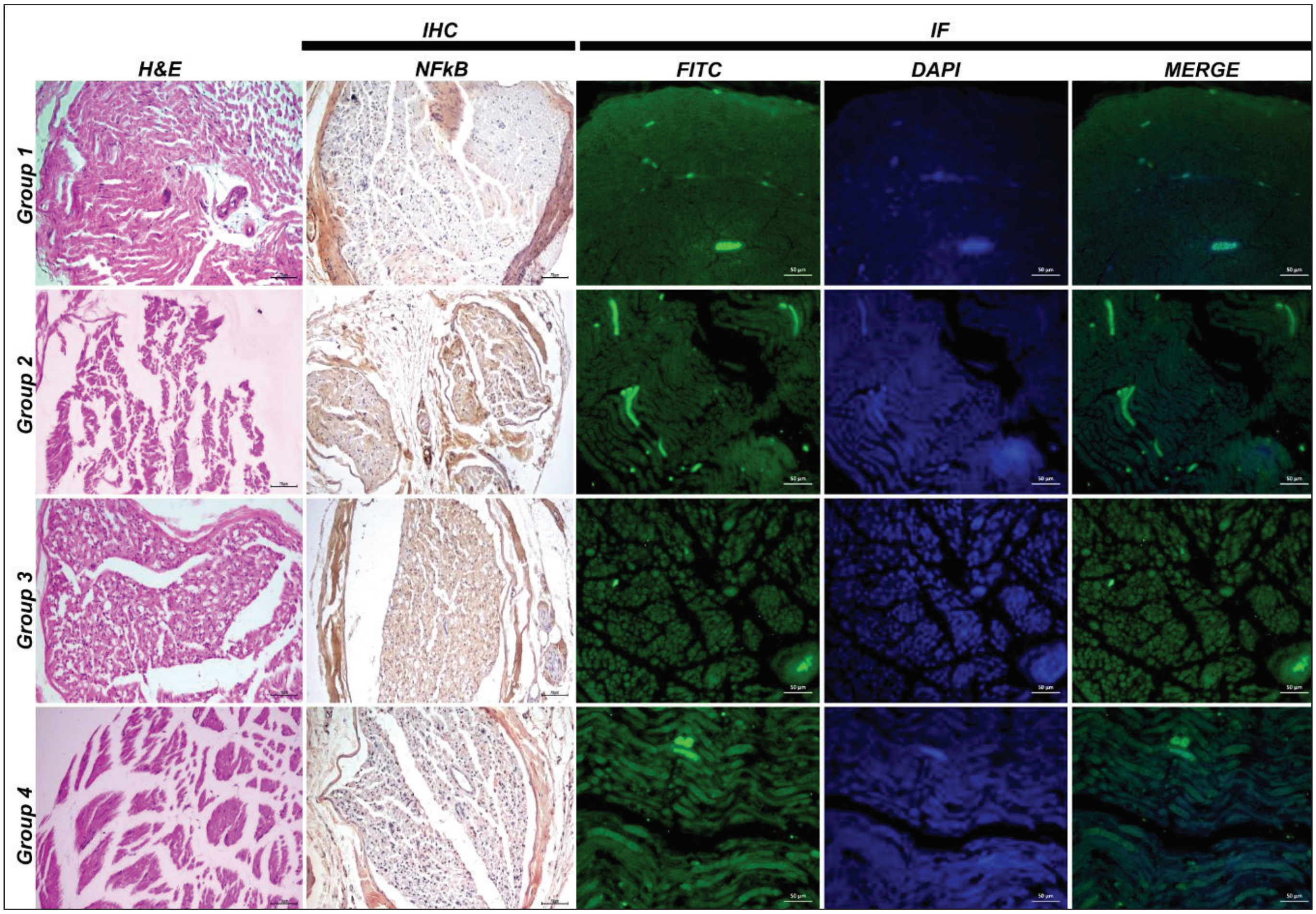

Figure 5: Sciatic nerve histopathological findings, Hematoxylin and Eosin, NF-κB expression IHC-P, Bar: 70 µm, NGF expression IF, Bar: 50 µm. **1:** Control group, **2:** sham group, **3:** Axonotmesis + Pregabalin 30 mg/kg, **4:** Axonotmesis + Gabapentin 30 mg/kg.

Biochemical Evaluation

Serum NGF, MBP, CNTF, and TGF-β levels rose significantly following peripheral nerve damage compared to those in the control group. Marker levels in the treated groups exhibited significant elevation compared to the untreated group. However, there was no significant difference between the two treatment groups (Figure 6).

DISCUSSION

Despite persistent efforts aimed at overcoming PNI, a long-standing health concern, genuinely satisfactory outcomes are still elusive. Although the peripheral nervous system (PNS) possesses an inherent repair and regeneration capacity, inadequate treatment of PNI can result in significant clinical challenges. Consistent with previous research, we employed axonotmesis created with a clamping technique to induce peripheral nerve damage in a rat sciatic nerve model (1). Hirakawa et al. reported that regeneration in axonotmesis injury began to be clearly observed on the fifth day and was partially completed on the 21st day (16). Warner et al. stated that the use of these two drugs in the first month was effective in nerve healing. An experimental period of 28 days was therefore adopted in the present study (36).

The comprehensive biochemical, histomorphological, immunohistochemical, electrophysiological, and clinical evaluations performed using this widely adopted axonotmesis model revealed positive results of both pregabalin and gabapentin on nerve healing.

Studies investigating the potential neural regenerative effects of pregabalin and gabapentin are relatively scarce. We identified two such studies focusing on the effect of pregabalin on nerve healing. In one, Whitlock et al. observed subjective clinical improvements in patients receiving pregabalin following traumatic nerve injury, although that effect did not seem to accelerate nerve regeneration (37). In a later investigation, Çelik et al. obtained histopathological, functional, and molecular evidence demonstrating that pregabalin treatment in axonotmesis and anastomosis groups enhanced nerve regeneration

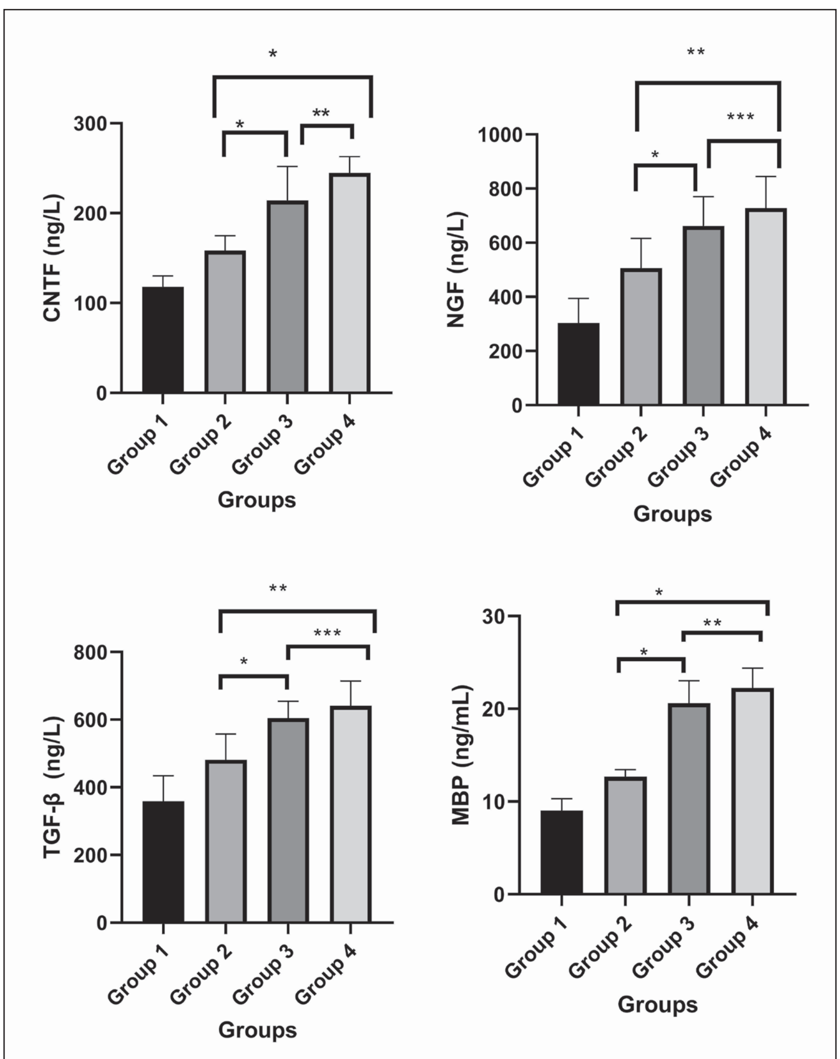

Figure 6: An evaluation of the groups' ELISA results, expressed as mean ± SD. **1:** Control, **2:** Axonotmesis, **3:** Axonotmesis + Pregabalin 30 mg/kg, **4:** Axonotmesis + Gabapentin 30 mg/kg. CNTF level (*p<0.001, **p=0.063); NGF level (*p=0.033, **p=0.002, ***p=0.613); TGF-β level (*p=0.007, **p<0.001, ***p=0.732); MBP level (*p<0.001, **p=0.265).

and elevated SFI values in PNIs by activating the anti-inflammatory cytokine TGF-β1. Those authors also indicated that a pregalbin dosage of 30 mg/kg was more efficacious than one of 60 mg/kg (10). Kardeş et al. investigated the histopathological effects of a 30 mg/kg dose of gabapentin administered for varying durations. Their findings suggested that gabapentin may be capable of use as an additional therapy to primary surgical interventions following peripheral nerve injury (18). In the light of these studies, we compared 30 mg/kg doses of pregabalin and gabapentin in the present research.

The evaluation of nerve recovery following PNI often relies on well-established methods including walking path analysis and SFI calculations (22). EMG measurements are also a valuable tool for assessing nerve impairment motor conduction levels during the post-therapeutic recovery phase (29). The present study also performed electrophysiological examinations in order to obtain deeper insights into nerve damage healing.

The EMG values in this study corroborated total functional loss, while SFI outcomes derived from walking path analysis demonstrated the effectiveness and sufficiency of the induced damage. Calculations performed on the seventh day after surgery confirmed the successful application of the axonotmesis injury model. By day 28, notable improvements were observed in SFI values and EMG measurements in the treated groups. These findings collectively underscore the effectiveness of pregabalin and gabapentin as agents facilitating functional recovery after nerve injury.

Growth factors encourage nerve healing, regeneration, and tissue regrowth and have emerged as a promising option for PNI repair. Immunohistochemical and biochemical evaluations were conducted to assess NGF levels. NGF, a neurotropic factor, plays a pivotal role in nerve regeneration, axonal growth, cellular proliferation, and differentiation. Previous research has demonstrated that elevated NGF expression within autografts enhances neuronal survival and significantly improves both motor and sensory axonal growth (14). Shen et al. reported elevated NGF levels following PNI in a rat sciatic nerve injury model (32). Li et al. proposed that administering exogenous NGF injections at the wound site in a sciatic nerve injury model encourages early-stage axon regeneration and remyelination (21). Moattari et al. assessed the positive impact of NGF-treated mesenchymal stem cells on the regeneration of transected sciatic nerves using SFI values, EMG amplitude measurements, and histological evaluations (26).

The activity of neuropoietic cytokines has been correlated with the survival, differentiation, and axonal regeneration of neurons within the PNS (30). CNTF has been linked to axonal regeneration, increased levels being observed following nerve damage. Yuan et al. observed elevation in CNTF levels after early nerve repair in a rat model of sciatic nerve injury (39). Similarly, another study suggested that CNTF plays an important role in promoting nerve axon regrowth and maturation during peripheral nerve regeneration (24). In the present investigation, serum CNTF and NGF levels were higher in the treated groups than in the sham group, although no significant variation was observed among the treatment groups. This rise in the levels of CNTF and NGF, pivotal neurotrophic cytokines in nerve healing, further confirms the efficacy of pregabalin and gabapentin therapy following peripheral injury, with no discernible superiority of one over the other.

The process of axonal regeneration is heavily dependent on myelination for the rapid transmission of nerve impulses. MBP, a crucial structural component of myelin formation in the PNS, has been implicated in nerve regeneration and the mitigation. Camara et al. established a rat sciatic nerve injury model and observed enhanced myelination in the 60 mg/kg gabapentin-treated group, marked by significantly elevated MBP levels compared to the untreated group (8). In the present study, serum MBP levels were significantly higher in the treated groups compared to the untreated nerve-damaged group. Within 48 hours of injury, denervated myelinating Schwann cells downregulate the expression of genes encoding various myelin associated proteins, including MBP (3). The present study, MBP levels in the treated groups in particular exhibited significant elevation compared to the untreated group.

TGF-β plays an important role in the regulation of neuronal survival and repair processes in the nervous system. It is involved in numerous major physiological processes, including inflammation, and affects nerve development and regeneration. The major effect of TGF-β is to inhibit the synthesis of monocytes, macrophages, and proinflammatory cytokine (33). Li et al. examined the effect of TGF-β on Wallerian degeneration after rat sciatic nerve injury. Their study data showed that TGF-β was expressed in the injured rat sciatic nerve and that inhibition of TGF-β expressions led to apoptosis (20).

Celik et al. reported that their histopathological, functional and molecular findings after sciatic nerve injury showed that 30 mg/kg pregabalin treatment in their axonotmesis and anastomosis groups improved nerve regeneration and increased SFI in PNI by activating the anti-inflammatory cytokine TGF-β (10). In the present study, the percentages of TGF-β staining in the nerve damage and treated groups were significantly higher than those in the untreated group.

Recent research has shown that NF-κB plays a role in conditions such as autoimmune disorders and inflammatory and neurodegenerative diseases (40). Previous studies have investigated PNI, neuroinflammation and NF-κB expression (9,38). In this study, NF-κB expression levels after PNI were consistent with the current literature, and examination of sciatic nerve tissues using immunohistochemical staining revealed severe NF-κB expression in the sham group compared to the treatment groups.

A powerful inflammatory response may develop following nerve trauma. Significant histopathological changes occur both proximal and distal to the affected area following injury. Degenerative changes, encompassing morphological, chemical, and functional alterations, are collectively known as Wallerian degeneration when a nerve fiber is severed or traumatized for various reasons, including trauma or experimental manipulation (7). Histopathological evaluations in this study included Wallerian degeneration, necrosis and inflammation. Decreased Wallerian degeneration, necrosis, and inflammation were observed in the two treatment groups. This revealed that both gabapentin and pregabalin exhibited histopathological anti-inflammatory and neuroprotective effects in PNI, thus contributing to neurological recovery.

There a number of limitations to this study. We employed a xylazine-ketamine combination before each EMG recording. Despite being considered safe, this may cause muscle relaxation at higher doses. However, we do not think that this factor significantly impacted the EMG recordings, in the light of the low intraperitoneal doses used on all rats. Based on the previous literature, this first study in this field involved a dosage of 30 mg/kg, although future research could usefully explore a wider range of doses for more comprehensive insights.

The outcomes of our analyses effectively corroborated the neuroprotective and anti-inflammatory properties of oral pregabalin and gabapentin administration. However, no discernible superiority was observed between the two agents in terms of these effects. This comparability may allow clinicians to selects treatments based on factors such as accessibility.

CONCLUSION

Both pregabalin and gabapentin appear to be of potential use in the treatment of PNI. However, further clinical and experimental studies showing the effects of these drugs at differing dosages are clearly needed. Additionally, considering the effects of these two drugs on neuropathic pain and inflammation, their use in nerve damage appears to be extremely beneficial. Such investigations may yield enhanced clarity and understanding in terms of the therapeutic potential of pregabalin and gabapentin in the context of PNI.

Declarations

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Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author by reasonable request.

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AUTHORSHIP CONTRIBUTION

Study conception and design: NK, IA, MC

Data collection: NK, IA, EL, SO, SY, BG

Analysis and interpretation of results: EL

Draft manuscript preparation: IA, EL

Critical revision of the article: BI

Other (study supervision, fundings, materials, etc...): NK, EL, BP, DK

All authors (NK, IA, EL, SO, SY, BP, BG, MC, DK, BI) reviewed the results and approved the final version of the manuscript.

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