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Research Paper
Nerve Regeneration

The effect of metformin on inferior alveolar nerve regeneration following crush-type injury

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Abstract. Peripheral nerve injuries often cause significant sensory and motor dysfunction, but current treatments provide limited neurosensory recovery. Beyond its antidiabetic use, metformin shows promise as a neuroprotective and regenerative agent by modulating autophagy and reducing inflammation. This study aimed to investigate the potential therapeutic effects of metformin on inferior alveolar nerve (IAN) regeneration following crush-type injury in a rat model. Thirty male Sprague–Dawley rats were randomly divided into metformin, placebo, and control groups. A crush injury was performed on the IAN in all but the control group. The metformin group received daily intraperitoneal 5 mg/kg injections for 10 days. Regeneration was assessed on day 28 by histological and immunohistochemical analyses, and functional recovery was evaluated using the von Frey test at day 0 (preoperative baseline) and on postoperative days 1 (T1), 7 (T7), 10 (T10), and 28 (T28). Histologic results showed reduced inflammation, oedema, fibrosis, and degeneration, and greater regenerated nerve fibre density in the metformin group. Immunohistochemical analysis showed a marked elevation in GAP-43 expression in the metformin group compared to the placebo and control groups ($P < 0.05$). Von Frey results showed gradual improvement in mechanical sensitivity in the metformin group, suggesting better recovery. Metformin showed neuroregenerative effects on crushed IAN in rats by promoting repair, increasing GAP-43, and reducing inflammation. These findings support its therapeutic potential in peripheral nerve injury and warrant further research for clinical application.

Keywords: Nerve regeneration; Metformin; Mandibular nerve; Crush injuries; Gap-43 protein.

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Peripheral nerve injuries occur frequently; however, efforts to understand the devastating functional and psychological effects and to develop evidence-based treatment methods have taken place only recently. The

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acceleration of research on neurotrauma in the mid-twentieth century, the development of animal models, and the introduction of the surgical microscope marked significant turning points in the diagnosis and management of nerve injuries[1].

Peripheral nerve injuries result in partial or complete loss of motor, sensory, or autonomic function. Sensory/motor changes are most commonly observed following traumatic nerve injuries, and they can occur in acute or chronic forms depending on the type of injury, such as compression, stretching, crushing, or cutting[2].

The inferior alveolar nerve (IAN), which contains sensory fibres, is highly susceptible to injury during lower jaw surgeries due to its location within the inferior alveolar canal (IAC), and such injuries can lead to significant patient morbidity. Therefore, IAN injuries are clinically significant and require careful management to prevent long-term sensory and functional impairments[3,4].

The regenerative capacity of peripheral nerves is limited, and, during the period required for functional recovery, permanent alterations may occur in the somatosensory cortex of the brain. These changes can disrupt signal transmission, potentially leading to irreversible impairments in nerve function at the central level[5].

The development of non-surgical treatment protocols used in the early stages following peripheral nerve injury may reduce the need for invasive surgery subsequently, and may also increase the success rates of future treatment(s).

Following peripheral nerve injury, the accumulation of damaged biomolecules (e.g. neuropeptides) in the affected area disrupts the regenerative capacity of Schwann cells in nerve tissue repair. Among the molecular mechanisms involved in peripheral nerve regeneration and repair, the autophagy mechanism plays a crucial role in facilitating the removal of damaged biomolecules intracellularly and extracellularly, thereby ensuring the effective progression of the regenerative process[6].

Metformin, in addition to being an anti-diabetic agent, is suggested to protect neural tissue from various neurotoxins and prevent neurodegeneration. Furthermore, it is believed to accelerate axon regeneration by modulating the intracellular autophagy mechanism, thereby contributing to nerve healing[6,7].

The literature reveals that there is limited research on conservative treatment methods used in cases of nerve injury and agents that may be effective in the regeneration process, and no definitive success has been achieved in the complete restoration of sensory function with these protocols. The aim of this study is to investigate the effects of metformin, an agent known to accelerate autophagy, on IAN regeneration following crush-type peripheral nerve injury, through histological analysis and neurosensory and behavioural testing[8].

Materials and methods

Animals

Thirty healthy 8–10-week-old male Sprague–Dawley rats, with an average weight of 250 ± 50 g, were used in this study. The animals were maintained on a 12 h light/dark cycle, with a stable temperature of 22–25°C and consistent humidity levels of 65–70%. The housing environment was regularly cleaned and ventilated.

The subjects were randomly assigned to three groups, each consisting of 10 rats (Table 1). Animals were randomly assigned to the groups using a simple randomization (lottery) method, in which each animal was assigned a unique number and group allocation was determined by drawing lots. To minimize potential confounders, the order of treatments and measurements was randomized, and cage positions were rotated on a regular basis. Group allocation was known only to the investigator performing the surgical procedures. The experimental animals were administered 5 mg/kg of metformin intraperitoneally for 10 consecutive days[6]. The drug, supplied in sterile powder form (Ant Teknik), was prepared daily in sterile distilled water (1 mL) according to the manufacturer's recommendations, with the dosage adjusted to 5 mg/kg based on each animal's body weight.

The exclusion criteria were defined as mortality due to anaesthesia-related complications, animal death, excessive haemorrhage, surgical site infection, significant weight loss resulting from impaired food or water intake, and systemic toxicity associated with the administered treatment.

Surgical procedures

The surgical procedures were performed under general anaesthesia using 50 mg/kg ketamine hydrochloride (Ketalar; Pfizer, New York, NY, USA) and 10 mg/kg xylazine hydrochloride (Rompun, Bayer, Leverkusen, USA) administered intramuscularly. After the induction of anaesthesia, the subjects were positioned in lateral recumbency on the surgical table with their right jaw positioned superiorly and the heads were immobilized. No procedures were performed on the contralateral side to enable comparison with the intact side in the behavioural testing protocol. The right mandibular area was shaved and disinfected with a povidone–iodine solution. After subcutaneous administration of 1 mL of maxicaine (40 mg/mL articaine, 0.005 mg/mL epinephrine hydrochloride) for local anaesthesia at the operation site, an incision 10 mm in length was made 2 mm inferior to the lower border of the right mandible [9]. Following the skin incision, the muscles were dissected to expose the mandible from the ramus to the mental foramen, including the mandibular foramen. Starting from where the antilingula is prominent inferior to the mandibular foramen, a bone window was created using a piezoelectric surgical tip under saline irrigation extending anteriorly to the ramus (Fig. 1). Subsequently, a crush-type injury was induced on the IAN extending beneath the bone window by applying pressure with fine flat #5 jeweller's forceps for 30 s[10,11]. The surgical site was

Table 1. Experimental groups and treatment protocols.

Group	*n*	Treatment protocol
1 (metformin)	10	Exposed to a crush-type nerve injury and subsequently administered metformin
2 (placebo)	10	Exposed to a crush-type nerve injury, followed by administration of 0.9% isotonic sodium chloride
3 (control)	10	Underwent surgical exposure without nerve injury, followed by closure and observation without any pharmacological intervention

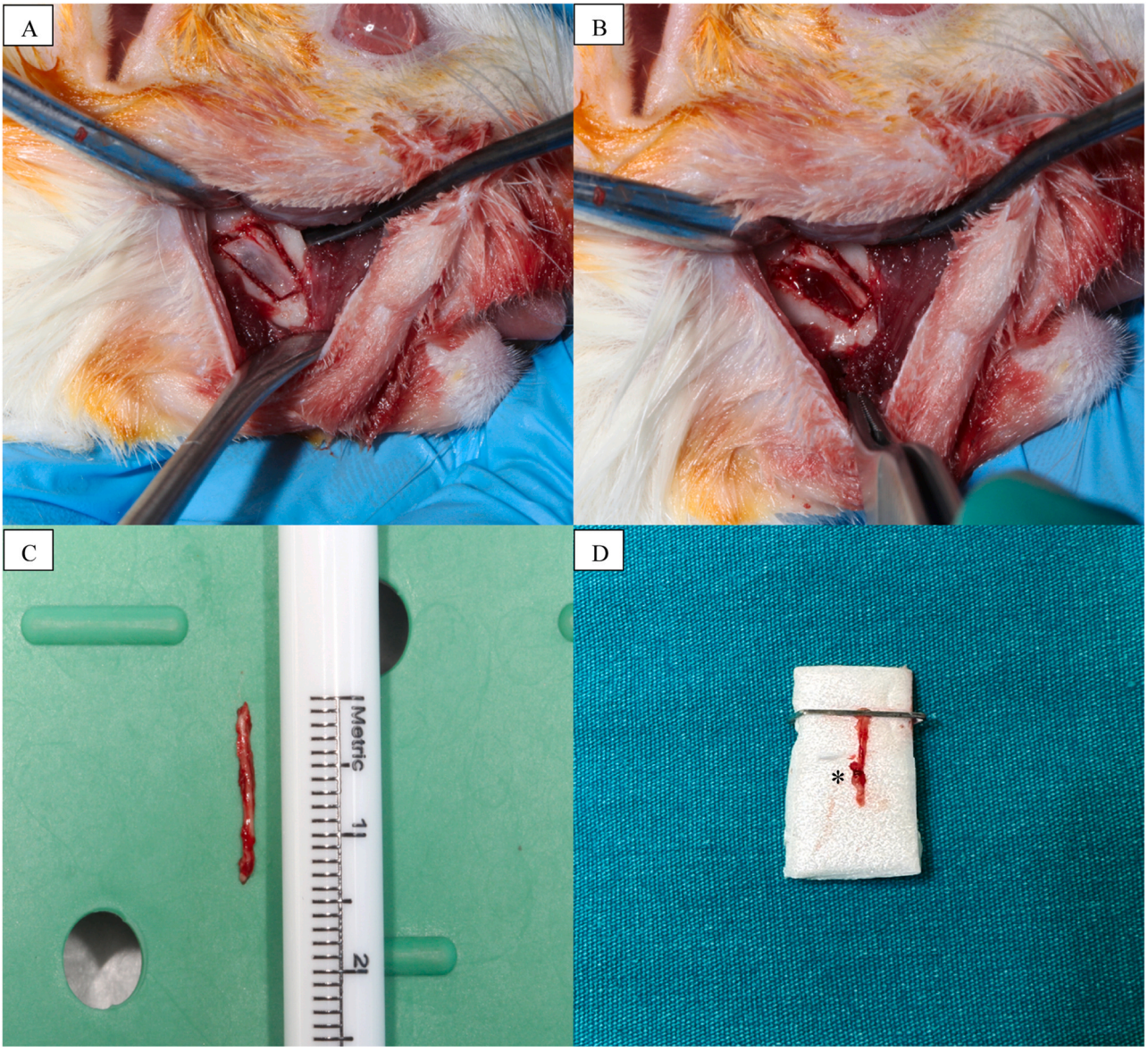

Fig. 1. Representation of the surgical margins and the harvested inferior alveolar nerve samples. (A) The dimensions and anatomical limits of the bone window created to access the inferior alveolar nerve were standardized across all groups. (B) Removal of the bone window and exposure of the inferior alveolar nerve. (C) Obtaining nerve tissue sample following death. (D) Positioning of the nerve section for histological examination (*crush-type injury site).

sequentially closed with 4/0 polyglactin 910 (Vicryl; Ethicon, Raritan, NJ, USA) sutures in a layered fashion including muscle, subcutaneous tissue, and skin.

On day 28, the experimental animals were killed under high-dose anaesthesia with 50 mg/kg ketamine hydrochloride (Ketalar) and 10 mg/kg xylazine hydrochloride[12]. Nerve samples were collected to evaluate the effects of metformin on the IAN, following established protocols (Fig. 1)[6,9,13]. Following euthanasia, the nerve injury site was exposed, and tissue samples were excised in 10 mm segments, ensuring that the injured nerve segment remained centrally positioned (Fig. 1).

Immunohistochemical and histological analysis

The nerve tissue samples were fixed in a 10% buffered formalin solution, and underwent routine tissue processing. From the resulting paraffin blocks, 3-µm-thick sections were prepared and stained with haematoxylin and eosin (H &E) and Masson's Trichrome. The stained sections were subsequently examined under an Olympus BX60 light microscope. For immunohistochemical staining, the GAP-43 antibody was applied. GAP-43 expression was considered the primary outcome measure of the study.

Tissue samples were prepared as both transverse and longitudinal sections. In the obtained nerve samples, histopathological assessments included oedema, inflammation (characterized by leucocyte, monocyte, and lymphocyte aggregation), fibrosis, degeneration, and regeneration[14–16]. Digital images of the sections were analysed using the Olympus AnalySIS 5 imaging software to ensure precise measurements. Histological evaluation was

performed by an investigator who was blinded to the group allocation. All histological and immunohistochemical procedures and evaluations were performed at the Department of Oral Pathology, Faculty of Dentistry, Istanbul University.

Von Frey test

The contact detection neurosensory test, frequently employed in clinical practice, was adapted for laboratory usage to evaluate neurosensory deficits in experimental animal models. To evaluate sensory recovery, the mechnoreceptor response threshold in rats was measured using a series of von Frey filaments. Prior to testing, the rats were kept in a quiet, dark environment to ensure acclimatization and relaxation. The testing procedure was conducted in a blinded manner, with the investigator unaware of the group assignments. To evaluate mechanical sensitivity, a set of five calibrated Semmes–Weinstein monofilaments (von Frey hair test; Stoelting, Wood Dale, IL, USA) arranged in a logarithmic series was employed. Each rat was first placed in a plastic enclosure to acclimate, after which the von Frey filaments were applied to the skin area surrounding the left and right lower jaw. The monofilaments used had $\log_{10}$ stiffness values of 2.83 (0.070 g), 3.22 (0.160 g), 3.61 (0.407 g), 4.08 (1.202 g), and 4.31 (2.000 g). The assessment began with the 0.407 g filament as the starting point for stimulus application.

The smallest force to which the subject responded was considered the 'withdrawal threshold'. To ensure accurate recording of behavioural responses, the experiments were conducted in a quiet environment by a blinded independent observer at day 0 (preoperative baseline), and on postoperative days 1 (T1), 7 (T7), 10 (T10), and 28 (T28), during the morning hours.

To prevent desensitization to the stimulus, testing commenced with the filament exerting the lowest pressure, and, if no response was elicited, the filament diameters were progressively increased. Each filament was applied for 5 s, with a total of three applications at 30 s intervals, ensuring that different points were stimulated. The threshold value was recorded as the average of the three responses[17,18]. Failure to respond to the strongest stimulus 4.31 (2.000 g) was considered to be the cut-off value.

Positive responses were defined as follows: slowly turning the head or rapid head withdrawal; spontaneous and rapid lateral head movements; one or multiple ipsilateral head movements directed toward the stimulated orofacial area; and actively attacking the stimulus object, including biting or grasping movements[19].

Statistical analysis

Based on a-priori power analysis with a statistical power of 80% and a significance level of $\alpha = 0.05$, it was determined that a minimum of 10 subjects per group was required. Data obtained in this study were analysed using IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA). Given the non-normal distribution of the data, the Wilcoxon signed-rank test was utilized for comparisons between measurement time-points, while the Mann–Whitney *U*-test was applied for intergroup comparisons. $P < 0.05$ was considered statistically significant.

Results

Immunohistochemical analysis

In the metformin-treated group, the expression of GAP-43 used for immunohistochemical evaluation was significantly higher compared to the control and placebo groups ($P = 0.0001$). Moderate GAP-43 immunoreactivity was observed in the placebo group. The expression of this antibody in the placebo group was found to be significantly lower compared to the level observed in the metformin-treated group ($P = 0.0001$). The expression of the GAP-43 antibody was found to be significantly lower in the control group compared to the placebo and metformin-treated groups ($P = 0.0001$) (Fig. 2; Table 2).

Histological analysis

Histological analysis revealed significant differences in oedema, inflammation, fibrosis, degeneration, and regeneration across the groups. The metformin-treated group exhibited significantly lower levels of inflammation, oedema ($P = 0.0001$), and fibrosis ($P = 0.001$) compared to the placebo group. The density of degenerated cells was also notably reduced, while the density of regenerated cells was significantly higher ($P = 0.0001$) (Fig. 3; Table 2). In the placebo group, histological sections revealed degenerated nerve bundles and oedema. Inflammatory reactions were prominent and dystrophic calcifications were observed (Fig. 3). Damaged or injured nerves exhibited demyelination, evidenced by vacuolated axons and fragmented axons. Oedema, inflammation ($P = 0.0001$), and fibrosis ($P = 0.001$) scores were significantly higher in the placebo group compared to the metformin group. Furthermore, the density of degenerated cells was markedly higher, while the density of regenerated cells was significantly lower than in the metformin-treated group ($P = 0.0001$) (Fig. 3; Table 2). The control group nerve structure seemed similar to that of normal and healthy individuals (Fig. 3). The levels of oedema and inflammation were significantly lower compared to the other two groups ($P = 0.0001$). Similarly, fibrosis, degeneration, and regeneration rates were also significantly lower in this group (Fig. 3; Table 2).

Von Frey test

In the metformin-treated group, the mechanical withdrawal threshold values at T1 ($P = 0.004$), T7 ($P = 0.003$), and T10 ($P = 0.020$) were significantly higher than those at T0. However, at T28, the withdrawal threshold values did not differ significantly from those at T0. Notably, the withdrawal threshold at T1 showed a significant increase, and this trend persisted until T10 (Table 3). In the placebo group, the mechanical withdrawal threshold values at T0 were significantly lower compared to those measured at T1 ($P = 0.006$), T7, T10, and T28 ($P = 0.007$; Table 3). In the control group, no significant differences were observed between the mechanical withdrawal threshold values across all time-points (Table 3). When comparing the right (operated) and left (non-operated) within groups, the metformin group showed significantly higher values on the right side at T1 ($P = 0.002$) and T7 ($P = 0.016$) compared to the left side. However, no significant difference was observed at T10 and T28 (Table 4). In the placebo group, results on the right side were significantly higher across all time-points (T1, T7: $P = 0.003$; T10, T28: $P = 0.001$; Table 4). In the control group, no significant difference was found between the right and left sides at any time-point (Table 4). On the left side a significant difference was found between the three

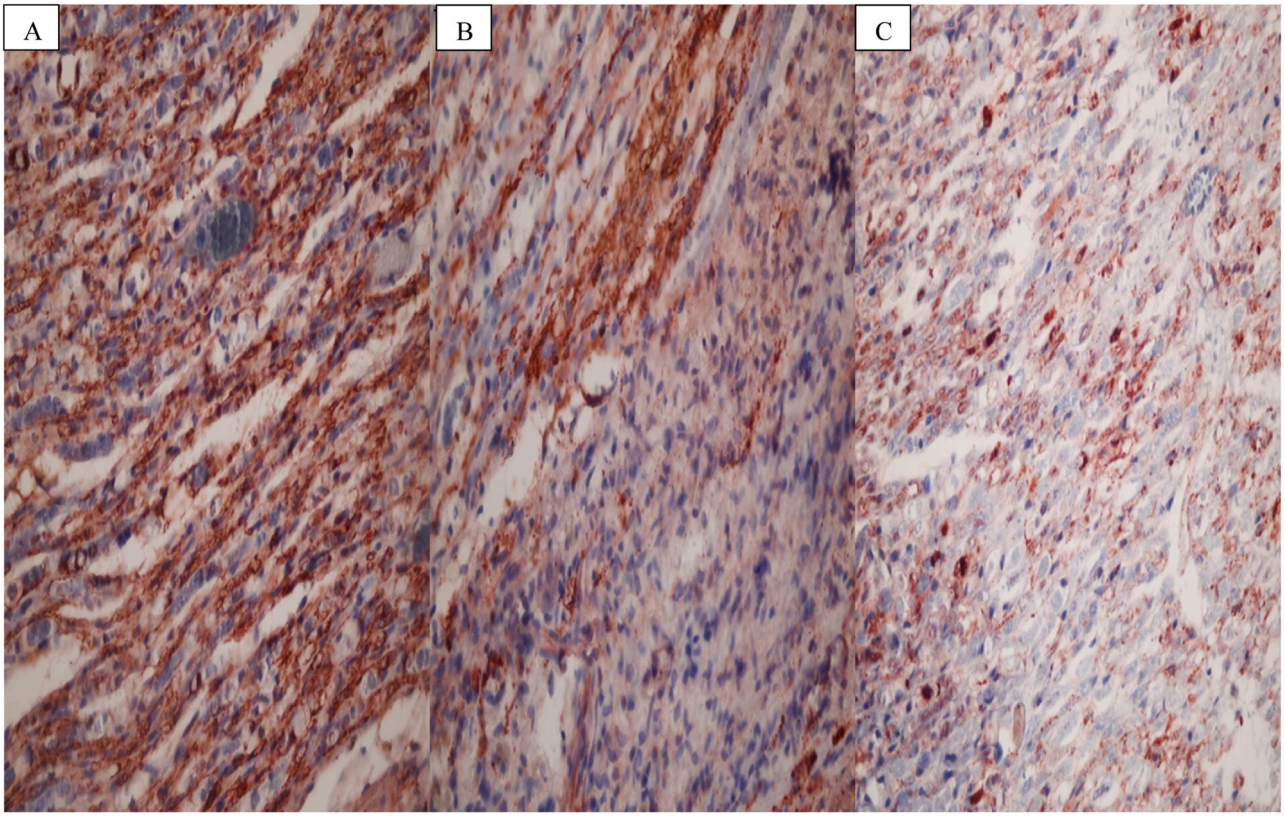

Fig. 2. Representative photomicrographs of Gap-43 immunohistochemical staining of rat inferior alveolar nerve sections (×400). (A) Group 1 (metformin), showing extensive expression. (B) Group 2 (placebo), showing moderate to severe immunoreactivity to Gap-43. (C) Group 3 (control), showing mild Gap-43 expression.

groups at T0 (P = 0.032), T1 (P = 0.013), and T10 (P = 0.040). At T0, the left-side values in the metformin and control groups were significantly higher compared to the placebo group (Table 5).

Discussion

Despite being a significant global clinical challenge, the non-surgical treatment of peripheral nerve injuries remains limited. Millions of cases are reported annually, with as many or more unreported, with suboptimal recovery or regenerative failure contributing substantially to patient morbidity and loss of quality of life. Accordingly, research continues to explore the molecular mechanisms underlying peripheral nerve regeneration, aiming to develop novel therapeutic strategies that enhance nerve regeneration[20]. Owing to its anatomical accessibility and sufficient length, the sciatic nerve containing both motor and sensory fibres has been the primary focus of most previous animal studies investigating the effects of therapeutic agents on nerve injuries. Nonetheless, it is important to recognize that findings derived from motor nerve models may not directly apply to purely sensory nerves such as the IAN, since the regeneration processes and recovery mechanisms differ between motor and sensory nerve fibres[21].

Given the complexity of peripheral nerve regeneration, therapeutic strategies that enhance the cellular microenvironment and support Schwann cell

Table 2. Histological evaluations.

	Group 1 (metformin)	Group 2 (placebo)	Group 3 (control)	P-value[a]	P-value[b]		
					Group 1 vs Group 2	Group 1 vs Group 3	Group 2 vs Group 3
Gap-43	2.60 ± 0.52	1.30 ± 0.48	0.30 ± 0.38	0.0001*	0.018*	0.002*	0.016*
Oedema	0.70 ± 0.67	2.00 ± 0.67	0.20 ± 0.27	0.0001*	0.003*	0.025*	0.0013*
Inflammation	0.60 ± 0.70	1.50 ± 0.53	0.00 ± 0.00	0.0001*	0.025*	0.001*	0.0001*
Fibrosis	0.70 ± 0.48	1.30 ± 0.82	0.00 ± 0.00	0.001*	0.022*	0.001*	0.0001*
Degeneration	1.00 ± 0.00	2.60 ± 0.52	0.60 ± 0.52	0.0001*	0.041*	0.035*	0.011*
Regeneration	2.40 ± 0.52	0.60 ± 0.70	0.00 ± 0.00	0.0001*	0.003*	0.001*	0.025*

Values are mean ± standard deviation.
[a]Mann–Whitney U-test test; Group 1 vs Group 2 vs Group 3.
[b]Pairwise comparison; Group 1 vs Group 2 vs Group 3.
*Statistically significant (P < 0.05).

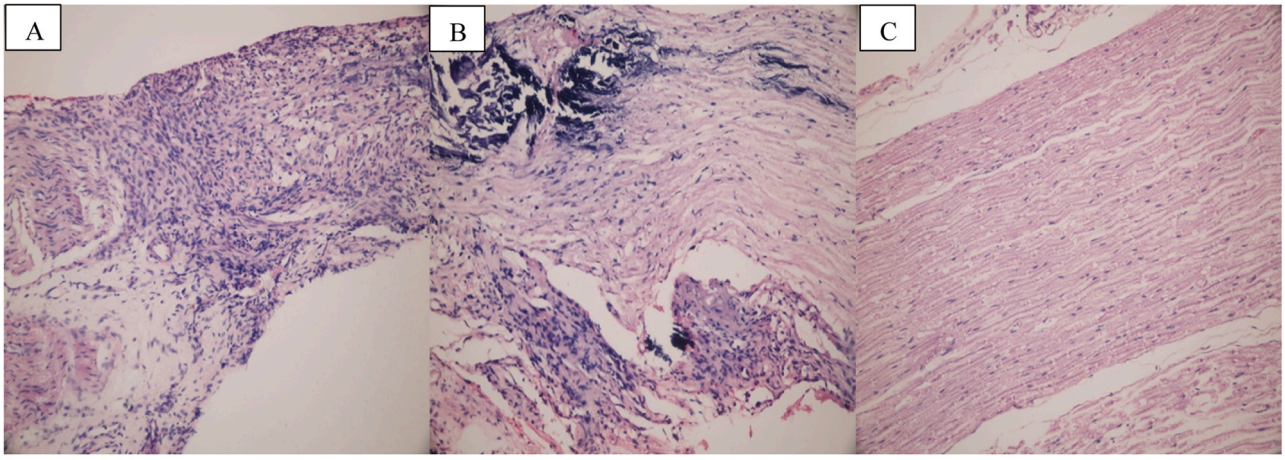

Fig. 3. Representative longitudinal sections (haematoxylin and eosin) ×200 from the three study groups. (A) Group 1 (metformin), showing regenerated nerve bundles with remyelination. (B) Group 2 (placebo), showing oedema, severe degeneration, extensive fibrosis, and dystrophic calcifications. (C) Group 3 (control), showing normal nerve structure.

functionality have attracted significant interest[22]. In the present study, metformin, a well-established activator of autophagy, was shown to exert a pronounced regenerative effect in a crush-type peripheral nerve injury model. Its administration was associated with improved histological and behavioural outcomes compared to both the placebo and control groups, suggesting that metformin promotes axonal repair and functional recovery through different mechanisms that extend beyond glycaemic control.

A growing body of evidence underscores the neuroregenerative potential of metformin and other autophagy-based therapeutic interventions in the context of peripheral nerve repair[6,23]. Alterations in autophagic flux and their impact on nerve regeneration have been extensively investigated, revealing that enhanced autophagy is associated with reduced cell death, improved myelin sheath integrity, and better motor function recovery[22–24]. In line with these findings, experimental studies have demonstrated that the activation of the autophagy system contributes to peripheral nerve regeneration by preventing neurodegeneration[25–27].

Although the underlying molecular mechanism responsible for metformin's neuroprotective action is still unknown, GAP-43 is an axonal phosphoprotein that is highly expressed during developmental stages and reappears in response to axonal regeneration. Its role in peripheral nerve repair has been well documented in the literature[28–31]. Liu et al.[6] and Wang et al.[32] reported a significant increase in GAP-43 levels in metformin-treated groups, which facilitated axonal growth. In a study conducted on diabetic rats with sciatic nerve injury, it was reported that the density and mean diameter of myelinated axons were significantly higher in the metformin-treated groups at all time-points[33]. Furthermore, compared to the control and placebo groups, the metformin-treated group exhibited a significantly higher density of regenerative cells, along with an increased expression of GAP-43, a key marker of axonal growth and differentiation. These findings underscore the potential of metformin to directly support axonal growth, highlighting its pivotal role in nerve repair strategies.

Inflammation, oedema, and fibrosis also play a significant role in shaping the healing response. Following nerve injury, the inflammatory response initiates a cascade of events, including oedema formation and fibroblast activation, ultimately leading to fibrotic tissue development. While a certain degree of inflammation is essential for the clearance of cellular debris and the activation of repair mechanisms,

Table 3. Von Frey test results, right (operated) side: correlation between different time-points within each group.

		P-value				
Group	Time (days)	T0	T1	T7	T10	T28
1 (metformin)	T0	–	0.004*	0.003*	0.020*	1.000
	T1	0.004*	–	0.157	0.020*	0.004*
	T7	0.003*	0.16	–	0.025	0.003
	T10	0.020*	0.020*	0.025*	–	0.020*
2 (placebo)	T0	–	0.006*	0.007*	0.007*	0.007*
	T1	0.006*	–	0.32	0.32	0.32
	T7	0.007*	0.32	–	1.0	1.0
	T10	0.007*	0.32	1.0	–	1.0
3 (control)	T0	–	0.10	0.10	0.10	1.0
	T1	0.10	–	1.0	1.0	0.10
	T7	0.10	1.0	–	1.0	0.10
	T10	0.10	1.000	1.0	–	0.10

*Statistically significant ($P < 0.05$).

Table 4. Von Frey test results for the right (operated) and left (non-operated) sides within each group.

Time (days)	Group	Right	Left	*P*- value
T0	1	2.30 ± 0.52	2.50 ± 0.71	0.67
	2	2.30 ± 0.82	2.40 ± 0.84	0.94
	3	3.00 ± 0.94	3.10 ± 0.32	0.95
T1	1	3.70 ± 0.48	2.50 ± 0.71	0.002*
	2	3.70 ± 0.67	2.40 ± 0.84	0.003*
	3	3.40 ± 0.52	3.30 ± 0.48	0.65
T7	1	3.50 ± 0.53	2.60 ± 0.84	0.016*
	2	3.80 ± 0.42	2.50 ± 0.97	0.003*
	3	3.40 ± 0.70	3.20 ± 0.42	0.34
T10	1	3.00 ± 0.94	2.60 ± 0.84	0.32
	2	3.80 ± 0.42	2.40 ± 0.84	0.001*
	3	3.40 ± 0.70	3.20 ± 0.42	0.34
T28	1	2.30 ± 0.67	2.60 ± 0.70	0.42
	2	3.80 ± 0.42	2.60 ± 0.70	0.001*
	3	3.00 ± 0.94	3.10 ± 0.32	0.93

Values are mean ± standard deviation.
Group 1: metformin; Group 2: saline; Group 3: no drug treatment (control).
*Statistically significant ($P < 0.05$).

Table 5. Von Frey test results for the right (operated) and left (non-operated) sides and statistical comparisons between groups over time.

Time (days)	Side	Group 1 (metformin)	Group 2 (placebo)	Group 3 (control)	*P*-value
T0	R	2.30 ± 0.67	2.30 ± 0.82	3.00 ± 0.94	0.094
	L	2.50 ± 0.71	2.40 ± 0.84	3.10 ± 0.32	0.032*
T1	R	3.70 ± 0.48	3.70 ± 0.67	3.40 ± 0.52	0.24
	L	2.50 ± 0.71	2.40 ± 0.84	3.30 ± 0.48	0.013*
T7	R	3.50 ± 0.53	3.80 ± 0.42	3.40 ± 0.70	0.28
	L	2.60 ± 0.84	2.50 ± 0.97	3.20 ± 0.42	0.078
T10	R	3.00 ± 0.94	3.80 ± 0.42	3.40 ± 0.70	0.094
	L	2.60 ± 0.84	2.40 ± 0.84	3.20 ± 0.42	0.040*
T28	R	2.30 ± 0.67	3.80 ± 0.42	3.00 ± 0.94	0.001*
	L	2.60 ± 0.70	2.60 ± 0.70	3.10 ± 0.32	0.081

Values are mean ± standard deviation.
Mann–Whitney *U*-test; Group 1 vs Group 2 vs Group 3.
*Statistically significant ($P < 0.05$).

excessive or prolonged inflammation disrupts the regenerative microenvironment, thereby impairing axonal growth and remyelination[34]. Oedema contributes to increased endoneurial pressure, which compromises microcirculation and results in ischaemic damage to the nerve tissue[35]. Moreover, uncontrolled fibrotic responses lead to scar tissue formation that not only physically obstructs axonal regeneration but also alters the composition of the extracellular matrix, further hindering functional recovery[36]. It has been found that early treatment with metformin for 7 days was sufficient to resolve oedema in mice[37]. In this study, the lower levels of inflammation, oedema, and fibrosis observed in the metformin-treated group suggest that metformin supports peripheral nerve regeneration by suppressing inflammatory responses and reducing tissue degeneration. Moreover, the persistence of inflammatory findings at the end of the experiment in the placebo group indicates the anti-inflammatory effect of metformin.

Axonal regeneration is not equal to functional recovery, because the function will not recover until the nerve regenerates through the injured area. Functional recovery constitutes a key endpoint that complements cellular and molecular improvements in peripheral nerve repair. In daily practice, the Medical Research Council Scale is commonly used to assess the degree of sensory and motor recovery following nerve injuries[38]. It provides a standardized and semi-objective framework for evaluating functional improvement. In experimental animals, one of the most commonly used methods in the literature to clinically evaluate sensory–motor recovery is the assessment of responses to mechanical stimuli using von Frey monofilaments, which apply calibrated pressure to the skin to elicit a withdrawal response[39,40].

Ma et al.[33] reported that in a rat sciatic nerve injury model, mechanical withdrawal thresholds were significantly lower in the metformin-treated group compared to the placebo group, indicating enhanced sensory responsiveness. Similarly, Kim et al.[41] demonstrated, in a diabetic neuropathy model, that tactile sensation, as assessed by the von Frey test, was preserved in the metformin-treated group. In this study, the von Frey filament test was employed to assess the sensory outcomes of nerve regeneration. In the group treated with metformin, behavioural test results obtained on day 28 returning to baseline levels suggest that clinical recovery has occurred. By contrast, the significant deviations from baseline values observed at earlier timepoints are likely attributable to the predominant inflammatory and oedematous responses during the acute phase of injury, which may have temporarily hindered the regenerative process. Also,

the outcomes observed in the placebo and control groups were consistent with the corresponding histological findings. These findings demonstrate that metformin not only promotes tissue regeneration but also contributes to clinical recovery by attenuating early inflammatory responses, thereby functionally supporting sensory nerve repair.

Although the von Frey filament test is widely used in experimental animals to assess mechanical sensitivity, it is difficult to fully control environmental variables[42]. The accuracy of such tests can be influenced by various factors, including the observer's experience, the animal's adaptation to the testing environment, differences in threshold values, and external conditions[43]. In the present study, differences in baseline behavioural scores between groups on the non-operated side were considered to potentially result from the aforementioned limitations reported in the literature[44]. Therefore, to enhance the reliability of the results, direct intergroup comparisons of right–left side differences were avoided, and only intragroup evaluations were interpreted. Moreover, excessive expression of GAP-43 particularly in the central nervous system has been associated with abnormal synaptic remodelling and sensory disturbances[45]. Additionally, studies conducted in Parkinson disease models have produced conflicting results regarding the neuroprotective effects of GAP-43 up-regulation[46]. In this context, while our findings suggest that metformin promotes regeneration by increasing GAP-43 expression, the potential adverse effects of overexpression should not be overlooked. Defining the optimal expression range of GAP-43 is essential and should be the focus of future research.

Although a well-established animal model was employed in the present study, it is important to acknowledge that findings derived from animal research cannot be directly extrapolated to human conditions. Differences in physiology and anatomy may influence the observed outcomes; therefore, further clinical investigations are warranted to confirm the translational relevance of these findings.

The findings of this study demonstrate that metformin possesses therapeutic potential in the regeneration of the inferior alveolar nerve. Its ability to promote axonal repair, reduce inflammation, and support the recovery of mechanical sensory function highlights its regenerative and anti-inflammatory properties. These findings indicate that metformin contributes to nerve regeneration through multiple mechanisms, and they provide a scientific basis for future research investigating its potential as a neuroregenerative agent.

Ethical approval

This study was approved by the Bezmialem Vakif University Animal Experiments Ethics Committee (2021-227-1).

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Competing interests

None.

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Patient consent

Not required.

References

1. Stevens AR, Belli A, Ahmed Z. Neurotrauma-from injury to repair: clinical perspectives, cellular mechanisms and promoting regeneration of the injured brain and spinal cord. *Biomedicines* 2024; **12**(3).
2. Menorca RM, Fussell TS, Elfar JC. Nerve physiology: mechanisms of injury and recovery. *Hand Clin* 2013;**29**:317–30.
3. Thaufiq Ahamed MI, Jayakumar N, Neelakandan RS. Incidence of neurosensory deficits following surgical removal of mandibular third molars: a prospective clinical study. *Int J Res Med Sci* 2019;**7**(5).
4. Valmaseda Castellon E, Berini Aytes L, Gay Escoda C. Inferior alveolar nerve damage after lower third molar surgical extraction: a prospective study of 1117 surgical extractions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; **92**:377–83.
5. Ito T, Toriumi T, Otake K, Okuwa Y, Tanaka S, Arai Y, Kurita K, Honda M. Performance of Schwann cell transplantation into extracted socket after inferior alveolar nerve injury in a novel rat model. *J Oral Sci* 2020;**62**:402–9.
6. Liu L, Tian D, Liu C, Yu K, Bai J. Metformin enhances functional recovery of peripheral nerve in rats with sciatic nerve crush injury. *Med Sci Monit* 2019; **25**:10067–76.
7. Boland B, Yu WH, Corti O, Mollereau B, Henriques A, Bezard E, Pastores GM, Rubinsztein DC, Nixon RA, Duchen MR, Mallucci GR, Kroemer G, Levine B, Eskelinen EL, Mochel F, Spedding M, Louis C, Martin OR, Millan MJ. Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. *Nat Rev Drug Discov* 2018; **17**:660–88.
8. Zuniga JR, LaBanc JP. Advances in microsurgical nerve repair. *J Oral Maxillofac Surg* 1993;**51**(1):62–8.
9. Diker N, Caglayan B, Helvacioglu F, Kilic E. The effect of systemic rifampicin treatment on inferior alveolar nerve regeneration in rats following crush injury. *Eur J Oral Sci* 2020;**128**:183–9.
10. Bridge PM, Ball DJ, Mackinnon SE, Nakao Y, Brandt K, Hunter DA, Hertl C. Nerve crush injuries—a model for axonotmesis. *Exp Neurol* 1994;**127**.
11. Umansky D, Hagen KM, Chu TH, Pathiyil RK, Alzahrani S, Ousman SS, Midha R. Functional gait assessment using manual, semi-automated and deep learning approaches following standardized models of peripheral nerve injury in mice. *Biomolecules* 2022;**12**(10).
12. Charbonneau R, Niel L, Olfert E, Von Keyserlingk M, Griffin G. CCAC guidelines on Euthanasia of animals used in science. *Can Council Anim Care* 2010:7–26.
13. Feng X, Yuan W. Dexamethasone enhanced functional recovery after sciatic nerve crush injury in rats. *BioMed Res Int* 2015;**2015**:627923.
14. Gao W, Tong D, Li Q, Huang P, Zhang F. Dexamethasone promotes regeneration of crushed inferior alveolar nerve by inhibiting NF-kappaB activation in adult rats. *Arch Oral Biol* 2017;**80**:101–9.
15. Wang L, Zhao Y, Cheng X, Yang Y, Liu G, Ma Q, Shang H, Tian L, Lei D. Effects of locally applied nerve growth factor to the inferior alveolar nerve histology in a rabbit model of mandibular distraction osteogenesis. *Int J Oral Maxillofac Surg* 2009;**38**:64–9.
16. Zhao YH, Zhang SJ, Yang ZH, Liu XC, Lei DL, Li J, Wang L. Relationship of distraction rate with inferior alveolar nerve degeneration–regeneration shift. *Neural Regen Res* 2018;**13**:360–5.
17. Ma J, Liu J, Chen Y, Yu H, Xiang L. Metformin promotes axonal regeneration and functional recovery in diabetic rat model of sciatic nerve transection injury. *NeuroSci* 2022;**3**:366–75.

18. Tena B, Escobar B, Arguis MJ, Cantero C, Rios J, Gomar C. Reproducibility of electronic von Frey and von Frey monofilaments testing. *Clin J Pain* 2012;**28**:318–23.
19. Li J, Ma K, Yi D, Oh CD, Chen D. Nociceptive behavioural assessments in mouse models of temporomandibular joint disorders. *Int J Oral Sci* 2020;**12**:26.
20. Alvites R, Rita Caseiro A, Santos Pedrosa S, Vieira Branquinho M, Ronchi G, Geuna S, Colette Mauricio A. Peripheral nerve injury and axonotmesis: state of the art and recent advances. *Cogent Med* 2018;**5**(1).
21. Hakimiha N, Dehghan MM, Manaheji H, Zaringhalam J, Farzad-Mohajeri S, Fekrazad R, Moslemi N. Recovery of inferior alveolar nerve by photobiomodulation therapy using two laser wavelengths: a behavioral and immunological study in rat. *J Photochem Photobiol B* 2020;**204**:111785.
22. Zhou K, Zheng Z, Li Y, Han W, Zhang J, Mao Y, Chen H, Zhang W, Liu M, Xie L, Zhang H, Xu H, Xiao J. TFE3, a potential therapeutic target for spinal cord injury via augmenting autophagy flux and alleviating ER stress. *Theranostics* 2020;**10**:9280–302.
23. Inada T, Sato H, Hayashi Y, Hitomi S, Furukawa A, Ando M, Oshima E, Otsuji J, Taguchi N, Shibuta I, Tsuda H, Iwata K, Shirota T, Shinoda M. Rapamycin accelerates axon regeneration through Schwann cell-mediated autophagy following inferior alveolar nerve transection in rats. *Neuroscience* 2021;**468**:43–52.
24. Li R, Li D, Wu C, Ye L, Wu Y, Yuan Y, Yang S, Xie L, Mao Y, Jiang T, Li Y, Wang J, Zhang H, Li X, Xiao J. Nerve growth factor activates autophagy in Schwann cells to enhance myelin debris clearance and to expedite nerve regeneration. *Theranostics* 2020;**10**:1649–77.
25. Kosacka J, Nowicki M, Bluher M, Baum P, Stockinger M, Toyka KV, Klöting I, Stumvoll M, Serke H, Bechmann I, Klöting N. Increased autophagy in peripheral nerves may protect Wistar Ottawa Karlsburg W rats against neuropathy. *Exp Neurol* 2013;**250**:125–35.
26. Marinelli S, Nazio F, Tinari A, Ciarlo L, D'Amelio M, Pieroni L, Vacca V, Urbani A, Cecconi F, Malorni W, Pavone F. Schwann cell autophagy counteracts the onset and chronification of neuropathic pain. *Pain* 2014;**155**:93–107.
27. Qu L, Liang X, Gu B, Liu W. Quercetin alleviates high glucose-induced Schwann cell damage by autophagy. *Neural Regen Res* 2014;**9**:1195–203.
28. Abe N, Borson SH, Gambello MJ, Wang F, Cavalli V. Mammalian target of rapamycin (mTOR) activation increases axonal growth capacity of injured peripheral nerves. *J Biol Chem* 2010;**285**:28034–43.
29. Aigner L, Arber S, Kapfhammer JP, Laux T, Schneider C, Botteri F, Brenner HR, Caroni P. Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. *Cell* 1995;**83**:269–78.
30. Zhang Y, Bo X, Schoepfer R, Holtmaat AJ, Verhaagen J, Emson PC, Lieberman AR, Anderson PN. Growth-associated protein GAP-43 and L1 act synergistically to promote regenerative growth of Purkinje cell axons in vivo. *Proc Natl Acad Sci USA* 2005;**102**:14883–8.
31. Bomze HM, Bulsara KR, Iskandar BJ, Caroni P, Skene JH. Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons. *Nat Neurosci* 2001:38–43.
32. Wang H, Zheng Z, Han W, Yuan Y, Li Y, Zhou K, Wang Q, Xie L, Xu K, Zhang H, Xu H, Wu Y, Xiao J. Metformin promotes axon regeneration after spinal cord injury through inhibiting oxidative stress and stabilizing microtubule. *Oxid Med Cell Longev* 2020:9741369.
33. Ma J, Liu J, Yu H, Chen Y, Wang Q, Xiang L. Beneficial effect of metformin on nerve regeneration and functional recovery after sciatic nerve crush injury in diabetic rats. *Neurochem Res* 2016;**41**:1130–7.
34. Gu D, Xia Y, Ding Z, Qian J, Gu X, Bai H, Jiang M, Yao D. Inflammation in the peripheral nervous system after injury. *Biomedicines* 2024;**12**:1256.
35. Burnett MGZE. Pathophysiology of peripheral nerve injury: a brief review. *Neurosurg Focus* 2004;**16**(5).
36. Ghosh NKD, Madduri S, Guzman R. Fibrosis and regulation of nerve regeneration in the peripheral and central nervous systems. *CNS Neurol Disord Drug Targets* 2020;**19**:560–71.
37. Das V, Kroin JS, Moric M, McCarthy RJ, Buvanendran A. Early treatment with metformin in a mice model of complex regional pain syndrome reduces pain and edema. *Anesth Analg* 2020;**130**:525–34.
38. Wang Y, Sunitha M, Chung KC. How to measure outcomes of peripheral nerve surgery. *Hand Clin* 2013;**29**:349–61.
39. Deuis JR, Dvorakova LS, Vetter I. Methods used to evaluate pain behaviors in rodents. *Front Mol Neurosci* 2017;**10**:284.
40. Chaplan SRBF, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 1994;**53**:55–63.
41. Kim SH, Park TS, Jin HY. Metformin preserves peripheral nerve damage with comparable effects to alpha lipoic acid in streptozotocin/high-fat diet induced diabetic rats. *Diabetes Metab J* 2020;**44**:842–53.
42. Chesler EJWS, Lariviere WR, Rodriguez-Zas SL, Mogil JS. Influences of laboratory environment on behavior. *Nat Neurosci* 2002;**5**:1101–2.
43. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev* 2001;**53**:597–652.
44. Bourquin AF, Süveges M, Pertin M, Gilliard N, Sardy S, Davison AC, Spahn DR, Decosterd I. Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse. *Pain* 2006;**122**(1–2). [14.e1–14].
45. Woolf CJ, Reynolds ML, Molander C, O'Brien C, Lindsay RM, Benowitz LI. The growth-associated protein GAP-43 appears in dorsal root ganglion cells and in the dorsal horn of the rat spinal cord following peripheral nerve injury. *Neuroscience* 1990;**34**:465–78.
46. Chung D, Shum A, Caraveo G. GAP-43 and BASP1 in axon regeneration: implications for the treatment of neurodegenerative diseases. *Front Cell Dev Biol* 2020;**8**:567537.

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