

# Exenatide promotes regeneration of injured rat sciatic nerve

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## Abstract

Damage to peripheral nerves results in partial or complete dysfunction. After peripheral nerve injuries, a full functional recovery usually cannot be achieved despite the standard surgical repairs. Neurotrophic factors and growth factors stimulate axonal growth and support the viability of nerve cells. The objective of this study is to investigate the neurotrophic effect of exenatide (glucagon like peptide-1 analog) in a rat sciatic nerve neurotmesis model. We injected 10 µg/d exenatide for 12 weeks in the experimental group ( $n = 12$ ) and 0.1 mL/d saline for 12 weeks in the control group ( $n = 12$ ). We evaluated nerve regeneration by conducting electrophysiological and motor functional tests. Histological changes were evaluated at weeks 1, 3, 6, and 9. Nerve regeneration was monitored using stereomicroscopy. The electrophysiological and motor functions in rats treated with exenatide were improved at 12 weeks after surgery. Histological examination revealed a significant increase in the number of axons in injured sciatic nerve following exenatide treatment confirmed by stereomicroscopy. In an experimentally induced neurotmesis model in rats, exenatide had a positive effect on nerve regeneration evidenced by electromyography, functional motor tests, histological and stereomicroscopic findings.

**Key Words:** nerve regeneration; sciatic nerve; glucagon like peptid 1; electromyography; stereomicroscopy; histology; neural regeneration

## Introduction

Peripheral nerves are the axonal extensions of the motor neurons of the anterior horn of the spinal cord, the sensory neurons of the dorsal ganglia, and the sympathetic neurons of the sympathetic ganglia (Shenaq and Kim, 2006; Winograd and Mackinnon, 2006). Damage to peripheral nerves results in partial or complete dysfunction. After peripheral nerve injuries, a full functional recovery usually cannot be achieved despite the standard surgical repairs (Brushart, 1998; Madison et al., 1999). Development of new technologies, widespread use of surgical microscope, use of tension-free and anatomically sutured nerve endings with epineural or perineural stitches, and discovery of the molecular mechanisms of nerve degeneration and regeneration processes all have led to more successful results in the field of surgical nerve repair. However, there is still no treatment method for nerve damage that enables sensory and functional nerve recovery to the same level as before injury (Lundborg, 2000; Shenaq and Kim, 2006; Hsu and Stevenson, 2015; Tseng et al., 2015). Besides, permanent dysfunction of only one peripheral nerve, for example, ulnar nerve in the hand may lead to serious functional limitations. Therefore, it is important to accelerate nerve regeneration after injury.

Neurotrophic factors and growth factors stimulate axonal

growth and support the viability of nerve cells. Glucagon-like peptide (GLP-1) is a polypeptide hormone composed of 30 amino acids, which is secreted by the endocrine cells of the small intestine (Luo et al., 2016). GLP-1 receptors (GLP-1Rs) have been detected in the central and peripheral nervous systems; GLP-1 is thought to exert neurotrophic effects through these receptors (Shenaq and Kim, 2006). GLP-1 is also known as a potent incretin and its injections may cause a weight loss (Liu et al., 2011). GLP-1 is contraindicated in renal dysfunction and if the creatinine clearance less than 30 mL/min, its usage is not recommended.

Exendin-4 is the best GLP-1 analogue in nature. It is derived from the saliva of glia monster (Göke et al., 1993). Exenatide is the synthetic analogue of exendin-4, which is currently used subcutaneously in the treatment of type 2 diabetes and approved by Food and Drug Administration (FDA) (Buse et al., 2004; Gallwitz, 2005).

Recent studies with GLP-1 analogues documented both amelioration of central degenerative changes and prevention of peripheral nerve degeneration in diabetic rats (Seddon et al., 1943; Liu et al., 2011; Chen et al., 2012). Another recent study explored the effect of exendin-4 on peripheral nerve regeneration in rats with crush sciatic nerve injury. They reported significant changes in functional, electrophysiological

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and histological parameters in just 4 weeks after use of exendin-4 (Liu et al., 2011). However, to the best of our knowledge, there are no previous studies investigating the effects of currently used GLP-1 analogue, exenatide, on peripheral nerve regeneration.

A stereomicroscope produces a truly three-dimensional image which documents the regeneration process better. A stereomicroscope cannot magnify an image as much as a compound microscope, which allows seeing the hole tissue without any preparation. To the best of our knowledge, stereomicroscopic follow-up of neuronal tissue was not performed in former studies with GLP-1 analogues.

The objective of this randomized controlled study was to investigate the neurotrophic effect of exenatide in a sciatic nerve injury model from the viewpoints of electrophysiological, behavioral, histomorphological and stereomicroscopic changes.

## Materials and Methods

### Ethics statement

This study was approved by Institutional Ethics Committee, Local Ethical Council for the Animal Experiments (Referral No. 2011-175; date: 11/04/2011) and performed in strict accordance with the International Council for Laboratory Animal Science guidelines. Approval for the study was obtained from the Local Ethical Council for the Animal Experiments of the institutional ethics committee.

### Animals

We used male Sprague-Dawley rats aged 12–16 weeks and weighing 190–220 g. The animals were kept under standard experimental conditions (21–22°C, 55–65% humidity, 12-hour light/dark cycle). They were fed commercial rat chow pellets and were allowed water and food *ad libitum*. These animals were randomly divided into an experimental group and a control group, with 12 rats in each group.

In the experimental group, following induction of sciatic nerve transection injury, Exenatide (AstraZeneca, Wilmington, DE, USA) was injected subcutaneously from the back skin at 10 µg/d for 12 weeks. To detect the harmful effects, we injected the maximum dose (10 µg/d) for human therapeutic usage (Buse et al., 2004; Gallwitz, 2005).

In the control group, after establishing sciatic nerve injury models as above, normal saline was injected subcutaneously at 0.1 mL/d for 12 weeks.

### Surgical procedure

All rats were anesthetized by intraperitoneal administration of 50 mg/kg ketamine HCl (Alfamine, Egevet, Turkey) and 10 mg/kg xylazine (Alfazyne). The same orthopedic surgeon (EK) performed the same surgical procedure on the right and left hindlimbs in all rats in both groups using the same microsurgery equipment (Bahadır Medical Equipment Inc., Turkey). An oblique incision of 1 cm was made on the right thigh posteriorly with a No. 15 scalpel. After the blunt dissection of the biceps femoris muscle, the sciatic nerve was exposed and completely transected at the mid-thigh level

with microsurgical scissors (Bahadır Medical Equipment Inc.). The dissected part of the biceps femoris muscle was sutured using 4/0 vicryl, and the skin was closed with 4/0 prolene. The procedure was terminated after wound antiseptis with povidone-iodine solution. For the left hindlimb, the sciatic nerve was completely transected, forming a gap of 1 cm at the mid-thigh level.

### Assessment methods

Weight, blood glucose level (at 0 hours or 3 hours after exenatide administration), motor function, and electromyography (EMG) records were assessed separately in all rats at 1, 3, 6 and 9 weeks after surgery. One rat was randomly selected from each group for stereomicroscopic evaluation, after which both of them were sacrificed for histological evaluation of the sciatic nerve. At 12 weeks, EMG records and motor strength tests were performed and all rats underwent stereomicroscopic evaluation. Thereafter, rats were sacrificed and the sciatic nerves were evaluated histologically.

#### *Evaluation of muscle strength of hindlimbs*

Using the motor power measurement device (OE-Turkey) (Figure 1), the climbing angle, an indicator of the muscle strength of the hindlimbs, was measured. The rat was released onto the device when the angle was 0 degrees. The angle was gradually increased, and the last angle at which the rat was able to cling to the device was recorded. This procedure was repeated three times for each rat, and the highest value was recorded.

#### *Electrophysiological evaluation*

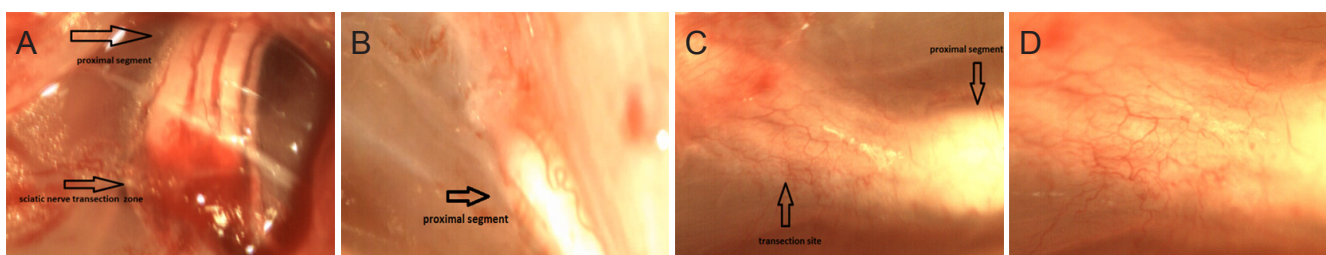
EMG was performed for each rat before and at 1, 3, 6, 9, and 12 weeks after surgery. For electrophysiological measurements, a computer-aided, MP30, nerve conduction velocity measurement kit (Biopac Systems, Inc., Goleta, CA, USA) was used. The proximal pole was placed 10 mm distal to the sciatic notch while the distal pole was put in the second interdigital space. At 9 and 12 weeks after surgery, both the second interdigital space and the distal part of gastrosoleus muscle were used as the distal pole. The latency and the amplitude values of compound muscle action potential (CMAP) were recorded and statistically analyzed.

#### *Stereomicroscopic evaluation of the sciatic nerve*

Using the stereomicroscope (45× magnification) (SOIF ST6024-B1) and a video recorder (3.2× magnification) (MD30, Image Transfer-China), video and photo recordings were taken at a magnification of 7× and 120×, respectively.

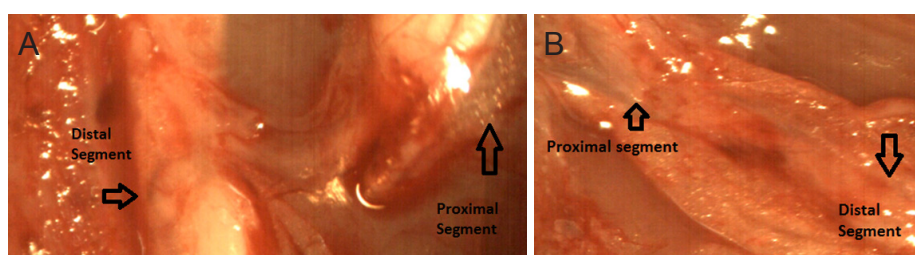
#### *Histological evaluation*

After the completion of electrophysiological recordings, one rat from each group was sacrificed. At 12 weeks after surgery, the remaining rats were sacrificed for light microscopy. Sciatic nerve sections of 3 µm thickness were obtained using a microscope (Leica RM 2145, GMI Inc., USA) and stained with hematoxylin-eosin. After washing with water and clean-



**Figure 2 Stereomicroscopic observation of injured sciatic nerve in rats.**

(A) Experimental group (32× magnification): At 1 week after surgery, rat right sciatic nerve showed no sprouting at the transection zone. (B) Control group (24× magnification): At 1 week after surgery, rat left sciatic nerve showed no decrease in blood supply or retraction of the proximal segment (arrow). (C) Experimental group (32× magnification): At 12 weeks after surgery, there was more than 50% integration of the distal and proximal segments of the right sciatic nerve, with good vascularization at the transection zone. (D) Control group (40× magnification): At 12 weeks after surgery, poor adhesion was observed at the distal segment.



**Figure 3 Stereomicroscopic observation of the right sciatic nerve transection site of the experimental group at 3 weeks after surgery.**

(A) 32× magnification; (B) 24× magnification.



**Figure 1 The muscle strength device used to measure the maximum degree at which the rat cannot roll from the device.**

ing with xylene for histopathological examination, the mean number of axons in the samples was counted under a light microscope (Olympus America, Inc., San Jose, CA, USA).

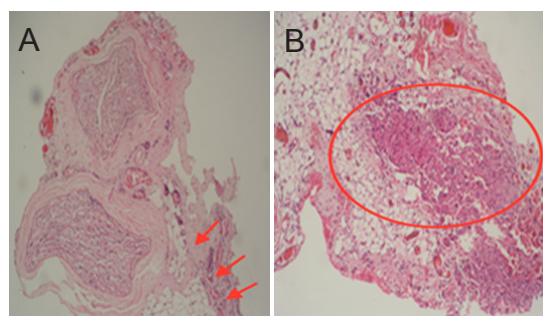
### Statistical analysis

Parametric variables were compared using one-way analysis of variance, whereas the values within the groups were compared using the parametric paired *t*-test. A *P*-value < 0.05 was considered statistically significant. Descriptive statistics are presented as the mean ± SD. All analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA), with a confidence level of 95%.

## Results

### Body weight and blood glucose level

There was no significant difference in body weight between experimental and control groups at 1, 3, 6 and 9 weeks after surgery (*P* > 0.05). No significant difference in body weight was found between experimental and control groups at 0



**Figure 4 Histological evaluation of the right sciatic nerve by hematoxylin-eosin staining at 12 weeks after surgery.**

(A) Control group (24× magnification); (B) Experimental group (48× magnification). Epineurial fibrosis was reduced in the experimental group (oval) than in the control group (arrows).

or 3 hours after administration of exenatide (experimental group) or saline (control group) (*P* > 0.05) (Table 1).

### Muscle strength of hindlimbs

The experimental group showed greater muscle strength of hindlimbs than the control group at 1 and 12 weeks after surgery (*P* < 0.01). There was no significant difference in muscle strength of hindlimb between the experimental and control groups at 3, 6 and 9 weeks after surgery (Table 2).

### Electrophysiological function of the sciatic nerve

Before surgery, the sciatic nerves of rats in both groups were assessed bilaterally using EMG and all were intact. EMG results showed that there were no responses in the second interdigital space in the right and left hindlimbs in the experimental and control groups at 1, 3 and 6 weeks after surgery. At 9 and 12 weeks after surgery, there was no response in the second interdigital space in the right and left hindlimbs of rats in both groups. At 9 weeks after surgery, there was no

**Table 1 Blood glucose level (mg/dL) at 0 or 3 hours after administration**

	Control group	Experimental group	P value
Baseline	1.0±24.2	9.6±12.7	0.443
Day 2	7.1±29.2	-0.8±25.7	0.410
Day 7	16.0±32.9	-15.6±30.8	0.056
Day 14	-20.2±41.6	-12.6±27.4	0.684
Day 21	1.9±36.3	-4.0±40.2	0.696
Day 40	9.8±17.4	0±24.9	0.515
Day 56	-1.6±17.4	9.3±32.1	0.279
Day 77	-6.6±24.6	-10.3±34.4	1.000

Data were expressed as the mean ± SD and analyzed using one-way analysis of variance.

**Table 3 Electromyography results**

	Latency (ms)		Amplitude (mV)	
	Week 9	Week 12	Week 9	Week 12
Experimental group				
Right sciatic nerve	1.19±0.02	1.04±0.01	12.44±1.33	12.47±1.62
Left sciatic nerve	1.25±0.02	1.01±0.03	8.86±1.70	11.29±0.44
Control group				
Right sciatic nerve	1.22±0.02	1.16±0.03	6.99±0.90	8.34±0.42
Left sciatic nerve	1.27±0.04	1.32±0.03	6.10±1.53	7.75±0.68
P	0.14	0.12	0.001	0.35

Data were expressed as the mean ± SD and analyzed using one-way analysis of variance.

significant difference in latency of CMAP between experimental and control groups ( $P > 0.05$ ), whereas the amplitude of CMAP of the right hindlimb was significantly greater in the experimental group than in the control group ( $P < 0.01$ ). There was no significant difference in terms of latency or amplitude of CMAP of the left hindlimb between these two groups (both  $P > 0.05$ ). At 12 weeks after surgery, there was no EMG response from the distal part of the gastrosoleus muscle in either group. The amplitude and latency of CMAP of the proximal part of the gastrosoleus muscle in the left and right hindlimbs of rats in the experimental group were significantly greater than in the control group (Table 3).

### Stereomicroscopic characteristics

#### 1-week imaging

The images taken at week 1 revealed retraction in the transection site in the right sciatic nerve (the transected side), with an extensive increase of vascularization in the proximal segment, particularly in the experimental group. In the right sciatic nerve, there was no nerve sprouting, though a formation of a blurred fibrous band between the distal and the proximal nerve segments was observed (Figure 2A, B). There was an increase in the gap size in the left sciatic nerve in both the experimental and control groups.

#### 3-week imaging

In the images taken at 3 weeks, vascularization increased at the transection site, in particular in the proximal segment,

**Table 2 Muscle strength of hindlimbs (angle (°))**

	Experimental group	Control group	P value
Week 1	60.0±3.9	53.3±2.5	0.001
Week 3	57.5±3.5	54.6±3.5	0.132
Week 6	59.4±3.0	56.7±2.5	0.094
Week 9	58.8±2.3	59.4±3.2	0.798
Week 12	65.0±2.9	54.3±1.9	0.001

Data were expressed as the mean ± SD and analyzed using one-way analysis of variance. The muscle strength of hindlimbs was measured using the motor power measurement device. The climbing angle is an indicator of the muscle strength of hindlimbs.

**Table 4 Quantitation of axon numbers in the left and right sciatic nerves by histological evaluation at 3 and 12 weeks after surgery**

	Experimental group	Control group
3 weeks after surgery		
Right sciatic nerve	498.4±28.7*	327.5±53.4
Left sciatic nerve	646.6±26.2*	202.5±18.3
12 weeks after surgery		
Right sciatic nerve	477.2±27.6*	318.6±14.1
Left sciatic nerve	588.4±24.1*	249.6±21.4

Data are expressed as the mean ± SD. \* $P < 0.05$ , vs. control group (one-way analysis of variance).

which was more prominent in the experimental group. Nerve sprouting had started in the right sciatic nerves of both experimental and control groups, and the sprouting exhibited a wider area and a greater diameter in the experimental group than in the control group. There was no obvious sprouting in the left sciatic nerve with a 1-cm gap. Moreover, in the distal segment of the left sciatic nerve, diameter and vascularity of the sprouting decreased due to degeneration (Figure 3).

#### 9-week imaging

In the images taken at 9 weeks after surgery, nerve sprouting greatly increased in both control and in particular experimental groups. A gel-like lining was maintained between the proximal and the distal poles of the transection site in the experimental group. The gel-like lining was less prominent in the right sciatic nerve in the control group and in the left sciatic nerve in the experimental group. Nerve sprouting in the left sciatic nerve was observed in both the experimental and control groups. In the experimental group, although there was no integrity between the segments with sprouting, sprouting was more prominent.

#### 12-week imaging

In the images taken at 12 weeks after surgery, in the right sciatic nerve, the vascularization of the segmental integrity provided by sprouting was increased compared to that at 9 weeks after surgery, and more than 50% integration between distal and proximal poles was observed. There was nerve sprouting in the proximal segment of the left sciatic nerve, but no integration with the distal segment was observed. In

the distal segment, diameter and vascularization reduced. However, there was no significant difference in this respect between the experimental and control groups (**Figure 2C, D**).

### Histological changes of the sciatic nerve

At the end of 3 weeks after surgery, following Wallerian degeneration of the right and left sciatic nerves, the number of axons was decreased, all of which were more prominent in the control group than in the experimental group. There was significant difference in the number of axons between the experimental and control groups. At the end of 12 weeks after surgery, there was significant difference in the number of axons between the right and left sciatic nerve in the experimental group ( $P < 0.05$ ; **Table 4**). Moreover, epineural fibrosis was reduced in the experimental group than in the control group (**Figure 4**).

### Discussion

Peripheral nerves are likely to be injured in many different ways, such as mechanical (compression, stretching, crushing), thermal, chemical, and ischemic injuries (Seddon, 1943). The main objective of treatment after the injury is to achieve neuronal integrity and re-enable the nerve function and conduction. The purpose of this experimental study was to investigate the neurotropic effect of exenatide (a GLP-1 analogue) in a rat sciatic nerve injury model and to determine the electrophysiological, behavioral, histomorphological and stereomicroscopic changes. Our study showed that use of exenatide accelerated peripheral nerve regeneration from the electrophysiological, behavioral, histomorphological and stereomicroscopic viewpoints without affecting body weight and blood glucose level.

There is strong evidence that numerous peptides have been identified to have effects on the viability and the regeneration capacity of the nerve tissue (Madison et al., 1999; Lundborg, 2000; Hsu and Stevenson, 2015). These so-called neurotropic factors affect the different phases of nerve regeneration positively. Luo et al. (2016) investigated the role of insulin-like growth factor-1 in the treatment of tibial nerve injury in rat models. They found that local insulin-like factor-1 administration reverses age-related declines in recovery and they might consider available adjuvant therapy to the current treatment modalities.

GLP-1 is an insulin tropic hormone secreted by the endocrine L cells of the human intestine post-prandial (Perry et al., 2007). By radioimmunoassay and immunohistochemical methods, immunoreactivity consistent with GLP-1 has been shown in human and rat brains, spinal oblongata, pons, and sciatic nerve. GLP-1 has anti-apoptotic properties in the hippocampal nucleus of (Avcı et al., 2002). After this discovery, the potential of GLP-1 to protect the central nervous system from the neurodegenerative damage has become a matter of interest (Luo et al., 2016).

Yamamoto et al. (2013) demonstrated in a rat sciatic nerve injury model that exendin-4 did not affect blood glucose levels. In this study, blood glucose level was measured in rats before and 3 hours after surgery. There was no significant

difference in blood glucose level or hypoglycemia between the experimental and control groups. The use of an anti-diabetic drug in peripheral nerve injury in clinical practice may lead to hesitation because of side effects including possible hypoglycemia and body weight loss. The neutral effect of exenatide on blood glucose levels compared to control group in our study is important and encouraging regarding drug safety (Ahmed et al., 1999).

Kaimal et al. (2012) observed in patients with type 2 diabetes mellitus that a GLP-1 analogue led to an average weight loss of 6.6 kg by reducing the rate of gastric emptying and due to its intestinal effects. Ohki et al. (2016) also reported weight reduction is a benefit of treatment with GLP-1 analogues in diabetic patients. However, we observed weight gain in both groups, without a statistically significant difference between the groups. This is also another important safety indicator for clinical use in future. When exenatide is planned to use in patients with peripheral nerve injury, weight change is not a possible side effect in the light of our results.

In experimental studies examining the peripheral nerve regeneration, both morphological and functional examinations are recommended (Luis et al., 2007). The best test to show the recovery after nerve injury, to the best of our knowledge, has not been clarified in experimental models. However, there are various evaluation parameters, such as gait analysis, EMG, climbing apparatus, histological evaluation, and foot withdrawal reflex (Rivli and Tator, 1977).

Electrophysiological tests are frequently used in the evaluation of peripheral nerve regeneration. These tests are based on the magnification of the action potentials in nerve fibers caused by the stimulation of the muscle fibers, using an amplifier (Wolthers et al., 2005). In this study, we documented a statistically significant increase in the amplitude of right hindlimbs at 9 weeks and there were significant differences in both amplitude and latency of CMAP of the left and right hindlimbs at 12 weeks between the experimental and control groups.

Wolthers et al. (2005) demonstrated that as axonal regeneration increases and remyelination progresses, the synchronization and the amplitude increase due to the increased change and response in the fibers. Baykal et al. (2002) reported that the time elapsed between the latency values and the start of contraction potentials is an important indicator of myelination. In our study, EMG recordings at 9 weeks, in particular at 12 weeks, have shown that exenatide increased myelination and total axonal synchronization in nerve regeneration, resulting in faster nerve regeneration.

To evaluate the motor function of rats in this study, the climbing angles were monitored and recorded at 0, 1, 3, 6, 9, and 12 weeks after surgery (Fisher and Peduzzi, 2007; Luis et al., 2007). The climbing angle was significantly greater in the experimental group at 1 and 12 weeks than in the control groups. Findings from a 12-week observation indicate that the sciatic function had recovered to a great extent. The data concerning functional evaluation indicates that

electrophysiological changes in nerve regeneration can reflect motor function capacity which is the most important endpoint regarding peripheral nerve injuries in daily clinical practice.

The incidence of auto-cannibalization is closely affected by the type of nerve damage, and it is more often reported in complete nerve lacerations (Lundborg, 2000; Hsu and Stevenson, 2015; Luo et al., 2016). Distal hyperalgesia and neuropathic pain are the reasons for auto-cannibalization (Lundborg, 2000; Luo et al., 2016). Martins et al. (2006) reported auto-cannibalization at a rate of 42%, which is quite high. However, in our study, no cases of auto-cannibalization were observed in the experimental group, whereas it was encountered at a rate of 25% in the control group. This was most likely due to the neuroprotective effect of exenatide on the peripheral nerves, which inhibits hyperalgesia and auto-cannibalization. Besides assessing nerve functions functionally and electrophysiologically, we aimed to monitor regeneration stages of the nerve. We used a Soif stereomicroscope (45× magnification) and an MD30 video recorder (3.2× magnification). These records showed subjectively that at 1 week after surgery, the right sciatic nerve (the transected side) had retraction in the transection site, with an extensive increase of vascularity in the proximal segment. Formation of a blurred fibrous band between the distal and the proximal segments was observed more prominently in the experimental group than in the control group. On the stereomicroscope images taken at 3 weeks, nerve sprouting in the transection site was present. At 9 weeks after surgery, a gel-like lining was maintained at the regeneration site in the experimental group, whereas no integrity was observed in the control group. At 12 weeks after surgery, vascularization of the gelatinous sheath was excessively increased, so axons in the sheath were clearly observed.

Results from this study revealed a faster degeneration in the distal pole of the nerve of neuromesis model with a 1-cm gap in the left sciatic nerve. At 12 weeks after surgery, the proliferation was more irregular over a wide range, with a severe degeneration of the distal poles. In the right sciatic nerve, which was transected and showed regeneration, the healing was subjectively more rapid and regular, and the proliferation and the integrity between the distal and the proximal pole were achieved earlier, in rats treated with GLP-1 than in control rats. However, in the left sciatic nerve, there was no significant difference in the phase of regeneration between the experimental and control groups. Also in the left sciatic nerve, there was no significant difference in stereological stereomicroscopic changes, which should be followed up for a longer time period. This study adds much to document the regenerative capacity of exenatide on tissue level according to these stereomicroscopic findings. To the best of our knowledge, this is the first study in literature which demonstrated the accelerated peripheral nerve regeneration after use of exenatide on the tissue level. We documented better regeneration in an injured sciatic nerve in the absence of surgical repair. Much better healing is expected when exenatide is applied after surgical repair

because anatomical integrity will already be provided by surgery.

Luis et al. (2007) and Rizli and Tator (1977) also used a sciatic nerve model with a defect. They used hematoxylin-eosin staining to measure axon diameter, myelin thickness and nerve density to evaluate nerve regeneration. In this study, histological evaluation at 3 and 12 weeks revealed Wallerian axonal degeneration bilaterally in the sciatic nerve in both the experimental and control groups. However, the number of axons in the experimental group was greater than in the control group. The number of newly formed axons was also increased significantly in the experimental group than in the control group both on the right and the left sciatic nerves at 12 weeks. In this study, we investigated the effects of 10 µg/d exenatide on sciatic nerve injury in rat models. Future research is needed to investigate the effects of exenatide at different doses. Another limitation of this study is the follow-up period and another incretin mimetic drug will be added as a control group. Further studies with longer follow-up periods are required to demonstrate the efficacy of exenatide in severe nerve injury.

In conclusion, in an experimentally induced neurotmesis model in rats, GLP-1 had a positive effect on nerve regeneration as evidenced by EMG, stereomicroscopic and histological findings and motor functional tests. The drug seems to have no effects on body weight and blood glucose levels. The absence of auto-cannibalization in the experimental group implies that exenatide accelerates the healing of sensory symptoms and helps to reduce pain. These findings have important implications for use of exenatide in the treatment of peripheral nerve injury in future.

**Author contributions:** EK and AB conceived the study. EK performed surgeries, measured blood glucose level, and took stereomicroscope images. BG performed electromyography and motor function tests and wrote the paper. OE participated in electromyography and physiological tests. FO carried out histological evaluation. All authors approved the final version of this paper.

**Conflicts of interest:** None declared.

**Plagiarism check:** This paper was screened twice using CrossCheck to verify originality before publication.

**Peer review:** This paper was double-blinded and stringently reviewed by international expert reviewers.

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