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Original Article

The Effect of Exanatide on Recovery of the Facial Nerve Crush Injury in the Rat

Begüm Yılmaz[1], Ayşe Aslı Şahin Yılmaz[2], Kadri İla[3], Reyhan Sürmeli[4], Murat Hakan Karabulut[5], Mehmet Sürmeli[6]

[1]Department of Otolaryngology, University of Health Sciences, Istanbul Kartal Dr Lutfi Kirdar City Hospital, Istanbul, Türkiye
[2]Department of Otolaryngology, Sağlık Bilimleri University (University of Health Sciences), Istanbul Kartal Dr Lutfi Kirdar City Hospital, Istanbul, Türkiye
[3]Department of Otolaryngology, Vanity Cosmetic Surgery Hospital, Istanbul, Türkiye
[4]Department of Neurology, Umraniye Education and Research Hospital, Istanbul, Türkiye
[5]Department of Pathology, Umraniye Education and Research Hospital, Istanbul, Türkiye
[6]Department of Otolaryngology, Acibadem Atasehir Hospital, Istanbul, Türkiye

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BACKGROUND: Exenatide, a glucagon-like peptide-1 receptor agonist, has been shown to promote functional recovery and nerve regeneration after nerve crush injury. The aim of this study was to investigate the effect of exenatide on facial nerve regeneration in traumatic facial paralysis.

METHODS: This prospective, randomized animal study included 28 healthy female Sprague–Dawley rats divided into 4 groups of 7 rats each: control group, sham group, 2.5 µg/rat/day exenatide-treated group, and 10 µg/rat/day exenatide-treated group. Exenatide was injected subcutaneously for 14 days. Crush injury was applied to the left facial nerve in all groups except the sham group. Functional assessment, electrophysiological, histopathological, and immunohistochemical examinations were performed after crush injury.

RESULTS: The blink reflex score was higher in 10 µg exenatide-treated rats than in other rats at week 1. At weeks 3 and 4, the vibrissae movement score was higher in exenatide-treated rats than in untreated rats. Electromyographic assessments performed at weeks 2 and 4 showed higher amplitude values and shorter latency periods in the exenatide treatment groups. Exenatide was found to have a dose-dependent effect on reducing the severity of vasoconstriction, and this effect was statistically significant. When comparing the exenatide treatment groups with the control group, a significant reduction in the incidence of severe macrovacuolation and an increase in myelin thickness were observed ($P < .05$).

CONCLUSION: Functional, electrophysiological, and histopathological evaluations have shown that exenatide treatment has a positive and accelerating effect on facial nerve recovery in traumatic facial paralysis.

KEYWORDS: Crush injury, exenatide, facial nerve, functional recovery, neuroprotective agents

INTRODUCTION

Facial paralysis leads to severe functional, cosmetic, and social problems, such as limitation of facial movements, synkinesis, limitation of eye closing, asymmetry in the oral commissure, and nasal valve collapse.[1] Injury to the facial nerve represents the second most prevalent underlying factor contributing to facial paralysis.[2]

Sunderland classifies facial nerve injury into 5 stages. Crushing injuries are referred to as axonotmesis, which involves damage to the axon and the surrounding myelin tissue, while the epineurium remains intact.[3] Crushing induces oxidative stress, generating free radicals that lead to pathological changes in the endoneurial microvessels, causing endoneural ischemia or hypoxia.[4] Spontaneous regeneration may occur following peripheral nerve crush injuries; however, the extent and quality of functional recovery can vary considerably.[3,4] While there is no established pharmacological treatment to enhance nerve regeneration, various agents have been investigated for their potential neuroprotective and neuroregenerative effects.[1,3-7]

Corresponding author: Begüm Yılmaz, e-mail: drbgmylmz@gmail.com
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"Glucagon-like peptide" (GLP-1) is a hormone known for its insulinotropic effects, secreted by L cells of the endocrine system within the intestinal mucosa. Exendin-4 is a GLP-1 analogue. Exenatide, a synthetic derivative of exendin-4, is an Food and Drug Administration (FDA)–approved medication administered subcutaneously to manage type 2 diabetes. Research has identified GLP-1 receptors in the central and peripheral nervous systems, indicating GLP-1 promotes neurotrophic effects by interacting with these receptors.[6] Repeated injections of exendin-4 have shown functional recovery and nerve regeneration effects for nerve crush injury. Studies on sciatic nerve injury in rat models have shown GLP-1 positively affects nerve regeneration through electromyography (EMG), stereomicroscopic examinations, histological analyses, and motor function tests.[6]

So far, the effects of exenatide on the recovery of facial nerve crush injuries in rats have not been investigated. This study aims to investigate how exenatide impacts the functional, histopathological, and electrophysiological recovery of the facial nerve in rats, hypothesizing that it could accelerate recovery and be clinically useful for treating facial nerve injuries.

METHODS

Animal Groups and Treatments

This research received approval from the Ethics Committee of Acıbadem Mehmet Ali Aydınlar University (Approval no: ACUHADYEK 2021/21; Date: 12.03.2021). The research followed the ethical standards and regulations outlined by the "International Council for Laboratory Animal Science."

The study utilized 28 healthy female "Sprague–Dawley" rats, between the ages of 12 and 16 weeks, weighing between 210 and 290 grams. The animals were housed under controlled conditions with a 12-hour light/dark cycle, humidity levels maintained at 55%-65%, and a constant temperature of 21-22°C. They were given a standard rodent diet with continuous access to food and water throughout the study. The rats were housed in 8 separate cages, each accommodating 3 to 4 individuals, and were assigned in a randomized manner to 1 of the 4 study groups: (1) Control group (n = 7): Received a daily dose of 0.1 mL physiological saline. (2) Sham group (n = 7): The left facial nerve was exposed without applying any crush injury. (3) 2.5 µg/day Exenatide treatment group (n = 7). (4) 10 µg/day exenatide treatment group (n = 7). While only the left facial nerve was exposed in the sham group, a crush injury was induced in the same region in the other groups.

Exenatide or saline was injected subcutaneously for 14 days after the crush injury. On day 28, the rats were killed by decapitation.

Surgical Technique and Treatment

Left facial nerve crushing was performed under general anesthesia. Isoflurane was applied as the inhalation method for general anesthesia, with the minimal alveolar concentration (MAC) value adjusted to 3%. During the induction phase of anesthesia, the MAC value stabilized between 3% and 5%. After the left ear area was shaved and prepared sterile, a 5 mm horizontal incision was made in the subauricular area. The layer below the skin was meticulously dissected along the subauricular incision, followed by the localization of the facial nerve via blunt dissection.[8] A 1 mV electrical stimulus was delivered to the main trunk of the facial nerve utilizing the "NIM-Response® 3.0 System (Medtronic Xomed, Inc., Jacksonville, FL, USA)" to evaluate the vibrissal muscle response. Following this stimulation, the main trunk of the nerve was subjected to a standardized crush injury by applying a clamp for a duration of 1 minute. Following this procedure, the proximal segment of the crushed region was stimulated with a 2 mV nerve stimulator to assess the loss of hemifacial movement (Figure 1). The wound was closed using a single-layer technique with absorbable sutures to ensure proper healing. One day after facial nerve injury, there was no response to the left blink reflex examination and the left vibrissal hair was observed to be motionless and in a posterior position.

Functional Recovery Testing

Functional evaluation was performed at the first, second, third, and fourth weeks after the surgical procedure. Five-minute videos of the rats were recorded using an iPhone SE device (Apple Inc., Cupertino, CA, USA). Video recordings of the rats were made from above on a black background, while the animals were gently stabilized manually or with the support of a soft towel to prevent excessive head and body movement. To evaluate the eye blink reflex, compressed air was administered using a 5 cc syringe and a 21 gauge needle tip (Figure 2).

Functional evaluation was performed through video recordings and assessed by a single blinded rater. The analysis included scoring of eye blinking, vibrissae orientation, and vibrissae movement using the Facial Movement Scoring Scale as described by Seth et al.[9] This scale rates each parameter on a 5-point scale. Eye blinking: 1: Absence of eye blinking or closure; 2: Presence of orbicularis oculi muscle contraction without a blinking reflex; 3: Approximately 50% eye closure through blinking reflex; 4: Approximately 75% eye closure; 5: Complete eye closure with a normal blinking reflex. Vibrissae movement: 1: No movement with vibrissae in posterior position; 2: Slight shivering, vibrissae remain in posterior position; 3: More pronounced shivering with continued posterior positioning; 4: Normal movement, vibrissae still posterior; 5: Symmetrical movement with vibrissae in anterior position.

Electrophysiological Test

Electromyography recordings were obtained using the "Medelec Synergy Nicolet AT2 EMG/EP system (Natus Medical Inc., Middleton, WI, USA)" to assess neuromuscular activity. Left facial nerve EMG

MAIN POINTS

- Exenatide accelerated functional recovery in rats with facial nerve crush injury, particularly improving blink reflex and vibrissae movement scores at earlier stages compared to the control group.
- Electrophysiological assessments revealed significantly higher compound muscle action potential amplitudes and shorter latency times in Exenatide-treated groups, indicating enhanced neuromuscular regeneration.
- Histopathological analyses demonstrated reduced vascular congestion and macrovacuolization and increased myelin thickness in Exenatide groups, supporting its dose-dependent neuroprotective effects on peripheral nerve healing.

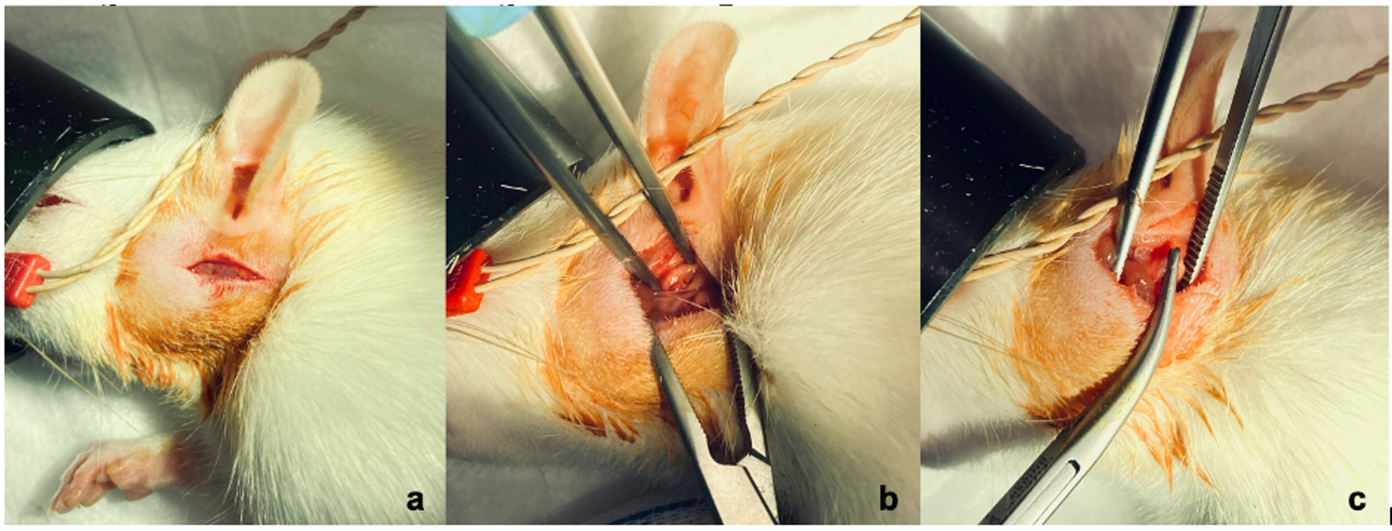


Figure 1. Surgical procedures in rats used in this study after the left ear area was shaved and prepared sterile, a 5 mm horizontal incision was made in the area 5 mm subauricular. b The main trunk of the left facial nerve is explored. c The main trunk of the facial nerve was crushed with a clamp.

recordings of the rats were made 2 and 4 weeks after the surgical procedure. The EMG recordings were blindly evaluated by a single neurologist. The vibrissal muscle was equipped with 2 monopolar needle electrodes, a dorsal ground electrode was placed in the dorsal region, and a bipolar stimulating electrode was positioned adjacent to the stylomastoid foramen, where the facial nerve exits. Supramaximal stimulation was given and "compound muscle action potential" (CMAP) signals were recorded with EMG. Amplitude in millivolts (mV) and latency times in milliseconds (ms) were determined from CMAP recordings. In the second week after the surgical procedure, the amplitudes and latency times of the sham group were compared with the other groups, and these measurements were used as a basis against which the records of the fourth week after the surgical procedure could be compared.

Histological and Immunohistochemical Analysis

The tissues were fixed in 10% formaldehyde buffer solution and then sectioned into 3 mm thick slices. The samples preserved in paraffin blocks were sectioned into 4 µm thick slices and subsequently stained with Giemsa and hematoxylin-eosin dyes for detailed histological analysis. In the examination performed by the pathologist under a light microscope, all samples were thoroughly evaluated for vascular congestion, macrovacuolization, and myelin sheath thickness. The degrees of vascular congestion and macrovacuolization were categorized as none, mild, moderate, and severe. Additionally, the axon thickness in the myelin sheath was classified into very thin, thin, and normal categories.[10]

Tumor necrosis factor-alpha (TNF-α) antibodies were diluted at a ratio of 1/400, and Cleaved Caspase-3 antibodies were diluted at a ratio of 1/50. The immunohistochemical staining procedure was performed using the "Ventana Benchmark Ultra" system and Ventana Ultraview Dab Kit (Tucson, Arizona, USA). When evaluating TNF-α and Caspase-3, the extent of staining and intensity of staining were rated as absent/focal/diffuse, none/mild/moderate/strong respectively.

Statistical Analysis

To analyze the data, the "Number Cruncher Statistical System 2007" (NCSS, Kaysville, Utah, USA) software was used. Descriptive methods summarized and interpreted the study findings. These included

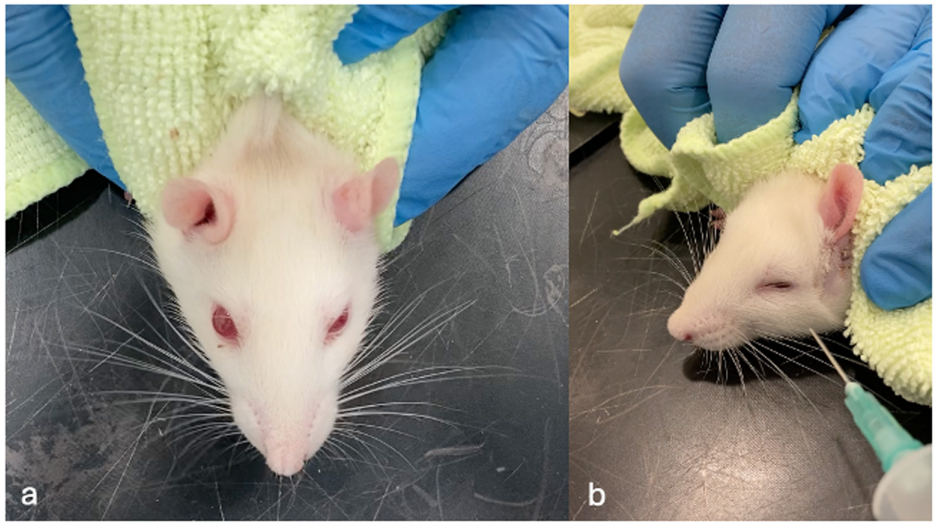


Figure 2. Rat stabilization and eye blink reflex assessment setup.

standard deviation, mean, frequency, median, minimum, maximum values, and percentage ratios. Graphical analyses and the "Shapiro–Wilk test" determined data normality. The "Kruskal–Wallis test," with the "Dunn–Bonferroni test," evaluated non-normally distributed variables across groups, while the "Friedman test" assessed within-group analyses. Comparisons between pairs used the "Wilcoxon signed-rank test" with Bonferroni correction. For non-normally distributed variables within groups, the Wilcoxon signed-rank test was applied. The "Fisher–Freeman–Halton exact test" was employed to compare categorical data. When a statistically significant difference was identified, post-hoc pairwise group comparisons were conducted using the Mann–Whitney *U*-test, applying a Bonferroni adjustment for multiple testing. A *P* value under .05 was considered indicative of statistical significance.

RESULTS

There were no intraoperative and postoperative complications. In the blood sugar monitoring, a statistically significant decrease in glucose levels was observed on the 14th day post-surgery when comparing the exenatide treatment groups with the control and sham groups. However, no hypoglycemia developed in the rats throughout the experiment. No notable difference was detected in the weight measurements throughout the study.

Functional Assessment

Blink Reflex Scores

During the first postoperative week, the sham and 10 µg Exenatide groups demonstrated significantly higher blink reflex scores compared with the control group ($P = .001$ and $P = .049$, respectively). However, there was no significant difference between the control and 2.5 µg exenatide groups ($P = .971$). Likewise, no significant difference was observed between the sham and 10 µg groups ($P = .699$). These findings indicate that the 10 µg exenatide dose, but not the 2.5 µg dose, accelerated functional nerve recovery at the first postoperative week (Figure 3a).

Vibrissae Movement

In analysis of vibrissae movements, no notable difference emerged between sham and 10 µg exenatide groups by the second week. In weeks 3 and 4, no significant differences in vibrissae movement scores were detected comparing the sham group to the 2.5 µg or 10 µg exenatide group. However, a significant difference between the control and sham groups was still evident and persisted throughout the fourth week. Results showed 10 µg exenatide treatment resulted in earlier recovery than 2.5 µg exenatide treatment at week 2 ($P = .042$). At the fourth week, more noticeable recovery was evident in 2.5 µg and 10 µg exenatide groups compared to saline ($P = .004$, $P = .023$). However, no significant difference was observed between the 2 exenatide-administered groups (Figure 3b) (Table 1).

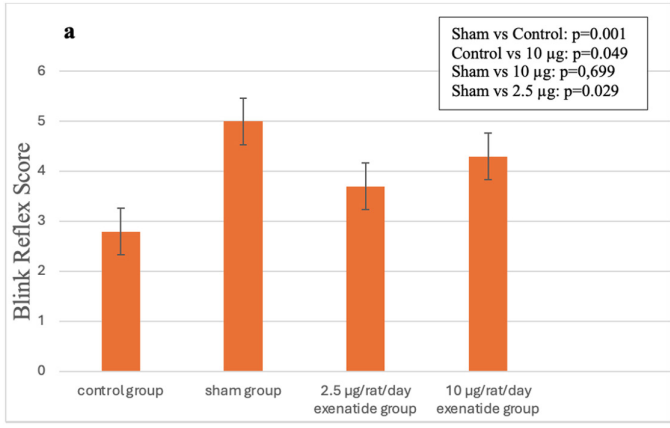


Figure 3. a. Blink Reflex Score at first week; b. Vibrissae Movement Score at fourth week.

Table 1. Facial Nerve Functional Score

Facial Nerve Functional Score	Postoperative First Day	First Week	Second Week	Third Week	Fourth Week
Control group					
Blink reflex	1	2.85 ± 0.69	4.57 ± 0.53	5	5
Vibrissae movement	1	2	2.71 ± 0.49	3.71 ± 0.49	3.71 ± 0.49
Sham group					
Blink reflex	5	5	5	5	5
Vibrissae movement	5	5	5	5	5
2.5 µg/rat/day exenatide group					
Blink reflex	1	3.71 ± 0.48	4.86 ± 0.38	5	5
Vibrissae movement	1	2	3.43 ± 0.53	4.29 ± 0.49	4.86 ± 0.38
10 µg/rat/day exenatide group					
Blink reflex	1	4.29 ± 0.76	5	5	5
Vibrissae movement	1	2	4.14 ± 0.69	4.57 ± 0.53	4.71 ± 0.49

Electrophysiological Assessment

Latency

At the second week, no statistically significant difference was observed between the control group and the 2.5 µg exenatide group ($P = .064$). However, latency values were significantly different at the second week between the control and the 10 µg exenatide group ($P = .006$). At the fourth week, both exenatide groups showed significantly shorter latency times compared with the control group (2.5 µg: $P = .006$; 10 µg: $P = .025$).

Amplitude Values

At the second and fourth weeks, amplitude measurements showed a significant difference between the control group and the 2.5 µg exenatide group (second week: $P = .005$; fourth week: $P = .018$), as well as between the control group and the 10 µg exenatide group (second week: $P = .017$; fourth week: $P = .012$). In the control group, lower amplitude values and longer latency times were observed. However, no significant differences were found when comparing the 2.5 µg and 10 µg exenatide treatment groups with the sham group, nor between the 2 exenatide treatment groups themselves. As a result of electrophysiological investigations, it was shown that exenatide treatment had positive effects on nerve regeneration (Table 2).

Histological Assessment

Hematoxylin and Eosin Staining

Nerve regeneration was evaluated using vascular congestion, macrovacuolization,and myelin thickness parameters (Figure 4).

Vascular Congestion

The control group demonstrated moderate to severe vascular congestion in 6 out of 7 rats (85.8%) (Figure 4a). In the 2.5 µg exenatide group, 6 rats (85.7%) exhibited mild congestion (Figure 4c), whereas in the 10 µg exenatide group, only 4 rats (57.1%) showed mild congestion (Figure 4d). These results indicate a statistically significant difference, implying a dose-dependent effect of exenatide in reducing the severity of vascular congestion ($P = .008$). The control group was shown to have higher rates of moderate and severe vascular congestion.

Table 2. Electrophysiological Assessment

Groups		Latency (ms) 2. Week	Amplitude (mV) 2. Week	Latency (ms) 4. Week	Amplitude (mV) 4. Week
Sham	N	7	7	7	7
	Mean	2.44	0.45	2.63	1.14
	Standard deviation	0.44	0.40	0.40	0.41
	Median	2.40	0.40	2.55	1.00
	Minimum	1.82	0.08	2.28	0.60
	Maximum	3.18	1.10	3.45	1.80
Control	N	7	7	7	7
	Mean	3.30	0.12	3.37	0.63
	Standard deviation	0.56	0.21	0.58	0.29
	Median	3.25	0.03	3.34	0.70
	Minimum	2.51	0.01	2.61	0.08
	Maximum	4.13	0.60	4.08	0.90
2.5 µg/rat/day exenatide group	N	7	7	7	7
	Mean	2.28	0.66	2.50	1.31
	Standard deviation	1.10	0.17	0.42	0.56
	Median	2.40	0.70	2.55	1.30
	Minimum	0.83	0.40	2.08	0.30
	Maximum	3.70	0.90	3.28	1.90
10 µg/rat/day exenatide group	N	7	7	7	7
	Mean	2.40	0.41	2.63	1.37
	Standard deviation	0.63	0.23	0.46	0.42
	Median	2.40	0.40	2.66	1.50
	Minimum	1.09	0.10	2.03	0.60
	Maximum	2.92	0.80	3.23	1.90
P value (Control vs. 2.5 µg/rat/day exenatide group)		.064	.005	.006	.018
P value (Control vs. 10 µg/rat/day exenatide group)		.006	.017	.025	.012
P value (2.5 µg/rat/day exenatide group vs. 10 µg/rat/day exenatide group)		.748	.052	.798	.898

mV, milivolts; ms, milliseconds, n, size of the group. Mann–Whitney *U* and Bonferroni test.

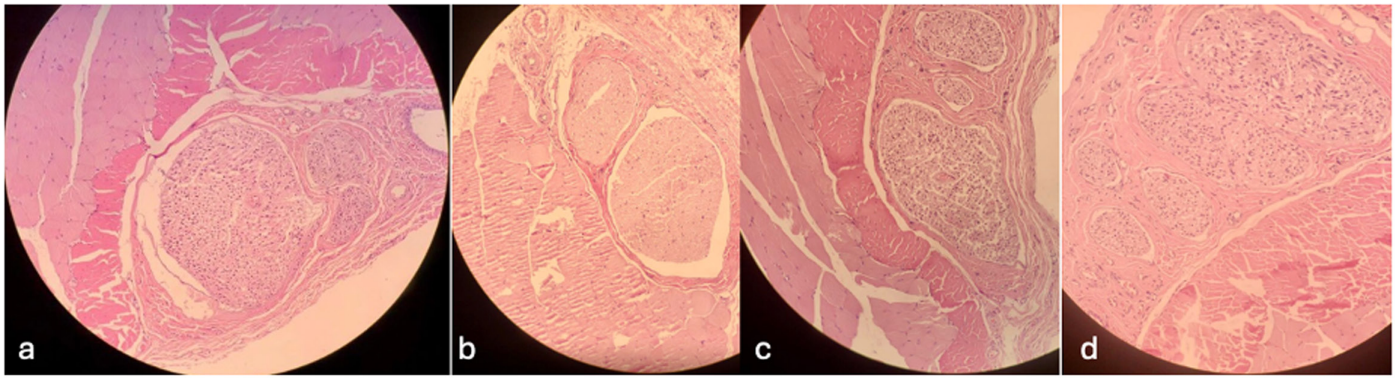


Figure 4. X200 magnification images under light microscopy after staining with Haematoxylin-Eosin a. Control group: mild vascular congestion, severe macrovacuolization, and markedly thin myelin sheath, b. Sham group: mild vascular congestion, no macrovacuolization, and normal myelin sheath thickness, c. 2.5 µg exenatide group: mild vascular congestion, moderate macrovacuolization, and thin myelin sheath, d. 10 µg exenatide group: mild vascular congestion, mild macrovacuolization, and thin myelin sheath.

Macrovacualization

In the control group, all 7 rats (100%) exhibited severe macrovacuolization (Figure 4a). Among the treated groups, the 2.5 µg exenatide group showed moderate macrovacuolization in 4 rats (57.1%) (Figure 4c), while the 10 µg exenatide group displayed severe macrovacuolization in 5 rats (71.4%) (Figure 4d). Notably, the incidence of severe macrovacuolization was significantly reduced in both exenatide-treated groups compared to the control group, suggesting a potential therapeutic effect of the treatment ($P = .001$).

Myelin Thickness

In the control group, the myelin layer was classified as very thin in all 7 rats (100%) (Figure 4a). In contrast, the sham group exhibited a myelin layer of normal thickness in all 7 rats (100%) (Figure 4b). Both the 2.5 µg and 10 µg exenatide groups showed a thin myelin layer (Figure 4c and d). Statistical analysis revealed significant differences in myelin thickness across the groups, suggesting a potential impact of exenatide on the myelin structure ($P = .001$) (Table 3).

Immunohistochemical Assessment

Immunohistochemical staining levels for TNF-α and caspase-3 were examined, revealing no staining in the sham group, while extensive staining was observed in crush injury groups. Nevertheless, analysis showed no significant differences between groups exposed to crush injury. Additionally, no differences in TNF-α and caspase-3 staining intensity were observed among crush injury groups. However, when TNF-α staining intensity was assessed, median staining intensity was higher in the 2.5 µg exenatide and 10 µg exenatide groups than in the control group. The intensity of TNF-α staining varied significantly across groups. In the control group, all 7 rats (100%) exhibited strong staining. In the 2.5 µg exenatide group, strong staining was observed in 5 rats (71.4%), while moderate staining was detected in 2 rats (28.6%). Similarly, in the 10 µg exenatide group, 4 rats (57.1%) displayed strong staining, and 3 rats (42.9%) showed moderate staining (Figure 5 and 6).

DISCUSSION

In cases of partial traumatic or non-traumatic facial paralysis, medical treatment is the preferred option. The aim of medical treatment is to reduce inflammation and edema. There is no definite treatment algorithm in the treatment of facial paralysis after crush injury. The best known medical treatment is steroid therapy. When nerve damage occurs due to crushing, various pathological changes occur. Neuroprotective pharmacological agents such as Ginkgo biloba, N-acetylcysteine, FK506, Ebselen, nimodipine and 4-aminopyridine, which aim to reduce oxidative stress and inflammation, have been evaluated for their efficacy in the treatment of peripheral nerve crush injuries.[1,3-5,11,12] There is limited information about the efficacy of other neuroprotective drugs.[3,4]

The role of exenatide in the treatment of traumatic facial paralysis was investigated in rats by crushing the facial nerve and inducing facial paralysis. In the study, functional analysis 1 week after traumatic facial nerve injury, 10 µg exenatide treatment showed favourable accelerated effect on recovery of blink reflex. At the second week after surgery, 10 µg exenatide treatment showed favourable accelerating effect on recovery of vibrissae movement. In the fourth week, both 2.5 µg and 10 µg exenatide were more effective than saline in improving vibrissae movement, but there was no significant difference between the 2.5 µg and 10 µg exenatide groups ($P = .001$).

Electrophysiological evaluation in the study showed a beneficial effect of exenatide on nerve regeneration. In second and fourth week EMG evaluations, statistically significant differences were found when the 2.5 and 10 µg Exenatide treatment groups were compared with the control group. However, no difference was observed between the 2.5 µg exenatide group and the control group for the 2-week latency times. Amplitude values were higher and latency times shorter than the control group in both treatment groups. Comparative analysis between the 2.5 µg and 10 µg exenatide treatment groups revealed no statistically significant differences. In a study by Yamamato et al,[7] sciatic nerve crush injury was induced and the efficacy of treatment with 2.5 µg/day exendin-4 for 14 days was evaluated. Consistent with the findings of the study, electrophysiological assessments in this research also demonstrated a statistically significant decrease in latency times within treatment groups.

Histopathological evaluations carried out in this investigation demonstrated the favorable influence of exenatide on facial nerve recovery. In comparison to the control group, both the 2.5 µg and 10 µg exenatide-treated groups displayed a marked reduction in vascular congestion and macrovacuolization, as well as a significant enhancement in myelin thickness. Notably, no statistically significant variations were found between the 2 exenatide dosage groups.

Table 3. Evaluation of Pathology Results by Group

		Group				
		Crush (+) Control Group, Administered 0.1 mL Saline/rat/day (n = 7)	Sham Group (With No Crush Injury) (n = 7)	Crush (+) 2.5 μg/rat/day Exenatide Group (n = 7)	Crush (+) 10 μg/rat/day Exenatide Group (n = 7)	*P*
Vascular congestion	None	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	[d].008**
	Mild	1 (14.3)	5 (71.4)	6 (85.7)	4 (57.1)	
	Moderate	3 (42.9)	0 (0.0)	1 (14.3)	3 (42.9)	
	Severe	3 (42.9)	0 (0.0)	0 (0.0)	0 (0.0)	
Macrovacuolization	None	0 (0.0)	7 (100.0)	0 (0.0)	0 (0.0)	[d].001**
	Mild	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)	
	Moderate	0 (0.0)	0 (0.0)	4 (57.1)	5 (71.4)	
	Severe	7 (100.0)	0 (0.0)	1 (14.3)	2 (28.6)	
Myelin thickness	Very thin	7 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	[d].001**
	Thin	0 (0.0)	0 (0.0)	7 (100.0)	7 (100.0)	
	Normal	0 (0.0)	7 (100.0)	0 (0.0)	0 (0.0)	
The prevalence of TNF-α staining	Absent	0 (0.0)	7 (100.0)	0 (0.0)	0 (0.0)	[d].001**
	Diffuse	7 (100.0)	0 (0.0)	7 (100.0)	7 (100.0)	
The prevalence of caspas-3 staining	Absent	0 (0.0)	7 (100.0)	0 (0.0)	0 (0.0)	[d].001**
	Diffuse	7 (100.0)	0 (0.0)	7 (100.0)	7 (100.0)	
The degree of TNF-α staining	None	0 (0.0)	7 (100.0)	0 (0.0)	0 (0.0)	[d].001**
	Moderate	0 (0.0)	0 (0.0)	2 (28.6)	3 (42.9)	
	Strong	7 (100.0)	0 (0.0)	5 (71.4)	4 (57.1)	
The degree of caspas-3 staining	None	0 (0.0)	7 (100.0)	0 (0.0)	0 (0.0)	[d].001**
	Moderate	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	
	Strong	5 (71.4)	0 (0.0)	7 (100.0)	7 (100.0)	

[d]Fisher Freeman Halton Test
**$P < .01$. n, size of the group.

These outcomes suggest exenatide administration contributes positively to peripheral nerve regeneration, highlighting its potential as a therapeutic agent in nerve repair. Similar studies were performed on the sciatic nerve. Kuyucu and colleagues evaluated 12 weeks of 10 μg/day exenatide on crushed sciatic nerve of rats. Functional evaluations of muscle strength indicated significant improvement in the control group compared to treatment groups, with statistically meaningful differences observed during initial and 12th-week assessments. Following surgical intervention, rats treated with exenatide exhibited improved electrophysiological functions at 12 weeks. Furthermore, histopathological analysis showed a significant increase in axon count in the exenatide-treated group.[6] Another study by Abrishami and colleagues evaluated the effects of medication on the recovery of infraorbital nerve paresthesia after injury. Daily injections of 10 μg exenatide were compared with 2 mg/kg methylprednisolone for 14 days after surgery and nerve trauma. Findings indicated the absence of inflammation in peripheral tissues was significantly more prevalent in the methylprednisolone-treated group. Conversely, indicators

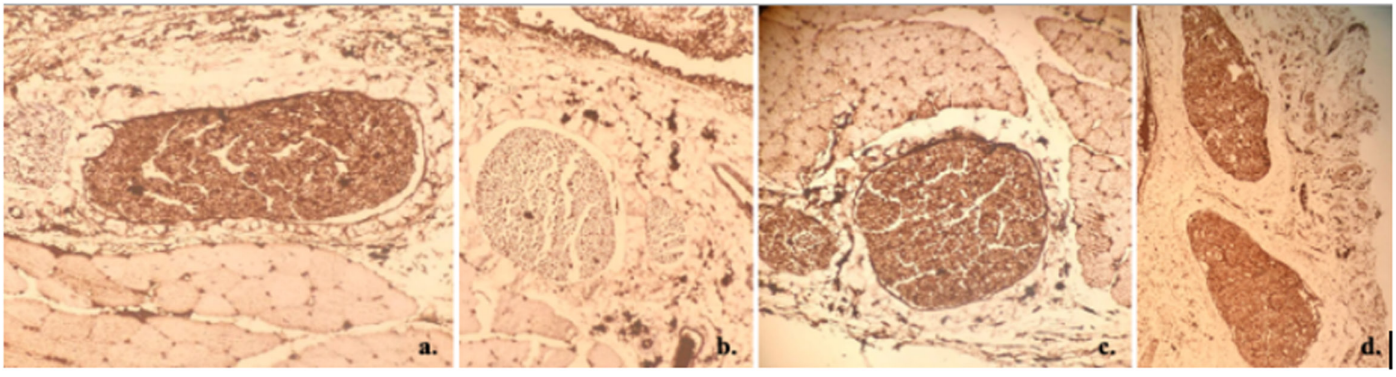


Figure 5. TNF-α immunohistochemical staining (×200). a. Control group b. Sham group c. 2.5 μg exenatide group d. 10 μg exenatide group. The Sham group shows negative staining, whereas the control, 2.5 μg exenatide, and 10 μg exenatide groups demonstrate diffuse strong TNF-α immunoreactivity.

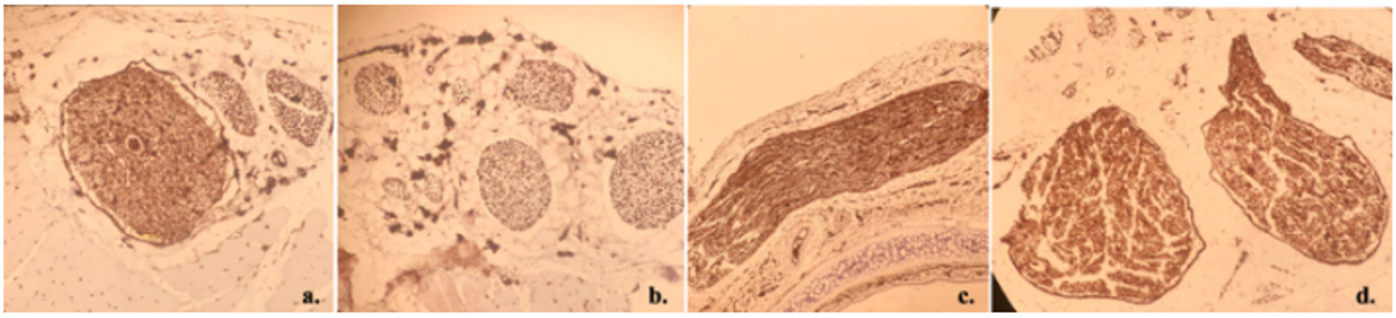


Figure 6. Caspase-3 immunohistochemical staining (×200). a. Control group b. Sham group c. 2.5 µg exenatide group d. 10 µg exenatide group. Similar to TNF-α staining, the Sham group exhibits negative staining, while the control, 2.5 µg exenatide, and 10 µg exenatide groups show diffuse strong Caspase-3 positivity.

like degeneration, intracellular inflammatory infiltrates, muscle damage, perineural fibrosis, granulation tissue, vascular proliferation, and perineural inflammatory cell infiltration were less common in the exenatide group.[13]

To demonstrate whether exenatide has an anti-inflammatory and anti-apoptotic effect, the extent and intensity of TNF-α and caspase-3 staining were evaluated. In the comparative analysis of these parameters, when the sham group was excluded, no statistically meaningful differences were identified among other groups. A statistically meaningful difference in diffuse staining was detected comparing nerve crush groups to the sham group. A comparison between the control group and exenatide-treated groups revealed a trend toward reduced severe staining and increased moderate staining in exenatide groups. However, although a difference was noted, it didn't reach statistical significance. Recent animal studies demonstrated pro-inflammatory cytokines, including TNF-α, play a significant role in pathological processes of injury-induced peripheral nerve damage.[14,15] Increased TNF-α expression and caspase-3 levels have been found after distal crush injury to rat dorsal root ganglia.[16] In a study by Sekiguchi et al,[17] proximal and distal crush injuries in the dorsal root ganglion of rats were compared. Results indicated damage to the distal region led to greater neuronal apoptosis and a marked rise in expression of TNF-α and caspase-3. A review of literature reveals no research specifically addressing the effects of Exenatide treatment on TNF-α and caspase-3 staining levels and intensities in crushed facial nerves.

The study has several limitations. Firstly, rat models may not accurately reflect the human condition. In addition, comparisons with steroid treatment were not made because the efficacy of exenatide in facial nerve palsy has never been studied. Furthermore, the rate of axon regeneration has been shown to be faster in rats than in humans.[18] Therefore, it is likely that EMG studies conducted in less than 2 weeks can provide more valuable data.

Exenatide, an FDA-approved molecule easy to administer, shows promise as a treatment for traumatic facial paralysis. Nevertheless, further research is needed to evaluate Exenatide's effectiveness compared to neuroprotective agents and steroid therapy, the most widely recognized treatment for traumatic facial paralysis. Additional studies are crucial to establish optimal dosage and treatment duration for exenatide in facial nerve injury.

CONCLUSION

In conclusion, Exenatide had a beneficial effect on the treatment of facial paralysis in an experimental rat model, improving both the rate and speed of facial nerve recovery. These findings clearly demonstrate that exenatide has promising potential as a neuroprotective agent for addressing facial paralysis.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Acıbadem Mehmet Ali Aydınlar University (Approval no: ACU-HADYEK 2021/21; Date: 12.03.2021).

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REFERENCES

1. Lindsay RW, Heaton JT, Edwards C, et al. Nimodipine and acceleration of functional recovery of the facial nerve after crush injury. *Arch Facial Plast Surg*. 2010;12(1):49-52. [CrossRef]
2. Lee LN, Lyford-Pike S, Boahene KD. Traumatic facial nerve injury. *Otolaryngol Clin North Am*. 2013;46(5):825-839. [CrossRef]
3. Rivera A, Raymond M, Grobman A, et al. The effect of n-acetyl-cysteine on recovery of the facial nerve after crush injury. *Laryngoscope Investig Otolaryngol*. 2017;2(3):109-112. [CrossRef]
4. Jang CH, Cho YB, Choi CH. Effect of Ginkgo biloba extract on recovery after facial nerve crush injury in the rat. *Int J Pediatr Otorhinolaryngol*. 2012;76(12):1823-1826. [CrossRef]
5. Yeh C, Bowers D, Hadlock TA. Effect of FK506 on functional recovery after facial nerve injury in the rat. *Arch Facial Plast Surg*. 2007;9(5):333-339. [CrossRef]
6. Kuyucu E, Gümüs B, Erbas O, et al. Exenatide promotes regeneration of injured rat sciatic nerve. *Neural Regen Res*. 2017;12(4):637-643. [CrossRef]
7. Yamamoto K, Amako M, Yamamoto Y, et al. Therapeutic effect of exendin-4, a long-acting analogue of glucagon-like peptide-1 receptor agonist, on nerve regeneration after the crush nerve injury. *BioMed Res Int*. 2013;2013:315848. [CrossRef]
8. Wang H, Fang F, Yi J, et al. Establishment and assessment of the perinatal mouse facial nerve axotomy model via a subauricular incision approach. *Exp Biol Med (Maywood)*. 2012;237(11):1249-1255. [CrossRef]

9. Seth R, Revenaugh PC, Kaltenbach JA, et al. Facial nerve neurorrhaphy and the effects of glucocorticoids in a rat model. *Otolaryngol Head Neck Surg*. 2012;147(5):832-840. [CrossRef]
10. Yildirim G, Kumral TL, Berkiten G, et al. The effect of coenzyme Q10 on the regeneration of crushed facial nerve. *J Craniofac Surg*. 2015;26(1):277-280. [CrossRef]
11. Toraman M, Külekçi Öztürk S, Uslu Coşkun B, et al. The effects of 4-aminopyridine and methylprednisolone on recovery of the facial nerve crush injury. *Eur Arch Otorhinolaryngol*. 2021;278(8):3057-3063. [CrossRef]
12. Şeneldir L, Gölcük VA, Tanyeri Toker G, Verim A, Güneş P. The Effectiveness of Ebselen in Facial Nerve Crush Injury: An Experimental Study. *J Int Adv Otol*. 2024;20(6):507-516. [CrossRef]
13. Abrishami M, Golestaneh A, Maleki L, et al. Efficacy of exenatide, ozone, and methyl prednisolone for the recovery of infraorbital nerve paresthesia after injury in rats. *Dent Res J (Isfahan)*. 2021;18:35. [CrossRef]
14. Sommer C. Zytokine bei neuropathischen Schmerzen Zytokine bei neuropathischen Schmerzen. *Anaesthesist*. 2001;50(6):416-426. [CrossRef]
15. Stoll G, Jander S, Myers RR. Degeneration and regeneration of the peripheral nervous system: from Augustus Waller's observations to neuroinflammation. *J Peripher Nerv Syst*. 2002;7(1):13-27. [CrossRef]
16. Kim SH, Nam JS, Choi DK, et al. Tumor necrosis factor-alpha and apoptosis following spinal nerve ligation injury in rats. *Korean J Pain*. 2011;24(4):185-190. [CrossRef]
17. Sekiguchi M, Sekiguchi Y, Konno SI, et al. Comparison of neuropathic pain and neuronal apoptosis following nerve root or spinal nerve compression. *Eur Spine J*. 2009;18(12):1978-1985. [CrossRef]
18. Celik B, Kara A, Guven M, et al. Effect of melatonin administration on nerve regeneration after recurrent laryngeal nerve injury. *An Acad Bras Cienc*. 2024;96(4):e20231149. [CrossRef]