Oxytocin mitigates peripheral nerve damage *via* Nrf2 and irisin pathway

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Abstract. – OBJECTIVE: Peripheral nerve injuries present challenges in achieving full functional restoration, necessitating effective therapeutic strategies. Oxytocin, known for its neuroprotective and anti-inflammatory properties, has shown potential in nerve recovery. This study aims to elucidate the role of oxytocin in nerve recovery *via* the nuclear factor erythroid 2-related factor 2 (Nrf2) and irisin pathways.

MATERIALS AND METHODS: Adult male Wistar rats (n=30) were subjected to surgical dissection of sciatic nerves and divided into Control, Surgery and Saline Group, and Surgery and Oxytocin (OT) group. Electromyographic (EMG) recordings, inclined plane tests, and histological assessments were conducted to evaluate nerve function, and Nerve growth factor (NGF) immunoexpression and axonal parameters were measured. Plasma irisin levels, nerve NGF, and Nrf2 levels were quantified.

RESULTS: The Surgery and Saline Group exhibited impaired EMG latency, amplitude, and inclined plane score compared to Controls, while the Surgery and OT Group demonstrated improved outcomes. Histomorphometric analysis revealed increased NGF immunoexpression, axon number, diameter, and reduced fibrosis in the Surgery and OT Group. Plasma irisin levels were higher following oxytocin administration. Additionally, nerve NGF and Nrf2 levels were elevated in the Surgery and OT Group.

CONCLUSIONS: OT administration mitigated nerve injury effects, promoting functional and histological improvements. Elevated NGF and Nrf2 levels, along with increased irisin, indicated the potential interplay of these pathways in enhancing nerve recovery. The results align with OT's neuroprotective and anti-inflammatory roles, suggesting its potential as a therapeutic intervention for nerve injuries. OT's positive impact on nerve recovery is associated with its modulation of Nrf2 and irisin pathways, which collectively enhance antioxidant defense and neurotrophic support and mitigate inflamma-

tion. These findings underline OT's potential as a therapeutic agent to enhance nerve regeneration and recovery. Further research is needed to elucidate the intricate molecular mechanisms and potential clinical applications of OT in nerve injury management.

Key Words:
  Peripheral nerve damage, Oxytocin, Nerve recovery.

Introduction

The vast network of nerves known as the peripheral nerve system (PNS) connects the central nervous system (CNS) and various parts of the body in a functional way[1,2].

Nerve defects can arise from various sources, including iatrogenic causes (related to medical or surgical interventions) and traumatic incidents[3,4]. A significant proportion of peripheral nerve injury (PNI) is correlated with unfavorable functional outcomes, inadequate nerve regeneration, and sensory and motor capabilities impairment. Subsequently, there is a subsequent occurrence of partial recuperation, muscular wasting, persistent discomfort, and significant debilitation[5]. Frequently, axons are required to undergo regeneration over considerable distances, progressing at a sluggish pace of 1-3 mm per day to reestablish connections and reach the motor endplates located distally[6]. Hence, the regenerative process requires significant time, particularly without any external intervention[7,8].

Various therapeutic strategies are currently available, such as surgical interventions, pharmacological treatments, and cell-based therapies, either independently or in conjunction with bio-

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materials in the shape of tube guides. However, despite the numerous treatment options, attaining an ideal result with complete functional restoration remains challenging.

For promoting nerve regeneration, pharmacological agents and immune system modulators have been studied[9-11]. Nevertheless, currently, there is a lack of pharmacological interventions in clinical practice that have been substantiated as efficacious in promoting nerve regeneration.

Oxytocin (OT), a neurohypophysis nonapeptide hormone, is relevant to social behavior across diverse species[12-14]. It is worth noting that OT was the first peptide hormone to have its structure elucidated[15]. The involvement of OT in the process of wound healing has been demonstrated through its ability to regulate stress responses. Moreover, many experimental studies have been documented in the existing literature, demonstrating the neuroprotective effects of OT. The neuroprotective effects of the OT hormone encompass various aspects such as social neuroprotection, resistance to oxygen-glucose deprivation, modulation of the immune system, anti-apoptotic properties, anti-inflammatory functions, and antioxidative capabilities[16-18].

Several molecules, including nerve growth factor (NGF), have demonstrated the ability to augment nerve regeneration and accelerate the axonal growth rate[19].

The nuclear factor erythroid 2-related factor 2 (NRF2) transcription factor pathway plays a crucial role in maintaining cellular redox balance by effectively counteracting the harmful effects of reactive oxygen species (ROS). Irisin is a myokine that is upregulated in response to exercise and has been shown to possess anti-inflammatory properties and potential benefits in the context of obesity. The regulation of glucose homeostasis is facilitated by irisin by converting white adipose tissue cells into brown adipose tissue cells, leading to energy expenditure. Irisin facilitated the upregulation of NRF2 expression. The activation of NRF2 leads to the upregulation of numerous genes involved in detoxification, cellular protection, and inflammation suppression. This is achieved by binding NRF2 to the antioxidant response element (ARE) located in the regulatory regions of these genes[20].

Although there are animal studies on the neuroprotective effect of OT, the mechanism of this effect is not fully known. In this study, we aimed to discover the nerve recovery effect of OT by NFR2 and irisin pathway.

Materials and Methods

Animals

The study utilized a sample of 30 adult male Wistar rats, with an average weight ranging from 200 to 210 grams. The animals were confined within enclosures and subjected to controlled environmental conditions, including a 12-hour alternation between light and darkness while being kept at a consistent room temperature of $22 \pm 2°C$. Throughout the study, the subjects were provided with a standard pellet diet and had unrestricted access to tap water. The Institutional Animal Care and Ethical Committee of the Demiroğlu Science University approved the study protocol with the assigned ethical number 23.09.2022/2723050720. The chemicals used in this study were sourced from Sigma-Aldrich Inc. (St. Louis, MO, USA) unless stated otherwise.

Experimental Protocol

In the study, thirty male Wistar rats were used. A total of 20 rats were selected as the experimental cohort for the study. Surgical dissection and subsequent repair of the sciatic nerves were conducted on each rat. A total of ten rats were selected to serve as the standard control group (n =10), and no surgical procedures or drug interventions were administered to this group. The experimental sample consisted of 20 rats divided into two groups: the Surgery and the Saline group and the Surgery and the OT group. The rats in both groups were randomly assigned to receive a placebo treatment, which involved the intraperitoneal administration of 1 ml/kg of a 0.9% sodium chloride solution. A group of rats, consisting of 10 individuals, were subjected to surgery and administered 0.1 mg/kg/day of OT (Synpitan Forte 5 I.U./Ml Deva Holding Corp. Istanbul, Turkey). The OT used in the experiment had a concentration of 10 Ü/ml and was administered intraperitoneally (i.p.). The administration of medications lasted 12 weeks. A motor function test was conducted after 12 weeks. Following the motor function test, electromyography (EMG) recordings were conducted.

After the study, all animals underwent euthanasia through cervical dislocation, following administration of anesthesia (Ketamin 100 mg/kg, Ketasol, Richterpharma AG Austria) and xylazine 50 mg/kg, Rompun, Bayer, Germany). Subsequently, blood samples were obtained *via*

cardiac puncture for biochemical analysis. Subsequently, sciatic nerve samples were collected to conduct immunohistochemical and biochemical analyses.

Electrophysiological Recordings

The rats underwent anesthesia using a combination of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg) (Alfasan International B.V. Holland). Electromyographic (EMG) studies were conducted on all groups after the study. The electromyography (EMG) data were collected from the right and left sciatic nerves on three occasions. The nerves were stimulated with supramaximal intensity (10 V) using a bipolar subcutaneous needle stimulation electrode (BIOPAC Systems Inc., Santa Barbara, CA, USA) placed at the sciatic notch. The stimulation parameters included a pulse duration of 0.05 ms, 1 Hz, and a frequency range of 0.5-5,000 Hz. The data were sampled at a rate of 40 kHz/s. Unipolar platinum electrodes were utilized to record compound muscle action potentials (CMAP) from 2-3 interosseous muscles. The data were analyzed using Biopac Student Lab Pro version 3.6.7 software (BIOPAC Systems Inc., Santa Barbara, CA, USA), with the parameters of distal latency and amplitude of compound CMAP. During the electromyography (EMG) recordings, the rectal temperatures of the rats were monitored using a rectal probe (H.P. Viridia 24-C, Hewlett-Packard Company, Palo Alto, CA, USA). The temperature of each rat was maintained at approximately 36-37°C using a heating pad. The experiments were conducted during the time frame of 10:00 a.m. to 14:00 p.m.

Assessment of Motor Function Via Inclined Plane Test

The motor performance of the rats was assessed using the inclined-plate test. In this experiment, the rat was positioned at an angle relative to the elongated axis of an inclined plate. The initial angle of inclination of the plate was 10 degrees. The incline angle was gradually incremented, and the highest angle at which the rat maintained its position for 5 seconds without falling was measured and recorded as the motor score. The angle of the inclined plate was measured three times in each rat to determine an average value.

Histology and Quantitative Histochemistry

The rats underwent intracardiac perfusion with a 4% formaldehyde solution to facilitate histological and quantitative immunohistochemical assessments. The distal segments located 10 mm away from the transected site of the sciatic nerves were excised. The paraffin-embedded sciatic nerves were sectioned into 5-micrometer slices using a microtome (Leica RM 2145, Nussloch, Germany) and stained with hematoxylin and eosin (H&E) to label the axons.

To ascertain the extent of fibrosis, the number of cells was quantified in a minimum of five randomly chosen regions. The calculation of the percent fibrosis score involved the division of the total cell count in the specific region by the count of enumerated cells.

Before conducting the immunohistochemical analysis, it was necessary to eliminate the endogenous peroxidase function of the samples by treating them with 10% H_2O_2 for 30 minutes. Following this step, the samples were blocked with normal goat serum (Invitrogen Thermo Fisher Scientific laboratory equipment NH, USA) at room temperature for 1 hour. Subsequently, the sections were incubated at a temperature of 4°C for 24 hours, utilizing a specific primary antibody (Santa Cruz Biotechnology, Dallas, TX, USA – diluted at a 1:100) targeting nerve growth factor (NGF). The rabbit immunoglobulin G-specific Histostain-Plus Bulk kit from Invitrogen (Carlsbad, CA, USA) was used to find antibodies, and 3,3'-diaminobenzidine (DAB) was used for further analysis. The slices were washed using phosphate-buffered saline and observed under an Olympus BX51 microscope (Shinjuku, Tokyo, Japan). The digital photographs were captured using an Olympus C-5050 camera (Shinjuku, Tokyo, Japan). The experimental procedure involved the utilization of quantitative immunohistochemistry on all experimental groups, with a total of six tissue slices obtained from each animal. Two researchers who were blind to the experimental conditions quantified the immune-positive Schwann cells and axons. This was achieved by employing a light microscope with varying magnifications of 20X. The data were presented as the mean value plus or minus the standard error of the mean.

The following parameters were quantified: the number of axons and the diameter of the axons. The cross-sections were acquired for these specific parameters by utilizing a digital counter with the assistance of a grid. This process was conducted in six randomly selected fields: one central and five peripheral fields. The magnification used for this procedure was set at 20X.

Nerve Biochemical Analysis

Following the sacrifice, both sciatic nerves were promptly excised and preserved at a temperature of -20°C until further biochemical analysis. The parts 10 mm from where the sciatic nerves were cut were mixed with a glass homogenizer and five times as much phosphate-buffered saline (pH 7.4). The resulting mixture was centrifuged at a force of 5,000 g for 15 minutes. Subsequently, the liquid portion of the mixture was gathered, and the overall protein concentration in the substances that were blended was assessed based on Bradford's technique, utilizing bovine serum albumin as a reference standard[21].

NGF and Nrf2 concentrations in the supernatants were quantified using commercially available Enzyme-Linked İmmunosorbent Assay (ELISA) kits designed for rats. Following the manufacturer's instructions, all animal samples were measured twice. The Absorbances were measured using a microplate reader (MultiscanGo et al. Laboratory Equipment, Ratastie, FI).

Measurement of Plasma Irisin level

Plasma irisin (Merck Sigma-Aldrich, Massachusetts, US) levels were measured using a commercially available ELISA kit (Abbott Laboratories, IL, USA). The plasma samples underwent a dilution of 1:2, and the irisin level was subsequently assessed in duplicate as per the instructions provided by the manufacturer.

Statistical Analysis

Statistical analysis was carried out with the SPSS 20 (IBM Corp., Armonk, NY, USA). The data are expressed as mean ± SD for each group. For multiple comparisons, one-way ANOVA was used. $p<0.05$ was considered statistically significant.

Results

The results of the electrophysiological and behavioral assessments are presented in Table I. The study involved the Normal Control Group, the Surgery and the Saline Group, and the Surgery and the OT Group.

EMG CMAP Latency (ms)

Compared to the Normal Control group (2.45 ± 0.08 ms), the Surgery and the Saline Group demonstrated a significant increase in CMAP latency (3.56 ± 0.16 ms, $p<0.05$). However, the Surgery and the OT Group showed no significant difference in CMAP latency (3.32 ± 0.09 ms) compared to the Normal Control group (Table I).

EMG CMAP Amplitude (mV)

The Surgery and the Saline Group exhibited a substantial decrease in CMAP amplitude (2.32 ± 0.07 mV) compared to the Normal Control group (13.1 ± 0.9 mV, $p<0.01$). In contrast, the Surgery and the OT Group demonstrated a partial restoration of CMAP amplitude (7.7 ± 0.3 mV) compared to the Surgery and the Saline Group ($p<0.05$) (Table I).

Inclined Plane Score (°)

The Surgery and the Saline Group showed a significant decrease in the inclined plane score (30.9 ± 2.6°) compared to the Normal Control group (86.4 ± 4.5°, $p<0.01$). Conversely, the Surgery and the OT Group displayed a significant improvement in the inclined plane score (65.3 ± 3.4°) compared to the Surgery and the Saline Group ($p<0.05$) (Table I).

These findings indicate that surgery and saline administration adversely affected the electrophysiological parameters (EMG CMAP latency and amplitude) and the behavioral outcome (inclined plane score). However, OT administration following surgery mitigated the adverse effects,

Table I. Electromyography and Inclaned plane scroe results.

	Normal control	Surgery and saline group	Surgery and oxytocin group
EMG CMAP latency (ms)	2.45 ± 0.08	3.56 ± 0.16*	3.32 ± 0.09
EMG CMAP amplitude (mV)	13.1 ± 0.9	2.32 ± 0.07**	7.7 ± 0.3#
Inclaned plane score (°)	86.4 ± 4.5	30.9 ± 2.6**	65.3 ± 3.4##

Measurements taken in different experimental groups related to surgery with oxytocine. Data are expressed as mean ± SEM. EMG: Electromyography, CMAP: compound muscle action potential. *$p < 0.05$ different from control group. **$p < 0.01$ different from control group. #$p < 0.05$ different from Surgery and Saline Group. ##$p < 0.01$ different from Surgery and Saline Group.

showing significant improvements in CMAP amplitude and inclined plane score compared to the Surgery and the Saline Group.

Figure 1 presents electromyography (EMG) recordings from the three study groups: A) the Control Group, B) the Surgery and the Saline Group, and C) the Surgery and the OT Group.

Control Group EMG

The EMG recording from the Control Group shows regular electrical activity with well-defined motor unit action potentials and consistent latencies and amplitudes (Figure 1).

Surgery and Saline Group EMG

In contrast, the EMG recording from the Surgery and the Saline Group displays altered electrical activity. The motor unit action potentials appear attenuated, and there is an observable increase in latency compared to the Control Group EMG (Figure 1).

Surgery and Oxytocin Group EMG

The EMG recording from the Surgery and the OT Group exhibits improvements in electrical activity compared to the Surgery and Saline Group. The motor unit action potentials show enhanced amplitudes, and latency is reduced compared to the Surgery and Saline Group EMG (Figure 1).

Table II presents the immunoexpression and histomorphometric analysis results in the three study groups: Normal Control, Surgery and Saline Group, and Surgery and OT Group.
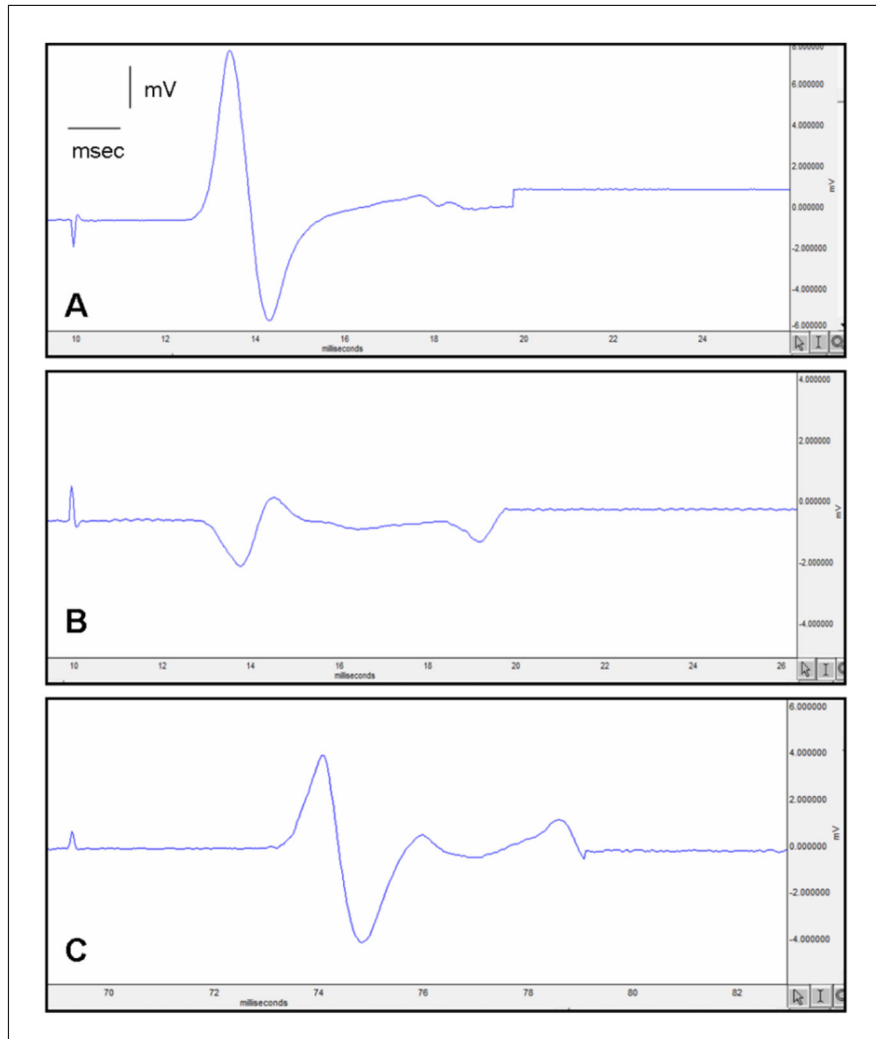

Figure 1. Electromyogram results. **A**, Control group EMG. **B**, Surgery and Saline Group EMG. **C**, Surgery and Oxytocin group EMG.

Table II. Axonal parametres of the groups.

	Normal control	Surgery and Saline group	Surgery and Oxytocin group
NGF immunexpression on Schwann cell (%)	88.9 ± 3.6	3.1 ± 0.8**	56.9 ± 3.3##
Total axon number	315.7 ± 10.2	19.3 ± 5.5 **	81.4 ± 6.2#
Axon diameter, μm	3.35 ± 0.09	1.79 ± 0.11*	2.88 ± 0.14#
Fibrosis score (%)	1.5 ± 0.3	82.1 ± 9.4**	13.1 ± 2.2##

Data are expressed as mean ± SEM. NGF: Nerve growth factor. *$p < 0.05$ different from control group. **$p < 0.01$ different from control group. #$p < 0.01$ different from Surgery and Saline Group. ##$p < 0.001$ different from Surgery and Saline Group.

NGF Immunoexpression on Schwann Cell (%)

Compared to the Normal Control group (88.9 ± 3.6%), the Surgery and Saline Group demonstrated a significant reduction in NGF immunoexpression on Schwann cells (3.1 ± 0.8%, $p<0.01$). However, the Surgery and OT Group showed partial restoration of NGF immunoexpression (56.9 ± 3.3%) compared to the Surgery and Saline Group ($p<0.001$) (Table II).

Total Axon Number

The Surgery and Saline Group displayed a substantial decrease in total axon number (19.3 ± 5.5) compared to the Normal Control group (315.7 ± 10.2, $p<0.01$). Conversely, the Surgery and OT Group exhibited an improvement in total axon number (81.4 ± 6.2) compared to the Surgery and Saline Group ($p<0.05$) (Table II).

Axon Diameter, μm

In comparison to the Normal Control group (3.35 ± 0.09 μm), the Surgery and Saline Group showed a significant reduction in axon diameter (1.79 ± 0.11 μm, $p<0.05$). On the other hand, the Surgery and OT Group displayed a partial recovery in axon diameter (2.88 ± 0.14 μm) compared to the Surgery and Saline Group ($p<0.01$) (Table II).

Fibrosis Score (%)

The Surgery and Saline Group exhibited a considerable increase in fibrosis score (82.1 ± 9.4%) compared to the Normal Control group (1.5 ± 0.3, $p<0.01$). However, the Surgery and OT Group demonstrated a significant reduction in fibrosis score (13.1 ± 2.2%) compared to the Surgery and Saline Group ($p<0.001$) (Table II).

Figure 2 displays representative images at 20x magnification, showing hematoxylin and eosin (H&E) staining along with NGF immunostaining in the three study groups: Normal Control, Surgery and Saline Group, and Surgery and OT Group.

Normal Control group

The images from the Normal Control group reveal intact nerve architecture with normal axon (a) and well-defined Schwann cells (arrow). NGF immunostaining shows robust expression, indicating healthy nerve tissue (Figure II).

Surgery and Saline Group

In contrast, the Surgery and Saline Group images demonstrate evident alterations. Increased fibrosis (f) indicates fibrotic changes in the nerve tissue. The axon, Schwann cells, and NGF immunoexpression appear significantly diminished (asterisk), suggesting impaired nerve regeneration and reduced neurotrophic factor expression following surgery with saline treatment (Figure 2).

Surgery and Oxytocin Group

The Surgery and OT Group images exhibit positive changes compared to the Surgery and Saline Group. There is an increase in the axon (a) density and well-preserved Schwann cells with NGF immunoexpression (arrow), indicating enhanced nerve regeneration and neurotrophic support with OT administration (Figure 2).

Table III presents the plasma irisin level, nerve NGF level, and nerve Nrf2 level results in the three study groups: Normal Control, Surgery and Saline Group, and Surgery and OT Group.

Plasma Irisin Level (pg/mg)

Compared to the Normal Control group (82.4 ± 1.5 pg/mg), the Surgery and Saline Group displayed a significant reduction in plasma irisin
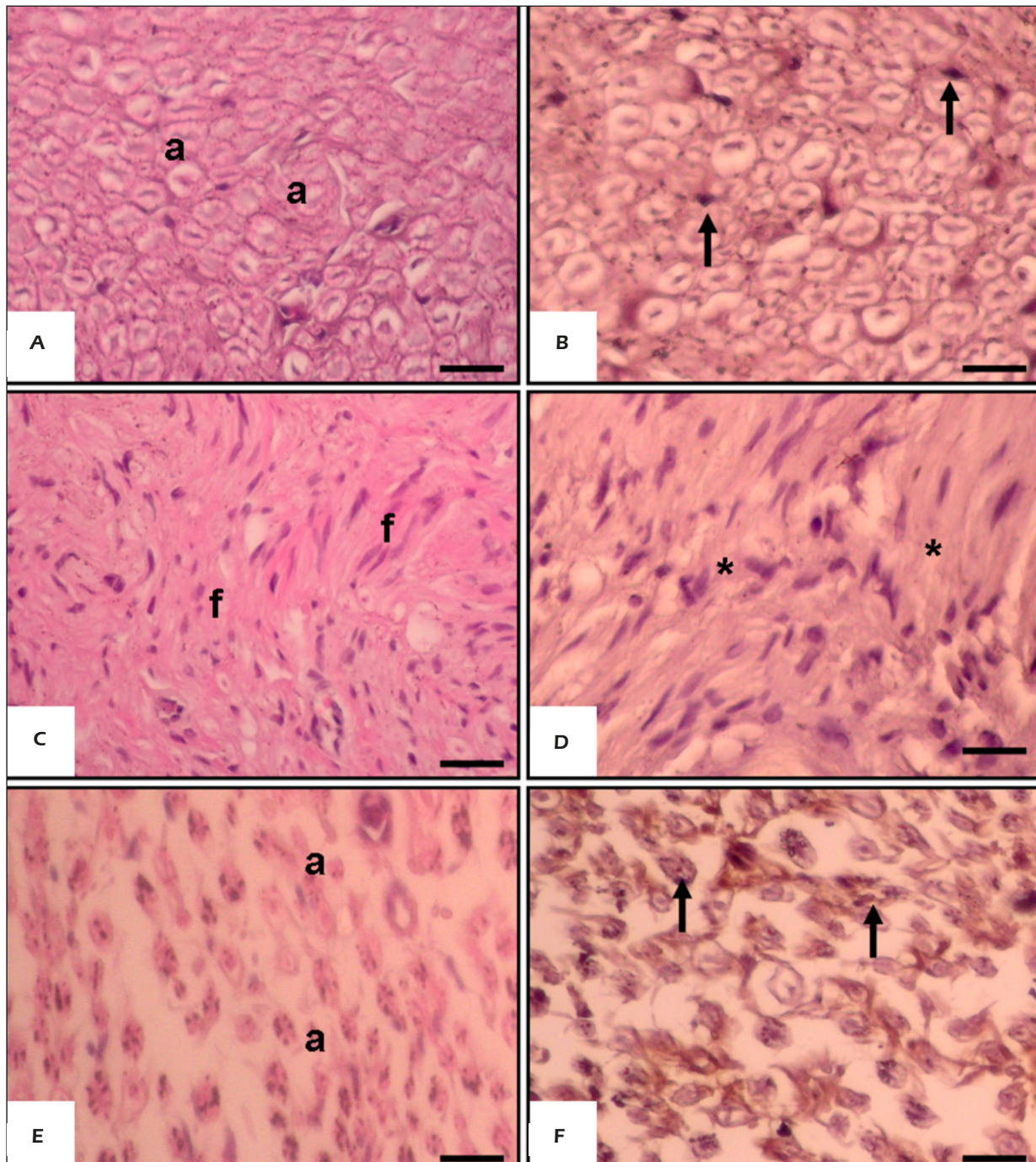
Figure 2. Histopathology of all study groups (×20 magnification). Hematoxylin & Eosine and NGF immunostaining. **A-B**, Normal Control group. Normal axon (a) and Schwann cell (*arrow*). **C-D**, Surgery and Saline Group. Increased fibrosis (f), very diminished axon, Schwann cell, and NGF immunoexpression (*asterisk*). **E-F**, Surgery and Oxytocin Group. Increased axon (a), Schwann cell, and NGF immunoexpression (*arrow*). Scale bar: 100 uM.

level (15.2 ± 0.7 pg/mg, $p<0.01$). However, the Surgery and OT Group demonstrated a partial restoration of plasma irisin level (46.8 ± 3.7 pg/mg) compared to the Surgery and Saline Group ($p<0.01$) (Table III).

Nerve NGF Level (pg/mg)

The Surgery and Saline Group exhibited a significant decrease in nerve NGF level (11.5 ± 1.6 pg/mg) compared to the Normal Control group (24.1 ± 1.03 pg/mg, $p<0.05$). Conversely,

Table III. Measurements taken in different experimental groups related to nerve injury and treatments with oxytocine.

	Normal control	Surgery and Saline group	Surgery and Oxytocin group
Plasma irisin level (pg/mg)	82.4 ± 1.5	15.2 ± 0.7**	46.8 ± 3.7##
Nerve NGF level (pg/mg)	24.1 ± 1.03	11.5 ± 1.6*	20.4 ± 0.5#
Nerve Nrf2 level (pg/g)	233.1 ± 4.5	115.9 ± 2.7**	182.3 ± 4.09##

Data are expressed as mean ± SEM. Nrf2: nuclear factor erythroid 2-related factor 2, NGF: Nerve growth factor. *$p < 0.05$ different from control group. **$p < 0.01$ different from control group. #$p < 0.05$ different from Surgery and Saline Group. ##$p < 0.01$ different from Surgery and Saline Group.

the Surgery and OT Group displayed a partial recovery in nerve NGF level (20.4 ± 0.5 pg/mg) compared to the Surgery and Saline Group ($p<0.05$) (Table III).

Nerve Nrf2 Level (pg/g)

In comparison to the Normal Control group (233.1 ± 4.5 pg/g), the Surgery and Saline Group showed a significant reduction in nerve Nrf2 level (115.9 ± 2.7 pg/g, $p<0.01$). On the other hand, the Surgery and OT Group demonstrated a partial restoration in nerve Nrf2 level (182.3 ± 4.09 pg/g) compared to the Surgery and Saline Group ($p<0.01$) (Table III).

Discussion

The study's results demonstrated that OT treatment following nerve injury played a pivotal role in improving electrophysiological parameters, such as EMG CMAP latency and amplitude and behavioral outcomes measured by the inclined plane test. These findings align with previous research suggesting that OT has a neuroprotective role in various injury models, including stroke and nerve injury[16,17,22,23]. The study results agree with Cho et al[24], who reported that OT alleviated cellular senescence and promoted neuroprotection through Nrf2 signaling. Additionally, the study's outcomes align with the findings of Zhu et al[23], who highlighted the neuroprotective effect of OT on vincristine-induced neurotoxicity. The improvement in motor function and electrophysiological parameters observed in the present study could be attributed to OT's anti-apoptotic, anti-inflammatory, and antioxidative capabilities, which collectively support nerve regeneration and recovery.

Wang et al[26] examined how OT affected autistic mice's behavior, oxidative stress, and inflammation. However, Stevenson et al[27] showed that OT prevents acute stress response dysregulation and glucocorticoid-induced oxidative stress. This suggests that OT may protect against oxidative stress. In early sepsis, Mehdi et al[28] suggested that OT and related peptide hormones could be effective as an anti-inflammatory treatment. This study suggests that OT may modulate the immune response, which could affect nerve healing by reducing inflammation. Nishimura et al[29] examined endogenous OT's anti-nociceptive and anti-inflammatory effects in rats. OT's anti-inflammatory effects may aid nerve recovery since inflammation slows healing. Gümüs et al[30] examined how OT affected nerve recovery in rat sciatic nerve damage models. OT improved nerve recovery functionally and histologically. This suggests OT may promote nerve regeneration and repair. Yuan et al[31] examined how OT inhibits LPS-induced inflammation in microglial cells and mice. This study highlights OT's anti-inflammatory effects in cells and animals. Szeto et al[32] examined inflammation-induced macrophage OT receptor regulation. Inflammation downregulated OT receptors, suggesting a negative feedback mechanism. This regulatory aspect may affect OT's effect on nerve recovery inflammation. Bale and Dorsa[33] explored the regulation of OT receptor gene transcription by NGF, cyclic AMP, and phorbol esters in cell lines. This study indicates a potential reciprocal relationship between OT and NGF signaling pathways. The fact that NGF can regulate the OT receptor gene transcription suggests a cross-talk between these two factors in cellular responses, which could be relevant in nerve recovery.

Furthermore, the histomorphometric analysis revealed that OT administration significantly increased NGF immunoexpression, total axon number, diameter, and reduced fibrosis score in the injured nerves. NGF is crucial in promoting axonal growth and nerve regeneration[19]. The results are consistent with studies that highlight

NGF as a potential therapeutic target to enhance nerve regeneration[9-11]. The correlation between OT and NGF levels could be attributed to OT's role in modulating cellular responses and promoting tissue repair, as supported by the findings of Condes-Lara et al[22] and Zhu et al[25], Bale and Dorsa[33] examined how NGF, cyclic AMP, and phorbol esters regulate OT receptor gene transcription in cell lines. This study suggests that OT and NGF signaling pathways may be reciprocal. The fact that NGF regulates OT receptor gene transcription suggests cellular responses between these two factors, which may be relevant to nerve recovery. Moreover, OT's positive impact on axonal density and Schwann cell preservation aligns with its known role in enhancing nerve regeneration[34]. The restoration of NGF levels and axonal parameters through OT administration substantiates its potential as a therapeutic intervention for peripheral nerve injuries.

In the context of the Nrf2 pathway, the study's results showed that OT treatment following nerve injury significantly increased nerve Nrf2 levels. This aligns with previous research indicating that Nrf2 activation is crucial for maintaining cellular redox balance and counteracting oxidative stress, thus promoting tissue repair[20]. The study results are consistent with Kobylinska et al[23], who suggested a potential association between OT and Nrf2 signaling pathways. The elevated nerve Nrf2 levels following OT administration indicate its role in enhancing the endogenous antioxidant defense mechanism, which likely contributes to the observed neuroprotection and tissue recovery.

Furthermore, the study highlighted the role of irisin in the context of nerve injury. The results indicated that OT treatment post-surgery led to an increase in plasma irisin levels. This finding is significant, as irisin has been associated with anti-inflammatory properties, metabolic regulation, and potential neuroprotective effects[23,25]. The study's outcomes are aligned with the observations of Wang et al[35], who demonstrated the potential of irisin in ameliorating lung ischemia/reperfusion injury and suppressing ferroptosis through the Nrf2/HO^{-1} signaling axis. The elevated irisin levels in response to OT administration suggest a possible crosstalk between OT and irisin pathways, which collectively contribute to neuroprotection and nerve regeneration.

Limitations
The mechanisms underlying the interactions between OT, Nrf2, irisin, and NGF need further

investigation, as the exact signaling pathways and molecular interactions remain fully elucidated. Moreover, the translation of findings from animal models to human patients requires careful consideration of potential differences in physiological responses.

Conclusions

This study demonstrates that OT administration following peripheral nerve injury mitigates the adverse effects on nerve regeneration and promotes neuroprotection. This effect might be attributed to OT's involvement in modulating the Nrf2 and irisin pathways, leading to enhanced antioxidant defense, neurotrophic support, and reduced inflammation. These findings suggest that OT could be a potential therapeutic agent for promoting nerve regeneration and functional recovery following peripheral nerve injury. Further research is warranted to unravel the precise molecular mechanisms underlying these effects and to translate these findings into clinical applications.

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Informed Consent
Not applicable.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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