The Evaluation of Estrogen and Tacrolimus on Experimental Sciatic Nerve Injury Following Bipolar Electrocautery in Animal Model

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Abstract

Iatrogenic peripheral nerve injuries (PNIs) cause neurogenic deficits because of limited regeneration potential of nerve and scar formation. This study evaluates effects of tacrolimus and estrogen on sciatic nerve healing following its lesion by bipolar electrocautery in rat. Twenty-five mature female Wistar rats were included into this study. The rats kept in same photoperiod twelve-twelve for one week. The rats divided into five groups as follow: Sham, DW (distilled water), Tacrolimus (Tac), Estrogen (Est), and Tacrolimus + Estrogen (Tac + Est). All rats anesthetized and sciatic nerve of left sciatic nerve was cauterized by bipolar-electrocautery except of rats in sham group. Treatments were given for 28 days after the injury; on day 28 clinical, electrophysiological, and histopathological evaluations were carried out. Rota-rod performance test, sciatic functional index (SFI), electromyography (EMG) latency and toe out angle (TOA) were carried out for evaluation of functional nerve recovery. Finally, the rats were killed humanly and sample of sciatic nerve tissues were submitted for histopathological studies on day 28. There did not find significant difference statistically in SFI ($p = 0.249$) among all groups. On rota rod tests, the Est group showed significant motor function improvement than the DW (distilled water), Tac, and Est + Tac groups ($p < 0.01$). Mean EMG latency in DW group was significantly longer than sham ($p < 0.001$), Tac ($p = 0.023$) and Est + Tac ($p = 0.012$) groups. Axonal swelling and inflammatory cell infiltration were less in the Tac and Est and Est + Tac groups to DW group ($p < 0.01$). There was no significant difference among Tac, Est, Est + Tac groups in EMG latency. Therefore, tacrolimus and estrogen solely showed neuroprotective role

based on histopathological results. Motor function improvement and less inflammation were significant statistically in Est and Tac groups, respectively. The finding of this investigation did not confirm the significant impact of the combination of estrogen plus tacrolimus in comparison to estrogen group and tacrolimus group in functional recovery and inflammation.

Keywords: Sciatic nerve, Estrogen, Tacrolimus, Electrocautery, Rat

1. Introduction

Iatrogenic peripheral nerve injuries (PNIs), a serious condition frequently leading to long-term functional deficits even with improvements by surgical techniques, were become a major problem in neurology of medicine and veterinary medicine (1). Scar formation often limits functional recovery by blocking the process of neural regeneration and myelination (2). The type and degree of the damage, patient age, lesion location, acute or chronic trauma, surgical techniques, and biomaterials and so on used in the healing process determine the outcome of peripheral nerve restoration (1). The main causes of these injuries are motor vehicle accidents, occupational injuries, trauma connected to combat, iatrogenic surgical procedures as electro-cauterization, anesthesia problems, and in tumor resections (3). Electrocautery is sometimes used to destroy nerves in the treatment of chronic sensory nerve pain that does not respond to medication.

Although PNIs are not fatal, they greatly lower patients' quality of life. Many therapeutic agents and procedures have been investigated to improve nerve regeneration and reduce scar formation (4). Recent researches have led to promising development in tissue engineering, 3D biomaterials, gene therapy, stem cell research, and pharmacological approaches and surgery (5). Despite many achievements, complete restore motor, sensory, or autonomic function has not yet been achieved (5). Medicines have always been the focus of attentions due to their lower cost and economic considerations.

Tacrolimus and estrogen are two medicines which are of interest due to their neuroprotective and neuroregenerative properties (6). Tacrolimus widely used in immunosuppressive treatment for

organ transplantation, it has also a neuroprotective role (6). Tacrolimus does its role via increasing axonal regeneration, stimulation of schwann cell proliferation, and acceleration of nerve elongation (7). Estrogen as an anabolic hormone has neuroprotective effect by controlling gene expression, improving neuronal stability, preserving neurons and also glial cells after trauma, and acceleration angiogenesis and neurogenesis (8). Although many studies on the effects of these drugs are being conducted, none of them have investigated their combined effect on functional and histopathological changes in experimental model of peripheral nerve damage by bipolar electrocautery. The aim of this study was to evaluate the effects of estrogen and tacrolimus administration alone or in combination on sciatic nerve regeneration and functional recovery following experimental bipolar electro-cauterization of the nerve in rat.

2. Materials and Methods

All experimental procedures were approved by the Research Ethics Committee of the Islamic Azad University, Science and Research Branch (Approval ID: IR.IAU.SRB.REC.1401.354).

2.1. Animals

Twenty-five female Wistar rats weighing 250 ± 25 g were conducted in this study. The animals were housed in specialized rat cages for acclimatization. They were kept under controlled conditions, including a stable temperature of 23 ± 3°C, humidity of 50 ± 5%, and a 12:12-hour light-dark cycle. All animals had ad libitum access to fresh tap water and a standard rat pellet diet (Behaver Co., Tehran, Iran).

2.2. Surgical Procedure

All rats were randomly divided into five groups as follows: sham, distilled water (Dw), estrogen (Est), tacrolimus (Tac), and estrogen + tacrolimus (Tac+ Est) groups. All rats were anesthetized using a combination of medetomidine (90 µg kg^{-1}, Syva, IM, Leon, Spain) and ketamine 10% (20 mg kg^{-1}, IM, Alfasan, Warburg, Germany). After anesthesia induction, left pelvic limb of all rats were clipped and prepared surgically with antiseptic agent. After positioning on right lateral recumbency, a two cm skin incision was made on lateral side of left pelvic limb. Then biceps femoris muscle dissected for sciatic nerve exposure under an operative microscope (Topcon

OMS 90, Tokyo, Japan). Three watts power snap touch of the left sciatic nerve of interested limb was cauterized using the bipolar electrocauthery mode (Kavandish System Company, Tehran, Iran) exception of rats of sham group. A 6-0 nylon suture (Suppa, Tehran, Iran) was placed on the biceps femoris fascia adjacent to the lesion to mark the injury site. Then muscular layer and skin were sutured with 5-0 polyglactin 910 and 5-0 nylon suture materials (Suppa, Tehran, Iran) by simple continues and simple interrupted patterns, respectively. Meloxicam (1 mg/Kg, s.c., Razak pharmaceutical labs Co., Tehran, Iran), and enrofloxacin (10 mg/Kg, i.m., laboratorios HIPRA, Girona, Spain) were injected post-operatively to all rats for one and three days as pain killer and antibiotic therapy, respectively. From day zero to 28, rats of the sham group did not receive any medicine; DW group received distilled water (s.c. Shahid Qazi, Tabriz, Iran), in the same volume as estrogen, Est group received estrogen (4 mg/kg, s.c., Tran hormone, Tehran, bran); Tac group received tacrolimus (5 mg/kg, p.o., Zahravi phormaceutical Co., Tehran, Iran) and Est + Tac group received estrogen and tacrolimus simultaneously as the same route and dosage.

2.3. Rota-Rod Performance Test

Rota-rod apparatus is for assessment of motor disabilities after functional recovery (Tajhiz gostar omid Iranian, Tehran, Iran). This test was carried out on day 28 post surgically. This device consists of four rods (9 cm width, 6 com diameter of a rod and 20 cm height) which were separated with a plastic sheet. The machine speed was adjusted on 15 rotate per minute and the test was measured during 3 minutes. The third time of falling of every rat was recorded by a sensor under the rods and then times were compared with each other.

2.4. Sciatic Functional Index (SFI)

SFI was performed on day 28 to evaluate motor functional recovery. For SFI assessment, the pelvic paws of the rats were coated with ink and walked along a 100 cm corridor lined with white paper. At least three clear footprints of each rat were recorded, and paw print measurements were utilized to calculate SFI as below formula: SFI= -38.8[EPL-NPL/NPL] + 109.5 [ETS-NTS/NTS] + 13.3 [EIT-NIT/NIT] – 8.8 in which; EPL is experimental (injured) paw length, NPL is normal paw length, ETS is experimental toe spread (distance between the first and fifth toes), NTS is normal toe spread, EIT is experimental intermediary toe spread (distance

between the second and fourth toes) and NIT is normal intermediary toe spread. SFI of zero indicates normal function, while an SFI of -100 represents complete loss of function.

2.5. Toe Out Angle (TOA)

External rotation of the leg is assessed with TOA and it can show functional improvement of the sciatic nerve. The rats were placed of a glass (30×21 cm) and limited from around to limiting more movements. The plantar aspect of paws was taken photo by a camera (Apple iphone 12 Pro Max-United state) which was fixed under the glass and in defined distance. Then the angle between direction of progression and a reference line was measured. A reference line is anatomically from the calcaneus to the tip of the third digit. Angles of affected and ipsilateral limb were recorded and compared between groups and in a group.

2.6. Electromyography

On day 28, the rats were sedated and affected sciatic nerve of left pelvic limb with mentioned procedure in surgical method was exposed, then the curved stimulus electrode (e Pulse, science beam, Tehran, Iran) was located about 10 mm proximal of trifurcation of sciatic nerve of the cauterized site which was determined by 6-0 nylon suture material as marker. The distal part of the electrode inserted longitudinally into the belly of the extensor digitorum longus muscle (EDLM) and the proximal part of the electrode inserted longitudinally into the belly of the gastrocnemius muscle. Characters of stimulating waves contain 1000 mA amplitude, 0.2 Hz frequency during 100 second and 20 times stimulation with the electrodes (e Wave, science beam, Tehran, Iran). Compound muscle action potential (CMAP) recording in the gastrocnemius muscle which contain tibial nerve compartment and CMAP recording in the extensor digitorum longus muscle which contain fibular nerve compartment after this stimulation was saved by computer program (e Trace analysis, Tehran, Iran). Latency time (millisecond) is the time between the stimulation of the nerve and the response of the muscle. Its delay response indicates the high level of damage and low healing of the nerve, and also the rapid response of this time indicates the low level of damage and high level of nerve regeneration. In all groups, electromyograms were recorded and the latency time was compared among them.

2.7. Histopathological Evaluation

All rats of each group were euthanized on day 28 using an overdose of anesthetic, humanely. The sciatic nerve of all rats was excised for histopathological analysis. All tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at a thickness of 6 µm at the lesion site for Hematoxylin & Eosin (H&E), masson's trichrome and toluidine blue stainings.

Perineurium formation, axonal swelling, axon count, inflammatory cell infiltration were studied by two blinded pathologists.

2.8. Statistical Analysis

All data were recorded as mean ± standard deviation (SD). Data were analyzed using SPSS software (version 26.0, Chicago, Illinois, USA). The comparison between groups was analyzed using one-way ANOVA, and statistical significance was determined at $p < 0.03$.

3. Results

All rats tolerated anesthesia and survived till the end of study. No infection and wound dehiscence were observed in surgical site. Neurological deficit was seen in left pelvic limb of all rats expect rats in sham group once after bipolar electro-cauterization. Significant muscular atrophy was observed in left pelvic limb of all rat expect of sham group in comparison to right pelvic limb.

3.1. Rota-Rod Performance Test

The one-way ANOVA revealed a statistically significant difference among the studied groups in terms of the rota-rod value ($F_{(4, 20)} = 163.26$, $p < 0.001$). Subsequent analysis using the Games-Howell test showed that the mean rota-rod performance in the sham group was significantly greater than in the other groups ($p < 0.001$). Furthermore, the mean rota-rod performance in the Est group was significantly greater than in the DW, Tac, and Est + Tac groups ($p < 0.01$). No significant differences were found in the other comparisons ($p > 0.05$) (Figure 1).

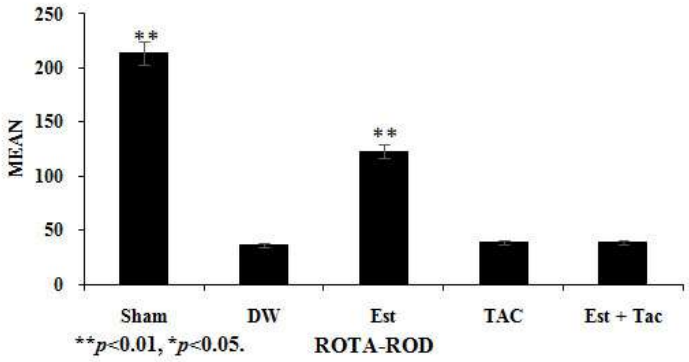
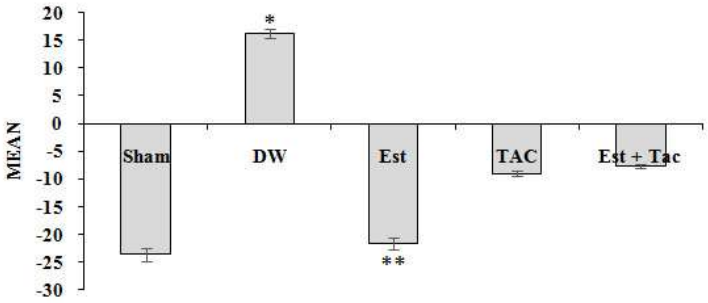


Figure 1. Comparison of mean rota-rod values among the studied groups. The sham group had significantly greater rota-rod performance than the other groups ($p < 0.01$). The mean performance of the Est group was significantly higher than the DW, Tac, and Est + Tac groups ($p < 0.01$).

3.2. Sciatic Functional Index (SFI)

Based on the data from the one-way ANOVA indicated no significant difference among the studied groups regarding the SFI value ($F_{(3,16)}= 1.51$, p=.249), statistically. Furthermore, the Games-Howell test analysis indicated that the mean SFI value in the DW group was



significantly higher compared to the sham group ($p = 0.021$) and the Est group ($p = 0.032$) (Figure 2).

Figure 2. Comparison of mean SFI values among the studied groups. The mean SFI in the DW group did not show any significant difference from the sham group and the Est group ($F(3,16)= 1.51$, p=.249).

3.3. Toe Out Angle (TOA)

The ANOVA test result indicated no significant difference statistically between the TOA means across the studied groups ($F_{(4, 20)}= 0.47$, $p = 0.755$) (Table 1).

Table 1. ANOVA results for comparing TOA in the study groups

variable	Sham n=5 M ± SD	DW n=5 M ± SD	Est n=5 M ± SD	TAC n=5 M ± SD	Est + Tac n=5 M ± SD	ANOVA	Post Hoc †
TOA	26.5 ± 13.01	20.00 ± 3.53	22.60 ± 5.77	18.5 ± 22.81	27.80 ± 10.87	$F_{(4,20)}$=.47, P=.755	Sham = DW Sham = Est Sham = Tac Sham = Est + Tac DW = Est DW = Tac DW = Est + Tac Est = Tac Est = Est + Tac Tac = Est + Tac

Note. M=Mean, SD=Std. Deviation.
†= pairwise comparison for five groups; if the Leven's test for homogeneity of variance was significant, Games-Howell was used as the post-hoc test; if not, Bonferroni was used

3.4. Electromyography (EMG)

The one-way ANOVA results revealed a statistically significant difference among the studied groups in term of the EMG latency value ($F_{(4,20)}$=21.15, p<.001). The data of the Games-Howell test indicated that the mean EMG latency in the DW group was significantly higher than that of the sham group (p < 0.001), the Tac group (p = 0.023), and the Est + Tac group (p = 0.012). Furthermore, the mean EMG latency in the Est group and the Est + Tac group was significantly longer than the sham group (p < 0.05) (Figure 3).

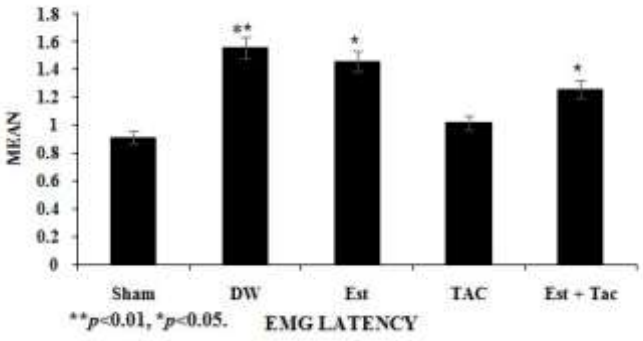


Figure 3. Comparison of mean EMG latency values among the studied groups.

3.5. Histopathological study

The perineurium is fully regenerated in all treatment groups, with no statistically significant differences in its regeneration among the groups (p=1.00). No inflammatory cells were detected in any of the rats in sham group. Rats in DW group showed a moderate grade of inflammatory cells, with prevalence between 50% and 75%. In the Est group, a mild grade of inflammatory cells (between 25% and 50%) was observed in three rats, while two rats exhibited a very mild grade (less than 25%). Similarly, in the Tac group, two rats exhibited a mild grade (between 25% and 50%) of inflammatory cells, and three rats showed a very mild grade (less than 25%). For the Est + Tac group, a moderate grade of inflammatory cells was noted in two rats, and a very mild grade (less than 25%) was observed in three rats. The results from the Mann-Whitney U test demonstrated that the grade of inflammatory cells in the sham group was significantly different compared to the other groups ($p < 0.01$). No axonal swelling was observed in any of rats in the sham group. In the DW group, moderate axonal swelling (between 50% and 75%) was identified in three rats, while mild swelling (between 25% and 50%) was detected in two rats. In the Est group, three rats exhibited mild axonal swelling (between 25% and 50%), and two rats showed very mild swelling (less than 25%). In the Tac group, mild axonal swelling (between 25% and 50%) was observed in two rats, and very mild swelling (less than 25%) was seen in three rats. Lastly, in the Est + Tac group, mild axonal swelling (between 25% and 50%) was noted in three rats, whereas very mild swelling (less than 25%) was present in two rats. The Mann-Whitney U test indicated that the sham group demonstrated significantly lower levels of axonal swelling compared to the other groups ($p < 0.01$). In terms of the average number of axons per field, all rats in the sham group showed a count similar to that of the normal nerve. The average number of axons in all five rats in the DW group was 50% of the normal nerve. For the Est and Tac groups, the average axon count was 75% of the normal nerve. In the Est + Tac group, the average number of axons was 50% in two rats and 75% of the normal nerve in three rats (Figure 4-6).

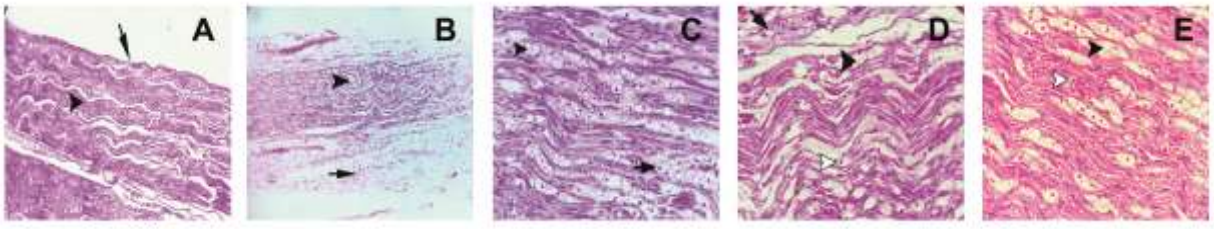


Figure 4. Longitudinal section of the sciatic nerve with hematoxylin and eosin staining, A. Sham group, perineurium (arrow) and axon (arrowhead) with normal order and without swelling (100X), B. DW group, severe swelling of axons (arrowhead) and severe inflammatory reaction (arrow) (100X), C. Est group, axon swelling (arrowhead) and inflammatory cells (arrow) (400X), D. Tac group, swelling of axons (black arrowhead), inflammatory cells (white arrowhead) and perineurium (arrow) (400X), E. Est + Tac group, swelling of axons (black arrowhead), inflammatory cells (white arrowhead) (400X).

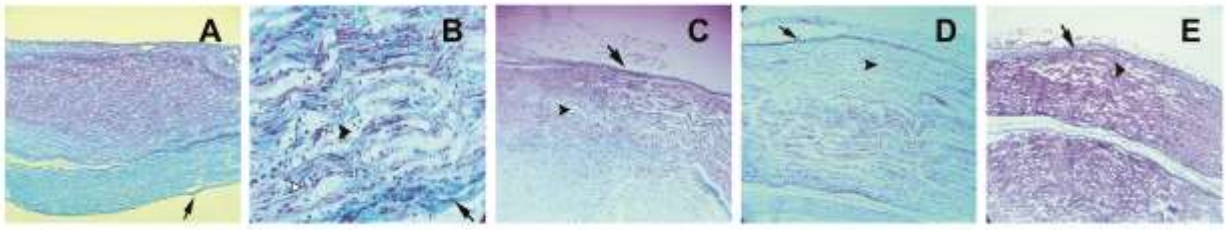


Figure 5. Longitudinal section of the sciatic nerve with masson's trichrome stain, A. sham group, normal epineurium (arrow) (100X), B. DW group, severe swelling of axons (black arrowhead), inflammatory cells (white arrowhead) and perineurium (arrow) (400X), C. Est group, significant swelling of axons (arrowhead) and normal perineurium (arrow) (100X), D. Tac group, significant swelling of axons (arrowhead) and normal perineurium (arrow) (100X), E. Est + Tac group, mild swelling of axons (arrowhead) and normal perineurium (arrow) (100X).

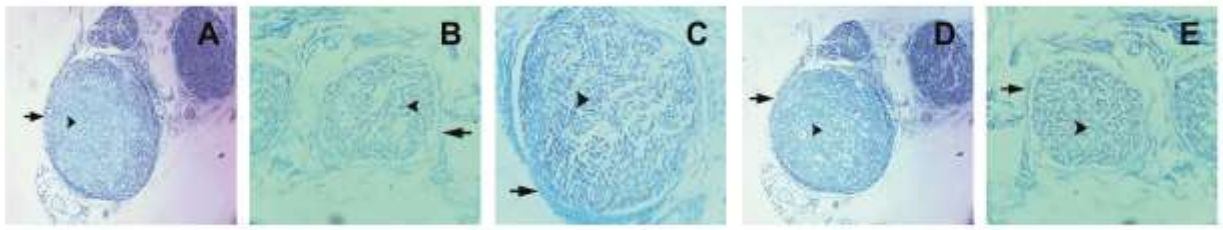


Figure 6. Cross section of the sciatic nerve with toluidine blue stain (100 X), A. sham group, perineurium (arrow) and axons (arrowhead) with normal order, B. DW group perineurium (arrow) and axons (arrowhead) less than normal, C. Est group, perineurium (arrow) and axons (arrowhead) less than normal, D. Tac group, perineurium (arrow) and axons (arrowhead) less than normal, E. Est + Tac group, perineurium (arrow) and axons (arrowhead) less than normal.

4. Discussion

Peripheral nerve injury happens in sequel of car accident, falling of height, injection of medicine, stretching of nerve plexus, iatrogenic or inadvertently by surgeon following ligation or electrocautery and so on (3). Following these causes, nerve suffered from loss of conduction of electrical impulse to organs and limbs (3). Bipolar-electrocautery of nerve during surgery is one of common cause that leads to neurologic deficit, inadvertently (9). Nerve damage caused by electrocautery is associated the generation of heat in nerve structure and peripheral tissues; and its pathogenesis is nearly different from neuron lesion by crushing that disrupts just myelin sheaths and axonal structure in various levels. Heat or thermal nerve injury is tissue irritation with multiple causes as iatrogenic (inadvertently) bipolar electrocautery (9). Nerve damage occurs in two general categories as primary and secondary lesions. Nerve is damaged by direct thermal injury (primary lesion), electro-chemical changes and damages, structural disruption of neuron and finally secondary injury cascade which happens after primary lesion (10). The difference in the degrees of injury following electerocautery and crushing is related to these mentioned mechanisms. Protein degeneration and axonal microtubules and neurofilaments coagulation, myelin sheath destruction and intracellular water loss, insufficiency of Na^+/k^+ neuron pumps, wallerian degeneration, scar formation as neuroma and glioma, thrombosis of arterioles and venules and finally prostaglandins and cytokines released in injury site and edema formation are the main pathologic changes of neuron following thermal injury by bipolar electrocautery (10). Our study was designed to reveal the neuroprotective effect of conjugated estrogen (Est) and tacrolimus (Tac) on sciatic nerve injury induced experimentally by bipolar electrocautery in a rat model. Authors of the present study chose conjugated Est and Tac based on their known neuroprotective and immune-modulatory effects on tissues (11). Despite known effect of Est and Tac, there is a paucity of data about their combined therapeutic impact on nerve

lesions, particularly following nerve injury by bipolar electrocautery. The authors presumed combination of Est and Tac could enhance functional improvement and histopathological recovery in female rat model with sciatic nerve injury induced by bipolar electrocautery. To evaluate the functional recovery of the sciatic nerve, the sciatic functional index (SFI) was one of the most known test for this purpose. Farahani's research revealed that significant functional improvements following administration of a combination of Tac and Est on crushed sciatic nerve injury in mice (1), while we found no significant change in SFI score, indicating that the functional deficits observed in the injured nerve by bipolar electrocautery did not show significant repair during 4 weeks. Jazinidorche et al; in other study showed anabolic agent as testosterone had positive effect on SFI index following experimental crushed sciatic nerve injury in rats (12). Their study agrees with our findings following estrogen administration on in SFI index in rats. Bipolar electrocautery could probably induce a different pattern of nerve damage. Although in this experimental study, we did not examine the extent and severity of nerve damage caused by bipolar electrocautery from a histopathological point of view, the results of the study over a 4-week period indicate that nerve damage caused by electrocautery can be more severe and extensive than the results of other studies on nerve damage following crushing. As stated earlier that thermal damage caused by electrocautery may result in a variety of more intricate pathophysiological processes, severe thermal burn of surrounding tissues and disruption of the blood-nerve supply, which can cause delay in repair (13).

According to the findings of the rota-rod performance test, our finding suggested that estrogen may have a positive role in enhancing motor coordination in rats. In comparison to estrogen, tacrolimus as immunosuppressive agent does not have direct and significant role on nerve regeneration and repair (14). Tacrolimus role on peripheral nerve regeneration is via its immune-modulatory effects, while estrogen plays significant role in regulating motor nerve function in the short term.

The EMG latency and TOA tests provide valuable information in DW group in the electroneurophysiology. The EMG latency test revealed significant alterations in nerve conduction properties following injury. Results showed significant prolonged latency in compared to the sham, Est, Tac, and Est + Tac groups. The correlation between Est and its role on motor function has been previously reported that a decline in serum estrogen level has critical

role in developing peripheral neuropathy in post-menopausal women (15). However, to the best of our knowledge, this study revealed significant difference in EMG latency following administration of tacrolimus in comparison to the other groups in our study. Although tacrolimus and estrogen reduced latency compared to the DW group, but they were unable to improve nerve conduction as much as rats in sham group. This finding suggests that these agents may have some neuroprotective effects, but they are insufficient to fully restore nerve function in the short term. We found a similar result in the TOA test. In contrast to the DW group, toe out angle improved partially after treatment in Tac, Est, and Est + Tac groups, but there was not significant difference statistically. This indicates that the combination of these drugs is not able to fully restore nerve function. The EMG and TOA findings demonstrated that nerve damage and its recovery despite considerable advances in therapy have not yet enabled complete recovery of damaged nerve following injury (16). Histopathological study showed new approach about the effects of estrogen and tacrolimus or their combination on regeneration and repair of the tissue. Inflammation cell infiltration, swelling of the axons, and density of the axons number were investigated at the end of our study. Perineurium regeneration was the same in all groups, with no statistical difference between the treatment and DW groups, which indicates that estrogen and tacrolimus had no effect on perineurium integrity. This finding confirmed the previous findings that have shown perineurial regeneration occurs in the early stage following nerve injury and to be less sensitive to treatments intended to be neuroprotective or to regenerate (17). Inflammatory cell infiltration was greatly different among groups. Inflammatory cells were not seen in sham groups at the end of the study, this is while the presence of inflammatory cells was intense and significant in the other groups. Tacrolimus, estrogen and its combination reduced inflammation. This reduction is in agreement with previous studies showing that anti-inflammatory activity of tacrolimus could decrease inflammation and provide a more suitable environment for nerve regeneration (18). Interestingly, the Est group showed a moderate level of inflammation, significantly more than the sham group and less than the DW group. This finding implies that estrogen may be have anti-inflammatory effect, but could not suppress inflammation in the early phases of nerve repair. Estrogen has been shown to have two effects: one is to reduce oxidative stress and the other modulates cytokines. However, estrogen effectiveness in inhibitory inflammatory response following nerve damage is not strong as much as the other anti-inflammatory agents as NSAID or corticosteroids (19). Tacrolimus have a more anti-

inflammatory property by inhibiting the activation of immune cells involved in the inflammatory cascade (20).

The effectiveness of tacrolimus is associated to the inhibition of immunity intervention and its neurotropic role. In our study, axonal swelling was observed in all groups except the sham group, which had no swelling. The DW group showed moderate swelling, reflecting tissue damage and lack of recovery of untreated nerve damage. Both tacrolimus and estrogen admistrations in Est, Tac and Est + Tac groups reduced axonal swelling compared to the DW group but cannot reduce swelling as much as the sham group. Our findings are consistent with Mansouri's study following 5 mg kg^{-1} tacrolimus administrated orally in crushed sciatic nerve in male mice. They showed that this dose of tacrolimus could reduce axonal swelling to the control group who did not receive tacrolimus (2). The studies from the past till now have shown that tacrolimus and estrogen have both neuroprotective characteristics by antioxidant and anti-inflammatory properties (14). The presence axonal swelling in the groups received estrogen and tacrolimus and their combination was explained that either the dosage and amount of the drugs were not appropriate or the duration of drug use was short to achieve acceptable results, since the anti-inflammatory and immunosuppressive properties of these two drugs have been confirmed in terms of nerve repair and regeneration.

We found interesting histopathological findings related to axonal density and count in this study. The sham group had axon counts like in a normal nerve. In contrast, the DW group had a significant reduction in axon density which indicated that distilled water is not capable of nerve regeneration after injury by bipolar-electrocautery. In this study, it was found that estrogen and tacrolimus were able to increase the number and density of axons compare to DW group based on histopathological evidence. However, the combination of these two medicines, like Est and Tac groups did not show acceptable results. These results were in accordance with previous research that has stated tacrolimus and estrogen abilities to promote nerve regeneration and neuroprotection by enhancing axonal survival, respectively (20). The difference in the amount of experimental nerve damage caused by crushing, ligation and electrocautery due to reversible or irreversible damage has been noted in many studies (21-22). The heat caused by electrocautery certainly destroys the myelin covering of nerve axons and, on the other hand, causes destruction

of the endoneurium of the sciatic nerve. If the irritation with electrocautery does not involve the endoneurium, there is a possibility of 90% recovery within 4 to 12 weeks (9-10).

According to the results of our study and the recovery of sciatic nerve function after four weeks of estrogen administration in rats in this group, it can be concluded that sciatic nerve irritation with bipolar electerocautery at intensity of 3 mA cannot damage the endoneurium layer. Because, according to the study of the other researches, the lack of recovery of sciatic nerve functions within four weeks in evidence that the nerve damage has reached the endoneurium layer by bipolar electerocautery.

Since there are few studies on the treatment of experimental sciatic nerve injuries following bipolar electrocautery compared to experimental nerve crush, further study is needed to substantiate the results of this study in order to justify the different results with nerve crush.

Finally, we could state that tacrolimus and estrogen solely show some level of neuroprotection, particularly in the regulation of inflammation and axonal edema, but they were not sufficient to fully restore nerve function and structure following extensive sciatic nerve injury by bipolar-electrocautery in this study. Estrogen and tacrolimus combined treatment had not priority to agent that used alone and could that combination of these two agents had not effective results in our experimental animal model study in rats. At last, we suggested further studying for long term with different dosages of these agents and in various administration routes.

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Authors' Contribution

NV: data collection, drafting the manuscript; HF: supervised the surgery process and manuscript writing; AJ: conducting the study; PM: supervised the histopathology slides.

Ethics

The research project was formally endorsed by the Ethics Committee of the Science and Research Branch of the Islamic Azad University (Approval ID: IR.IAU.SRB.REC.1401.354).

Conflict of interest

The authors declare no competing interest.

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Data Availability

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

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