Iran Red Crescent Med J. 2019 September; 21(9):e92610. doi: 10.5812/ircmj.92610.
Published online 2019 September 23. **Research Article**

Effect of Selenium and Electrical Stimulation on Sperm Parameters and Spinal Cord Repair: A Rat Model of Spinal Cord Injury

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Received 2019 April 26; **Revised** 2019 August 04; **Accepted** 2019 August 19.

Abstract

Background: Selenium (Se) and Electrical stimulation (ES) play an important role in maintaining the integrity of various body functions after spinal cord injury (SCI).
Objectives: This study aimed to investigate the effect of Se and ES on the sperm parameters and the repair of the damaged spinal cord in a rat model.
Methods: A total of 50 male Wistar rats were randomly divided into five groups (n = 10 per each group, including control, sham, SCI, Se, and ES. After SCI in the T10 space, the BBB and Von Frey test were used to evaluate the motor and sensory functions. Six weeks after the treatment, the assessment of sperm parameters was done.
Results: The treatment with Se, compared to the SCI group, significantly increased the sperm concentration (38.66 ± 14.81 vs. 18.50 ± 7.02, P < 0.001), motility (55.00 ± 3.0 vs. 8.33 ± 1.16, P < 0.001) and viability (81.66 ± 8.16 vs. 19.16 ± 1.06, P < 0.001). ES compared to the SCI group, significantly increased sperm motility (60.00 ± 6.32 vs. 8.33 ± 1.16, P = 0.001) and viability (70.83 ± 9.70 vs. 19.16 ± 1.06, P < 0.001). Se and ES revealed no significant effects on sperm morphology (P > 0.05). A significant increase was observed in the BBB locomotor Score and Von Frey test in the SE and ES groups compared to the SCI group and between the Se and ES groups. However, no significant difference was observed between the Se and ES groups in the Von Frey test.
Conclusions: The effect of Selenium on sperm parameters and motor function was superior than that of the Electrical stimulation, but the effective effect on repairing the sensory function was the same.

Keywords: Electric Stimulation, Rats, Selenium, Semen Analysis, Sperm Count, Sperm Motility, Spermatozoa, Spinal Cord Injuries, Spinal Cord Regeneration

1. Background

According to the estimation of the World Health Organization (WHO), approximately 250,000 - 500,000 spinal cord injuries (SCI) occur annually around the world (1). Males are four times more likely to have SCI than females (2). SCI often affects the fertility of men that one of the best-performing mechanisms of injury is the production of reactive oxygen species (ROS) (3). When the ROS level is higher than the antioxidant system of the semen, a reaction known as oxidative stress will occur (4). The ability of sperm function is characterized by the sperm number and motility. Several selenoproteins, including mitochondrial capsule selenoprotein (MCSeP) and selenophosphate synthase (SPS-2) are localized in testicular tissue (5). The antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) contribute to the reduction of ROS. Superoxide radicals are converted into hydrogen peroxide by SOD, and subsequently, hydrogen peroxide cleavage by CAT into oxygen and water to prevent DNA damage. The deficiency in Se intake, as well as other antioxidants, resulted in a decreased activity of antioxidant capacity and increased the production of free radicals (6). The importance of Se in the body, especially in animal species, is not yet completely comprehensive and more studies on the effects of Se may show a number of new biologically important processes (5). Many studies have been done in animals and the effects of Se supplementation have often been evaluated in combination with other antioxidants, but no valid conclusions have been drawn so far. In general, provid-

ing enough nutrients guarantees optimal reproduction in both males and females, while additional supplements appear to have a negative impact (7). It has been reported that supplementation of vitamin E and Se in Holstein Bulls show no effect on sperm parameters such as sperm count, viability and DNA integrity. However, supplementation of Vitamin E and Se improved testosterone levels and non-progressive motility of sperms (8).

Low or high concentrations of sperm Se has been reported to affect sperm count and motility negatively. The optimal concentration of sperm selenium (Se) has not yet been determined. Some evidence suggests that the metabolic defect in Se composition of sperm cells may be associated with human infertility (7). Se is also known to be a neuroprotective agent against some neurological diseases. Javdani et al. (2019) have reported that oral administration of the nano-Se decreases spinal inflammation due to the neuroprotective effects of Se (7).

Electrical stimulation (ES) is a safe outpatient procedure and has a good potential for producing sperm acceptable for artificial insemination (9). It has been shown that the ES at 50 HTZ for 4 minutes have a minimal adverse effect on sperm motility (10). Electrical stimulation can facilitate and improve the movement of the upper/lower extremities, along with the loss of other parts of the body's function through damage to the paraplegic muscles.

Electric stimulation, combined with foam dressing using surface electrodes accelerated the wound healing process in rats with SCI (11). Zhang et al. (2018) have argued that the early use of ES by locating the anode in the lesion site and the cathode at the end of the lesion reduces the damage potential and inhibits secondary damage (12). Several studies inspected the effect of Se on CNS and brain with a discrepancy in their results have been done. Despite numerous studies on the effects of ES on neurological repair, there is no consensus on the optimal frequency, duration, and length of training in patients with SCI. Badri et al. (2017) have shown that combination treatment of Es and evening primrose oil improve nerve function following sciatic nerve injury in male rats. They have shown that combined treatment with EPO and ES might increase the re-myelination of the sciatic nerve in rats' sciatic nerve injury, contributing to the recovery of sciatic nerve function (13).

2. Objectives

Therefore, given the results of this study on neuronal protection ES, this study was performed on SCI rat model to determine the effect of ES on neuronal protection. So in this study, for the first time, the effects of Se nutrition as well as ES on sperm parameters and repair of spinal cord injury were investigated.

3. Methods

The rat animal model was used for this research. This study was conducted at the Laboratory of Neuroscience Research Center of Tabriz University of Medical Sciences from January 30, 2018, to December 27, 2018.

3.1. Animals

A total of 50 male Wistar rats (weight 200 - 250g) were purchased from the pharmacology animal lab of Tehran University of Medical Sciences, Tehran, Iran. The animals had free access to tap water and commercial food, in a temperature 22 °C to 24°C with a 12 h light: 12 h darkness cycle one week before starting the study. All procedures of this study were in accordance with the rules and procedures of the Ethics Committee and the Helsinki Declaration on the use and care of animals (14) (Ethics Committee Approval Code of IR.TBZMED.REC.1395.65). In order to adapt animals to laboratory conditions, they were brought to the operating room three days before the surgery to get used to the laboratory. The sample size, according to a study by Asadi et al. (15) and taking into account the results obtained from this study, (M1 (Mean progressive mobility in the intervention group) = 75.83, M2 (Mean progressive mobility in the control group) = 63.28, SD1 = SD2 = 4.39, Two sided $\alpha = 0.05$, Power = 0.95 and Considering a potential sample loss of 10%) was determined 50 rats. We used block randomization with 5 or 10 block sizes. The animals were randomly divided into five groups (n = 10 for each group), including control (received no intervention), sham group (underwent only laminectomy), SCI group (underwent laminectomy, and SCI without any intervention), Se group (SCI and treated by Se), and ES group (SCI and treated by ES).

3.2. SCI Model

The groups (except for the control group) were anesthetized using isoflurane 2.5% inhalation. The spinal cord of the rat was completely visible by the incision of paravertebral muscles in the space of T10 and laminectomy was done. The sham group only underwent a laminectomy, and the opened area was stitched. After laminectomy, the rats in the groups of SCI, AST and Se were subjected to SCI experienced using a SCI model device (NsrcImpactor Homemade code: 90778) (16) for SCI in the studied groups, moderate injury (equivalent to 150 - 175 kilo dynes) was entered the T10 space to the spinal cord (Figure 1). To limit postoperative pain, 5 mg/kg/day of Metacam was injected at the first 24 - 48 hours after injury. Before the intervention, each animal was individually kept in a cage with thick straw to prevent non-surgical injuries of the recovery period. The pellet of animals was soaked and poured into the cage to prevent an extra movement of the animal while eating and thus any possible damage. Bottles with long nozzles were

used to give water. The cage was replaced every three days and washed with alcohol 70% to minimize the risk of infection.

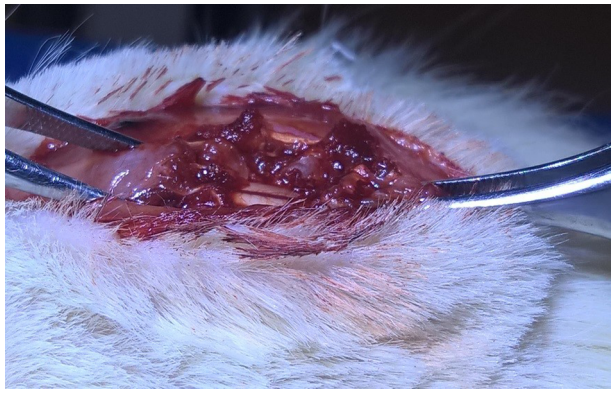

Figure 1. Cutting paravertebral muscle, laminectomy (T10 piece), and spinal cord are shown.

3.3. Treatment

Frothy eight hours after the injury, the group Se received 0.2 mg/kg of Se (Sodium selenite was purchased from Sigma-Aldrich Co. USA) for six consecutive days for six weeks via gavage (17). At the same time, the group ES experienced the ES in which two dipole electrodes were planted in two rostral and caudal regions relative to the SCI segment. After stitching these electrodes in the paravertebral muscles, artificial dura was placed in the spinal cord and sutured. The electrical stimulation was performed at a frequency of 100 HTZ (18). With the intensity of below threshold for one hour per day for 6 consecutive days for 6 weeks.

3.4. Sensory and Motor Scales

This assessment was started one week after the injury in the groups (Once a week for 4-minutes) and continued until five weeks later. The Basso, Beattie and Brenham (BBB) locomotor scale method was used to evaluate the motor function of rats, which has a score scale of 0 to 21. The scores of 0 and 21 were given if there was no spontaneous movement and normal locomotion and consistent coordination of gait with parallel hind paw placement, respectively. A score of 14 points was assigned when the animal displayed complete forelimb-hind limb coordination and plantar stepping with full weight support. This test was performed in an open enclosure of 90 mm diameter and 30 cm wall height (19, 20).

To determine the sensitivity of the skin to contact stimulates, different Von Frey (USA Stolting Co.) filaments in the range of 0.008 to 300 g were used. Each test was started by filament with minimum weight and in the lack of response to the minimum weight filaments, the filaments with higher weight were used. In the cases that responses were observed two consecutive times, the similar weights were recorded as Paw Withdrawal Threshold (PWT), and the test was finished (21, 22). This test was also performed along with the BBB scale (Once a week) for 5 weeks and the results were recorded. We had one observer in this study in all of the study period.

3.5. Sperm Parameters

After the end of the treatment, the rats were euthanized in each group by the exposure of the animals to 100% CO_2 in a chamber. In this chamber, 10% - 30% of the space was filled with CO_2 in one minute and the animal experienced rapid anesthesia with minimal stress (23). Finally, to confirm SCI, the spinal tissue sections were checked by a pathologist (Figure 2).

3.6. Sperm Count

The cauda epididymis was isolated and spliced into 2 mL of medium (Hams F10) containing 0.5% bovine serum albumin to release sperms and incubated for 5 min at 37°C (with 5% CO_2). The sperm count was done using the standard hemocytometric method and expressed in millions per milliliter (106 /mL) (24).

3.7. Sperm Motility

Sperm motility was immediately evaluated according to the WHO laboratory manual protocol (2010). A total of 10 mL of sperm suspension was placed onto Makler's counting chamber, and at least two hundred sperm were then evaluated for each rat. The motility of the sperm was analyzed in terms of the following motion patterns: progressive movement of sperm (PMS), non- progressive movement of sperm (NPMS), and non-movement of sperm (NMS) (24).

3.8. Sperm Viability

The sperm viability was evaluated by staining with eosin Y. Briefly, ten μL of sperm suspension was mixed with 0.5% eosin Y (w/v) stain solution on the microscope slides and then left for 30 s. Slides were viewed at 400× under a light microscope, The viability percentage of sperm was calculated at a magnification of 400 under a light microscope. Spermatozoa with dark pinked head and white or light pink head were considered as dead and live, respectively (25).

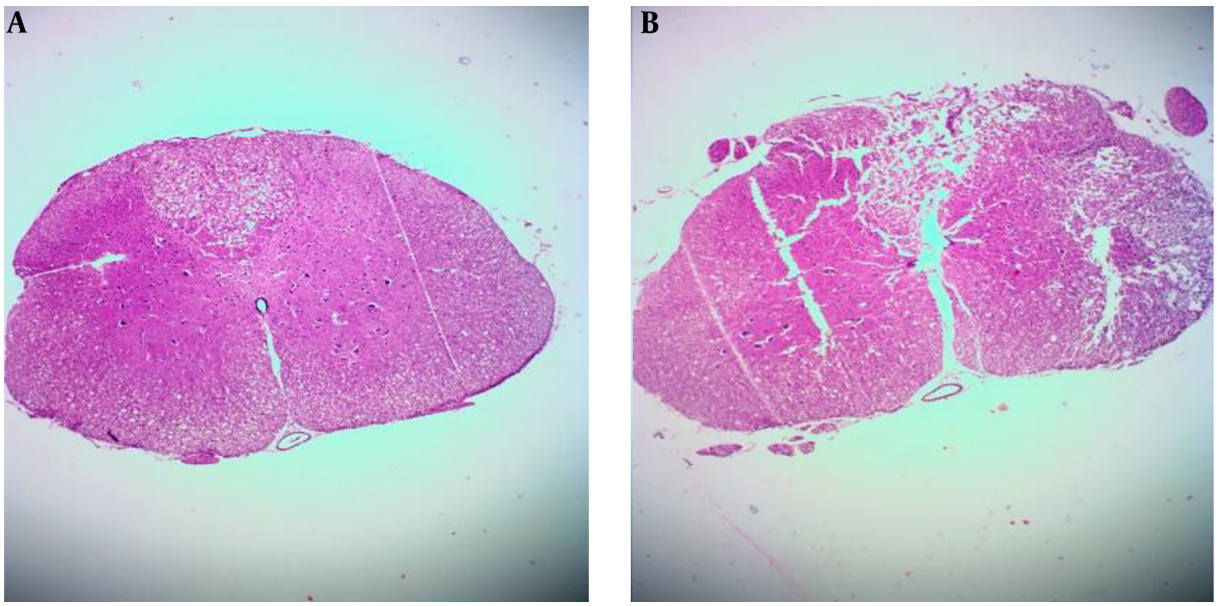

Figure 2. Histological sample of spinal cord in the control (A) and SCI (B) groups with a force of 150 Kilo dyne is shown.

3.9. Sperm Morphology

The normal and abnormal sperm morphology was classified by WHO (2010) protocol. About 200 sperms in each microscopic field were investigated and the mean percentage of sperm count with natural morphology was considered as a natural percentage of sperm morphology (25).

3.10. Statistical Analysis

The data were expressed as mean ± standard deviation (SD), and analyzed by IBM SPSS Statistics Software for Windows, version 21.0 (IBM Corp., Armonk, Ill., N.Y). The differences of means between the groups were analyzed by one-way ANOVA followed by the post hoc Tukey's test for multiple comparisons. For comparison within the groups, data were analyzed using repeated measure ANOVA. We checked repeated measures in terms of normality, sphericity, and randomness. The differences of variables were considered to be significant statistically at $P < 0.05$.

4. Results

4.1. Sperm Concentration

The Sperm concentrations (Sp. Conc, 10^6/mL) are shown in Table 1. A significant decrease was observed in the sperm concentration in the SCI group compared to the control (18.50 ± 7.02 vs. 43.66 ± 6.53, $P < 0.001$) and sham (18.50 ± 7.02 vs. 36.00 ± 4.15, $P < 0.001$) groups. Results indicated a significant increase in the sperm concentration in the Se-fed (0.2 mg/kg) group compared to the SCI group (38.66 ± 14.81 vs. 18.50 ± 7.02, $P < 0.001$). No significant difference was observed in ES (100 HTZ) group compared to the SCI group ($P > 0.05$) (Table 1).

4.2. Sperm Motility

The values for sperm motility (%), including the progressive movement of sperm (PMS), non- progressive movement of sperm (NPMS), and non-movement of sperm (NMS) are presented in Table 1.

The results indicated a significant decrease in the mean percentage of progressive movement of sperm in SCI group compared to the control (8.33 ± 1.16 vs. 83.33 ± 6.05, $P < 0.001$) and sham (8.33 ± 1.16 vs. 70.00 ± 20.00, $P < 0.001$) groups. Results indicated a significant increase in the sperm motility in the Se-fed and ES groups compared to those of the SCI group (55.00 ± 3.0.8 vs. 8.33 ± 1.16, $P < 0.001$) and (60.00 ± 6.32 vs. 8.33 ± 1.16, $P < 0.001$), respectively. No significant difference was observed between the Se and ES groups ($P > 0.05$).

No significant difference was seen in term of the mean percentage of NMS and NPMS in the control and other groups ($P > 0.05$) (Table 1).

4.3. Sperm Viability

The values for sperm viability (%) are shown in Table 1. A significant decrease was observed in the mean percentage of sperm viability in the SCI group compared to the control (19.16 ± 1.06 vs. 86.66 ± 8.16, $P < 0.001$) and sham (19.16 ±

Table 1. Concentration (Sp. Conc., 10^6/mL), Motility (%), viability (%), and Morphology (%) of Sperm Treated in Rats Spinal Cord Injury Model with Selenium and Electrical Stimulation[a]

Variables (Sperm Parameters)	Groups	N	Mean ± SD	Intervention Groups	N	Mean ± SD	P Value
Concentration (sperm/rat × 10^6)	Control	10	43.66 ± 6.53	Se	10	38.66 ± 14.81	0.32
				ES	10	26.00 ± 3.14	< 0.001
	Sham	10	36.00 ± 4.15	Se	10	38.66 ± 14.81	0.99
				ES	10	26.00 ± 3.14	0.17
	SCI	10	18.50 ± 7.02	Se	10	38.66 ± 14.81	< 0.001
				ES	10	26.00 ± 3.14	0.18
PMS (%)	Control	10	83.33 ± 6.05	Se	10	55.00 ± 3.0.8	0.04
				ES	10	60.00 ± 6.32	0.01
	Sham	10	70.00 ± 20.00	Se	10	55.00 ± 3.0.8	0.97
				ES	10	60.00 ± 6.32	0.85
	SCI	10	8.33 ± 1.16	Se	10	55.00 ± 3.0.8	< 0.001
				ES	10	60.00 ± 6.32	< 0.001
NPMS (%)	Control	10	13.33 ± 6.05	Se	10	24.16 ± 1.42	0.75
				ES	10	27.00 ± 6.70	0.30
	Sham	10	22.5 ± 1.83	Se	10	24.16 ± 1.42	0.97
				ES	10	27.00 ± 6.70	0.10
	SCI	10	28.33 ± 2.48	Se	10	24.16 ± 1.42	0.98
				ES	10	27.00 ± 6.70	0.99
NMS (%)	Control	10	5.00 ± 2.47	Se	10	20.83 ± 3.41	0.82
				ES	10	11.00 ± 5.47	0.91
	Sham	10	7.50 ± 1.57	Se	10	20.83 ± 3.41	0.92
				ES	10	11.00 ± 5.47	0.97
	SCI	10	46.66 ± 3.82	Se	10	20.83 ± 3.41	0.02
				ES	10	11.00 ± 5.47	0.01
Viability (%)	Control	10	86.66 ± 8.16	Se	10	81.66 ± 8.16	0.83
				ES	10	70.83 ± 9.70	0.06
	Sham	10	85.00 ± 6.32	Se	10	81.66 ± 8.16	0.96
				ES	10	70.83 ± 9.70	0.01
	SCI	10	19.16 ± 1.06	Se	10	81.66 ± 8.16	< 0.001
				ES	10	70.83 ± 9.70	< 0.001
Morphology (%)	Control	10	90.00 ± 5.47	Se	10	78.33 ± 11.25	0.19
				ES	10	70.00 ± 10.95	0.08
	Sham	10	87.50 ± 8.80	Se	10	78.33 ± 11.25	0.07
				ES	10	70.00 ± 10.95	0.68
	SCI	10	61.66 ± 14.71	Se	10	78.33 ± 11.25	0.06
				ES	10	70.00 ± 10.95	0.71

Abbreviations: ES, electrical stimulation; NMS, non-movement of sperm; NPMS, non-progressive movement of sperm; PMS, progressive movement of sperm; SCI, spinal cord injury; Se, selenium.
[a] P value < 0.05 was considered significant.

1.06 vs. 85.00 ± 6.32, P < 0.001) groups. Results indicated a significant increase in the sperm viability in the Se and ES groups compared to the SCI group (81.66 ± 8.16 vs. 19.16 ± 1.06, P < 0.001) and (70.83 ± 9.70 vs. 19.16 ± 1.06, P < 0.001), respectively. No significant difference was observed between the Se and ES groups (P > 0.05) (Table 1).

4.4. Sperm Morphology

The values for sperm morphology (%) are shown in Table 1. A significant decrease was seen in the mean percentage of sperm morphology in the SCI group compared to the control (61.66 ± 14.71 vs. 90.00 ± 5.47, P < 0.001) and sham (61.66 ± 14.71 vs. 87.50 ± 8.80, P = 0.001) groups. No significant difference was observed in the SCI group compared to the Se-fed and ES groups (P > 0.05) (Table 1).

4.5. The BBB Locomotor Score

The BBB locomotor Score was used to evaluate the locomotor function in rats from the first to fifth weeks after SCI. Repeated measure ANOVA was used to analyze within-group data during the study period. A significant difference was observed in BBB locomotor Score in the sham (P

< 0.001, F = 33.94), SCI (P < 0.001, F = 34.67), Se (P < 0.001, F = 120.29), and ES (P < 0.001, F = 345.73) groups during the study period. A significant decrease was observed in BBB locomotor score in the SCI group compared to the control (6.88 ± 0.90 vs. 17.38 ± 0.81, P < 0.001) and sham (6.88 ± 0.90 vs. 16.76 ± 0.44, P < 0.001) groups. A significant difference was observed in BBB locomotor Score in the Se-fed and stimulated groups compared to the SCI group on the first week (7.40 ± 0.51 vs. 5.9 ± 0. 87, P < 0.001 and 6.80 ± 0.78 vs. 5.9 ± 0. 87, P = 0.04, respectively). On the second week, (9.00 ± 0.47 vs. 6.40 ± 0.96, P < 0.001 and 8.40 ± 0.51 vs. 6.40 ± 0.96, P < 0.001, respectively), on the third week (13.60 ± 1.60 vs 7.00 ± 1.05, P < 0.001 and 9.70 ± 0.48 vs. 7.00 ± 1.05, P < 0.001, respectively), on the fourth week(15.50 ± 1.17 vs. 7.20 ± 0.91, P < 0.001 and 13.40 ± 0.51 vs. 7.20 ± 0.91, P < 0.001, respectively) and on the fifth week (16.50 ± 0.70 vs 7.90 ± 0.87, P < 0.001 and 15.40 ± 0.51 vs. 7.90 ± 0.87, P < 0.001, respectively) after the treatment. A significant increase was observed in BBB locomotor score in the Se-fed group in comparison to the ES group (12.40 ± 0.70 vs. 10.47 ± 0.42, P < 0.001) (Figure 3).

4.6. Von Frey Test

Different Von Frey filaments in the range of 0.008 to 300 g were used for the measurement and determination of the sensitivity of the skin to contact stimulates (Figure 4). A significant difference was observed in PWT in the control (P < 0.001, F = 30.83), sham (P < 0.001, F = 98.18), SCI (P < 0.001, F = 68.56), Se (P < 0.001, F = 16.05), and ES (P < 0.001, F = 75.20) groups during the study time.

A significant increase in PWT in the SCI group was observed in comparison with the control (267.80 ± 4.84 vs. 9.88 ± 0.44, P < 0.001) and sham (267.80 ± 4.84 vs. 29.94 ± 2.93, P < 0.001) groups from the second to the fifth week after treatment. The results indicated a significant decrease in PWT in the Se-fed and ES groups compared to the SCI group on the second week (230.00 ± 0.00 vs. 300 ± 0.00, P < 0.001 and 229.00 ± 7.37 vs. 300 ± 0.00, P < 0.001, respectively), on the third week (233.50 ± 16.67 vs. 300 ± 0.00 , P < 0.001 and 203.50 ± 12.92 vs. 300 ± 0.00 , P < 0.001, respectively), on the fourth week (198.00 ± 23.94 vs. 268.00 ± 26.99, P < 0.001 and 178.00 ± 13.16 vs. 268.00 ± 26.99, P = 0.04, respectively), and on the fifth week (159.50 ± 10.12 vs. 171.00 ± 9.94, P = 0.02 and 161.00 ± 11.00 vs. 171.00 ± 9.94, P = 0.04, respectively) after the treatment. No significant difference was observed between the Se and ES groups in the Von Frey test on the weeks of the study (P > 0.05) (Figure 4).

5. Discussion

Male reproductive system dysfunction in the patients with SCI is the result of the combination of dysfunction of erectile and ejaculatory as well as abnormal semen characteristics. Men with SCI seem to have elevated levels of ROS in their semen. It seems to be at least in part due to an increase in oxidative stress and leucocytospermia (26). In recent years, many studies have focused on the effects of oxidative stress, ROS and antioxidants on male reproductive systems (27). Antioxidants inhibit cellular damage processes mainly via their ability to scavenge the free radicals (28). Se is an essential trace element that plays an important role in the antioxidant, reproductive, endocrine, and immune system in animals and its deficiency causes fertility problems. On the other hand, ES can help facilitate and improve sexual functions after SCI (29). In this study, for the first time, the effect of Se on the sperm parameters and spinal cord repair was compared with ES after SCI. The results of this study indicate that Se (daily dose of 0.2 mg/kg), unlike the ES, significantly increases the concentration of sperm after SCI. According to our study, Se and ES (100 HTZ, the intensity of below threshold, and one hour per day) both equally were able to significantly increase the sperm motility and sperm survival in the rats with SCI, but could not make a difference in the sperm morphology. Khazaei Monfared et al. (2018) indicated that the SE, as an antioxidant affects the number of sperms and motility (30). Their findings are consistent with the present study. Although their study has been conducted on the effects of Se on varicocele and the present study was based on Se effects on SCI, but the main cause of infertility in both of varicocele and SCI may result from the high level of ROS in semen (31). Toman et al. (2016) reported that the administration of the Se and diazinon alone or in combination with each other caused significant changes in sperm motility. They have also found that dysfunction of sperm motility in the SE alone as well as SE in combination with diazinon leads to a decrease in fertility or induce infertility (32). This study contradicts with our study. In their experiment, the high dose of Se was used, while in our study, a low dose of Se (0.2 mg/kg) has been used. However, low doses of Se may improve the male reproductive dysfunctions resulted from ROS. A beneficial effect of supplementation with Se and vitamin E on the quality of sperm parameters has been previously reported. However, there was no positive effect of supplementation with the aforementioned antioxidants on the quality of sperm parameters in the human (33, 34). In these studies, the dose and duration use of SE was different from the present study. Based on our documents, the effect of Se on sperm parameters after SCI was not found to date. In our study, the ES did not affect sperm concentration. The most finding on sperm parameters in the patients with SCI is decreasing sperm motility and vitality, but sperm concentration is less affected after SCI (35). Saito et al. (1999) reported that the sperm motility near electrodes is decreased with high electric current

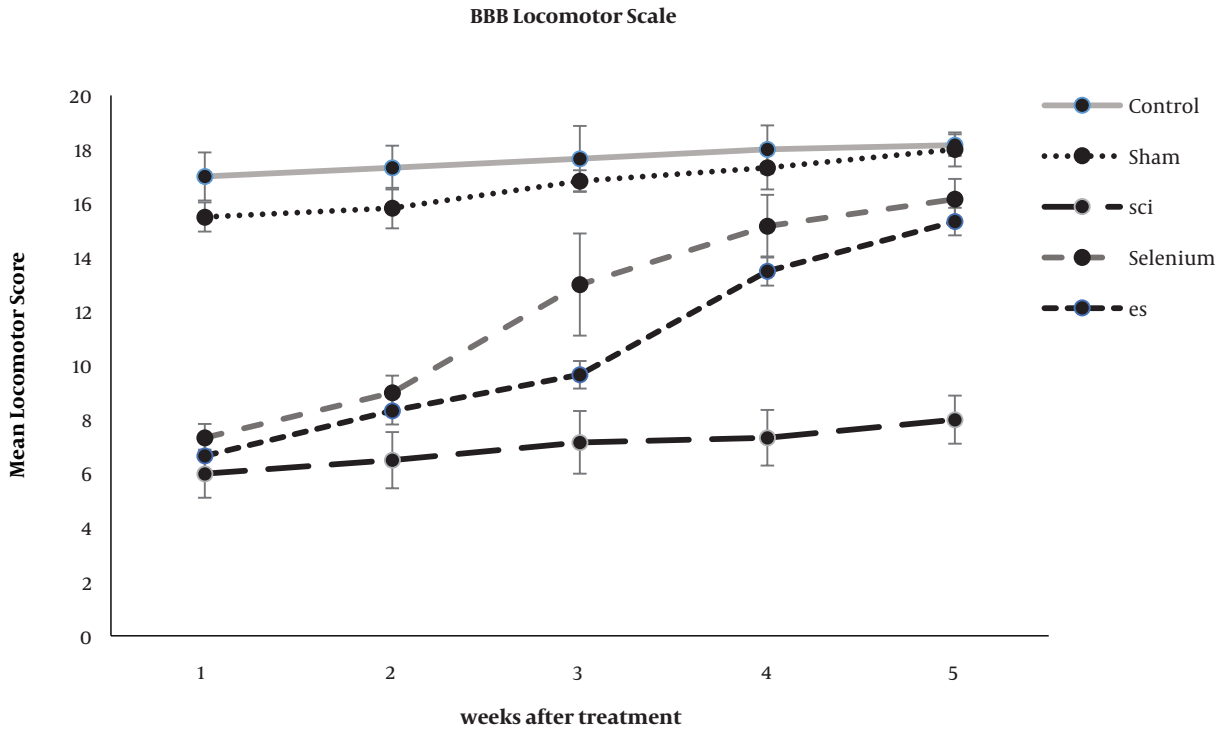

Figure 3. Line graph plotting changes in BBB locomotor behavior as a function of post-treatment time. Each point represents the group mean. Abbreviations: ES, electrical stimulation; SCI, spinal cord injury

flowed even in low electrical voltage conditions (10).

Moreover, sperm motility is lost with extremely high-voltage ES. However, it seems that ES has no effect on sperm motility during clinical rectal probe electroejaculation. In their study, rectal probe electroejaculation has used, but in the current study, the electrodes were placed in the paravertebral muscles, and with 100 HTZ, the intensity of below threshold, and one hour per day. One of the possible mechanisms of ES is to diminish the number of astrocytes in the injury area and changes in spinal cord blood flow after SCI that are able to produce ROS in response to damage. It has been suggested that ES has an effect on the sperm parameters by reducing astrocytes in the injury site (36).

SCI is a traumatic event that is usually associated with loss of sensory and motor functions as well as sexual dysfunction. It has been currently shown that Se has a clear role to protect and recovery of SCI. However, the protective mechanism of Se is yet to be cleared (37). So in this study, we sought to determine if Se and ES repair the spinal cord in a rat's SCI model. We have used BBB locomotors rating scale to assess the functional recovery and different Von Frey filaments for measurement and determine the sensitivity of the skin to contact stimulates following SCI in the rats. According to our study, both treatments with SE and ES could increase the BBB locomotors score within the groups during the study. Both treatments also could enhance the BBB locomotors score on the first to fifth weeks after SCI. But this increase in the Se group was higher than that of the ES group. Also, this study shows that both treatments with Se and ES decrease the PWT within the groups during the study. In addition, both treatments using Se and Es were able to reduce PWT on the second to fifth weeks after SCI. In this study, the effect of Se and ES on the PWT reduction was the same.

Yeo et al. (2008) showed that the administration of Se could recover the motor function by preventing secondary pathological events in traumatic SCI in a rat model (38). Their findings are consistent with the present study. The efficacy of Se may facilitate the development of novel drug targets for the SCI therapy (38). The results of studies have shown that Se treatment has been effective in improving locomotor function than that of ES. It seems (a) the disorders in the metabolism of membrane lipids may lead to cellular necrosis and the functional failure of the SCI, and (b) Se may protect the injured spinal cord tissue via the limitation of changes in membrane lipids (39). In this study, although both methods alone were effective on sperm parameters and damaged spinal cord repair, Se was better

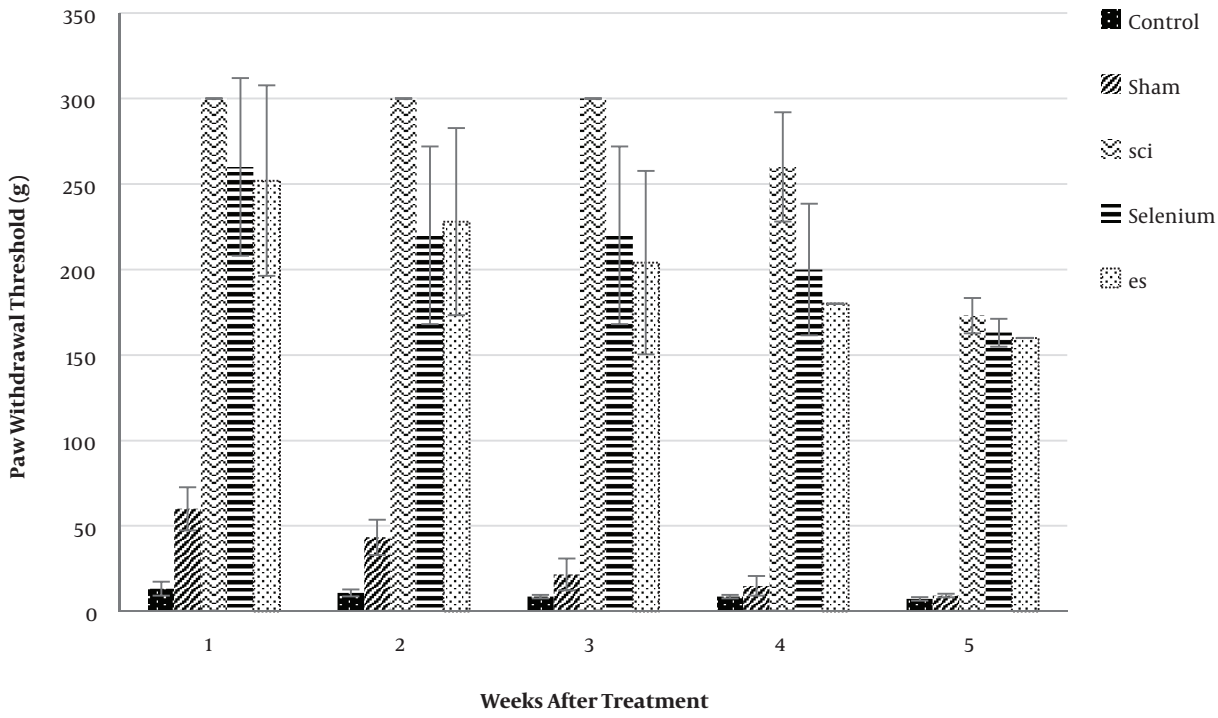

Figure 4. The changes of Paw Withdrawal Threshold as a sensitivity function of post-treatment time. Each point represents the group mean. Abbreviations: ES, electrical stimulation; SCI, spinal cord injury

than that of ES. This effect appears to be due to various antioxidant mechanisms. Considering different biological, pharmacological and various therapeutic methods, a co-administration would be significantly more effective than single therapy.

Iran is ranked as the second in SCI among 28 developing countries (40). Therefore, Iranian people are more exposed to complications from SCI. Since this problem affects most men in childbearing age and according to the current population growth policies, any improvement in this situation can be one of the strengths of our study. However, this study has been done in an animal model, and its generalization to humans needs further investigation.

5.1. Conclusions

In conclusion, the present study demonstrated that the Se and the ES could affect sperm motility and viability, but none had any effect on sperm morphology. The Se, unlike the ES, increased the mean sperm concentration. Both Selenium and Electric Stimulation were effective equally in repairing the sensory functions. However, motor function in the Se group was superior than that of the ES group.

Acknowledgments

The authors are grateful to the financial support of the Tabriz University of Medical Sciences, Neurosciences Research Center and Drug Applied Research Center and all those who helped us to do this research.

Footnotes

Authors' Contribution: Soheila Bani: Title selection, review of texts, preparation of required materials and materials, surgery, spermogram and sensory and motor tests, data collection and statistical tests, article writing. Jalal Abdolalizadeh: Supervise surgery and practical works, assist in writing articles and statistical tests. Iraj Lotfinia: Supervise surgery and practical tasks, assist in writing articles and statistical tests. Parviz Shahabi: With the first author, contribution in review of texts, preparation of required materials and materials, surgery, spermogram and sensory and motor tests, data collection and statistical tests, article writing. Amir Vahedy: Contribution in practical works and statistical tests. Meyssam Ghorbani: Contribution in surjury and article writing. Behnaz Sadeghzadeh: Contribution in spermogram and article writing.

Conflict of Interests: The authors declare that there is no conflict of interest.

Ethical Approval: All procedures of this study were in accordance with the rules and procedures of the Ethics Committee and the Helsinki Declaration on the use and care of animals (Ethics Committee Approval Code of IR.TBZMED.REC.1395.65).

Funding/Support: We have no grant number.

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