International Journal of Engineering & Technology, 8 (1.4) (2019) 384-394

SPC

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Effects of nerve regeneration therapy on functional recovery after nerve injury

Youn-Bum Sung[1], Jung-Ho Lee[2]*

[1]Department of Physical Therapy, Daegu University: 201, Naeri-ri, Jinlyang, Gyeongsan-si, Kyeongsangbuk-do, Republic of Korea. E-mail: playeryoon@naver.com
[2]Department of Physical Therapy, Kyungdong University: Bongpo-ri, Toseong-myeon, Goseong-gun, Gangwon-do, Republic of Korea. E-mail: ljhcivapt@naver.com*
**Corresponding author E-mail: Jung-Ho Lee, ljhcivapt@naver.com*

Abstract

The purpose of this study was to investigate the effects of treadmill exercises utilizing CIMT as performed by white rats, in which crushed sciatic nerves were induced, by time of measurement through neurological motor-behavior tests. The rats were randomly assigned to a control group, a CIMT group, and an exercise group after being affected by sciatic nerve damage. To evaluate the effects of the nerve regeneration therapy on motor nerves, the SFI of rats across all groups were measured after application of the treatment. The ladder walking test was used to measure the degrees of deficit or recovery of sensory functions. To analyze gait behavior following sciatic nerve damage recovery, the gait behaviors of rats in all groups were measured using the Dartfish program after application of the treatment. As the result, there was no statistically significant difference in all groups at 1, 7 days ($p>.05$), but there was a significant difference at 14, 21 days on SFI, ladder rung walking test, and ankle angle. In conclusion, the combined use of CIMT and exercise therapy had the greatest positive effect on nerve recovery after nerve injury.

Keywords: *Nerve Regeneration, Functional recovery, Nerve injury, Rat, CIMT*

1. Introduction

Peripheral nerves undergo a very slow therapeutic recovery process after being damaged and the degree of recovery is often shown to be incomplete[1]. Peripheral nerve damage reduces partial or total motor nerve, sensory nerve, and autonomic nerve control for the denervated areas and reduces functional activity capacity and quality of life due to said impaired sensory and motor abilities[2]. The areas innervated by the damaged nerves are accompanied by abnormal sensations such as spontaneous pain, mechanical allodynia, temperature hypersensitivity, hyperesthesia, and sympathetic pain. In addition, damage is caused to the nerves in the spinal ganglia and the anterior and posterior spinal horns over time and microcirculatory disturbances occur that block nourishment of the nerves, which results in impaired delivery functions and degeneration of innervated muscles[3][4]. If muscles are not normally innervated due to peripheral nerve damage, denervated muscle atrophy will occur as a result of decreased intramuscular protein synthesis and increased proteolysis[5]. Wallerian degeneration—in which axons and myelin sheaths at the distal side of a lesion are degenerated by Schwann cells and macrophages—occurs within 24 hours after peripheral nerve damage, resulting in a microenvironment favorable to neuronal response, axonal regrowth, and axon elongation. This stage of degeneration lasts one to two weeks[6].

To promote nerve regeneration and redistribution, axotomized nerves must be switched to the phenotype for the ability to survive and regenerate during an early stage of recovery. It is known that damage to peripheral nerves leads to the death of a large number of axotomized nerves. After damage to sciatic nerves, approximately 10–30% of sensory nerves in the dorsal root ganglia are known to die, while only 0–10% of post-endothelial nerve injury nerves are known to die, and only 0–10% of motor nerve cells die[7]. On the contrary, damage to the ventral root leads to the degenerative death of 50–80% of motor nerve cells over several weeks[8]. When posterior nerves among the sympathetic nerves have been damaged, approximately 50% of the nerve cells die within 3 days of the posterior nerve damage. When the anterior nerves of a nerve ganglion have been damaged, the nerve cells of the spinal cord decrease by 40–60%[9]. Nerve cell death after axonotmesis is affected by age, the degree of damage, and the distance between the cell body and the damaged area, mature nerves are less affected by the killing conditions than immature nerves and nerve cell bodies closer to damaged areas show a higher ratio of cell death[10]. If axon regeneration is suppressed, the nerve cell death process will last longer, causing more serious damage. Neurogenic vacuolization and apoptosis following nerve damage will gradually increase for one month and then decrease little by little for up to six months[11]. To date, studies have been actively conducted on the promotion of axonal regeneration, survival of nerve cells, and the maintenance of neurotrophic factors after nerve damage[12]. After peripheral nerve damage, the mature peripheral nervous system increases the availability of neurotrophic factors through autocrine and lateral secretion. Neurotrophic factors activate neuronal molecules and other factors necessary for axon reaction and regeneration, which leads to nerve recovery after damage to peripheral nerves[13].

Since disabilities due to peripheral nervous system damage have negative effects on independent functions necessary to engage in daily life activities, getting appropriate treatment is an important element that will affect the promotion of recovery. Treatment methods for peripheral nerve damage include invasive procedures such as injection therapy and surgical restoration, and non-invasive methods such as electrical stimulation therapy, exercise therapy, hydrotherapy, and medication[14][15]. An important element of treatment for peripheral nerve damage is the formation of an environment that can maximize neuroplasticity. Decreasing the use of the affected area can cause abnormal movement, decreased muscle strength, stiffness, soft tissue shortening, and pain. A clinically developed treatment to control this learned nonuse is Constraint Induced Movement Therapy (CIMT).

CIMT is defined as a therapy characterized by high-intensity training over a short period of time and an enhancement of the function and use of the affected side by restricting the movement of the unaffected side to better induce movement of the affected side[16]. CIMT has been reported to stimulate the sensory motor cortex in the uninjured cortical circuit and the area adjacent to the damaged area through continuous and repeated functional movement learning, thereby facilitating local terminal firing and reorganization of functional synapses to improve precise and delicate movements[16][17]. However, as patients may feel burdened by the training, experience psychological stress, and face safety issues, Page et al. (2001) performed a modified CIMT[18]. The modified CIMT was applied to constrain the unaffected side for only the five most active hours of a patient's day. The therapeutic effects of the modified CIMT were proven by comparing it to the therapeutic effects of other treatments[18][19]. Although most studies of CIMT have focused on improving upper limb function in patients with central nervous system damage, a more recent study indicates that gait ability and balance were improved through CIMT thanks to a reported improvement of upper limb function[20]. In addition, Rostami et al. (2016) reported that when CIMT was applied to patients with damage to the median or ulnar nerve, it was effective in treating peripheral nerve injuries[21]. Although many studies echo these reports, CIMT is not widely used as a therapeutic intervention in clinical practice at this time. This may be attributable to the fact that, despite the application of the modified CIMT, the problems mentioned above could not be completely resolved. Therefore, the purpose of this study was to investigate the effects of treadmill exercises utilizing CIMT as performed by white rats, in which crushed sciatic nerves were induced, in order to solve the ethical problem of being unable to apply CIMT to humans.

The purpose of this study was to investigate the effects of treadmill exercises utilizing CIMT performed by white rats with crushed sciatic nerves on nerve regeneration and functional recovery over time.

An important element for the treatment of peripheral nerve damage is the formation of an environment that can maximize neuroplasticity. Decreased use of the affected side can cause abnormal movements, muscle weakness, stiffness, shortening of the soft tissues, and pain. CIMT is a treatment method clinically developed to control this learned non-use. However, CIMT is not widely used as a therapeutic intervention in clinical practices at this time. This is considered attributable to the fact that, despite the application of a modified CIMT, problems such as the patient feeling burdened by the training, psychological stress, and patient safety issues could not be completely solved. Therefore, the purpose of this study was to investigate the effects of treadmill exercises utilizing CIMT as performed by white rats, in which crushed sciatic nerves were induced, by time of measurement through neurological motor-behavior tests in order to solve the ethical problem of being unable to apply CIMT to humans.

2. Main body

2.1. Subject

This study was conducted using 45 male Sprague-Dawley rats aged 8 weeks and weighing 250-300g. Sprague-Dawley rats aged 8 weeks belongs to the human adolescence, and a prior study using white rats usually uses the age of white rats to see the effects of a quick recovery. During the experimental treatment period, the temperature of the laboratory was maintained at 23 ± 2 ℃ and the humidity was maintained at 50 ± 5%. Four rats were housed per cage in the laboratory and light and dark cycles of 12 hours each per day were applied. The rats were randomly assigned to a control group (CG, n = 15) that would not to receive any therapeutic intervention after being affected by sciatic nerve damage, a CIMT group (CEG, n = 15) that would undergo treadmill exercises utilizing CIMT after being affected by sciatic nerve damage, and an exercise group (EG, n = 15) that would only undergo treadmill exercise after being affected by sciatic nerve damage. Three days after inducing crushed sciatic nerves in the rats, the first intervention method was implemented immediately after confirming the nerve damage. The rats were treated 5 times per week for 2 weeks with the aforementioned therapeutic intervention methods.

2.2. Experimental procedure

2.2.1 Induction of Crushed Sciatic Nerves

To put the rats under general anesthesia, Rompun and Zolethyl were mixed at a ratio of 1:1 and 2 ml of the mixture per 1 kg of body weight was injected intraperitoneally. After identifying the state of anesthesia with the presence or absence of avoidance responses following artificially induced pain, the rats were affixed to the operating table and hair in the region between the right hip joint and knee joint was removed. After removing the hair, the skin was incised about 2 cm in length, the sciatic nerves were isolated from the muscles surrounding them, and then the area before where they branch into the shin nerve and peroneal nerve—which is about 7 cm proximal to the ankle joint—was continuously pressed for 30 seconds using a pair of straight hemostatic forceps. To prevent damage due to the blade of the straight hemostatic forceps during compression, the forceps were covered with a soft plastic and disinfected with 70% alcohol before the crush was induced. To apply nerve damage to a certain area with the same force, a marking was made at a point 5 mm inward from the end of the hemostatic forceps and the same three-step pressure was applied to all rats[22].

Disinfection was performed to prevent post-crush infections. The wounds were sutured using animal suture threads. Thereafter, the animals were put into plastic cages—four animals were housed in each plastic cage and were introduced to the feeding room for stabilization.

2.2.2 Constraint Induced Movement Therapy (CIMT)

The CIMT intervention method in this study was applied to the unaffected hind leg that was not given a crushed sciatic nerve to prevent the overuse of the unaffected side during treadmill exercises, thereby inducing use of the affected side.

CIMT was applied by wrapping the unaffected hind leg—while maintaining the extension of the knee joint and the ankle joints—with a PET bottle to prevent excessive compression of the local region and wrapping the PET bottle with Kinesio tape to affix the bottle to the leg. Exercise on the treadmill was applied thereafter. The CIMT device was removed immediately after completion of the treadmill exercise to minimize stress.

2.2.3 Treadmill Exercise Treatment Method

The exercise intervention in this study was an exercise program applied to all groups except for the control group using a small animal treadmill(JD-A-09 type, JEUNGDO Bio & Plant Co., Ltd., Korea). In a study conducted earlier by Shepherd and Gollnick (1976), the VO2 max of Sprague-Dawley rats were measured with seven steps of speeds with in the range of 16-67 m/min and the researchers reported[23]. Based on the results of the study, to minimize stress and adverse effects from exercise intensity in this study, a low-intensity exercise program was applied with an intervention application time of 30 minutes, a gradient of 0°, and an exercise speed of 8 m/min.
All groups had a resting period of 3 days after the crushed sciatic nerve was induced; sciatic nerve damage was confirmed thereafter and the exercise program was applied to all groups except for the control group. The intervention was applied 5 times per week for 3 weeks and the exercise was performed during a dark cycle time zone (7:00 p.m.) when the rats were active to maintain their biorhythms and enhance the effects of training. To prevent the rats from ceasing to exercise, a device that would apply an electrical stimulation of 15 volts was installed at the back of the belt of the treadmill, thereby inducing continuous exercise(Fig. 1).

2.3 Result measurement method

2.3.1. Sciatic Functional Index (SFI)

To evaluate the effects of the treadmill exercise using CIMT on motor nerves, the SFIs of rats across all groups were measured at day 1, day 7, day 14, and day 21 after application of the treatment.
Chinese ink was applied to the soles of the rats, which were then induced to walk through a 50 cm long, 8 cm wide, and 10 cm high dark passage with white paper spread on the floor to obtain footprints. A dark room was crafted at the end of the passage in the direction of progression so that the rat could proceed forward. The rats were allowed to walk twice so that they could acquaint themselves with the direction of the passage and each rat was induced to walk three times repeatedly to ensure that footprints were left. In this case, all external elements that could affect the walking habits of the rats were removed[24].
For the footprint analysis of the rats, the distance from the heel to the third toe, the distance between the first and fifth toes, and the distance between the second and fourth toes were measured. Using these measured values allowed the SFIs to be calculated. The SFIs were near 0 in the case of normal rats and toward -100 as the damage would be recorded as more severe[25].

The calculation method for evaluating SFI is as follows.

PLF(print length factor) = (EPL – NPL) / NPL
TSF(toe spread factor) = (ETS – NTS) / NTS
ITF(intermediary toe spread factor) = (EIT – NIT) / NIT
PL : distance from the heel to the third toe, the print length
TS : distance from the first to the fifth toe, the toe spread
IT : distance from the second to the fourth toe, the intermediary toe spread

2.3.2. Ladder Walking Test

The ladder walking test was used to measure the degrees of deficit or recovery of sensory functions. This test was conducted by filming the rats with a video camera while they traversed a horizontally placed, 1 m long, 10 cm wide ladder with a consistent distance of 1.5 cm between cross bars[26].
As for the scoring method: 0 points were given when the rat could not step on the ladder and dropped its feet, 1 point was given when the rat slipped its weight-bearing foot off the ladder, 2 points were given when the rat slipped on its weight-bearing foot but could continue to traverse the ladder without dropping its foot or losing balance, 3 points were given when the rat could not bear weight although its feet remained on the ladder and quickly moved to other cross bars, 4 points were given when the rat quickly repositioned its foot in cases where one foot passed the targeted cross bar and stepped on another cross bar, 5 points were given when the big toe and heel of the weight-bearing foot were placed on the cross bar, and 6 points were given when the rat was completely bearing its weight with the middle region of its sole. When different score requisites occurred simultaneously, the lowest score was recorded. The cases that would result in 0, 1, or 2 points being given were defined as mistakes; a mistake score was obtained and recorded by dividing the total number of mistaken steps by the total number of steps and converting that value into a percentage. To measure only consecutive steps, the first, second, penultimate, and final step were excluded from the record[27].

2.3.3. Dartfish Program

To analyze gait behavior following sciatic nerve damage recovery, the gait behaviors of rats in all groups were measured using the Dartfish program at day 1, day 7, day 14, and day 21 after application of the treatment. The Dartfish program used for the gait analysis is software that can analyze and interpret movement and track an object's trajectory using video. This program is widely used in rehabilitation therapy, sports, diagnosis, and exercise prescriptions[28].
Before filming the videos, the coxofemoral region of each rat's hind leg was disinfected with 70% alcohol, shaved, and then the greater trochanter of the coxal articulation around the region where the sciatic nerve was crushed, the lateral epicondyle of the knee, the lateral ankle bone, and the metatarsophalangeal joint of the fifth toe were all marked with black dots. Each rat was induced to walk along a 100 cm long, 8 cm wide, and 10 cm high passage with one transparent side used to film the video. A dark room was made at the end of the passage to induce the rat forward. The rats were allowed to walk twice so that they could become acquainted with the direction of the passage and each rat was induced to walk three times repeatedly while the video was being filmed. The average values were used as re-

sults. Videos were filmed on the sagittal plane at a distance of 1 m from the passage using a 60 Hz digital video camera. From the videos filmed, the angles of the ankle joint on the affected side according to gaits were analyzed using the Dartfish program[29]. Only those videos that showed perfect gait cycles from the initial ground contact phase to the next initial ground contact phase were used as measured values in the gait analysis[30].

2.4. Data analysis

The results of the experiments obtained in this study were described as mean ± standard deviation. One-way ANOVAs were conducted for comparison and analysis between groups and between measurement times, and ex post facto tests were assessed using LSD. Statistical processing was performed using the PASW Win. 18.0 package and the level of statistical significance (α) was set to 0.05.

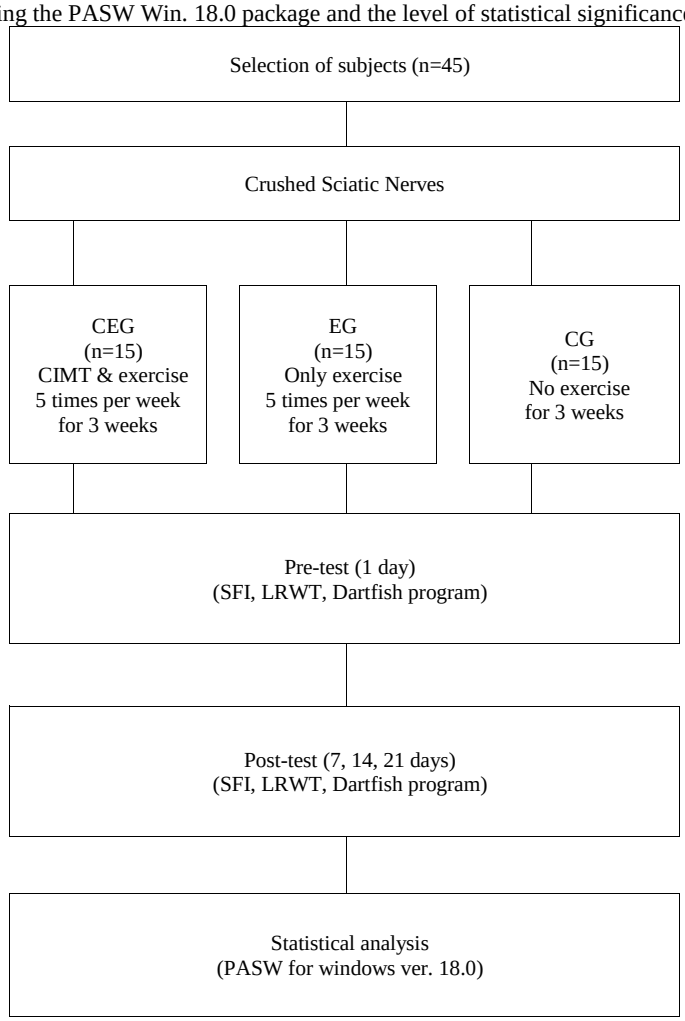

Fig. 1: flow chart

2.5. Results

2.5.1. Sciatic Functional Index (SFI)

The results of SFI assessment was no statistically significant difference in all groups at 1, 7 days ($p>.05$), but there was a significant difference at 14, 21 days ($p<.05$)(Table 1)(Fig. 2). The post hoc test showed significant differences among all groups at 14, 21 days ($p<.05$). Significant differences were found between CEG and EG, EG and CG ($p<.05$)(Table 2)(Table 3)(Table 4).

Table 1. Comparison of SFI in each group

	CEG	EG	CG	F	p
1 day	-82.49±5.99	-80.89±5.73	-81.63±5.19	1.341	.533
7 days	-66.34±6.91	-68.82±7.33	-72.03±4.87	5.127	.069
14 days	-32.87±7.01	-40.11±8.30	-47.39±5.77	31.533	.000*
21 days	-21.66±7.12	-31.38±8.88	-39.23±5.91	26.932	.000*

*p<.05
Unit : score
Mean±SD: mean±standard deviation
CEG: constraint-induced movement therapy & exercise group
EG: exercise group
CG: control group
SFI: sciatic functional index

Table 2. Comparison of SFI in CEG

	SFI	F	p
1 day	-82.55±5.99	241.413	.000*
7 days	-66.34±6.91		
14 days	-32.87±7.01		
21 days	-21.66±7.12		

*p<.05
Unit: score
Mean±SD: mean±standard deviation
CEG: constraint-induced movement therapy & exercise group
SFI: sciatic functional index

Table 3. Comparison of SFI in EG

	SFI	F	p
1 day	-80.89±5.73	201.061	.000*
7 days	-68.82±7.33		
14 days	-40.11±8.30		
21 days	-31.38±8.88		

*p<.05
Unit: score
Mean±SD: mean±standard deviation
EG: exercise group

Table 4. Comparison of SFI in CG

	SFI	F	p
1 day	-81.63±5.19	106.561	.000*
7 days	-72.03±4.87		
14 days	-47.39±5.77		
21 days	-39.23±5.91		

*p<.05
Unit: score
Mean±SD: mean±standard deviation
CG: control group
SFI: sciatic functional index

2.5.2. Ladder Walking Test

The results of ladder rung walking test was no statistically significant difference in all groups at 1, 7 days (p>.05), but there was a significant difference at 14, 21 days (p<.05)(Table 5)(Fig. 3). The post hoc test showed significant differences among all groups at 14, 21 days (p<.05). Significant differences were found between CEG and EG, EG and CG (p<.05)(Table 6)(Table 7)(Table 8).

Table 5. Comparison of LRWT on hind limb in each group

	CEG	EG	CG	F	p
1 day	99.11±0.93	99.09±1.22	98.46±1.03	0.452	.877
7 days	77.17±4.33	86.30±6.83	87.64±3.68	1.438	.157
14 days	36.12±7.54	46.83±9.47	50.74±7.47	13.441	.000*
21 days	7.34±3.50	18.76±4.31	32.11±11.60	9.633	.000*

*p<.05
Unit: %
Mean±SD: mean±standard deviation
CEG: constraint-induced movement therapy & exercise group
EG: exercise group
CG: control group
LRWT: ladder rung walking tes

Table 6. Comparison of LRWT on hind limb in CEG

	Error ratio	F	p
1 day	99.11±0.93	56.692	.000*
7 days	77.17±4.33		
14 days	36.12±7.54		
21 days	7.34±3.50		

*p<.05
Unit: %
Mean±SD: mean±standard deviation
CEG: constraint-induced movement therapy & exercise group
LRWT: ladder rung walking test

Table 7. Comparison of LRWT on hind limb in EG

	Error ratio	F	p
1 day	99.09±1.22	49.133	.000*
7 days	86.30±6.83		
14 days	46.83±9.47		
21 days	18.76±4.31		

*p<.05
Unit: %
Mean±SD: mean±standard deviation
EG: exercise group
LRWT: ladder rung walking test

Table 8. Comparison of LRWT on hind limb in CG

	Error ratio	F	p
1 day	98.46±1.03	45.878	.000*
7 days	87.64±3.68		
14 days	50.74±7.47		
21 days	32.11±11.60		

*p<.05
Unit: %
Mean±SD: mean±standard deviation
CG: control group
LRWT: ladder rung walking test

2.5.3. Dartfish Program

The results of ankle angle assessment was no statistically significant difference in all groups at 1, 7, 14 days (p>.05), but there was a significant difference at 21 days (p<.05)(Table 9)(Fig. 4). The post hoc test showed significant differences among all groups at 21 days (p<.05). Significant differences were found between CEG and EG, EG and CG (p<.05)(Table 10)(Table 11)(Table 12).

Table 9. Comparison of ankle angle on pre-swing in each group

	CEG	EG	CG	F	p
1 day	50.68±5.34	49.66±8.92	51.31±7.30	.961	.653
7 days	44.23±5.81	45.24±8.77	46.45±6.22	.473	.763
14 days	57.33±6.62	54.35±4.16	53.87±7.13	5.411	.064
21 days	101.75±8.11	83.76±7.73	72.97±12.43	12.682	.008*

*p<.05
Unit: °
Mean±SD: mean±standard deviation
CEG: constraint-induced movement therapy & exercise group
EG: exercise group
CG: control group

Table 10. Comparison of ankle angle on pre-swing in CEG

	Ankle angle	F	p
1 day	50.68±5.34	28.620	.000*
7 days	44.23±5.81		
14 days	57.33±6.62		
21 days	101.75±8.11		

*p<.05
Unit: °
Mean±SD: mean±standard deviation
CEG: constraint-induced movement therapy & exercise group

Table 11. Comparison of ankle angle on pre-swing in EG

	Ankle angle	F	p
1 day	49.66±8.92	22.111	.000*
7 days	45.24±8.77		
14 days	54.35±4.16		
21 days	83.76±7.73		

*p<.05
Unit: °
Mean±SD: mean±standard deviation
EG: exercise group

Table 12. Comparison of ankle angle on pre-swing in CG

	Ankle angle	F	p
1 day	52.23±6.32	11.381	.000*
7 days	46.45±6.22		
14 days	53.87±7.13		
21 days	72.97±12.43		

*p<.05
Unit: °
Mean±SD: mean±standard deviation
CG: control group

3. Discussion

This study was carried out to investigate the effects of treadmill exercise utilizing sciatic nerve constraint induced movement therapy on functional recovery and nerve regeneration in rats with crushed sciatic nerves.

The rat model with crushed sciatic nerves utilized in the experiment is a known model widely used for evaluating the effects and mechanisms of impaired peripheral nerve regeneration in the medical and physiotherapeutic fields[31].

Peripheral nerve damage brings about partial or complete loss of motor, sensory, and autonomic functions that are transmitted according to the size and severity of damage to the denervated segments because of the cell deaths in the axotomized neurons, the degeneration of nerve fibers distal to the damaged area, and the interruption of axon continuity. Peripheral nervous system damage results in major personal or social repercussions when viewed from a perspective of life with secondary problems, such as a substantial loss of function, permanent sensory and motor dysfunction, and neuropathic pain[2].

Nerve damage is classified according to the degree of histological damage and functional recovery. The nerve block recovers over a period of several weeks to several months due to the local damage to the myelin sheath leading to the blockage of nerve conduction. Axon damage causes Wallerian degeneration to the region distal to the damage and the prognosis is generally worse than nerve block, although there are some differences. Neurotmesis requires surgery in most cases because spontaneous recovery cannot be expected since the nerves were entirely severed[32]. When peripheral nerves have been damaged, dorsal root ganglion cells—which are the primary sensory nerve cells—begin to undergo histologically diverse changes, including the dissolution of Nissl bodies in nerve cell bodies, changes in neuropeptides such as substance P, along with apoptosis. The surviving nerve cells undergo regeneration and show an increased gene expression and protein production necessary for the generation of growth cones and axonal elongation required to form new connections with peripheral target organs. In addition, the surviving nerve cells temporarily promote the production of NGF and NGFR, like target organs, and form new cell receptors on the surfaces of cell membranes to promote axon regeneration[33].

Nerve damage due to ischemic injury and inflammatory reactions causes denaturation to adjacent areas. Even nerve cell bodies and damaged nerve cells begin to undergo a regenerative process because regeneration occurs by centering on surviving cells. Even once recovered, the functions of nerve cells cannot be completely regained, which eventually leads to significant disabilities. One of the changes caused by damage to peripheral nerves is that the axons, which are neurites, separate from cell bodies due to the damage, lose functions, and are gradually denatured. The substances liberated while axons are denatured sometimes initiate inflammatory reactions when they gather in macrophages. The damaged peripheral nerves have the ability to regenerate axons, which has been attributed to the fact that retinoic acid induces the synthesis of numerous cytokines that regulate the regeneration process[34].

Peripheral nerve damage not only causes changes in the nerves but also brings about changes in sensory nerve cells and motor nerve cells to promote the survival of nerves and the regeneration of axons in the damaged area, eventually enhancing functional recovery[35].

Among cases of damage to peripheral nerves, in those where the damage is due to pressure, the axons in marginal zones are severed in many cases whereas the nerve membranes that surround the nerves are not damaged in most cases. Thanks to this characteristic, the nerve membranes that have not been damaged will act as physiologic conduits in the regeneration of axons as targets, thus leading to axon function recovery close to normal levels over the regeneration process. The damaged area can be re-innervated through the regeneration of axons or the formation of side branches from surrounding undamaged axons. After peripheral nerves are crushed, a series of changes is induced in the damaged nerves and the nerve cell bodies in sensory and motor neurons, leading to the survival of nerves in the lesion regions and the regeneration of axons to achieve functional recovery. Such changes are triggered through the activation of the gene program and signal transmission processes in the local cells and cell adhesion molecules are expressed by the Schwann cells, and substrate glycoproteins of cells and fibroblasts increase, as changes in the nerves leading to the regeneration of axons[12][35].

After peripheral nerve damage, distal axons are disconnected from the nerve cell bodies and denatured, and then the phenotypic process known as nerve reactions and chromatolysis progresses. Wallerian degeneration provides the creation of a microenvironment at the distal region of the damage and causes the generation of nerve reactions and axon regrowth, which are essential for regeneration and axonal elongation. When the peripheral nerves have been damaged, the distal axons and myelin sheaths are degenerated due to Wallerian degeneration, and the proximal region is also affected. The final product of degeneration is removal by the joint action of Schwann cells and macrophages. The first stage of degeneration occurs within 24 hours after damage and progresses for about 1–2 weeks. The removal of myelin sheaths enables the removal of reproductive-inhibitory materials associated with them while maintaining their associated glycoproteins within the peripheral nerves. These processes occur because Schwann cells quickly separate myelin sheaths after axonotmesis as tyrosine kinase receptors take action. Once 2–3 days have passed since the initial damage was sustained, macrophages enter the nerves that have been denatured due to the stimulation by the leukemia inhibitors secreted from damaged Schwann cells and cytokines, such as interleukin[36].

Distal nerve Schwann cells are stimulated by proteins secreted by the axons that were separated due to the loss of axonal connections and by cytokines secreted by macrophages. Schwann cells show the highest increase 3 days after damage and then slowly decrease for 2–3 weeks. The rate of nerve regeneration is low at the beginning and the axons of damaged nerve cells are connected 3–4 days later. The regulation of nerve regeneration is influenced by various factors, but those factors in the damaged area carry the most significant effects, and axonal elongation requires appropriate actions by the neurotrophic factors produced by the action of an extracellular matrix and Schwann cells in the denatured nerve regions[37].

The sprouting of the nerves proceeds from the proximal to the distal axons, and due to this process, the number of nerve cells in the distal axons remains greater than the axons in the proximal segment for a long time. When the distal axons have been connected to peripheral tissues, a large number of axon buds are gradually localized. In this connection process, if some of the regenerative nerve fibers come into contact with distal axons in the wrong way, re-innervation becomes impossible. In the case of functional nerve regeneration, the recovery of distal nerve segments lost due to denaturation and re-innervation of target tissues acts as an important element. However, in the process of nerve regeneration, the reconstruction of nerves that perform normal functions does not occur when the general lesion area is serious. The diameter, conduction velocity, and excitability of those axons that have been regenerated after nerve damage and recovery are maintained at lower levels than normal for a long time. As a result, the recovery of organs where re-innervation occurred becomes incomplete and maladaptation often occurs. If a loss of nerve connection occurs in the lesion area, nerve regeneration restrictions are further exacerbated and as the gaps in the damaged area in-

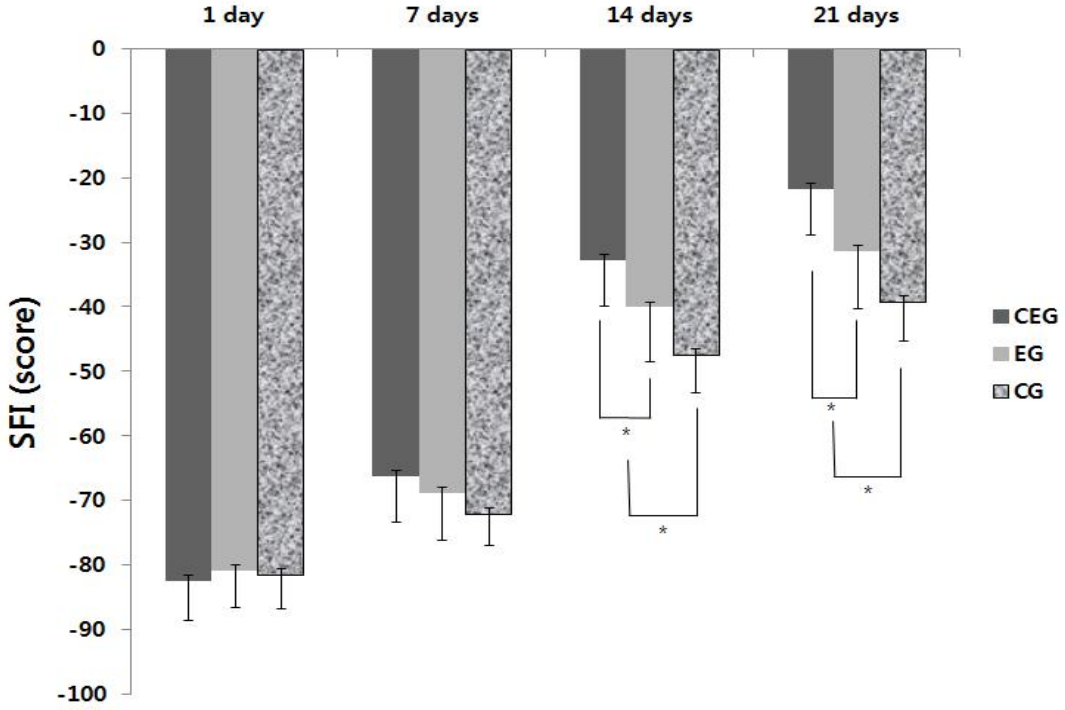

Fig. 2: Comparison of SFI in each group

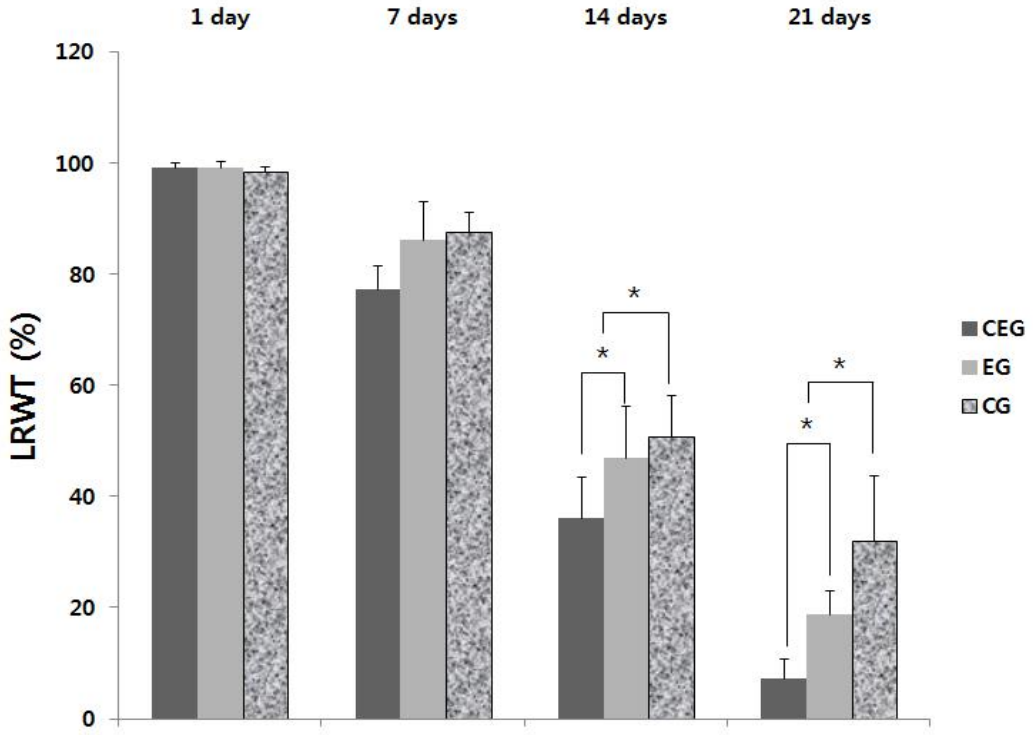

Fig. 3: Comparison of LRWT on hind limb in each group

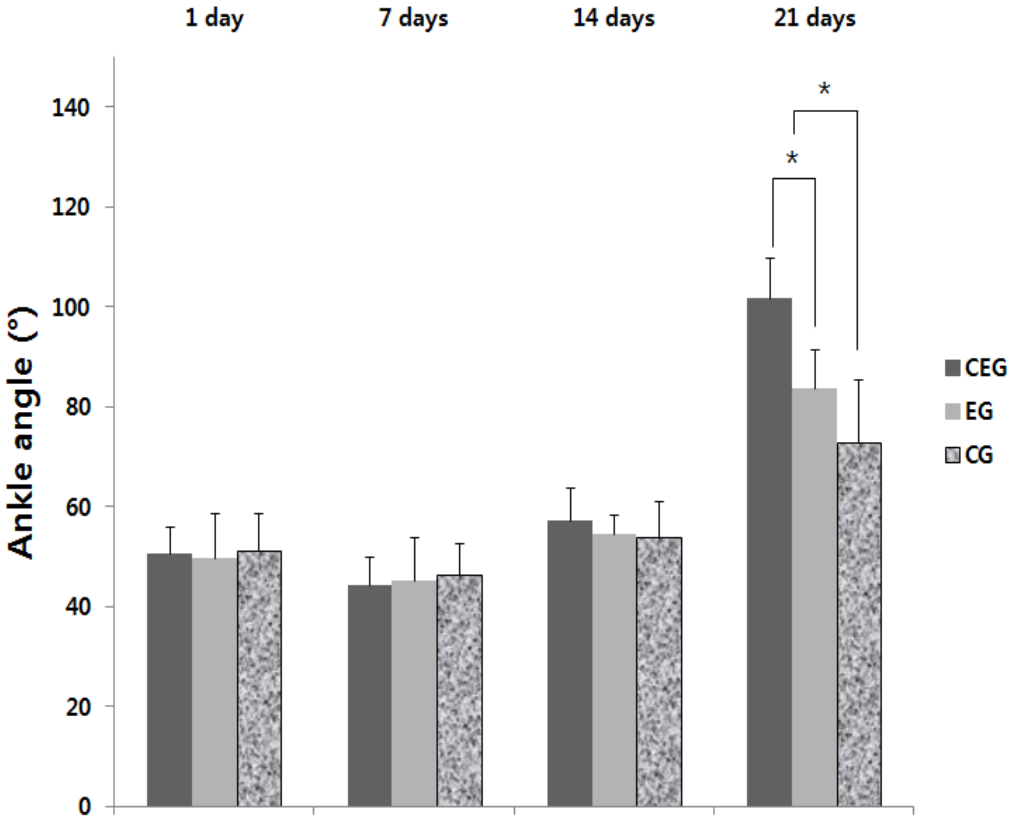

Fig. 4: Comparison of ankle angle on pre-swing in each group

crease, nerve regeneration decreases. Therefore, external stimulation methods that can assist the appropriate progression of nerve sprouting and adaptation should be used to enhance nerve regeneration ability[38].

Although the regions of peripheral nerve axon damage can be regenerated, re-innervation does not always result in the recovery of motor and sensory functions. There are special rules for the reconnection of damaged regions after nerve damage and regeneration. Nerve reconnection shows diverse patterns depending on the characteristics of the distal axons that are combined with the sprouts of the proximal axons and the morp developed by Taub and was based on hology of the terminal organs to which the damaged nerves are connected. The Schwann cells present in the motor nerves and sensory nerves selectively regulate axon regeneration methods through differences in the secretion of cell adhesion substances. Conversion into the phenotype for the ability to survive and regenerate axotomized nerves should occur at an early stage to promote nerve regeneration and re-innervation[39].

CIMT was developed by Taub and was based on behavioral neuroscience research[16]. The conventional CIMT is a rehabilitation program that constrains the unaffected side to limit its use for 90% of the patient's waking time for 2 weeks while engaging them in high-intensity training and task repetition of the affected side, thereby inducing the use of the affected upper limbs[16]. However, despite the positive effects of CIMT, the fact that patients feel the burden of this challenging training, experience stress, and face other safety problems were pointed out. Page et al. (2001) therefore implemented a modified form of CIMT. The modified CIMT was implemented for 30 minutes per session, 3 times per week for 10 weeks. The unaffected side was constrained only for the 5 most active hours of the subject's day. The therapeutic effects of the modified CIMT were proven through changes in effects before and after treatment and when compared with the therapeutic effects of other treatments[18][19].

Functional assessments related to peripheral nerve recovery are divided into those for motor nerve recovery and sensory nerve recovery. In relation to motor nerve recovery, SFI, which is a functional assessment for nerve damage in rodents, was known as the most reliable test method and used by previous study making functional assessments regarding regeneration after nerve damage possible. The SFI shows the ratio of insufficient functions when the normal gait is regarded as 0%. An SFI value of 0 indicates normal conditions and an SFI value of -100 indicates complete damage[24][25]. The SFIs obtained from the rats with crushed sciatic nerves per measurement period after the rats performed treadmill exercise using CIMT can be an objective index to evaluate the recovery of motor functions. In addition, the ladder walking test is a useful tool for assessing the waling ability of the forelegs and hind leg in relation to aging and damage to the motor system. The mistake scores of the ladder walking test show a significant correlation with the degree of neurological damage. Therefore, for the assessment of functional recovery, the SFI, the ladder walking test, and the angles of the ankle during walking were measured in this study. According to the results, in a comparison between the groups, the group that utilized CIMT showed statistically significant functional improvements compared to the control group at day 14 and day 21 of treatment. This indicates that the exercise group using CIMT and the exercise group subject to injection therapy further improved their walking ability compared to the control group. These results suggest that sensory fibers and motor fibers were effectively regenerated and re-innervated thanks to the stimulation of cell healing based on the principle of sensory motor integration therapy to improve functional activities.

The deconstruction of the ribosome bundles indicates chromatolysis, which is a morphologic change in the damaged nerve associated with anabolic reactions. Not using muscles due to chromatolysis and neuroparalysis is a cause of continuous innervated muscle atrophy after peripheral nerve damage and maximal atrophy occurs 2 weeks after damage. As such, chromatolysis is caused by biochemical changes, such as decreased DNA inhibition, increased RNA synthesis accompanied by cytoplasmic migration of nuclear RNA, and increased protein contents. Biochemical changes immediately after axonotmesis and morphological changes due to chromatolysis occur simultaneously. While the production of neurotransmitters and cytoskeletal proteins decreases, the enzymatic synthesis of pentose phos-

phate necessary for RNA synthesis increases. In order for the axotomized nerves to progress from the delivery stage to the regeneration stage, decreases in the synthesis of neurotransmission-related substances and appropriate changes in the expression of genes related to growth-related proteins and the components of nerve cell membranes are all necessary. In previous study, it was reported that the weights and cross-sections of the gastrocnemius muscle and the soleus muscle on the affected side significantly decreased compared to the unaffected side over time after nerve damage[5][32].

The limitations of this study include the fact that not all the experiment results conducted using rats can be generalized and the treatment program based on such experiments should be appropriately adjusted in terms of intensity and time if it is to be applied to humans. In addition, since the application of treadmill exercise using CIMT immediately after inducing a crushed sciatic nerve was difficult, only low-intensity exercises were performed. In addition, since CIMT could not be applied for a long period of time, it was applied only while exercises were being performed. Although walking patterns of white mouse and humans are different, improving the walking capacity of white mouse means improving their functional motor skills. To complement the limitations of this study, many more studies on CIMT applied to humans should be conducted.

4. Conclusion

In this study investigated the effect of exercise therapy combined with CIMT on neurological recovery after nerve injury. This study was conducted using 45 male Sprague-Dawley rats aged 8 weeks and weighing 250-300g. The rats were randomly assigned to a control group that would not to receive any therapy after nerve injury, a CIMT group that would undergo treadmill exercises utilizing CIMT after nerve injury, and an exercise that would only undergo treadmill exercise after nerve injury. The results of this study showed that there was no statistically significant difference at the beginning of SFI, ladder rung walking test, and ankle angle but there was a significant difference between the groups at 14th and 21st. In other words, exercise therapy combined with CIMT was able to maximize nerve recovery by inducing more nerve regeneration than CIMT alone. The results of this study can be used as a basic data to enable CIMT to be used as a method for neural recovery in clinical practice.

Acknowledgement

This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (No. 2017R1C1B5074613).

References

[1] Rochkind S, Nissan M, Alon M, Shamir M & Salame K (2001), Effects of laser irradiation on the spinal cord for the regeneration of crushed peripheral nerve in rats. *Lasers in Surgery and Medicine* 28, 216-219.
[2] Rosberg HE, Carlsson KS & Dahlin LB (2005), Prospective study of patients with injuries to the hand and forearm: Costs, function, and general health. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery* 39, 360-369.
[3] Ma CHE, Omura T, Cobos EJ, Latremoliere A, Ghasemlou N, Brenner GJ & Woolf CJ (2011), Accelerating axonal growth promotes motor recovery after peripheral nerve injury in mice. *The Journal of Clinical Investigation* 121, 4332-4347.
[4] Zimmermann M (2001), Pathobiology of neuropathic pain. *European Journal of Pharmacology* 429, 23-37.
[5] Aagaard P, Suetta C, Caserotti P, Magnusson SP & Kjær M (2010), Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scandinavian Journal of Medicine & Sciencein Sports* 20, 49-64.
[6] Perry VH & Brown MC (1992), Role of macrophages in peripheral nerve degeneration and repair. *Bio Essays* 14, 401-406.
[7] Tandrup T, Woolf CJ, & Coggeshall RE (2000), Delayed loss of small dorsal root ganglion cells after transection of the rat sciatic nerve. *The Journal of Comparative Neurology* 422, 172–180.
[8] Hoang TX, Nieto JH, Tillakaratne NJK & Havton LA (2003), Autonomic and motor neuron death is progressive and parallel in a lumbosacral ventral root avulsion model of cauda equina injury. *The Journal of Comparative Neurology* 467, 477-486.
[9] Tang H & Brimijoin S (2002), Death of preganglionic sympathetic neurons after surgical or immunologic lesion of peripheral processes. *Experimental Neurology* 177, 105–114.
[10] Ygge J (1989), Neuronal loss in lumbar dorsal root ganglia after proximal compared to distal sciatic nerve resection: aquantitative study in the rat. *Brain Research* 478, 193-195.
[11] Groves MJ, An SF, Giometto B & Scaravilli F (1999), Inhibition of sensory neuron apoptosis and prevention of lossby NT-3 administration following axotomy. *Experimental Neurology* 155, 284-294.
[12] Markus A, Patel TD & Snider WD (2002), Neurotrophic factors and axonal growth. *Current Opinionin Neurobiology* 12, 523-531.
[13] Boyd JG & Gordon T (2003), Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Molecular Neurobiology* 27, 277-323.
[14] Kuphal KE, Fibuch EE & Taylor BK (2007), Extended swimming exercise reduces inflammatory and peripheral neuropathic pain in rodents. *The Journal of Pain* 8, 989-997.
[15] Rochkind S, Drory V, Alon M, Nissan M & Ouaknine GE (2007), Laser phototherapy (780nm), a new modality intreatment of long-term incomplete peripheral nerve injury: a randomized double-blind placebo-controlled study. *Photomedicine and Laser Surgery* 25, 436-442.
[16] Taub E, Miller NE & Novack TA (1993), Technique to improve choronic motor deficit after stroke. *Archives of Physical Medicine and Rehabilitation* 74, 347-354.
[17] Rossini PM & Pauri F (2000), Neuromagnetic integrated methods tracking human brain mechanisms of sensorimotor areas 'plastic' reorganisation. *Brain Research Reviews* 33, 131-154.
[18] Page SJ, Sisto S, Levine P, Johnston MV & Hughers M (2001), Modified constraint induced therapy: a randomized feasibility and efficacy study. *Journal Rehabilitation Research and Development* 38, 583-590.
[19] Page SJ, Sisto S, Levine P & McGrath RE (2004), Efficacy of modified constraint induced movement therapy in chronic stroke: A single-blinded randomized controlled trial. *Archives of Physical Medicine and Rehabilitation* 85, 14-18.
[20] Genevieve PZ & Sue W (2012), Effects of Constraint-Induced Movement Therapy on Gait, Balance, and Functional Locomotor Mobility. *Pediatric Physical Therapy* 24, 64-68.
[21] Rostami HR, Akbarfahimi M, Hassani MA, Akbarinia AR & Samani S (2016), Occupation-based intervention versus rote exercise in modified constraint-induced movement therapy for patients with median and ulnar nerve injuries: A randomized controlled trial. *Clinical rehabilitation* 31, 1087-1097

[22] Bozkurt A, Deumens R, Scheffel J, O'Dey DM, Weis J, Joosten EA, Führmann T, Brook GA & Pallua N (2008), Catwalk gait analysis in assessment of functional recovery after sciatic nerve injury. *Journal of Neuroscience Methods* 173, 91-98.
[23] Shepherd RE & Gollnick PD (1976), Oxygen uptake of rats at different work intensities. *Pflügers Archiv* 362, 219-222.
[24] Wu YH, Lun JJ, Chen WS & Chong FC (2007), The electrophysiological and functional effect of shock wave on peripheral nerves. In 29th Annual International Conference of the IEEE. *Engineering in Medicine and Biology Society*, 2369-2372.
[25] Inserra MM, Bloch DA & Terris DJ (1998), Functional indices for sciatic, peroneal, and posterior tibial nerve lesions in the mouse. *Microsurgery* 18, 119-124.
[26] Riek BM, Henrich NP, Metz GA & Reymann KG (2004), Detection of chronic sensorimotor impairments in the ladder rung walking task in rats with endothelin 1 induced mild focal ischemia. *Journal of Neuroscience Methods* 137, 227-233.
[27] Metz GA & Whishaw IQ (2002), Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task toe valuate fore- and hind limb stepping, placing, and co-ordination. *Journal of Neuroscience Methods* 115, 169-179.
[28] Hayes HB, Chang YH & Hochman S (2012), Stance-phase force on the opposite limb dictates swing-phase afferent presynaptic inhibition during locomotion. *Journal of neurophysiology* 107, 3168-3180.
[29] De Ruiter GC, Spinner RJ, Alaid AO, Koch AJ, Wang H, Malessy MJ & Windebank AJ (2007). Two dimensional digital video ankle motion analysis for assessment of function in the rat sciatic nerve model. *Journal of the Peripheral Nervous System* 12, 216-222.
[30] Borel S, Schneider P & Newman CJ (2011), Video analysis software increases the interrater reliability of video gait assessments in children with cerebral palsy. *Gait & posture* 33, 727-729.
[31] Varejao AS, Meek MF, Ferreira AJ, Patrício JA & Cabrita AM (2001), Functional evaluation of peripheral nerve regeneration in therat: walking track analysis. *Journal of Neuroscience Methods* 108, 1-9.
[32] Seddon HJ (1943), Three types of nerve injury. *Brain* 66, 237-288.
[33] Heuman R, Korsching S, Bandtlow C & Thoenen H (1987), Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. *Journal of Cell Biology* 104, 1623-1631.
[34] Schwab ME (2000), Neurobiology. Finding the lost target. *Nature* 403, 259-260.
[35] Fawcett JW & Keynes RJ (1990), Peripheral nerve regeneration. *Annual Review of Neuroscience* 13, 43-60.
[36] Tofaris GK, Patterson PH, Jessen KR & Mirsky R (2002), Denervated Schwann sells attract macrophages by secretion of leukemia inhibitory factor(LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *The Journal of Neuroscience* 22, 6696–6703.
[37] Verdú E & Navarro X (1998), The role of Schwann cell in nerve regeneration. *Understanding Glial Cells*, 319–359.
[38] Krarup C, Archibald SJ & Madison RD (2002), Factors that influence peripheral nerve regeneration: An electrophy siological study of the monkey median nerve. *Annals of Neurology* 51, 69-81.
[39] Sung YB, Lee JH (2018), Effects of Nerve Regeneration Therapy on SFI in Nerve Injured Rats. *International Journal of Bio-Science and Bio-Technology* 10, 7-12.