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The Neuroprotective Effects of Alcoholic Extract of *Levisticum Officinale* on Alpha Motoneurons' Degeneration After Sciatic Nerve Compression in Male Rats

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ABSTRACT

As peripheral neurons are injured, several inflammatory factors will be released from the fiber of the injured nervous leading to morphological and biochemical changes in the injured point. This changes ultimately results in the Wallerian degeneration of the cell bodies of these neurons in the spinal cord which are involved in retrograde reaction. Levisticum officinale is one of the herbs that has been traditionally used for treatment of many diseases. Many studies have confirmed the anti-inflammatory and anti-oxidant effects of this plant. It is expected that the extract of Levisticum officinale would have a significant role in the restoration of nervous system injuries. The purpose of this study was to investigate the effects of the alcoholic extract of Levisticum officinale on the neuron protection of the sciatic nerves of injured rats. In this study, the ethanolic extract was obtained from Levisticum officinale. Then the rats were randomly divided into 5 groups, each containing 6 rats, including two control and compression groups and three treatment groups treated with 50, 75 and 100 mg/kg doses of the extract. Sciatic nerve in compression and treatment groups was exposed to compression for 60 seconds, and the ethanolic extract was injected intraperitoneally in the first, second and third weeks. The spinal cord of the rats were sampled and the neurons of the samples were counted using a dissector method after tissue passaging and staining. Meanwhile, the density of motoneurons was measured. T-test analysis was performed by using SPSS software. The density of α motoneurons in compression group decreased significantly as compared to the control group ($p<0.001$). The density of α motoneurons in all treatment groups with 50,75 and 100 extract doses was significantly increased as compared to the compression group ($p<0.01$). The highest neuron protection was observed in the treatment group with 100 mg/kg dose as compared to the compression group. Conclusion: Ethanolic extract of Levisticum officinale has neuron protective and restorative effects on the injuries of the peripheral nerves such as compression.

Keywords: *Levisticum officinale*, Neuron protection, α motoneurons, Wallerian degeneration

INTRODUCTION

When the cell body of neurons is destroyed or detached from their respective axon due to illness, injury or other factors, Wallerian degeneration occurs in the distal part of the axon, destroying the axons and their myelin sheath [1]. When Wallerian degeneration occurs, the distal part of the axon becomes swollen and chaotic during a few days and neurofibrils rapidly disappear, followed by the breaking of axon into smaller pieces. in the early days after degeneration, myelin sheaths of nerve fibers break into small elliptical pieces and gradually blow apart, and the axon residues and myelin sheaths become destroyed by macrophages [2][3]. If the damage is high, the effects of injuries, backward to the cell body, would cause central degeneration of the nerve and bodies break. This process is called chromatolysis. Furthermore, the nucleus shifts from the central position to the side position [4]. This process is faster in the peripheral nerves and all the steps are completed in a few weeks, while completion takes several months in the central nervous system. In addition, in an intact neuron cell body, healing of peripheral nerves progresses remarkably in contrast to the central nervous system, [3][5][21].

Levisticum officinale is one of the most important herbs and medicinal plants that has been used for a long time. Roots, fruits and leaves of this plant have medicinal characteristics. Alcoholic extract of this plant is produced for treatment of kidney stones and urinary tract infections in the pharmaceutical industries. The most important compounds identified in different parts of the plant are: Z-ligustilide, β-Phellandrene, α-terpinyl acetate, E-ligustilide and flavonoids like Quercetin [6]. Flavonoids are effective in destroying free radicals. Due to the presence of Quercetin in *Levisticum officinale*, the antioxidant effect is obvious. One of the most important roles of antioxidant compounds is their anti-inflammatory effect. So far, the neuroprotective effect of the extract of this plant has not been studied. So with regard to anti-inflammatory effect of this plant, we decided to examine the effect of the ethanolic extract of this plant on Wallerian degeneration of the sciatic nerve [6][7].

Axons in the peripheral nervous system (PNS) can ease the process of restoration but this process in the central nervous system (CNS) often fails. Regeneration of axons in the central nervous system is not completely successful and shows poor healing response to injury when the environment is harnessed towards the growth of axons, But peripheral neurons can easily follow the process of restoration [3][8]. It is believed that motor neurons get trophic factors from the target organs and without these factors the preservation is not possible. The loss of the protective effect of such factors because of denervation is likely to lead neurons towards exclusion from trophic factors and in most cases they lead to cell death [9][10]. Related research shows that the degeneration of the pre ganglion may be a general phenomenon that occurs after peripheral nerve injury. Cutting dorsal root L4 - L6 of spinal nerves in rats begin the process of degeneration in the affected area and extend to the dorsal column at about 3 mm per hour [11].

Followed by sciatic nerve compression, many biological events occur in cellular and molecular level such as apoptosis, increase of the entry of calcium into neurons, release of excitatory neurotransmitters like glutamate, formation of free radicals and activation of inflammatory processes [12]. Studies have shown that apoptosis occurs after sciatic compression and expression level of many genes increases (apaf1, BAX, Caspase 3,9). TNF-α is known as one of a Wallerian degeneration starters, which is aninflammatory cytokine that starts systemic inflammation and its concentration increases after peripheral nerve axon injury [2][12].

Flavonoids also inhibit production of inflammatory cytokines like IL6, IL1 and TNFα [16][17].Some of the flavonoids also inhibit NF-kB pathway. Inhibiting this pathway causes a decrease in TNF-α production [18][19]. Quercetin is one of the flavonoids in this plant that has anti-inflammatory effect in vivo. Quercetin inhibits inflammatorycytokines through weakening NF-kB and p38.This component also causes a decrease in miloperoxydase and oxidative stress and inhibits apoptosis [16][20]. In a research, the amount of Quercetin was measured between 32 plants and the amount of Quercetin in *Levisticum officinale* was the highest level as compared to the 32 plants [7][13].Studies on *Levisticum officinale* have proven very important effects on several cases. In many of such studies, the ingredients of these plants have been investigated, for both therapeutic and functional applications.

In 2003, researchers succeeded in determining the composition and the amount of poliphenolic compounds in the different parts of *Levisticum officinale*[13]. The compounds found in this plant justifies the effects observed. In a study conducted in 2007 in Iran, it became clear that the essential oil from *Levisticum officinale* was characterized by high quantity of monoterpens (98.3%). The main components in the oil wereβ-Phellandrene (42.5%) andα-terpineol (27.9%) [14]. Mirjalili et al. in 2010 reported composition and antibacterial effects of the essential oil of

this plant. The researchers in this study determined the amount of many materials in flowers and fruits at different developmental stages. The current study also showed that *Levisticum officinale* has a strong antibacterial effect. This was a very important result [6]. In 2011, Serkan Sertelet al. showed this plant has an antiproliferative activity. This study proved that this plant has an anticancer effect because of its anti-inflammatory compounds [15]. Some studies showed anti-inflammatory effects of this plant on kidney inflammations. Flavonoids existing in *Levisticum officinale* can justify this anti-inflammatory effects on kidney inflammations [22].

MATERIALS AND METHODS

In this study, Wistar albino rats were used. The rats at approximately 3 months and weighing 250 to 300 grams were prepared from Animals Research Center of Zahedan University of Medical Sciences, Zahedan, Iran. All the rats were kept for one week to adapt to new conditions in animals' room in University of Sistan and Balouchestan, Zahedanin the same condition as standard with a temperature of 21 to 25 ° C and 12 hours of light and 12 hours darknesslight-cycle. Then the rates were randomly divided into five groups with 6 animals in each group:

Control group: In this group, surgery was performed and only skin and muscle of the right thigh was opened but the compression of the sciatic nerve was not affected and only normal saline was injected.

Compression group: This group was subjected to surgery with severe compression of the sciatic nerve in the region of the right thigh (for 60 seconds with cord blood pence grade 2). The group was intraperitoneal injected with normal saline.

Treatment group A, B and C: In these groups, severe compression of the sciatic nerve in the region of the right thigh was performed (like the compression group) and ethanolic extract at a dose of 50, 75 and 100 mg/kg was injected to groups A, B and C respectively for three times (one injection for each week).

Twenty-eight days after the rats were injected with different doses of the extract, perfusion was performed. For this purpose, the animals' chest area was cut, as the triangle of the heart of animal was clearly visible. Then the needle of perfusion machine that contained the fixator (formalin 10 %)was entered into the aorta through left ventricle. In this way, the fixator flows the entire body of the animal and the animal organs can be fixed in the shortest time. The head and the skin of the animals were separated and the spinal cord segments related to sciatic nerve (L4-L5 andS1-S3) were sampled. Then the samples were entered into tissue passage stages. After that, trim operation was performed by a microtome machine and the samples were subjected to staining with Toluidin blue- erythrosin.

Dissector method was used to count the density of neurons. This method consists of two parallel cutting that have a known distance. Neurons are counted in the frame of reference. For analysis of the raw data, some parameters such as£Q (total neurons counted in a sample)،£Frame(Total number of sampled)V dissector (the volume of the sample) were calculated by the formula: V dissector = A Frame × H (A Frame as area of sampling frame and H as the distance between two slices or a thickness of each cut)) and Mean±SE are required. Numerical Density (NDwas) calculated by:£Q frame × dissector For statistical analysis, t-test and one way Anova test with significance level $P<0.05$ were used and were plotted using charts Excel software.

RESULTS AND DISCUSSION

The results of the study on effects of alcoholic extract of *Levisticum officinale* on neuronal density in the anterior horn of the spinal cord in treatments, compression and control groups as counting neurons are presented in the tables 1 to 6 as mean ± standard deviation.

Table 1: Neuronal Density in Control group

Neuronal Density (ND)	Neurons Number ΣQ	Dissector Number(Σ frame)	Sample
1750	63	30	C1
1527	55	30	C2
1722	62	30	C3
1666	60	30	C4
1607	58	30	C5
1722	62	30	C6
1665.66±77.59	60	30	Means ± SD

Table 2:Neuronal Density in Compression group

Neuronal Density (ND)	Neurons Number ΣQ	Dissector Number(Σ frame)	Sample
722	26	30	C1
833	30	30	C2
777	28	30	C3
777	28	30	C4
690	25	30	C5
722	26	30	C6
753.5±47.33	27	30	Means ± SD

Table 3:Neuronal Density in Treatment group A

Neuronal Density (ND)	Neurons Number ΣQ	Dissector Number(Σ frame)	Sample
1167	42	30	C1
1083	39	30	C2
1250	45	30	C3
1083	39	30	C4
1222	44	30	C5
1194	43	30	C6
1166.5±64.23	42	30	Means ± SD

Table 4:Neuronal Density in Treatment group B

Neuronal Density (ND)	Neurons Number ΣQ	Dissector Number(Σ frame)	Sample
1360	49	30	C1
1444	52	30	C2
1527	55	30	C3
1502	54	30	C4
1387	50	30	C5
1444	52	30	C6
1444±58.53	52	30	Means ± SD

Table 5: Neuronal Density in Treatment groupC

Neuronal Density (ND)	Neurons Number ΣQ	Dissector Number(Σ frame)	Sample
1502	54	30	C1
1416	51	30	C2
1416	51	30	C3
1556	56	30	C4
1583	57	30	C5
1527	55	30	C6
1500±64.37	54	30	Means ± SD

Table 6: Comparing of Neuronal Density in all groups

Treatment C	Treatment B	Treatment A	Compression group	Control group	Sample
1502	1360	1167	722	1750	C1
1416	1444	1083	833	1527	C2
1416	1527	1250	777	1722	C3
1556	1502	1083	777	1666	C4
1583	1387	1222	690	1607	C5
1527	1444	1194	722	1722	C6
1500±64.38	1444±58.53	1166.5±64.23	±47.33 753.5	1665.66±77.59	Means ± SD

Neuronal density in compression group in comparison to the control group was significantly decreased because of the retrograde reaction (compression group 753.5±57.33, control group 1665.66±77.59, $P<0.001$) (figure 1). Neuronal density in treatment group A with a dose of 50 mg/ kg alcoholic extract (1166.5±64.23) as compared to the compression group showed a significant increase (753.5±47.33, $P<0.05$). Density of alpha motoneurons of the anterior horn in treatment group B, with alcoholic extract of the plant at a dose of 75 mg/ kg (1444±58.53) as compared to the compression group (753.5±47.33) showed a significant increase ($P<0.01$). The most neuroprotective effect was observed in treatment group C with 100 mg/kg alcoholic extract as compared to both of the other treatment groups, and showed a significant increase as compared to the compression group (1500±64.38, 753.5±47.33, $P<0.01$) (figure 2).

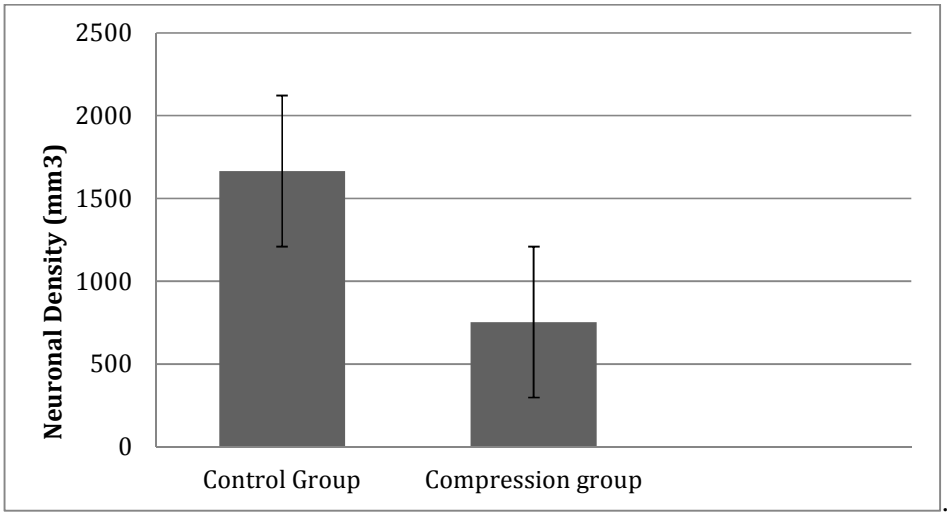

Figure 1:Density of alpha motoneurons of the anterior horn of the spinal cord of the control and compression groups

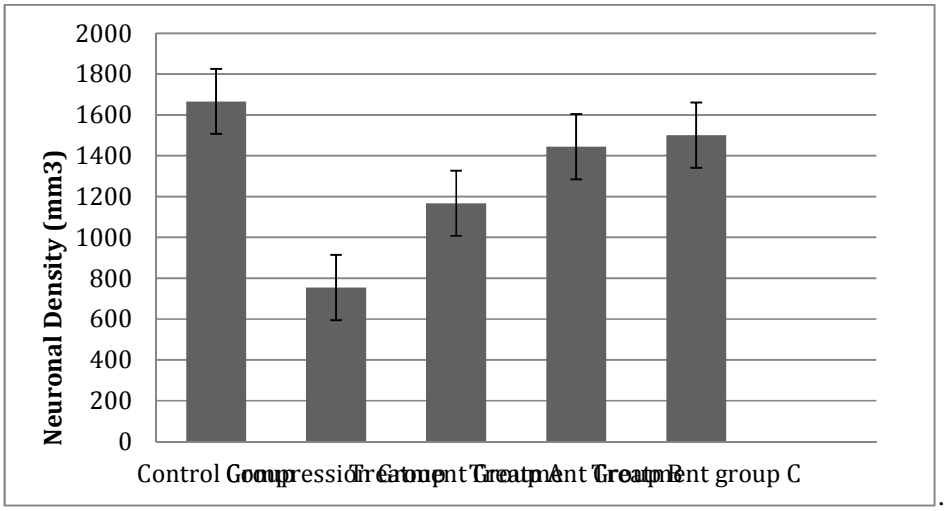

Figure 2: Neuronal densities of the control, compression and different treatment groups

According to previous related studies, this plant is a rich source of anti-inflammatory compounds that has therapeutic effects on some organs' inflammation. Also *Levisticum officinale* has strong anti-bacterial and anti-cancer therapeutic effect. Alcohol is used as skin disinfectant in combination with other solution or alone [23]. This study was designed, based on the results of the previous studies conducted. Based on the anti-inflammatory effects of this herb that has been demonstrated in previous studies, the results obtained in this study is in sufficient agreement with its reported medicinal potential. Existing anti-inflammatory compounds like Quercetin cause inflammation and suppression of nerve cells. However, recovery of the cell body and the fibrils of neurons in peripheral nervous system is repairable. This study demonstrated that this plant has a potential capacity of neurons protection.

CONCLUSION

According to this study, neuronal density in compression group as compared to the control group had a significant decrease. It means that the animal sciatic nerve compression leads to the creation of retrograde central degeneration to the cell bodies of motor neurons in the anterior horn of the spinal cord. Based on the results, the treatment groups with alcoholic (ethanolic) extract showed more neuronal density than the compression group and also injected dose-dependent effects were observed significantly. Additionally, the dose of 100 mg/kg had the highest effect, dose of 75 mg/kg had average effects and dose of 50 mg/kg had the least effect on restoration of nervous system injuries.

Therefore, the injection of alcoholic (ethanolic) extract of *Levisticum officinale* has both repair and restoration effects on peripheral nerves (such as sciatic nerve) injuries. Further studies by increasing the number of samples and increasing the number of injections will generate more accurate results and provide better evaluation of the effects of plant extract. Study on neuroprotective effects of the methanolic extract of this plant and also monitoring the behavioral tests in rats helps to examine the applicability of *Levisticum officinale* extracts for animal healthcare.

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