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The Effects of Root Aquatic Extract of *Salvia staminea* on Neuronal Density of Alpha Motoneurons in Spinal Cord Anterior Horn after Sciatic Nerve Compression in Rats

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Abstract: The aim of this study is carried out to examine the neuroprotective effects of root aquatic extract of *Salvia staminea* on neuronal density of motoneuron in spinal cord anterior horn in rats. Forty adult male Wistar rats were used and divided to five groups (control, compression, three experimental groups). In compression and experimental groups right sciatic nerve were highly compressed for 60 sec, assigned to experimental groups (Compression + aquatic extract of *Salvia* root injections (25, 50, 75 mg kg⁻¹, i.p., 4 time) (N = 8). After 4 weeks post-operative the lumbar segments of spinal cord were sampled, processed, sectioned serially and stained with toluidine blue (pH 4.65). By using stereological quantitative technique, the number of alpha motoneurons in the right horn of spinal cord were counted and compared with each other. Statistical analysis showed remarkable increase in the number of alpha motoneurons in the groups with dosage (50, 75 mg kg⁻¹) in compared to compression. Result shows that root aquatic extract could increase neuronal density motoneuron in anterior horn of spinal cord after sciatic nerve injury in rat.

Key words: *Salvia*, degeneration, sciatic nerve

INTRODUCTION

The cell body of neuron is the place to synthesize cytoplasmic members and surface membrane of all cell parts. When nervous fiber is cut or crushed degeneration wallerian is occurred then myelin is divided to geom like pieces. Finally, macrophages and shown's cells raid in to neurollema (shown) cover and swallow axonal myelin pieces (McPhail *et al.*, 2004).

The course of defeated axons destruction is different from wallerian process, because the destruction phagocytosis of these pieces in CNS is carried out at very lower speeds (Siniscalco *et al.*, 2007). The defeated pieces of myelinated axons can be seen even after months and phagocyte cell containing a parted pieces remain in the place even after years and show the place of destroyed fibers (Siniscalco *et al.*, 2007). Chromatolysis is a procedure following axonal defeat in neurons cellular body so some times this phenomenon is called axonal reaction (Baker and Hagg, 2005).

Chromatolysis or other cellular body's reactions are requirements for the correction of remained of the nucleus (Baker and Hagg, 2005), although, some neurons can correct their axon without making significant changes in cellular body's metabolism (Rosenfield and Paksima, 2001). But some neurons continue to correct their axon after the recovery of chromatolysis (Maier and Schwab, 2006).

Salvia was recognized and interesting in the collection of Greek and Romans herbal medicine from the ancient times. In middle ages, the European traditional medicine scientists were using *Salvia* to cure constipation (Capasso *et al.*, 2006), pestilence, cold, fever and hepatic malfunction, epilepsy and paralysis and it was prescribed for strengthen muscles and relieving nerves (Cheng *et al.*, 1999).

This plant entered Europe in 9th century and spread rapidly and then entered China. It useful medical features are: stimulating digestion (Capasso *et al.*, 2006) and secretion of bile. This plant has aromatic components and its essence is used in perfume making.

The basic ingredients of this genus are essences, ditrepens, tri-trepnoids, tanens, flavonoids, rezine, saponins and phenols. Besides, the basic component of *Salvia* species is salvanic acid (Ahmed *et al.*, 2004). Different species of *Salvia* have anti-oxidant effects and karmozeic acid, rosmarinic acid, rosemanol, karnosol, salvanic acid A and salvanic acid B are responsible for collection and removing free radicals (Mosaddik, 2003; Koo *et al.*, 2004).

The acetonic and metanolic extract of *Salvia aegyptica* has a painkilling and anti-inflammating *in vivo* (Imanshahidi and Hosseinzadeh, 2006). Phenolic material existing in *Salvia* is anti-inflammation and its flavonoids are anti-oxidant. The purpose of this research

is to examine neuroprotective effects of root aquatic extract and of *Salvia staminea* on neuronal density of alpha motoneurons in anterior horn of spinal cord after compression of sciatic nerve in rats.

MATERIAL AND METHODS

Materials: The *Salvia* roots (herbarium code 2676) was supplied by Islamic Azad University of Mashhad, Iran (2009).

Salvia root was grinded by a grinder system. The powder was kept in a cool and dry place until extraction time.

How to prepare extract: Aquatic extract of root powdered was prepared by Soxhlet apparatus method (Cicchetti and Chaintreau, 2009) and the extraction was carried on in the laboratory of herbal researches. Most of the requisite material of this project was supplied by the Sigma Company.

Method: Forty Wistar male rats were used in this research. These rats were purchased from animal part of Razi institute and were maintained in the house of Animals Biology Department Science Faculty with temperature of 21°C and light cycle of 12 h light and 12 h darkness in standard conditions. All animal could access enough water and food.

At first, forty male rats with below mentioned characteristics were classified in five groups.
Age: 3 month, weight: 300-350 g sex: male

Groups:

- Control (N = 8)
- Compression (N = 8)
- Compression + aquatic extract of *Salvia* root injections (25 mg kg⁻¹, i.p., 4 time) (N = 8)
- Compression + aquatic extract of *Salvia* root injections (50 mg kg⁻¹, i.p., 4 time) (N = 8)
- Compression + aquatic extract of *Salvia* root injections (75 mg kg⁻¹, i.p., 4 time) (N = 8)

How to carry out compression: Rats in each group were anesthetized under interaperitoneal injection of 0.24 cc of a mixture (1:2) of 10% ketamin and 2% xylazine. Then sciatic nerve of right leg at the top of thigh bone was compressed by use of locker pincers. After the compression the place was disinfected and patched by metal pincers (Behnam-Rasouli *et al.*, 2000). At the time of anaesthetize the rats kept warm. After becoming

conscious, the rats were carried to separate cages and kept in standard circumstances of light, temperature and moist.

They could consume enough water and specified food during the experiment. In care groups, the extract injection was carried out immediately after compression during 28 days (Each week one injection).

After 28 days following perfusion a block of the spinal cord segments L4 to L6 (approximately 8 mm length) was removed while sciatic nerve roots of both sides were still attached it. Since, the nervous tissues are very sensitive and autolysis rapidly. Besides fixators can not penetrate in spinal cord because of though cover around it. So, for better fixation, perfusion method was used. When perfusion finished, sampling of spinal cord was began. The spinal cord was completely separated to the end of horse tail in order to equally sampling in all samples and some samples 8 mm in length from 18 mm upper than the end of horse tail, then samples entered to passage stage, then entered to cutting stage and serially 7 Mm sections were prepared and colored with toluidine blue. Required photos from front horn of spinal cord for future studies were taken according their numbers. Two photos were taken from two serial sections, one of anterior horn right half of first section and another from anterior horn right half of second section. The magnitude of microscope in this stage was $5 \times 10 \times 2/5 = 100$.

In order to count neurons random systematic method was used and dissector method was used for counting particles (Tehranipour and Kabiri, 2009). To analyze raw data, parameters like: ΣQ , Σ frame, average, V dissector are required, these parameters are identified as below:

- ΣQ = The sum of counted neurons in one half sample
- Σ_{frame} = Sum of sampling times
- $V_{\text{dissector}}$ = Volume of sampling frame equals:

$$V_{\text{dissector}} = H \times A_{\text{frame}}$$

Where:

A_{frame} = The area of sampled frame

H = The distance between to cut or the thickness of each section

The numerical density of cells (NV) in the ventral horns of spinal cord was providing according to the equation:

$$NV = \frac{\Sigma a}{\Sigma \text{frame} \times V_{\text{dissector}}}$$

In this research, we use micrometer lam in order to measure the real area of dissector on the sample and in mm. The area of dissector equals:

$$V_{\text{dissector}} = 347/222 \times 347/222 \times 7 = 84394182 \text{ mm}^3$$

Statistical analyze: The ratio of numerical density of neurons in samples of spinal cord was then used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test.

RESULTS

The result of examining central degeneration after compression and examining neuroprotective effects of groups cared with root aquatic extract are presented as counting Alpha motoneurons in anterior horn of spinal cord in Table 1. The results show a significant difference between the neuronal density of anterior horn of spinal cord in compression and control groups ($p < 0.1$). The effects of the compression of sciatic nerve cause the destruction of cell bodies in motoneurons of spinal cord (Fig. 1).

The results dose not show a significant difference between the neuronal density in anterior horn of spinal cord in compression and treatment, with root aquatic extract groups ($p < 0.1$), although there is an increase in density of neurons in treatment with root aquatic extract in dose 50 and 75 mg kg⁻¹ in compare with compression group.

The finding show that there is not any increase in the neuronal density of alpha motoneurons in care group with root aquatic extract (dose 25 mg kg⁻¹) in compare with

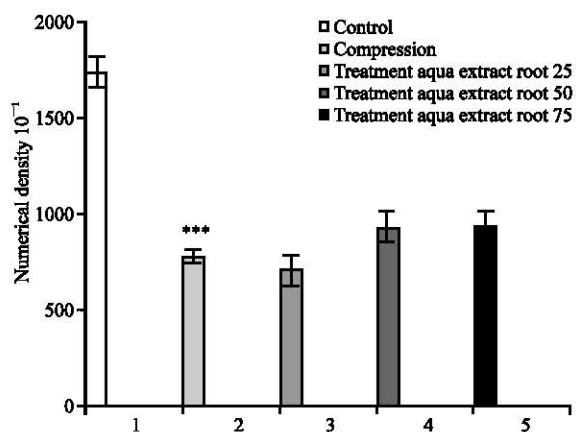


Fig. 1: The X-axis shows the different groups. The compression of the Neuronal density of Alpha motoneurons in the anterior horn of spinal cord in treatment with root aquatic extract in 3 different dosages (25, 50, 75 mg kg⁻¹) and the compression of the Neuronal density of Alpha motoneurons in the anterior horn of spinal cord in compression and control groups (***) $p < 0.1$

compression group (Table 1). It seems that the extract in this dose has less neuroprotective effects, but other doses (50, 75 mg kg⁻¹) have shown an increase in neuronal density in compare with compression group.

The statistical analysis of data does not show a significant difference between neuronal density in compression group and treatment groups with dose (25 mg kg⁻¹). Although, in treatment with (50, 75 mg kg⁻¹) there is a remarkable increase in neuronal density in compare with compression group but this increase is not significant. There is not any changes or different in number of alpha motoneurons in section from compression group and treatment with doses 75 mg kg⁻¹ (Fig. 2a, b).

Table 1: Neuronal density of alpha motoneurons in anterior hom of spinal cord in different groups

Groups	NV×10 ⁻⁹ (Mean±SE)
Control	1739.3±78.5
Compression	781.2±27.9
Treatment (25 mg kg ⁻¹)	713.0±79.4
Treatment (50 mg kg ⁻¹)	933.0±73.0
Treatment (75 mg kg ⁻¹)	950.0±70.0

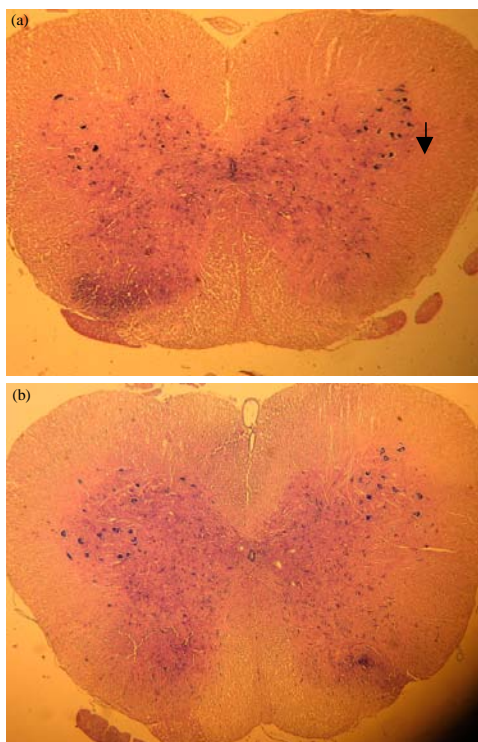


Fig. 2: The cross section of spinal cord (magnitude 50 x-toluidin blue). (a) Treatment with root aquatic extract. (b) Compression the nucleus of alpha motoneurons was showed with spikes

DISCUSSION

Examining the death phenomenon of neural cells during natural evolution, diseases and tissue damages has a long history. By the years 1940-1950, axotomy was introduced as the suggested pattern of neurons death and long term researches were carried out on neuron's reaction following axotomy.

In 1987, it is claimed that neurons death occurs after cutting the nerve too. It has proven that cutting sciatic nerve in rats newborns cause decrease in motoneurons of spinal cord (Nesic *et al.*, 2001).

The results show that in compression group the neuronal density was decreased in compare with normal group (Table 1). Snider caused death in neural cells by axotomy and claimed that this procedure is similar to natural death of neurons and younger cells are more sensitive to damage rather than mature cells (Nesic *et al.*, 2001). After cutting a nerve, the cell experiences some structural and morphologic changes similar to changes in neurons experiencing planned death. Nucleus not locating at the center, folding of nuclei's membrane and pekinosis of nucleus are some of these changes (Nesic *et al.*, 2001). Causing any pressure to axon or occurrence of axonic defeat induced changes in both distal and proximal parts because of breaking the connection of axon with neuron's body. In distal part, both axon and myelin cover is degenerated completely. In this defeat, emerging 2-3 days later, Endoneurim layers keep unchanged. Considering the ability of producing new myelin, schwann cells increase along degenerated fiber, so in distal part of defeated nerve, for myelinating in new branches of proximal part of nerve new circumstances is required (Rosenfield and Paksima, 2001).

In central nervous system there is no collagen cover or basic membrane around nerves and oligodendrocytes are not able to multiply so axonic new branches (of proximal part) are not able to grow because of lack of a channel or cover to locate in totally, the correction is closely related to factors like grow stimulating materials or grow preventing materials (Imaizumi *et al.*, 2000; Yiu and He, 2006). The most important difference between the sequences of damage environmental neural system and central neural system is reformation of axons. The cutted axons of one nerve, grow rapidly again and connect to related organs. When axons of brain or spinal cord are cut, the proximal part of cutted part begin to grow and send same of its branches to the damaged part, but this growing ends after two weeks. This kind of use less grows exists in central neural system of mammals, birds and reptiles (McPhail *et al.*, 2004).

The circumstances initiating correction of neural system's neurons are not completely recognized, but the role of factors increasing neural life or protecting axonic growth and prepare appropriate circumstances for correction, should not be ignored.

These factors consist of fibroblastic growth factors secreting from shown cells and macrophages that their synthesis is stimulated by cytokines and sticky molecules. Other neurotrophic factors affecting on correction are growth factor (NGF), growth factor originating from brain (BDNF), insulin-like growth factor (IGF) of integrin, laminin, collagen and fibronectin (Imaizumi *et al.*, 2000).

Many trophical factors affect on correcting nerves and many studies are carried out on potential role of growth factor (Oudega and Hagg, 1999).

Many researches show that cells death after compression or chronic contraction of sciatic nerve CCI are planned cellular death or apoptosis. In many experiments was emphasis on the pre- apoptotic genes or apoptosis genes roles. For example after sciatic nerve injury some of these factor such as factor of apoptotic (apaf-1), bax, caspase 3 and 9 have been activated (Sakurai *et al.*, 2003; Li *et al.*, 2006). In heavy damages, apoptosis can cause second degeneration in damage place and demyelization of neural channels (Crompton, 2000). This research was showed that the compression occurred on sciatic nerve reduced the neuronal density of spinal cord anterior horn. There is a significant difference between the neuronal density of compression group and control group ($p < 0.05$).

Following sciatic nerve injury, the generation of free radicals causes apoptosis in the cell body of a neurons of spinal cord (Siniscalco *et al.*, 2007). Antioxidants inactive free radicals and prevent apoptosis in spinal cord neurons. Different species of *Salvia* have anti oxidant effects and karnosic acid, rosmarinic acid, rosmarinol, karnosol, salvinol, salvamic acid b are responsible for collection and removing free radicals (Mosaddik, 2003).

Anti- oxidant effects of *Salvia miltioriza* root are because of components such as dihydrotanshinogen, tanshinogen and crypto tanshinogen (Kang *et al.*, 2003). Therefore, neuroprotective effects of root aquatic extract are due to its anti- oxidant effects (Fig. 1, 2). In treatment group with root aquatic extract doses ($50,75 \text{ mg kg}^{-1}$) the neuronal density are increased but this increased was not significant (Fig. 1). It shows that the root aquatic extract has some component that can induced neuroprotective effects.

In total administration of root aquatic extract after sciatic nerve injury can increased neuronal density in compare with compression groups.

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