NEUROPROTECTIVE EFFECT OF *SALVIA STAMINEA* ALCOHOLIC EXTRACT ON PERIPHERAL NERVE DEGENERATION AFTER SCIATIC NERVE COMPRESSION IN RATS

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Summary

There are extensive evidences those axonal processes of the nervous system (peripheral and/or central) may degenerate after nerve injuries. Wallerian degeneration and choromatolysis are the most conspicuous phenomena that occur in response to injuries. In this research the effect of root alcoholic extract of ***Salvia staminea*** on neuronal density of motoneurons in spinal cord anterior horn in rats was studied.

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 s, assigned to experimental groups (Compression + alcoholic extract of salvia root injections (25 ,50,75 mg/kg $^{-1}$, ip, 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 analyses 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 alcoholic extract could increase neuronal density motoneurons in anterior horn of spinal cord after sciatic nerve injury in rat.

Key words: Salvia, Degeneration, Sciatic nerve

Running title: salvia stamina effect on neuronal degeneration

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Introduction

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(1), pestilence, cold, fever and hepatic malfununction (2), epilepsy and paralysis and it was prescribed for strengthen muscles and relieving nerves (3).

This plant entered Europe in 9th century (4) and spread rapidly and then entered china. Its useful medical features (5) are: stimulating digestion and secretion of bile cytotoxicity (6). 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, flaunoids, rezine, saponins and phenols. Besides, the basic component of Salvia species is salvanic acid (7). Different species of Salvia have anti-oxidant effects (8) and karnozeic acid, rosmaric acid, rosemanol, karnosol, salvanic acid A and salvanic acid B are responsible for collection and removing free radicals (9).

The acetonic and metanolic extract of *Salvia aegyptica* has a painkilling and anti-inflammating in vivo (10,11). Phenolic material existing in Salvia is anti-inflammation and its flaonoids are anti-oxidant (12).

Within a few days following axotomy, changes occur in the cell bodies of most types of neurons. When a motor axon in a peripheral nerve is severed, a characteristic sequence of changes occurs (13). The distal of portion of the axon degenerates, as dose a short length of the proximal portion. Certain effects of axotomy-chromatolysis , atrophy and cell death result from the less of trophic substance produced by the target tissue and transported retrogradely along the axon to the cell body (14).

Wallerian degeneration is an important phenomena,which consists of the breakdown and phagocytosis of damaged axons and their myelin sheaths distal to the site of injury(15). The initial axonal breakdown occur rapidly undergoing granular degeneration of cytoskeletal structures via the action of proteases (16). This type of degeneration is remarkably slow in the mammalian CNS and takes several months to complete. All of these phenomena causes' neuronal death and decrease in number of intact neurons induce neurodegenerative disease (17).

The purpose of this research is to examine neuroprotective effects of root alcoholic extract 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

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

Animal subjects

Forty male, Wistar rats weighting between 300-350 g served as subjects for these experiments. All animals were housed individually and maintained on a 12/12 light/dark cycle, with lights on at 6.00h. Ambient temperature in the animal facility was kept at 22±2C$^{\circ}$ .Food and water was given ad libitum.

Extraction

Salvia staminea was collected from a reign around mashhad and was coded with Islamic Azad University of Mashhad, Iran herbarium (herbarium code 2676). For extraction 50g powder rhizome with 300 cc alcohol were mixed and extraction perform with Soxhlet apparatus (18). After obtaining extract, it was situated in oven with temperature ($45^{\circ}\pm2^{\circ}$) for 48 hours to remove solvent.

Groups

Forty rats divided into five groups:1) Control; 2)Compression ; 3)Compression + alcoholic extract of salvia root injections (25mgkg $^{-1}$, ip ,4 time); 4) Compression + alcoholic extract of salvia root injections (50mgkg $^{-1}$, ip ,4 time); 5) Compression + alcoholic extract of salvia root injections (75mgkg $^{-1}$, ip ,4 time) (N=8)

Surgery

Animals were anesthetized under interaperitoneal injection of 0.24 cc 92-of a mixture (1:2) of 10% ketamin and 2% xylazine.

Right sciatic nerve was exposed through a gluteal muscle splitting incision. At this location the nerve trunk was crushed for 60 seconds period between prongs of #5 clamp forceps. The muscle and skin were then closed with 14mm stainless steel sutures (19).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 day following perfusion a block of the spinal cord segments L4 toL6 (approximately 8mm 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 8mm 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 $5\times10\times2/5=100$.

In order to count neurons random systematic method was used and dissector method was used for counting particles (20).

Statistical analysis

The ratio of numerical density of neurons in samples of spinal cord was used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test. All data were presented as mean±S.E. Differences were considered statistically significant when $p<0.05$.

Results

The results indicate several facts: 1-The number of alpha motoneurons decreases in compression group in compare with control group.

2- The neuronal density (number of alpha motoneuron) in all experimental groups increased in compare with compression group (Table.1.)

Tab.1: **Neuronal density of Alpha motoneurons in anterior horn of spinal cord in different groups.Data are presented as mean±SEM (n=8 in each group).**

Groups	NV (Mean ±SE)
Control	1739.3 ±78.5
Compression	781.2 ± 27.9
Treatment (25mgkg$^{-1)}$	914 ±102
Treatment (50mgkg$^{-1)}$	870 ± 49
Treatment (75mgkg$^{-1)}$	973 ±49.8

3-This increase just in experimental group with doses (75mgkg $^{-1}$) was significant ($p<0.05$; Fig.1).

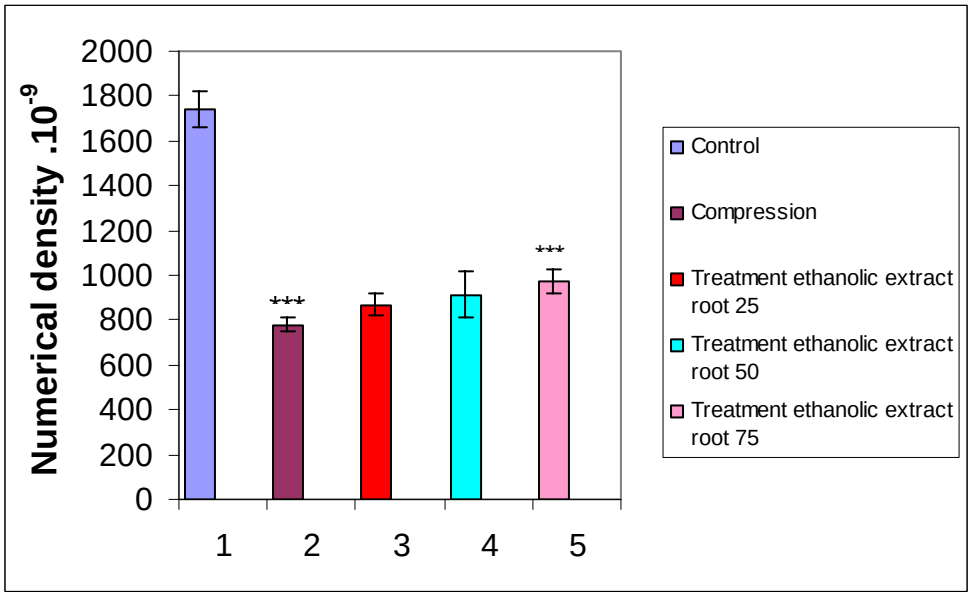

Fig.1: Comparison of Neuronal density of Alpha motoneurons in anterior horn of spinal cord in different groups. Data are presented as mean±SEM (n=8 in each group).
A) The compression of the neuronal density in treatment alcoholic extract root in 3 different dosages (25,50,75 mg/kg)with compression group.
B) The compression of the neuronal density in compression and control groups.(*p<0.05)**

4-The size and number of alpha motoneuron in experimental groups have remarkable increase in compare with compression group(Fig.2).

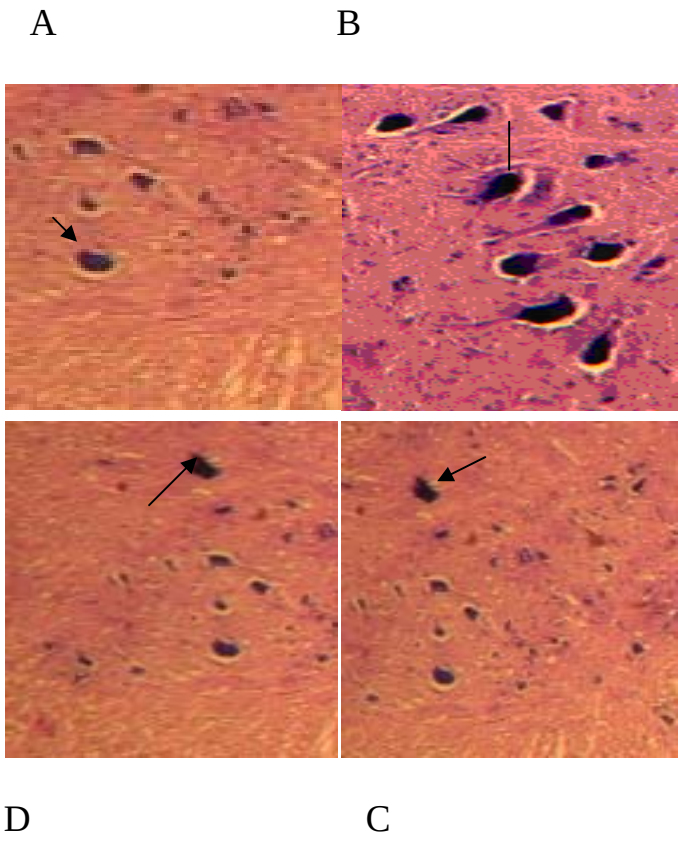

Fig.2:The cross section of spinal cord anterior horn (magnitude 50 x and painting in toloidin blue)
A: compression group
B: Ttreatment root alcoholic extract doses 75mgkg $^{-1}$
C: Treatment root alcoholic extract doses 25 mgkg $^{-1}$
D: Treatment root alcoholic extract doses 50mgkg $^{-1}$
The nucleuses of neuron were shown with spike.

Discussion

Neurodegenerative diseases are characterized by progressive dysfunction and death of neurons (21). Neurodegeneration may occur by apoptosis, necrosis or both (22). It is believed that there are many different mechanisms and neurochemical modulators responsible for the central nervous system damage, which may overlap temporarily (23). Progressive dysfunction and death of neurons characterize neurodegenerative diseases (24). There are some evidences supporting the hypothesis that some herbs may also exert neurotrophic actions. It provides neuronal differentiation and increase in neuritis outgrowth (25).

In 1994, Xiang and Kuang showed that *Salvia miltiorriza* root, stops neuronic death by prevention of relieving glutamate (26).This plant is specie similar to our specie in this research. So it is possible that *Salvia staminea* extract has neuroprotective effects on injured neurons and the results of this research show this hypothesis. It was shown that the neuronal density of motoneurons in compression group decreased in compare with control group (Table.1).But in all treatment groups with root alcoholic extract the neuronal density was increased in compare with compression group (Fig.1). It may be concluded that there is some component in root alcoholic extract of *Salvia staminea* that protected neurons of death after sciatic nerve injury or may be this component has had some roles in regeneration phenomena and act as a neurotrofic factors. As was shown in (Fig.2), in experimental group with doses (75mgkg $^{-1}$) soma size of alpha motoneurons was increased in compare with compression group.

In 2005 Wang showed that the bax gene in cared cells with Mpp + (ion 1- methyl 4 – phenil piraniuyum) is increased , while treating cells with salvanic acid (a diterepen existing in salvia root affect on bcl2), the activation of bax gene reduce and increase bcl2 level. The studies have shown that Mpp+ can initiate relieving Cytochrom c from mytocondria and activate apoptosis cascade while salvanic acid induce a decreases relieving of Cytochrome (c) and stop apoptosis (27).These studies are coordinate with our results.

The essence of Tanshionon II B is a primitive active component of root *Salvia miltirriza* (danshen). In 2008, the neuropratective effects of this material and it's mechanism on cortical rat's neurons were examined (28). Apoptosis in vitro was carried out on cortical rat neurons by staurosporin and apoptotic morphological changes such as folding cells and membrance inflation and etc were observed (29). In fact the treatment with tanshionone II B stopped apoptosis. The material used as apoptosis initiator can cause increase in gene of bax and decrease of gene bcl2 in neurons. The low regulation of bcl2 gene cause later separation of bcl2, bax and hemodimer bax form and apoptosis occur. This study showed that tanshionone IIB cause high regulation of gene bcl2 and prevents apoptosis.

Probably root alcoholic extract has effective materials that act as antiapoptosis(30). The root alcoholic extract in doses (75mgkg $^{-1}$) showed better anti- apoptosis effects(Table 1 and Fig.1).Following sciatic nerve injury, the generation of free radicals cause apoptosis in the cell body of a neurons of spinal cord(31). Antioxidants materials inactive free radicals and prevent spinal cord neurons of apoptosis. Different species of Salvia have anti oxidant effects for example karnosiic acid, rosmaric acid, rosemanol, karnosol, salvinol, salvanic acid b are responsible for collection and removing free radicals.

Anti- oxidant effects of *Salvia miltioriza* root are because of components such as dihydrotanshinogen, tanshinogen and crypto tanshinogen (32).Therefore neuroprotective effects of root alcoholic extract are due to its anti- oxidant effects.

we can conclude that the root alcoholic extract of this plant prevent cellular death of neurons after compression of sciatic nerve because of anti – apoptosis and anti-oxidant effects and many other effects that were not established.

So these data confirm that there is potential neuroprotective role for root alcoholic extract with doses (75mgkg $^{-1}$). However, still a remarkable decrease in the number of intact neurons in experimental groups when compared to the control group has remained.

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