The neuroprotective effects of *Prosopis farcta* pod aqueous and ethanol extracts on spinal cord α-motoneurons neuronal density after sciatic nerve injury in rats

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Abstract : Following crush injury to the sciatic nerve changes occur in the cell bodies of the most types of neurons. Knowledge of the physiological roles of Prosopis farcta (Banks& Sol. Fabaceae) plant has opened up new strategies in the treatment of neurological diseases. This study examined the neuroprotective effects of aqueous and ethanol extracts of Prosopis farcta pod on **α**-motoneuron neuronal density in rats. Ethanol and aqueous extracts was produced by using soxhlet method. Sixty-four male Wistar rats were divided to eight groups (control, compression and six experimental groups). In compression and experimental groups right sciatic nerve were highly compressed for 60 s, assigned to experimental groups (compression + aqueous extract of Prosopis farcta injections (25 ,50,75 mgkg $^{-1}$, ip , 2 time) and (compression + ethanol extract of Prosopis farcta injections (25 ,50,75 mgkg $^{-1}$, ip , 2 time) (N=8).After 4 weeks 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 α- motoneurons were counted and compared with each other. Neuronal density showed significant difference in compression and control groups (P<0/001). There was a meaning full difference between compression group and all treatment groups (P<0/001). The Prosopis farcta pod aqueous and ethanol extracts have neuroprotective effect because of antioxidant rule. [Mahtab Mollashahi, Maryam Tehranipour, Jina Khayyatzade, Bibi ZahraJavad Moosavi**. The neuroprotective effects of *Prosopis farcta* pod aqueous and ethanol extracts on spinal cord α-motoneurons neuronal density after sciatic nerve injury in rats**. *Life Sci J* 2013;10(2s):293-297] (ISSN:1097-8135). http://www.lifesciencesite.com. 50

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Introduction

When a motor axon in a peripheral nerve is severed, a sequence of changes occur [1] that cause the distal portion of axon to degenerate. The certain effects of axotomy is chromatolysis, atrophy and cell death that were resulted from the less of trophic substance produced by the target tissue and transported retrogradely along the axon to the cell body [2]. Neurodegenerative diseases are characterized by progressive dysfunction and death of neurons. Neurodegeneration may occur by apoptosis, necrosis or both. It is believed that there are many different mechanisms and neurochemical modulators responsible for the central nervous system damage, which may overlap temporarily [3].

The most important factors contributing to neuronal cell death are genetic factors, glutamate mediated excitotoxicity leading to disturbances in intracellular calcium and sodium metabolism, mitochondrial dysfunction, oxidative stress, growth factor with drawl, cytokines and toxins [4]. Following crush injury to the sciatic nerve, changes occur in the cell bodies of the most types of neurons [5], if the neuron successfully regenerates its axon restores connections with other cells, the cell body usually returns to its former appearance. But failure to contact a new target cell leads to atrophy and death. Studies on neural development have identified several neurotrophic factors that are released by the targets of neurons and retrogradly transport to neuronal cell body. These factors are necessary and important for neuronal survival and growth [6]. Plants have been used for medical purposes for many centuries. Different cultures have tried different kinds of plants for traditional treatment. After producing of chemical medications in 16th century, use of plants medications decreased gradually. However, due to a least side effect, trend to use of plants medications have been increased recently [7].The genus Prosopis comprises almost 50 species, 25 of which are on the list of federal noxious weeds. Prosopis are most often spiny trees or shrubs predominantly well adapted to hot, arid climates. It has been distributed in Southwest Asia, Africa; predominantly America, from western North America to Patagonia. Only four species is native to Southwest Asia and Africa. The remaining species in the genus are native to Mexico and South America, with the center of polymorphism in Argentina [8].There is a long history of traditionally using the extraction of Prosopis farcta plant for treatment of pains in Sistan and bluchestan Province. Decoction of Prosopis farcta has also been used traditionally to reduce cardiac or chest pain in this province .There is no report to show an academic research (in vitro, in vivo), to evaluate the neuroprotective effect of this plant so far. This study

aimed to evaluate the effect of Prosopis farcta ethanol and aqueous extracts on neuronal density in spinal cord after sciatic nerve injury in rats .

Materials and methods

The Prosopis farcta was collected from zahedan and coded with Dr. A.Gafari (herbarium code 1952) that was supplied by Islamic Azad University of Mashhad, Iran (2012). Prosopis farcta pod was grinded by a grinder system. The powder was kept in a cool and dry place until extraction time.

Extract preparation

Aqueous and ethanol extracts of pod powdered was prepared by Soxhlet apparatus method [9] 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.

Animal and housing conditions

Sixty-four male, Wistar rats weighting between 300-350g (Razi, Iran) were used in this study. All rats were housed in standard plexiglass cages, (three to four rats per cage) and a prior to 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. All procedures were in accordance with the local guidelines for the care and use of laboratory animals and were approved by the Islamic Azad University Mashhad branch (2012).

Groups

1. Control (sham) (N=8)

For baseline measurement in this group on the right hind limb an operation was performed which exposed the sciatic nerve without compression.

2. Compression group (N=8)

In this group after operation the right hind limb sciatic nerve was compressed.

3. Compression + aqueous extract of Prosopis farcta pod injections (25mgkg $^{-1}$, i.p. ,2 time) (N=8)

4. Compression + aqueous extract of Prosopis farcta pod injections (50mgkg $^{-1}$, i.p. ,2 time) (N=8)

5. Compression + aqueous extract of Prosopis farcta pod injections (75mgkg $^{-1}$, i.p. ,2 time) (N=8)

6. Compression + ethanol extract of Prosopis farcta pod injections (25mgkg $^{-1}$, i.p. ,2 time) (N=8)

7 Compression + ethanol extract of Prosopis farcta pod injections (50mgkg $^{-1}$, i.p. ,2 time) (N=8)

8. Compression + ethanol extract of Prosopis farcta pod injections (75mgkg $^{-1}$, i.p. ,2 time) (N=8)

These animals were compressed and simultaneously extraction was injected [10].

Compression method

Rats in each group were anesthetized under an initial dose of 60mg kg^{-1}ketamine ip and 6 mg kg^{-1} xylazine ip. Then sciatic nerve of right leg at the top of thigh bone was compressed by use of locker forceps for 30 seconds [11]. 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 15 days (Each week one injection).

Sampling

After 28 day following perfusion a block of the spinal cord segments L4 to L6 (approximately 8mm length) was removed while sciatic nerve roots of both sides were still attached it. The nervous tissues are very sensitive and autolysis rapidly. So for better fixation, perfusion method was used. When perfusion was finished, sampling of spinal cord was begun. The spinal cord was completely separated to the end of horse tail in order to equally sampling in all samples. After a block of the spinal cord segments L4 to L6 (approximately 8mm length) was removed, processed, sectioned serially (7 Mm) and stained with toluidine blue (pH 4.65). For future studies required photos from spinal cord anterior horn was taken. 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 5×10×2/5=100. Systematic random method was used in order to counting neurons[12].To analyze the data, parameters like: ∑ Q, ∑ frame, average, V dissector are required, these parameters are identified as below:

$\sum_{Q}$ = the sum of counted neurons in one half sample.

$\sum_{frame}$ = Sum of sampling times.

$V_{dissector}$ = volume of sampling frame equals:

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

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 [13]:

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

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

$V_{dissector}$ =347/222×347/222 ×7=84394182 mm^3

Statistical analyze

The ratio of numerical density of neurons in spinal cord samples was used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test with ($p<0.05$).

Results

The effects of Prosopis farcta pod extract on the numbers of neurons in the right anterior horn region of spinal cord at 28 days after sciatic nerve compression in rats are shown in Figurs1 and 2. The control (sham) group revealed normal neuronal cells but in compression group the numerical density had decreased. As has shown in figures, sciatic nerve crush resulted in massive neuronal damage manifested has a significant 50% decrease in the number of normal appearing neurons ($P<0.05$).

In all compressed and treatment with ethanol extract (25, 50,75mgkg $^{-1}$) injected group the neuronal density is obviously increased and has significant differences ($P<0.05$) in comparison to compression groups (Fig.1).

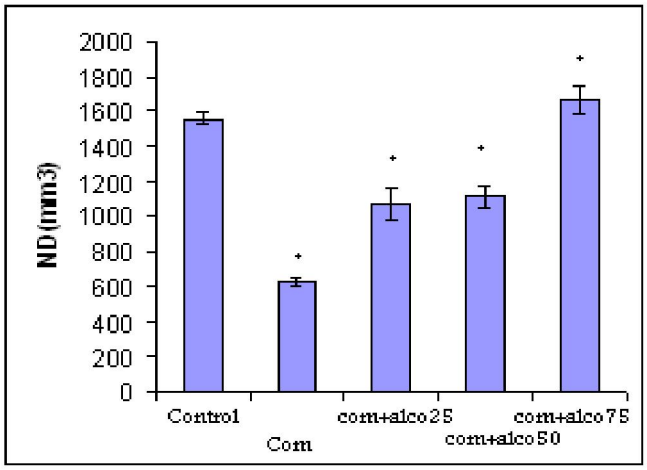


Fig.1: Effects of compression and alcoholic extract on the number of intact neurons of right ventral horn of spinal cord in rat. Results are expressed as Mean± SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey- kramer multiple comparisons test. Significantly different between control and compression also compression and compression +alcoholic25, compression + alcoholic50, compression +alcoholic75 injected groups have been showed**.** In all compressed and treatment with aqueous extract (25, 50,75mgkg $^{-1}$) injected group the neuronal density has had increased significantly ($P<0.05$) in comparison to compression groups (Fig.2).

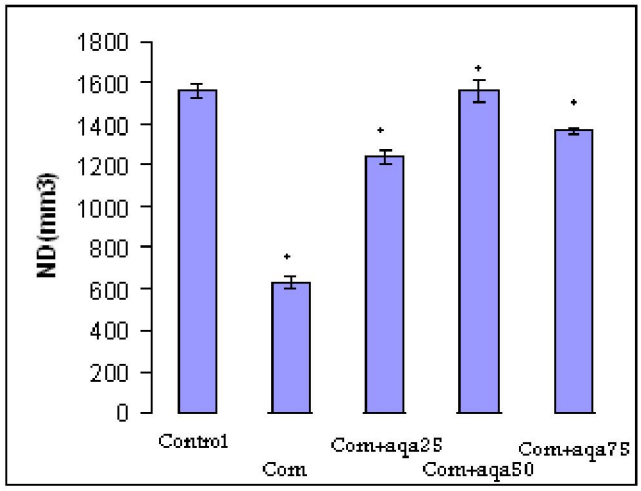


Fig. 2: Effects of compression and aqueous extract on the number of intact neurons of right ventral horn of spinal cord in rat. Results are expressed as Mean± SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey- kramer multiple comparisons test. Significantly different between control and compression also compression and compression+ aqueous25, compression+aqueous50, compression+aqueous75 injected groups have been showed**.**

All photos are shown that in experimental groups the neurons have very normal shape in compare with compression group (Fig.3).

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.Our findings demonstrate that Prosopis farcta [1]**.** pod extracts play an important role in the maintenance and repair of the nervous system after injury or disease. There is a remarkable change in the number of α-motoneurons in different groups. Animals were treated with extract immediately after compression of sciatic nerve (for 2 weeks) resulted in a significant ($P<0.05$) increase in the number of intact neurons, respectively as compared to compression groups (Fig1, 2). Data was suggested that aqueous extract of Prosopis farcta increase the numerical density more than groups that were treated with ethanol extracts. May be effective component of this plant are soluble in water more than alcohol. We have demonstrated that the Prosopis farcta [1] pod extracts has therapeutic and repairing effects in the spinal cord that protecting motor neurons from atrophy after the death of neighboring motor neurons. Previous

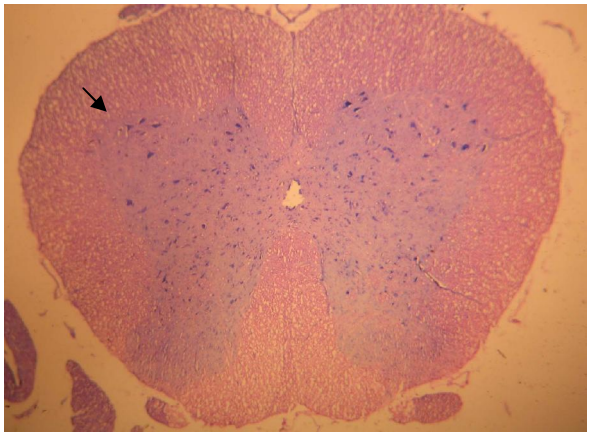
researches showed that neurons death occurs after cutting the nerve too. It was proven that cutting sciatic nerve in rats newborns cause decrease in motoneurons of spinal cord [14]. The results show that in compression group the neuronal density was decreased in compare with normal group (Fig.1). Snider caused death in neural cells by axotomy and claimed that this procedure is similar to natural death of neurons that younger cells are more sensitive to damage rather than mature cells [15]. 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 nucleus membrane and pekinosis of nucleus are some of these changes [2]. It is clear that 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 myeling making in new branches of proximal part of nerve new circumstances is required [16].The circumstances initiating neurons repair 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 [17].

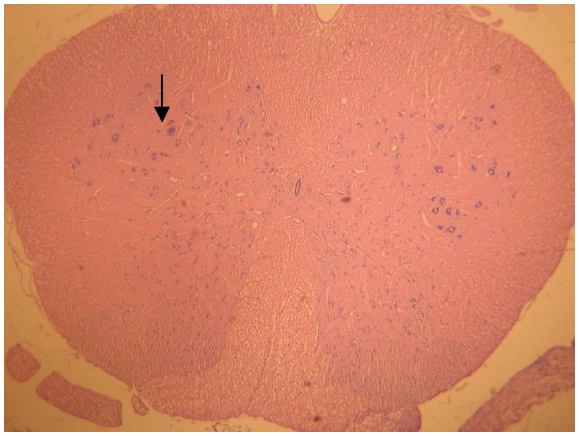
Many trophical factors affect on repairing nerves and many studies are carried out on potential role of growth factor [6]. Some of the component exists in prosopis faracta extracts have essential rule in protection nerves of injury as inflammation substrata [18]. They act as neurotropic factor or survival factor [8]. Although it is not clear that which component does this effect but result indicates the neuroprotective effect of this extract.

Many researches show that cells death after compression or chronic contraction of sciatic nerve CCI are planned cellular death or apoptosis [19]. In many experiments on the pre- apoptotic genes or apoptosis genes roles were emphasized [20]. For example after sciatic nerve injury some of these factor such as apoptotic factor (apaf-1), bax, caspase 3 and 9 have been activated [21]. In damages, apoptosis can cause second degeneration in damage place and demylined of neural channels [22]. 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 neural density of compression group and control group ($p<0.05$).

Following sciatic nerve injury, generation of free radicals causes apoptosis in the cell body of spinal cord neurons [23,24]. Antioxidants substant inactive free radicals and prevent apoptosis in spinal cord neurons. Different species of prosopis farcta have anti oxidant effects that are responsible for collection and removing free radicals [25]. Therefore antioxidant effect of prosopis farcta induce neuroprotective rule.

Fig.3: The cross section of spinal cord (magnitude 50 x -toloidin blue).Left panel: treatment with aqueous extract .Right panel: compression. The nucleus of α- motoneurons were showed with spikes**.**





Conclusion

In total administration of prosopis farcta pod aqueous and ethanol extracts after sciatic nerve injury can increase neuronal density in compare with compression groups. Then prosopis farcta pod aqueous and ethanol extracts have neuroprotective effect.

Acknowledgment

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