THE EFFECT OF *PROSOPIS FRACTA* POD ALCOHOLIC EXTRACT ON NEUROGLIA DENSITY AFTER SCIATIC NERVE INJURY IN RATS

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Summary

Following the sciatic nerve injury neuroglias were proliferated and act as macrophages. One of the problems in central nervous system defeats is the return of these defeats to cellular body of those neurons which their axons are destroyed. These effects arrive to the point of defeats as retrograde and cause central degeneration in spinal cord. ***Prosopis farcta*** has antioxidant and ant diabetic effect. The aim of this study is carried out to examine the effects of alcoholic extract of ***Prosopis farcta*** pod on neuroglia density after sciatic nerve injury in rats.

Thirty two adult male wistar rats were used and divided to four groups (control, compression and two experimental groups). In compression and experimental groups right sciatic nerve were highly compressed for 60 s, assigned to experimental groups (Compression + alcoholic extract of ***Prosopis farcta*** injections (50 ,75mgkg $^{-1}$, ip , 2 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 neuroglia in the right horn of spinal cord were counted and compared with each other. Statistical analyses showed remarkable increase in the number of neuroglia in the compression and all experimental groups($p<0.05$). lt shows that administration of ***prosopis farcta*** pod alcoholic extract after sciatic nerve injury cannot decreased neuroglia density in compare with compression groups.

Key words: prosopis farcta, neuroglia, density, sciatic nerve

Running title: prosopis fracta alcoholic extract on neuroglia density

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Introduction

The positive microglial cells respond to the sciatic nerve injury becoming activated and macrophage and express MHCII molecules (1). Such activated microglia apparently may serve as neurosupportive cells, providing neuroprotection and scavenging cellular debris in response to the injury (2).

Injured nerves regenerate slowly and often over long distances (3). Prolonged periods for regenerating nerves to make functional connections with denervated targets prolong the period of isolation of the neurons from the target (chronic axotomy) and of the denervation of Schwann cells in the distal nerve pathways (chronic denervation)(3). In an animal model, we demonstrated that prolonged axotomy and chronic denervation severely reduce the regenerative capacity of neurons to less to 10% (4). Concurrent reduction in neurotrophic factors, including brain- and glial-derived neurotrophic factors (BDNF and GDNF) in axotomized neurons and denervated Schwann cells, suggest that these factors are required to sustain nerve regeneration(5). Findings that exogenous BDNF and GDNF did not increase numbers of neurons that regenerate their axons in freshly cut and repaired rat nerves, but did increase the numbers significantly after chronic axotomy, are consistent with the view that there is sufficient endogenous neurotrophic factor supply in axotomized motoneurons and denervated Schwann cells to support nerve regeneration but that the reduced supply must be supplemented when target reinnervation is delayed (6).

Early peripheral lesions may deprive central neurons of necessary trophic factors, accentuate naturally occurring central cell death, and thereby result in smaller central representations. Or, smaller central representations may be produced by competitive interactions of deprived with adjacent intact pathways. In addition, throughout all stages of development, the capacity for reorganization may be spatially limited and depend on the size or pattern of the peripheral injury (7).
There is a long history of traditionally using the extraction of *Prosopis farcta* plant for treatment of pains in Ilam Province. Decoction of *Prosopis farcta* has also been used traditionally to reduce cardiac or chest pain in this province (8).The antioxidant activity of these plant's extracts and their potential rule in radical scavenging agreed with their potential use by Jordanian population as a traditional anti-diabetic agents (9,10).
There is no report to show an academic research (*in vitro, in vivo*), to evaluate the protective effect of this plant so far. This study aimed to evaluate the effect of *Prosopis farcta alcoholic* extract on neuroglia density after sciatic nerve injury in rat.

Material and Methods

The ***prosopis farcta*** (herbarium code 1952) was supplied by Islamic Azad University of Mashhad, Iran (2010).

Animal subjects

Thirty two 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°. Food and water was given ad libitum.

Extraction

prosopis farcta was collected from a reign around Zahedan and was coded with Islamic Azad University of Mashhad, Iran herbarium (herbarium code 1952). For extraction 50g powder pod with 300 cc methanol were mixed and extraction perform with Soxhlet apparatus (11). After obtaining extract, it was situated in oven with temperature (45°±2°) for 48 hours to remove solvent.

Groups

Thirty two rats divided into four groups:1) Control; 2)Compression ; 3)Compression +alcoholic extract of ***prosopis farcta*** injections (50mgkg -1 , ip ,2 time); 4) Compression +alcoholic extract of ***prosopis farcta*** injections (75mgkg -1 , ip ,2 time). (N=8)

Surgery

Animals were anesthetized under intera peritoneal injection of a mixture of ketamin and xylazine (6,66mgkg -1).

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 #5clamp forceps. The muscle and skin were then closed with 14mm stainless steel sutures (12).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 cannot 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 and 8mm samples at the injured area (L4-L6) 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 5×10×2/5=100.

In order to count neuroglia cells random systematic method was used and dissector method was used for counting particles (13).

Statistical analysis

The ratio of numerical density of neuroglia cells in samples of spinal cord was used as an index of neuroglia cells 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 neuroglia cells increases in compression group in compare with control group ($p<0.05$).

2- The neuroglia cells density (number of neuroglia cells) in all experimental groups increased in compare with control group and very near to compression group (Table.1).

Table.1: Neuroglia cells density in anterior horn of spinal cord in different groups. Data are presented as mean± S.E. (n=8 in each group).

Groups	NV (Mean ±S.E.)
Control	56 ± 2
Compression	101 ±2
Treatment (50mgkg-1)	105 ±1
Treatment (75mgkg-1)	118 ±3

3- The neuroglia density in treatment groups is very similar with compression group.

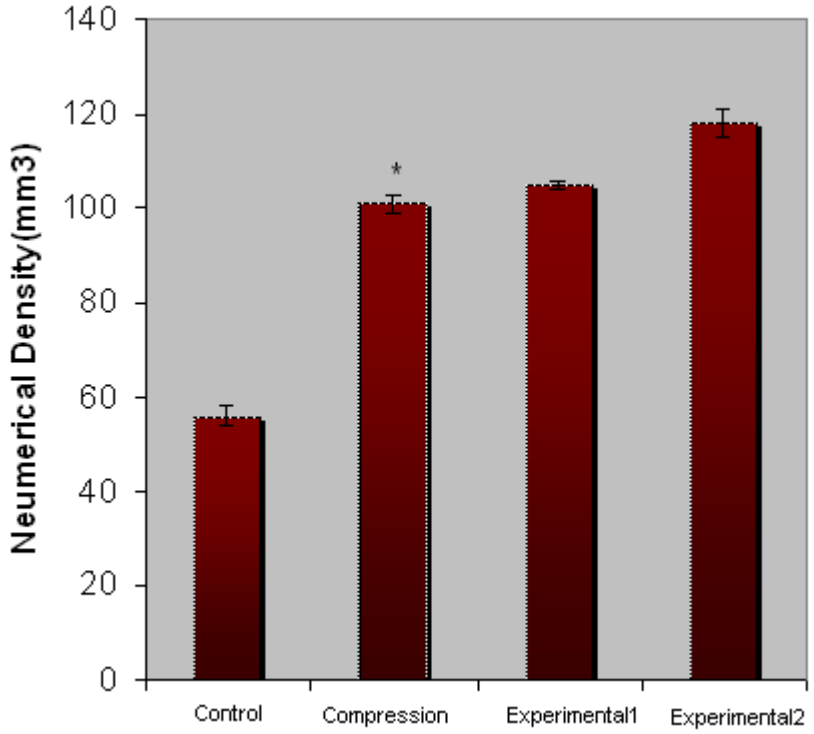

Fig.1: Comparison of Neuroglia cells density in anterior horn of spinal cord in different groups. Data are presented as mean± S.E. (n=8 in each group).
A) The compression of the neuroglia cells density in treatment alcoholic extract in 2 different dosages (50, 75 mg/kg) with compression group.
B) The compression of the neuroglia cells density in compression and control groups.($p<0.05$)*

4-The size and number of Neuroglia cells in experimental groups have remarkable increase (Fig.2).

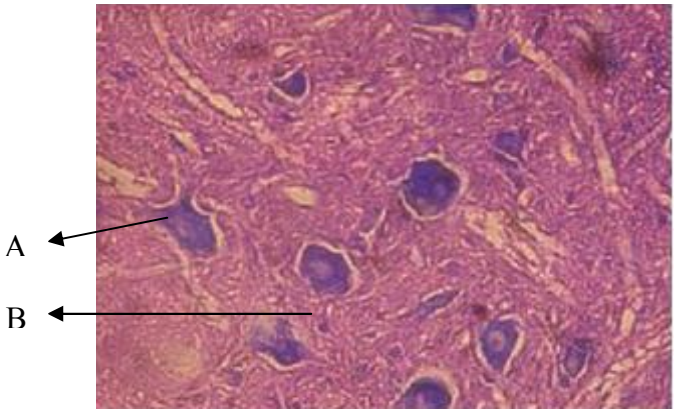

Fig.2: The cross section of spinal cord anterior horn (magnitude 400x And painting in toloidin blue)A: Alpha motoneuron B: Neuroglia cell

Discussion

There are some evidences supporting the hypothesis that some herbs may also exert neurotrophic and inflammation actions (14). It provides neuronal differentiation and increase in neuritis outgrowth. This plants act by prevention of relieving glutamate (15).

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(16). 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 myelin making in new branches of proximal part of nerve new circumstances is required (17).

In these phenomena neuralgias proliferated and act as macrophage cells (18). In addition increasing of inflammation proceeding is coordinated with proliferated of neuroglia cells. Then if some component could inhibit these phenomena, degeneration of central nervous system was decreased.

So it is possible that ***prosopis farcta*** alcoholic extract has protective effects on injured central nervous system cell and the results of this research show this hypothesis. It was shown that the neuroglia density in compression group increased in compare with control group (Table.1).The neuroglia density in treatments groups is very near to comperssion group. There is not any meaningful different between experimental and compression group in neuroglia density. It may be concluded pod alcoholic extract of ***prosopis farcta*** cannot protect neuroglia from proliferation after sciatic nerve injury. May be this component has had some roles in regeneration phenomena and act as a neurotrophic factors (Fig.2).

These factors consist of fibroblastic growth factors secreting from Schwann cells and macrophages that their synthesis is stimulated by cytokines and sticky molecules (19). 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 (20).

Following sciatic nerve injury, the generation of free radicals causes apoptosis in the cell body of spinal cord neurons (21). Antioxidants inactive free radicals and prevent apoptosis in spinal cord neurons. Different species of ***prosopis farcta*** have anti

oxidant effects and are responsible for collection and removing free radicals (9).Therefore protective effects of alcoholic extract are due to its anti- oxidant role.
In total administration of ***prosopis farcta*** pod alcoholic extract after sciatic nerve injury cannot decreased neuroglia density in compare with compression groups.

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