ANTI INFLAMMATION EFFECT OF CANNABIS SATIVA LEAVES ALCOHOLIC EXTRACT ON NEUROGLIA DENSITY AFTER SCIATIC NERVE INJURY IN RATS

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Summary

Inflammations phenomena happen following the sciatic nerve injury. In this time neuroglia 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. Cannabis sativa has antioxidant effect. The aim of this study is carried out to examine the effects of alcoholic extract of Cannabis sativa leaves 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 Cannabis sativa injections (25,50 mg/kg , 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 decreased in all experimental groups(p<0.05). It shows that administration of Cannabis sativa leaves alcoholic extract after sciatic nerve injury can decreased neuroglia density in compare with compression groups because of antioxidant effect.

Key words: Cannabis sativa, neuroglia, density, sciatic nerve

Running title: Cannabis sativa alcoholic extract on neuroglia density

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Introduction

*Cannabis* is an annual, dioeciously, flowering herb. The leaves are palmate compound or digitate, with serrate leaflets. *Cannabis* normally has imperfect flowers, with staminate "male" and pistilate "female" flowers occurring on separate plants (1).

*Cannabis* plants produce a unique family of terpeno-phenolic compounds called cannabinoids, which produce the "high" one experiences from smoking marijuana. The two cannabinoids usually produced in greatest abundance are cannabidiol (CBD) and/or Δ⁹-tetrahydrocannabinol (THC), but only THC is psychoactive (2). A synthetic form of the main psychoactive cannabinoid in *Cannabis*, Δ⁹-tetrahydrocannabinol (THC), is used as a treatment for a wide range of medical conditions (3).

In the United States there has been considerable interest in its use for the treatment of a number of conditions, including glaucoma, AIDS wasting, neuropathic pain, treatment of spasticity associated with multiple sclerosis, and chemotherapy-induced nausea," the agency has not approved "medical marijuana" (4). Clinical trials have shown the efficacy of cannabis as a treatment for cancer and AIDS patient (5).

Potential therapeutic uses of cannabinoid receptor agonists include the management of spasticity and tremor in multiple sclerosis/spinal cord injury, pain, inflammatory disorders, glaucoma, bronchial asthma, cancer, and vasodilatation that accompanies advanced cirrhosis. CB(1) receptor antagonists have therapeutic potential in Parkinson's disease (5).

Proliferation of glial cells is one of the hallmarks of CNS responses to neural injury. These responses are likely to play important roles in neuronal survival and functional recovery after central or peripheral injury. The positive microglial cells respond to the sciatic nerve injury becoming activated and macrophage and express MHCII molecules (6). Such activated microglia apparently may serve as neurosupportive cells, providing neuroprotection and scavenging cellular debris in response to the injury (7).

Injured nerves regenerate slowly and often over long distances (8). 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) (8). In an animal model, we demonstrated that prolonged axotomy and chronic denervation severely reduce the regenerative capacity of neurons to less than 10% (9). 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 (10). 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 (11).
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 (12).

Then according to antioxidant effect of this plant this study aimed to evaluate the effect of *Cannabis sativa* alcoholic extract on neuroglia density after sciatic nerve injury in rat.

Material and methods

The *Cannabis sativa* (herbarium code 2521) 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±2°C. Food and water was given ad libitum.

Extraction

*Cannabis sativa* 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 (13). 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 *Cannabis sativa* injections (25mgkg -1 , ip ,2 time); 4) Compression +alcoholic extract of *Cannabis sativa* injections (50mgkg -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 (14). 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 $5 \times 10 \times 2/5 = 100$.

In order to count neuroglia cells random systematic method was used and dissector method was used for counting particles (15).

**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 ($p<0.05$) (Fig.1).

![Fig.1. Compression of the neuroglia cells density in compression and control groups ($p<0.05$). Data are presented as mean± S.E. (n=8 in each group).](image)

2- The neuroglia cells density (number of neuroglia cells) in all experimental groups decreased in compare with compression group ($p<0.05$) (Table.1) but this decreased is not meaningful (Fig.2).
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).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NV (Mean ±S.E.)</th>
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<tbody>
<tr>
<td>Control</td>
<td>2669 ±192</td>
</tr>
<tr>
<td>Compression</td>
<td>4976 ±180</td>
</tr>
<tr>
<td>Treatment (25mgkg⁻¹)</td>
<td>4597 ± 98</td>
</tr>
<tr>
<td>Treatment (50mgkg⁻¹)</td>
<td>4628 ± 138</td>
</tr>
</tbody>
</table>

Fig.2: Comparison of Neuroglia cells density in anterior horn of spinal cord in 2 different dosages (25, 50 mg/kg) with compression group.. Data are presented as mean± S.E. (n=8 in each group).

4-The size and number of Neuroglia cells in experimental groups have remarkable decrease in compare with compression group (Fig.3).
Cannabis sativa (marijuana) have been used both medicinally and recreationally for many centuries. Recent advances in the knowledge of its pharmacological and chemical properties in the organism, mainly due to Delta (4)-tetrahydrocannabinol, and the physiological roles played by the endocannabinoids have opened up new strategies in the treatment of neurological and psychiatric diseases (5).

There are some evidences supporting the hypothesis that some herbs may also exert neurotrophic and inflammation actions (16). It provides neuronal differentiation and increase in neuritis outgrowth. This plants act by prevention of relieving glutamate (17).

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 (18). 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 (19).

In these phenomena neuralgias proliferated and act as macrophage cells (20). 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.

Evidence has accumulated over the last few years suggesting that endocannabinoid-based drugs may potentially be useful to reduce the effects of neurodegeneration. In fact, exogenous and endogenous cannabinoids were shown to exert neuroprotection in a variety of in vitro and in vivo models of neuronal injury via different mechanisms,
such as prevention of excitotoxicity by cannabinoid CB1-mediated inhibition of glutamatergic transmission, reduction of calcium influx, anti-oxidant activity, activation of the phosphatidylinositol 3-kinase/protein kinase B pathway, induction of phosphorylation of extracellular regulated kinases and the expression of transcription factors and neurotrophins, lowering of cerebrovasoconstriction and induction of hypothermia. The release of endocannabinoids during neuronal injury may constitute a protective response (21, 22, 23).

So it is possible that cannabis sativa 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). But in all treatment groups with alcoholic extract the neuroglia density was decreased in compare with compression group (Fig.2).

It may be concluded that there is some component in alcoholic extract of cannabis sativa that protected neuroglia from proliferation after sciatic nerve injury or may be this component has had some roles in regeneration phenomena and act as a neurotrophic factors (Fig.3).

These factors consist of fibroblastic growth factors secreting from Schwann cells and macrophages that their synthesis is stimulated by cytokines and sticky molecules (24). 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 (25).

Following sciatic nerve injury, the generation of free radicals causes apoptosis in the cell body of spinal cord neurons (26). Antioxidants inactive free radicals and prevent apoptosis in spinal cord neurons. Different species of cannabis sativa have anti-oxidant effects and are responsible for collection and removing free radicals (27). Therefore protective effects of alcoholic extract are due to its anti-oxidant role.

In total administration of cannabis sativa alcoholic extract after sciatic nerve injury can decrease neuroglia density in compare with compression groups and have had anti-inflammation effect.

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References


