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THE NEUROPROTECTIVE EFFECT OF HYDRO-ALCOHOLIC EXTRACTS OF *CANTHARELLUS CIBARIUS* ON NEURONAL DENSITY OF ALPHA MOTONEURONS IN SPINAL CORD ANTERIOR HORN AFTER SCIATIC NERVE COMPRESSION IN RATS

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ABSTRACT

This aim of this study is carried out to examine the neuroprotective effects of hydro-alcoholic extract of *Cantharellus cibarius* on neuronal density of motoneuron in spinal cord anterior horn in rats. This experimental research, was carried out on 30 male Wistar rats. Rats were divided into five groups each consisting six member. A: control, B: Compression, C: Compression + treatment with 50,75,100 mg\kg Hydro alcoholic extract. 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 (100 mg\kg) in compared to compression. Result shows that hydro-alcoholic extract could increase neuronal density motoneuron in anterior horn of spinal cord after sciatic nerve injury in rat.

KEYWORDS: *Cantharellus cibarius*, Sciatic Nerve, Alpha motoneuron, Neuroprotective

INTRODUCTION

Spinal cord injury is one of the most severe disabilities had a major impact on one's life and his life is in a limited range. Only in America there are more than two hundred thousand people with spinal cord injury and between 10,000 to 11,000 new cases are added every year (DeVivo *et al*., 2003). When a nerve gets cut off communication with the cell body with axon is cut and the distal part of the injury to the end begins degenerating at the same time. In addition degeneration the first Node of Ranvier continues towards the proximal . This process is called Valerian degeneration (DeVivo *et al*., 2003). If the damage in the proximal portion is severe, the impact to the lesion back is developed into the cell body and is the cause of central degeneration. (destruction of the cell body). The Nissl bodies are broken and are scattered throughout the cytoplasm, called chromatolysis. Also, the nucleus from its central position, shifts towards the cell environment and the cell body is swollen due to osmotic changes (Coleman *et al*.,1998). Damage to neurons causes a series of molecular and cellular responses associated with an important role in the success of the process of nerve regeneration.

Signals generated as a result of injury or pressure on the damaged neurons have an important role in the induction of transcription factors and cytokine response that causes inflammation in the affected area followed by expression of growth factors, neuropeptides, and molecules involved in intercellular interactions plays an important role in initiating the process of recovery and gradual improvement in inflammation (Schlaepfer and Bunge, 1973). Axonal injury can cause changes in inflammation at the peripheral nerve injury, these changes are diagnosed with the arrival of white blood cells to the injury. In topical inflammation, white blood cells move towards infection and immediately start a direct interaction between the cell body of the neuron and white blood cells occurs Although the changes of the inflammation is gradual the regeneration is associated with the fast speed of axon. Some growth factors are involved in the inflammation process, such as a lack of macrophage colony-stimulating factor (MCSF) reduces microglial cells and reducing the number of lymphocytes but has no apparent effect on the axons regeneration (Schwartz *et al*.,1981) . If a nerve fiber cuts of there is a change both in the peripheral nervous and the central nervous. If the changes is intense in nature it will lead to cell death (Perry *et al*., 1990).

Use of a substance that at this stage could reduce the severity of lesions or to intensify the process of regeneration, could be a solution to many neurological problems. The presence of the fungi are one of the great divine blessings in

nature. Fungi have numerous advantages. They have a variety of vitamins, proteins (essential amino acids) including the minerals (Hardenack, 1997). Drugs were derived from fungi, unlike chemical drugs have no side effects and their impact on the human body is far more than chemical drugs (Hardenack, 1997). Chanterelle (*Cantharellus cibarius*) come from the family of funnel fungus. This fungus grows in the province of Mazandaran and in their dialect is called Zardkyja. Which means "yellow girl". These fungi contain essential minerals and vitamins needed by a body such as niacin, pantothenic acid, vitamin B, vitamin D (Hardenack, 1997). (Mattila *et al*.,2003). (Reddy et al., 2002).

Figure 1. ***Cantharellus cibarius***

This fangus has Anti-oxidant, Antidiabetic and Anti-inflammatory effect (Jennemann, *et al*., 2001). This funus is benefit for eye inflammation, night blindness, and prevent dry skin and mucous membranes due to reduced discharge. It can also cause respiratory system resistance against infectious diseases. The extract is used to treat swelling and wounds. Ethanol extracted from the fungus has an inhibiting effect on tumors in rat (Jennemann, *et al*., 2001).Multiple pharmacological effects of fungi have led us to perhaps a possible and useful factor involved in the use of stimulating regeneration of sciatic nerve compression in rats. Accordingly, this study aimed to investigate The neuroprotective effect of hydro-alcoholic extracts of *Cantharellus cibarius* (Figure 1), on neuronal density of alpha motoneurons in spinal cord anterior horn after sciatic nerve compression in rats.

MATERIAL AND METHOD

Chanterelle (*Cantharellus cibarius*), is collected from Neka forest area southeast of the Caspian Sea at a height of 150 meters above sea level with a tilt toward the north and dried in the incubator and was powdered. From the fungi powder with the soxhlet extractor (model H626) the hydro-alcoholic was extracted. First 50 grams drained powder was put into filter paper and placed in the extractor. Then, 150 cc distilled water and 150 cc 96% ethanol was poured in the container device. As Thermo bag, slowly heated solvent (water and alcohol) extract Chanterelle also heated and mixed with the solvent goes back to the balloon (Ying *et al*., 1987). Thus, the total volume of solution is not reduced. Extraction took 10 hours to ensure that all solvent soluble is extracted from the dried powder .Finally 4.00 g dried extract was yielded, that 2.50 g of hydro-alcoholic extract, for injecting to rats was put aside. Thirty male Wistar rats weighting 250-300 g received from Ferdowsi Medical University of Mashhad, Mashhad, Iran. were used in this study. The rats were held in department of biology, Islamic Azad University of Mashhad at 22-24 °C, humidity of 50%, and a cycle of 12 hours light- dark. Moreover, all rats had access to sufficient food and water. 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 (2015).

Male Wistar rats were divided into 5 groups (6 rats per group): A (control or sham): For baseline measurement in this group on the right hind limb an operation was performed which exposed the sciatic nerve without compression. B (Compression group): In this group after operation the right hind limb sciatic nerve was compressed. C (compression + injection of 50 mg/kg hydro-alcoholic extract of *cantharellus cibarius*), D (compression + injection of 75 mg/kg hydro-alcoholic extract of *cantharellus cibarius*), E (compression + injection of 100 mg/kg hydro-alcoholic extract of *cantharellus cibarius*) These animals were compressed (Cicchetti, 2009). and simultaneously extracts was injected i.p., 2 times. The rats were anesthetized under intraperitoneal injection of 60 mg/kg Rompun and 6 mg/kg Ketamine After removing animal hair on right femur, skin was cut for 2-3 cm and femoral muscle underwent surgery in order to find sciatic nerve. In the next stage, compression of sciatic nerve was done for 60 seconds using locker pincers (second lock) (Tehranipour and Ghamyari, 2010). After compression, the injured part was sterilized and stitched. In experimental groups the first injection of extract was done immediately after compression. After consciousness, the rats were transferred into separate cages and kept under standard conditions. Second injection of extract was done one week after the first injection.(Behnam-Rasoli, 2000). After 28 days of compression, animals' tissues were fixed using perfusion method and then sampling was performed from lumbar spinal cord. Spinal cord was taken out of spinal column to cone medullary end and then after going 18 mm up cone medullary, 8 mm samples were provided. Samples were kept in fixator for two weeks and then entered tissue passage which included three steps: dehydration of tissue (using alcohol), transparency (using xylene), and soaking in paraffin. Cutting was carried out with microtome set, so that 7 micron cuts were transferred to slides. The work continued until 30 slides were prepared. The slides were then stained with toluidine blue (Tehranipour *et al*.,2010) In the next step, some photos were taken from spinal anterior horn in the right part of slides. Dissector method was used to count alpha motor neurons of right anterior horn. In this method neurons are counted in a reference framework. When a particle is in the reference framework but is not present in the next frame (next consecutive cut), it is counted. However, if a neuron is present in both frameworks, it is not counted (Tehranipour *et al*.,2009),(Sterio,1984).

Neuron density was estimated as below:

$ND=\Sigma Q/\Sigma$ frame $\times$V dissector
In which: ΣQ: total neurons counted in one sample
Σ frame: total number of sampled cases in a sample
V dissector: volume of sampling frame
which equals:
V dissector= A frame × H
A frame: the area of sampling frame
H: distance between two consecutive slices, or depth of each cut.
Data were analysed using Minitab 13 software, Tukey and ANOVA statistical tests (for binary comparison). Tests significance level was considered $P<0.05$.

RESULTS

In this study, the neuroprotective effect of hydro-alcoholic extracts of *Cantharellus cibarius* were individually investigated on spinal motoneurons degeneration after sciatic nerve compression in rats. The result of comparing density of alpha motor neurons in spinal anterior horn between group A (control), B (compression) and C, D, E (experimental groups) was explained in table 1. The extract with 100 mg\kg was reported as the most effectiveness.

Table 1: the study of difference in Neuronal Density of Alpha motoneurons in Spinal cord Anterior Horn of these five groups.

Group name	Paired t-test experimental		
	SE Mean	Mean (Nv)	P-value
Control	30.7	1634.2	0.000
Compression	34.1	612.0	0.000
hydro-alcoholic extract 50 mg/kg	36.6	1175.7	0.000
hydro-alcoholic extract 75 mg/kg	32.1	1161.8	0.000
hydro-alcoholic extract 100 mg/kg	46.3	1590.6	0.000

Data analysis shows significant difference in neuronal density between compression groups with (control, compression, hydro alcoholic 50,75,100 mg/kg) groups.

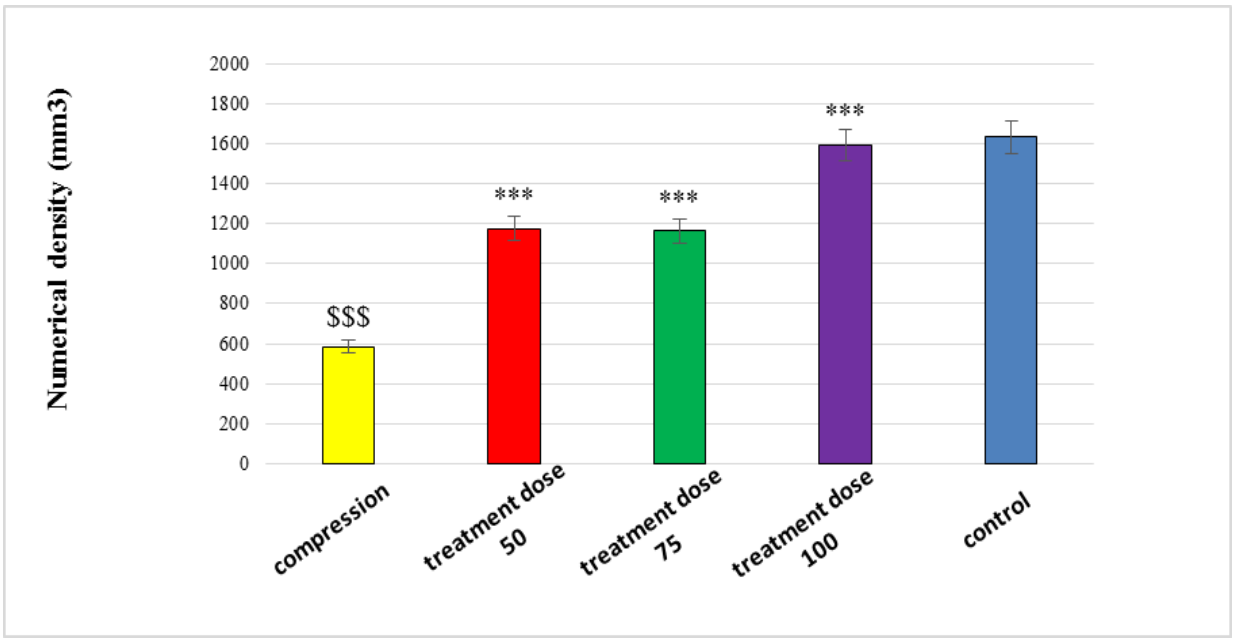

Figure 1: Alpha motoneurons neuronal density compression in the right part between compression and other group (Control, hydro alcoholic 50,75,100) (n=6).

In each group numbers represent neuronal density average standard deviation.
*****: significant difference indicates P<0.001 (comparison treatment groups with compression group). $$$: significant difference indicates P<0.001(Comparison compression group with control group)**

So neuronal density in experimental groups with hydro alcoholic extract (50, 75, 100 mg/kg) has been meaningfully increased compared with compression.

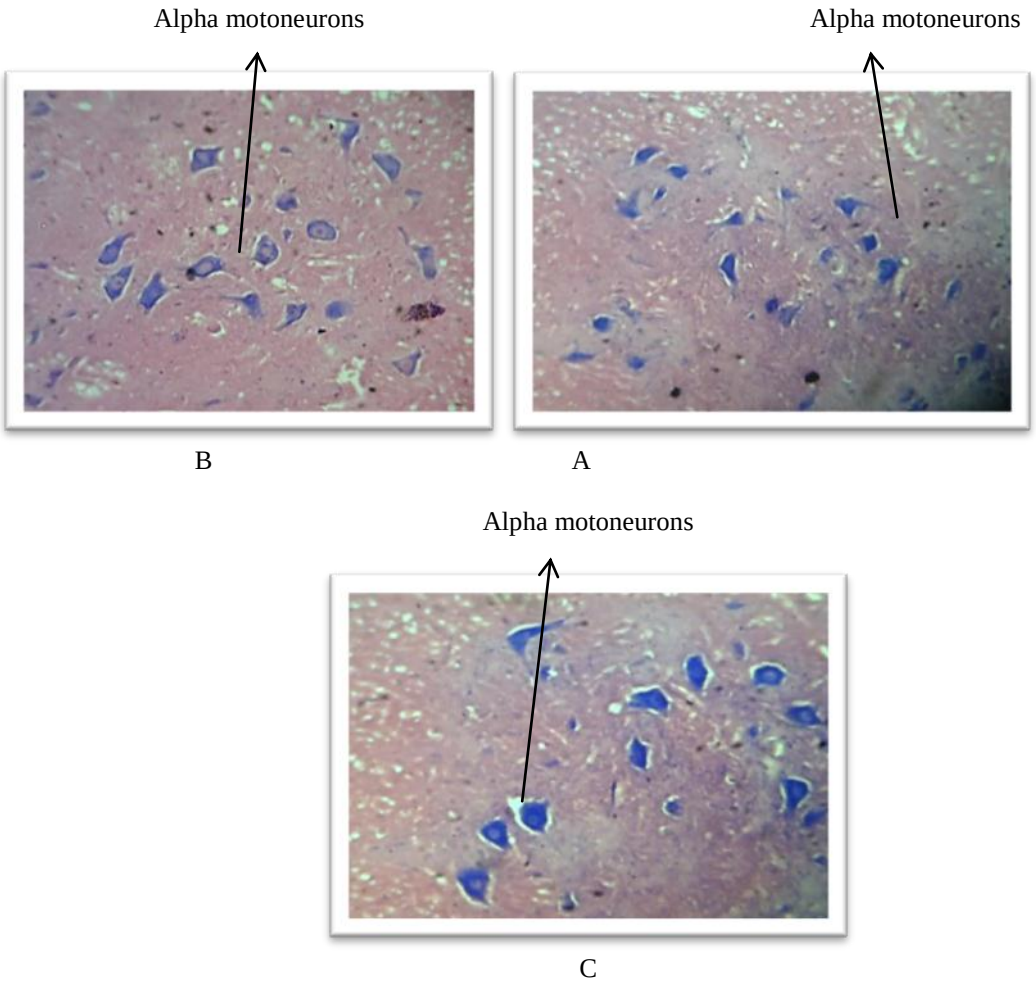

Fig.1: Cross-sectional representation of spinal and alpha motor neurons of spinal anterior horn in the right part of different groups (toluidine blue staining, zoom X 1600) A: control, B: compression, C: compression + treatment with a dose of 100 mg/kg hydro alcoholic extract.

In experimental groups with a dose of 100 mg/kg hydro alcoholic (*Cantharellus cibarius*) extract, due to existence conservation products the changes are slight and form of cell are neary in normal condition.

DISCUSSION

The result of the present study showed that neuronal density in the compression group (B) was significantly lower than control group (A) ($P<0.001$). In the Ferrer and Planas study cutting nerve fiber in peripheral nerve and central nerve resulted in changes and Caused cell death (Ferrer and Planas, 2003). In study of Scott and Foote, severe compression of nerve and severe degeneration of axonal proximal section, caused central degeneration effects retrograde towards the cell body of motor neurons in anterior horn of the spinal cord. The injuries to the nerve fibre in the distal part of neurons caused the Wallerian degeneration along with calcium influx and histological changes such as axonal fragmentation and myelin disruption (Scott and Foote,1981). The apoptosis along with denervation which is an

example of neuronal cell death due to the structural changes can lead in neuronal death (Behnam-Rasouli *et al*., 2000). This study shows Sciatic nerve compression cause neuronal density reduction in anterior horn of the spinal cord in Rat according to other scientists and hydro alcoholic extract of *Chantharellus Cibarius* with dose of 50, 75, 100 mg/kg in rat with sciatic nerve compression cause significantly increased number of Alpha motoneuronal density in anterior horn of the spinal cord. Probably it is through anti-oxidant and anti-inflammatory properties of this fungus extract. In the process of nerve compression , inflammatory was active and causing harmful chemical enviroment and further damage (Jamalpoor *et al*., 2012). Probably hydro alcoholic extract of *Chantharellus Cibarius* haveing anti-inflammatory effects and preventing of injury progress. So that in present study experimental groups slowed progress of injury. According to the Nathan study and colleagues, denaturation of proteins is the main cause of inflammation. The *Chantharellus Cibarius* extract affects on the membrane of cell, like red blood cell and lysosome, that causes of membrane stability and plays a significant role in the inflammatory process (Nathan, 2002). Existing Pyrogallol in *Chantharellus Cibarius* extract cause of reduced inflammation at the injuty area, so that this substance at the primary phase after the injury the secretion of inflammatory cytokine tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) is significantly suppressed (Moro *et al*.,2012)

Therefore, another effective mechanism that by hydro alcoholic extract of *Chantharellus Cibarius* after sciatic nerve compression prevents from cell death, by reducing the inflammation at the site of injury, and probably for this reason in experimental groups neuronal density was significantly increased. And however much time was spend regeneration extent and extract effect increased until the amount of neuronal density in experimental groups after 28 days is closer to control group (diagram1). Another effective factor in cell death is free radicals, followed by mechanical damage or sciatic nerve compression, increased production of free radicals, Overproduction of these radicals causes damage to cell function. The extracts of *Chantharellus Cibarius* causes removal of free radical at the damaged site. For example, removal of the (DPPH) radicals has been proven (Barros *et al*.,2008) Compounds stock in the *Chantharellus Cibarius* extract such as flavonoids, alkaloids, terpenoids, saponin and phenols each having a specific function, phenols and gallic acid, flavonoids and polysaccharide stock in the fungus extracts contains strong anti-oxidant activity. In this study, maximum anti-oxidant property is due to the phenolic and flavonoids compounds. The result of the present study shows that hydro alcoholic extract of *Chantharellus Cibarius* has been neuroprotective factor that probably might be used to treat a variety of neurodegenerative diseases.

CONCLUSION

The increased neuronal density in experimental groups compared to the compression group indicated the stock compounds in this fungus has regeneration effect on the central nervous system and in addition to reducing the severity of degeneration process, it speeds up the regeneration process. The injections of different concentrations of hydro-alcoholic extract of *Chantharellus Cibarius* resulted in neuroprotection activity in vivo. The effective result of *Chantharellus Cibarius* depends on the dose, which is considered the best for regeneration in the 100 mg/kg dose.

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