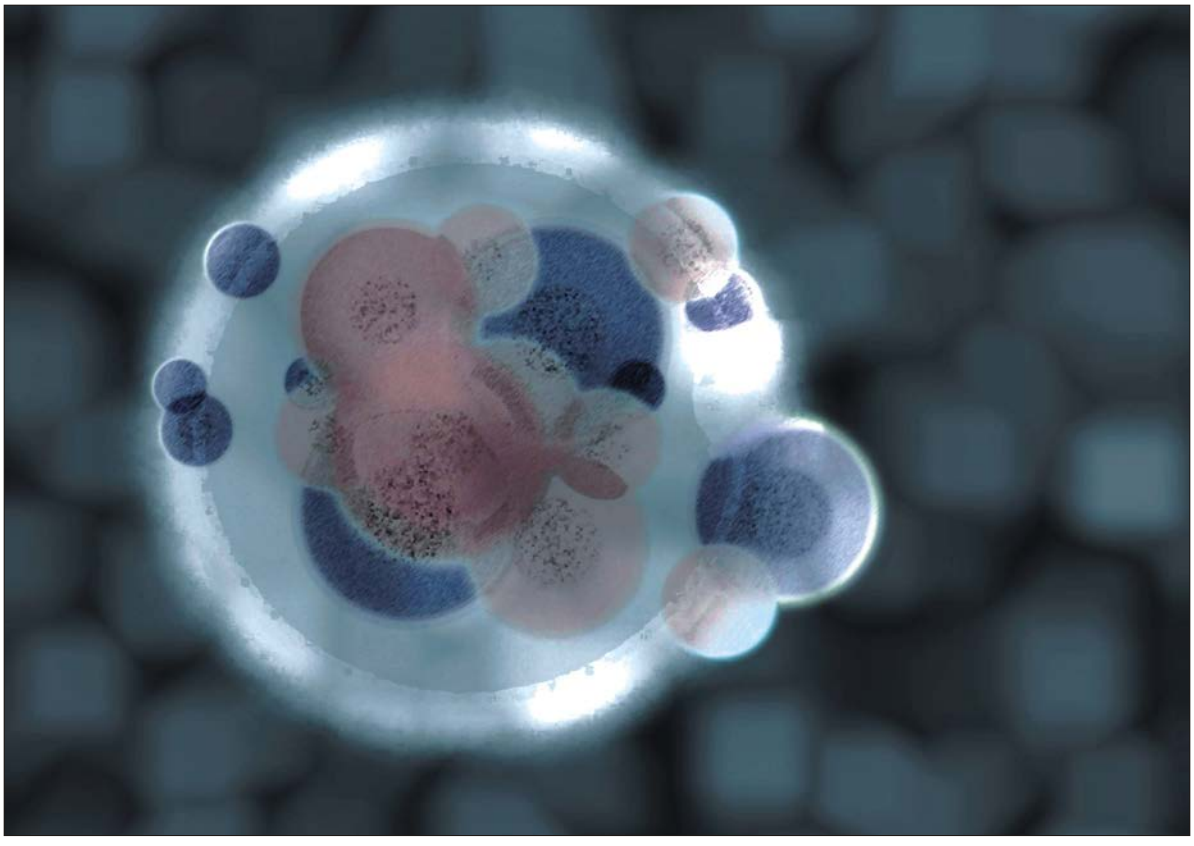

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Neuroprotetive Effect of *Curcuma longa* Alcoholic Extract on Peripheral Nerves Degeneration after Sciatic Nerve Compression in Rats

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Abstract: Wallerian degeneration in the CNS and PNS consists of degradation and phagocytosis of axons and their myelin sheath distal to the site of injury. The present study was undertaken to evaluate the possible antioxidant neuroprotective effect of *Curcuma longa* extract on neuronal death after sciatic nerve injury in rat. Treatment of *Curcuma longa* extract (100 mg kg^{-1}, i.p.) at three different times (immediately, 3, 6 and 9 day after compression) significantly ($p<0.05$) reduced neuronal damage. The present study demonstrates that *Curcuma longa* extract treatment attenuates sciatic nerve injury and oxidative stress that's following it. Because of antioxidative effect of *Curcuma longa* extract; treatment with this immediately or even delayed until 24 h may have the potential role to be used as a protective agent in neurodegenerative process.

Key words: *Curcuma longa*, neuroprotective, compression

INTRODUCTION

Curcumin is a natural polyphenol used in ancient Asian medicine (Strimpakos and Sharma, 2008). Curcumin, a yellow pigment extracted from the rhizome of the plant *Curcuma longa* (Zingiberaceae) has been shown to have antioxidative properties due to the presence of chain breaking or H donating phenolic groups in it's molecular structure (Bala *et al*., 2006). Curcumin is widely used as a food additive and also herbal medicine throughout Asia (Bala *et al*., 2006). Curcumin use as a therapy for malignant and inflammatory diseases and it's potential use in the treatment of degenerative neurologic diseases (Cheng *et al*., 2001), cystic fibrosis and cardiovascular diseases (Strimpakos and Sharma, 2008) and cancer (Li and Lin-Shia, 2001). The potential neuroprotective actions of this substance were discovered during a screening of its potential to protect against the adverse effects from high doses of alcohol, which revealed positive results. Since them, studies have indicated potential benefits for Alzheimer's disease and parkinson's disease based on laboratory models (Masterman and Cummings, 2005). Turmeric is used as a tonic and as a blood purifier. Its role in the treatment of skin diseases and its ability to soften rough skin resulted in the prolific use of turmeric in topical creams and bath soaps in India. Tumeric is also used in home remedies in the treatment of cuts, wounds, bruises and sprains. It is use as an anti-inflammatory and antimicrobial agent has been recognized for more than a century (Joe *et al*., 2004). The importance of turmeric in medicine took a new twist when it was discovered that the rhizome of *Curcuma longa* is very rich in phenolics, whose structures have been identified as curcuminoids. Phenolics are known to possess antioxidant properties (Joe *et al*., 2004). When a motor axon in a peripheral nerve is severed; a characteristic sequence of changes occurs (Ro *et al*., 2007). The distal 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 (Wang *et al*., 2007). 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 (Perrin *et al*., 2005). This type of degeneration is remarkably slow in the mammalian CNS and takes several months to complete. The initial axonal breakdown occur rapidly undergoing granular degeneration of cytoskeletal structures via the action of proteases. However, the removal of the axonal and myelin debris is very slow. The clearance of myelin after injury may be important for axon regeneration in the CNS because of the presence of axon growth inhibitors in myelin (Cole *et al*., 2004). 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 modulatos responsible for the central nervous system damage, which

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may overlap temporarily (Bialek *et al*., 2004). Neuron survives depends on many factors. Soluble survival factors may be supplied by the postsynaptic target, by neighboring nerve and glial cells, or by the circulatory system. Neurons also depend upon the synaptic contacts that they receive and differentiation leads to atrophy or death. These diverse signals are referred to as trophic factors, because one cell is nourished or sustained by another (Perrin *et al*., 2005). The aim of this study is to evaluate the possible antioxidant neuroprotective effect of curcumin on neuronal death after sciatic nerve injury in rat.

MATERIALS AND METHODS

All experiment was conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2009).

Animal subjects: Thirty 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 6.00 h. Ambient temperature in the animal facility was kept at 22±2°C. Food and water was given *ad libitum*.

Extraction: *Curcuma longa* was collected from a reign around mashhad and was coded with Islamic Azad University of Mashhad, Iran herbarium. For extraction 50 g powder rhizome with 300 cc alcohol were mixed and extraction perform with Soxhlet apparatus (Cicchetti and Chaintreau, 2009). After obtaining extract, it was situated in oven with temperature (45±2°C) for 48 h to remove solvent.

Surgery: Animals were anesthetized under intraperitoneal injection of 0.24 cc 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 30 sec period between prongs of No. 5 clamp forceps. The muscle and skin were then closed with 14 mm stainless steel sutures (Behnam-Rasouli *et al*., 2000).

Groups

- **Controls (N = 6):** For baseline measurement in this group on the right side an operation was performed which exposed the sciatic nerve but did not disturb it (Just for induced stress effect of operation)
- **Compression or sham-operated controls groups (N = 6):** In this group after operation the right sciatic nerve was crushed
- **Compression + curcumin extract injections (100 mg kg^{-1}, i.p., 3 time) (N = 6):** In this animal Coordinated with sciatic nerve crush, Curcumin extract was injected three times during 28th day (Every nine day one injection)
- **Compression + curcumin extract injections (100 mg kg^{-1}, i.p., 6 time) (N = 6):** In this group animal were given curcumin extract six times after compression (Every 5th day one injection)
- **Compression + curcumin extract injections (100 mg kg^{-1}, i.p., 9 time) (N = 6):** In this group animal were given curcumin extract nine times after compression (Every 3rd day one injection)

At the selected post-operative time (4 weeks), rats were anesthetized and intracardially perfused with formaldehyde. Immediately following perfusion a block of the spinal cord segments L4 toL6 (approximately 8 mm length) was removed while sciatic nerve roots of both sides were still attached it. The spinal blocks were placed in the same fixative for post sampling fixation overnight and then processed and embedded in paraffin. The blocks were sectioned serially at 7 mm. A uniform random sampling scheme was employed so that about 10 sections from each block were sampled. With each section thus selected its immediately preceding neighbor was also collected. Sections were stained with toluidine blue staining method with special buffer of acetic acid, sodium acetate and distilled water (pH = 4.65).

After permanent mounting the number of motoneurons in right sides of ventrolateral regions of the spinal cord ventral horns (L4 to L6) were determined, using stereological counting technique; physical dissector (Tehranipour and Kabiri, 2009). The dissector principle was used to determine the umbers of motoneurons in each section. Form each section and it's adjacent neighbor two photos were taken, one from each section with a final magnification of 100. A two-dimensional unbiased counting frame was overlaid in a uniform, random manner on to regions of any two photos taken from right sides of both sections. Those cell nuclei selected by the frame on the reference plane but not appearing on the adjacent look-up frame section were deemed to have their tops in the volume described by the product of the area of the counting frame and the distance between sections. These nuclei were counted (Q) to provide the numerical density of cells (NV) in the ventral horns of spinal cord according to the equation:

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

where, Σa is the sum of counted neurons, H is the depth of the dissector equal to the section thickness (7 μ) and a (frame) is the scaled area of the dissector frame.

Statistical analysis: The ratio of numerical density of neurons in samples of spinal cord was then used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test.

RESULTS AND DISCUSSION

The effects of curcumin extract treatment on the numbers of intact neurons in the right ventral horn of spinal cord region (Fig. 1) at 28th days after sciatic nerve compression in rats are shown in Fig. 2 and 3.

The control group revealed healthy neuronal cells (Fig. 2) amounted by 1400±400 Intact neurons (Fig. 3) Sciatic nerve crush resulted in massive neuronal damage manifested as a significant ($p<0.05$) 25% decrease in the number of normal appearing neurons (Fig. 1, 2).

Animal treated with curcumin extract immediately after compression (Sciatic nerve injury) and continued for 4 week resulted in a significant ($p<0.05$) increase in the number of intact neurons, respectively as compared to compression group (Fig. 2, 3).

The comparison of normal-appearing neurons counts between animals treated with curcumin at different times confirmed no significant difference. However, when control group was compared to treatment groups still a significant decrease in the number of intact neurons has remained.

Curcumin's neuroprotective role has been recently demonstrated in a few studies (Ghoneim *et al*., 2002). For example, Manikandan *et al*. (2004) have evaluated the free radical-scavenging and neuropotective potential of the manganese complexes of curcumin (Manikandan *et al*., 2004). Other has demonstrated the neuroprotective effects of curcumin against the effects of middle cerebral artery occlusion (Thiyagarajan and Sharma, 2004).

Free radical mediated damage to biological systems is recognized as the initiating agent for many diseases, such as cardiovascular diseases, cancer and arthritis. Tumeric and its constituents show beneficial effects on these diseases and on other illnesses (Al-Omar *et al*., 2006). It has been well known that the survival and functional maintenance of neurons is clearly dependent upon retrogradly transport of neurotrophins. Peripheral nerve transaction or crush, blockade axonal transport and therefore might produce chromatolysis and cell death (Perrin *et al*., 2005). It is known that clinical motor function deteriorates in a delayed manner after sciatic nerve compression. We show that 40% of motor neurons were

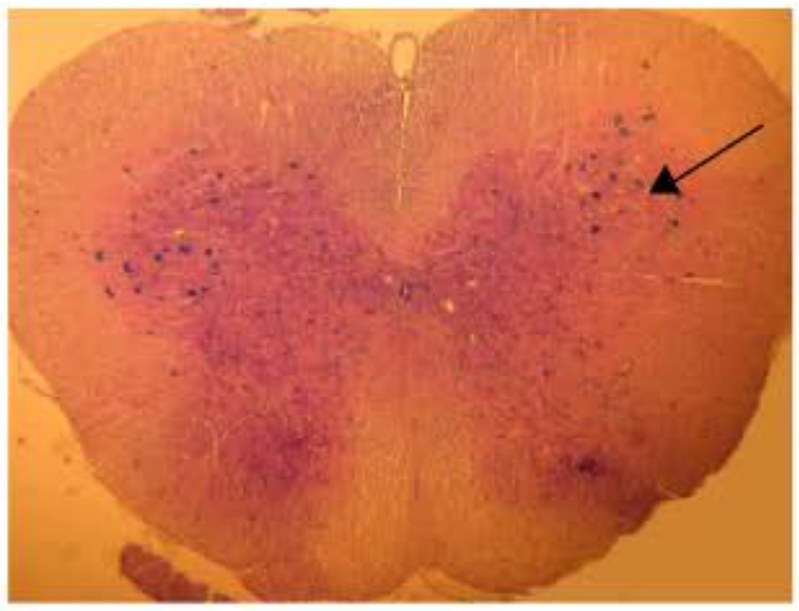

Fig. 1: Photomicrographs illustrate neurons of the anterior horn of spinal cord stained with tuloidin blue and eosin at magnification of (20x) 28 days after injury. Arrow shows alpha motor neurons

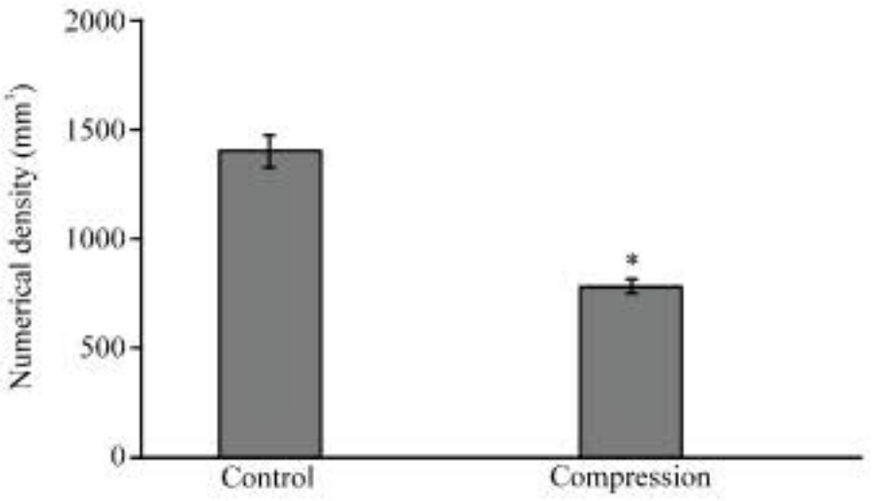

Fig. 2: Comparison between control and compression groups. Results are expressed as Mean±SD (n = 8)

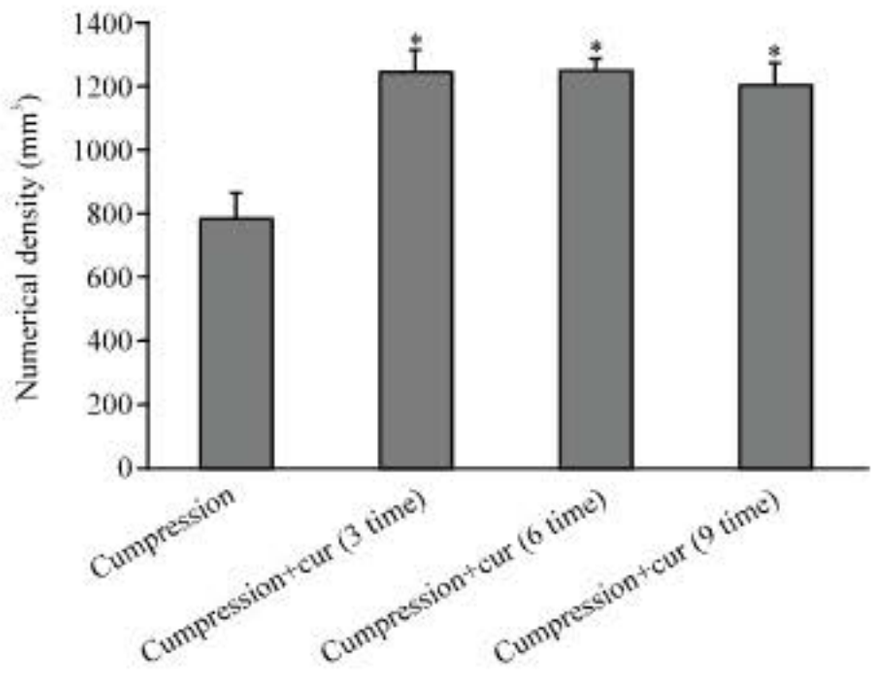

Fig. 3: Effects of compression and Curcumin extract on the number of intact neurons of right ventral horn of spinal cord in rat. Results are expressed as Mean±SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparisons test

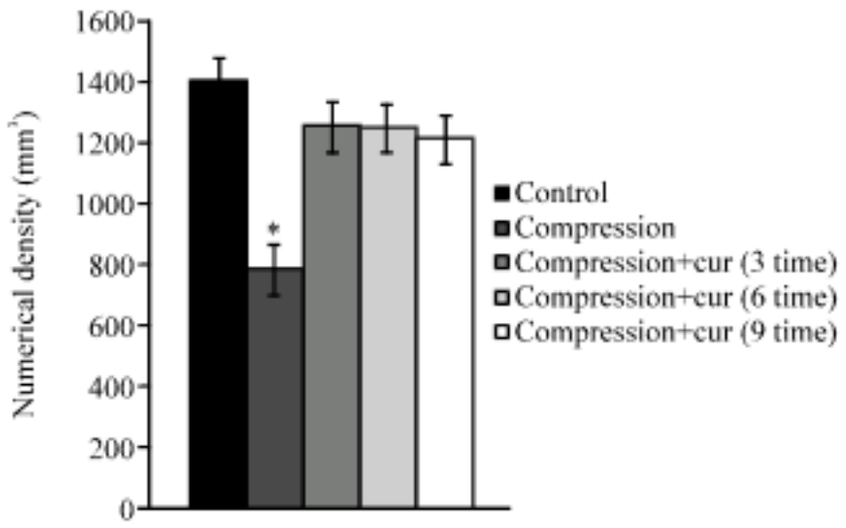

Fig. 4: Comparison between control and compression and treatment groups. Results are expressed as Mean±SD (n = 8)

selectively lost after compression (Fig. 2) and the motor neuronal loss could be the reason for the delayed neurological dysfunction.

Progressive dysfunction and death of neurons characterize neurodegenerative diseases (Baker and Hagg, 2005). There are some evidences supporting the hypothesis that curcumin may act protectively in some neurodegenerative disorders (Sicotte *et al*., 2007). 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 (Singh *et al*., 2008). In addition after compression, inflammatory process was started some inflammatory mediators, such as bradykinine, prostaglandin and serotonin enhance the excitability of normal and injured neurons. Researchers suggest that inflammation is a critical factor for progressing degeneration process. Tissues inflammation factors were surround surface of injured sciatic nerve after chronic compression. In this situation existence of Anti inflammation factors could suppress degeneration. Curcumin extract has Anti inflammation role (Wang *et al*., 2005) and could reduce the rate of degeneration as happen in this study. In all treatment groups, there is a remarkable changes in number of alpha motoneurons in compare to compression group ($p<0.05$) (Fig. 3).

Although, the number of intact alpha motoneuron in all treatment groups was increased but in treatment group with does (100 mg kg^{-1}, i.p., 3 time) the best result was seen. In this group the mean of alpha motoneuron after compression is very near to control group (Fig. 4). Finally, histological and stereological assessment showed that Curcumin with a dose of 100 mg kg^{-1} attenuated neuronal damage after sciatic nerve compression. This dose in rat is equivalent to approximately a total dose of 1.25 g in adult man weighing 70 kg taking in consideration the differences in surface areas and weights between the two species. Curcumin is relatively safe in human. If curcumin extract provides neuroprotection against sciatic nerve injury in humans, as seen in rats, curcumin treatment would act to save a number of patients from CNS damage.

CONCLUSION

The tumeric spice has been used for many centuries mainly as a food, additive, primarily because of its golden yellow color. It was used as a tonic for improving health and in various combinations for the treatment of diseases, such as the common cold. The major breakthrough in realizing the medicinal value of tumeric came with the isolation of phenolics called curcuminoids, of which curcumin is the major constituent. In total the most important mechanism that we can suggest for such effect is antioxidative and anti inflammation functions of curcumin extract. It is concluded that curcumin extract with the protective role is clinically beneficial in the cases of neuronal death that result from sciatic nerve injury.

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