Neuroprotective effects of *Equisetum telmateia* in rat

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Abstract

Equisetum telmateia (Equisetaceae) seems to have anti inflammatory and antioxidant properties. In the present study, the neuroprotective effects of organic and inorganic silica were investigated on spinal cord alpha motoneuron of rats after injury of sciatic nerve. After highly compression of sciatic nerve in 42 Wistar rats, the injured rats were divided into sham (n= 6) and two experimental groups which each were divided into 3 subgroups (n= 6). The first subgroups received 3, 6 or 9 injections (15 mg/kg/injection, ip) of horse tail extract and the second subgroups received 3, 6 or 9 injections (6 mg/kg/injection, ip) of sodium meta silicate, respectively. The first injection was made after sciatic nerve injury and the others by 72 hours intervals. After a month, the rats were sacrificed and their spinal cord lumber segment sampled, processed for histological preparation and analyzed stereologicaly (the *dissector* technique) for estimation of numerical density of alpha motoneurons. The results showed significant decrease in the numerical density of alpha motoneurons in shams ($p < 0.05$) and no significant differences between experimental and control groups. This may suggest the neuroprotective effects of silica on the survival of alpha motoneurons.

Key words: neuroprotective, antioxidant, *Equisetum telmateia*, horsetail, rat

Introduction

The medicinal properties of aerial parts of Equisetaceae family in the treatment of acne, rheumatism, pain in broken bones, diuretic, expectorant, kidney stones and in strengthening hair, skin and nails (Uzun et al., 2004) have made these plants useful natural drug in traditional medicine. The hydroalcoholic extract of *E. arvense* stem has an antinociceptive property, which is not related to the opioid system, and also anti inflammatory effect in mice (Do Monte et al., 2004). Chronic administration of the hydroalcoholic extract from stems of *E. arvense* improves the cognitive deficits in aged rats, and this effect can be due, at least in part, to its antioxidant action (Dos Santos et al., 2005a). Between three species of Equisetaceae, *E. arvense*, *E. ramosissimum* and *E. telmateia*, it has been shown that the *E. telmateia* extract demonstrates the most relevant scavenger and antioxidant properties (Stajner et al., 2006).

Traumatic events of intense mechanical compression of the mammalian peripheral nerves lead to axotomized motoneurons regenerate their axons and if this happens shortly after nerve injury, the cell body usually returns to its former appearance (Seniuk, 1992). Failure to contact a new target cell leads to the neuronal atrophy and death (Crouch et al., 1994). Secondary injury, which is partly due to oxygen radicals released from neutrophils, further contributes to worsening of CNS function (McTigue and Tripathi, 2008; Bagdatoglu et al., 2002; Marin et al., 1998). In rat after peripheral axotomy, some motoneurons survive and undergo typical reactive changes typical for chromatolysis while the others undergo changes that lead to cell death (Behnam Rassouli et al., 2000).

In the hope of promoting the survival rate of neurons and axonal regeneration, this paper explains a possible supportive effect of extract from a horsetail plant. Since *E. telmateia* has most relevant scavenger and antioxidant properties this study was designed, by comparing the beneficial effects of SM by HT, to explore whether this property is due to HT high silica content.

Material and methods

Animal: Fortyeight male Albino Wistar rats weighing 250-300 g (supplied by Razi Institute,

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Mashhad, Iran) were used in the study. At the time of injury the animals were two to three months old. The animals were housed in plastic cages in the animal house and given rat chow (Javaneh Khorassan, Iran) and tap water *ad libitum* and maintained under conditions of controlled lighting (lights on: 6 a.m. to 6 p.m.) and ambient temperature 22±2°C. Animals were cared for and handled in accordance with the Iranian Society of Animal Care (member of International Animal Care Society).

Sciatic nerve injury process: At the time of surgery all rats were anesthetized for sterile surgery by intera peritoneal injection of 0.2 ml of a mixture (1:2) of 10% ketamin (Bayer, Germany) and 2% xylazine (Boxtel, Holland). After exposure of the left sciatic nerve through a gluteal muscle splitting incision, the sciatic nerve of 42 rats were crushed for 30 seconds period between prongs of #5 clamp forceps (Behnam Rassouli et al.,2000). On the remaining 6 rats, a sham operation was performed which exposed the sciatic nerve but did not disturb it. The muscle and skin were then closed with 14 mm stainless steel sutures.

Experimental design: The lesion rats were then divided into control (n=6) and two treating groups; horsetail stem extract (HT) and sodium metasilicate (SM) treated groups. The HT group were further divided into 3 subgroups (n=6); receiving 3, 6 or 9 injections (13.65 mg/kg, ip), respectively. Also the SM group further divided into 3 subgroups (n=6) and received 3, 6 or 9 injections (6 mg/kg, ip), respectively. The first injection was made after sciatic nerve injury and the others by 72 hours intervals. At the end of experimental period (one month) the animals were anesthetized and transcardially perfused with 10% formaldehyde. Immediately following perfusion, the L4 to L6 spinal segments with associated dorsal roots of sciatic nerve were dissected and post fixed for 2 h or overnight. The spinal blocks were processed for histological preparation and embedded in paraffin and then sectioned serially at 7 µm diameter. To sample the sections, a uniform random sampling scheme was employed so that about 10 sections from each block were sampled (Gundersen and Jensen, 1987). Sections were stained with toluidine blue with special buffer of acetic acid 1N (1 ml), sodium acetate 1N (1 ml) and distilled water (98 ml), pH 4.65. After permanent mounting, the numerical density (Nv) of motoneurons in the left and right sides of ventrolateral regions of spinal cord were estimated, using stereological counting technique; the physical *dissector* (Sterio, 1984; Gundersen, 1986; Cruz Orive, 1987).

Preparation of sodium metasilicate: In order to prepare the sodium metasilicate solution, 180 mg sodium metasilicate powder (Aldrich Chemical Co.) was dissolved in 100 ml of distilled water and the pH of the solution neutralized (pH=7.4) by adding 1N hydrochloric acid. Since the administrational dose of silica is varies up to 40 µg/g rat chow (Seaborn and Nielsen, 2002, 1994) we administrated a supplemental dose of 6 mg/kg/injection SM. Therefore, by using a sterile syringe and 22 gauge needle approximately 1 ml of the solution was injected intra peritoneally in every injection.

Plant material and preparation of aqueous extract: Horsetail was collected from Syah Roodbar forest in the north of Iran during the summer of 2005 and identified by Mr. Jouharchi, Herbarium Centre, Ferdowsi University of Mashhad, as *Equisetum telmateia* (voucher no. 31401). The leaves and stems of the plant were separated, dried and homogenized to a fine powder and then stored at room temperature in opaque screwtop jars until use. In order to prepare an aqueous extract of leave and stem, 5 g powdered materials were placed in 250 ml boiling (distilled) water for 2 hours and concentrated to half of the volume by boiling in a water bath. The suspensions were filtered (Whatman no. 1) and the filtrated volumes adjusted to 130 ml with distilled water and neutralized (pH=7.4) by adding 1N NaOH. To determine the amount of silica, as various compounds in crude extracts, a sample of each extract was analysed by Atomic Absorption Spectrometry (AAS) method in the Analytical Chemistry Lab., Dept. of Chemistry, Ferdowsi University of Mashhad, in acetylene flame and N2O and C2H2. The results showed that the amount of silica in the stem of horsetail is higher than leaves (Figure 1). Therefore it was decided to treat the injured rats by stem extract. After that the amount of dried material in the above horsetail stem extract was measured (4.55 mg/ml). Since the administrational dose of the horsetail stem extract varies between 10 to 400 mg/kg body weight (Dos Santos et al., 2005b; Do Monte et al., 2004) the dose selected for the treatment of animals was 4.55 mg/kg body weight/day (or 13.65 mg/kg body weight/injection).

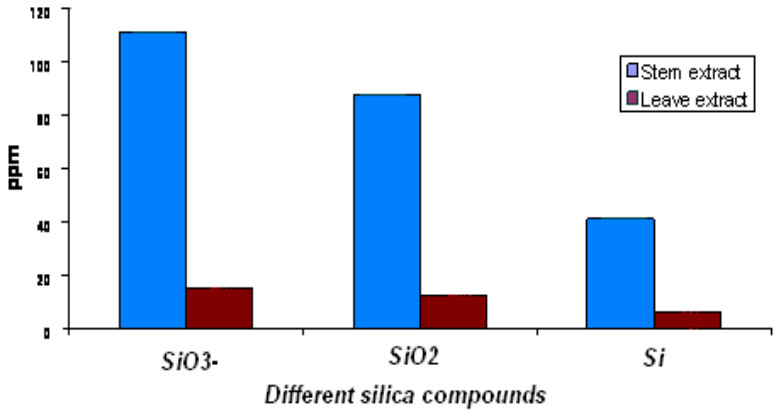

Figure 1. The amount (ppm) of different silican compound in the extract of Equisetum telmateia stem and leave, measured by the Atomatic Absorption Spectrometry technique.

Statistical analysis: One-way single factor ANOVA was used to compare more than two groups followed by student test (Microsoft Office Excel software) to detect differences between groups. For all test, $P<0.05$ was considered statistically significant. All results are expressed as mean ± S.E.M.

Results

The results of the Atomic Absorption Spectrometry assays are presented in Figure 1. These data showed that the total amount of different silica compounds (SiO3 --, SiO2 and Si) in the stem extract of *Equisetum telmateia* is higher than leaves (the total amount silica was 240.42 ppm vs. 32.71 ppm). The results of the estimation of numerical density of alpha motoneurons in the ventral horn of L4 to L6 segments of spinal cord are indicated in table 1. As seen in the table after sciatic nerve crush, the spinal ventral horn motoneuron counts show a decline in number (962.72 /mm3 in controls vs. 1466.13/mm3 in shams). Statistical analyses show that the reduction in the motoneurons of controls, when compared with shams and all experimental groups, is significant ($p<0.05$) (table 1). Although the numerical density of motoneurons in all experimental groups is lower than control group but comparison of numerical density among control and all experimental groups was only significant ($p<0.05$) in HT treated subgroups who received 3 and 6 injections of extract (table 1). Also inter group comparison of numerical density of motoneurons among the similar HT and SM treated subgroups as well as intra group comparison of numerical density between different HT treated subgroups and between different SM treated subgroups showed no significant differences.

Table 1. The numerical density (no/mm^3) of motoneurons in control, sham and experimental groups at 30th post operation day.

		Sodium meta silicate (6mg/kg/injection)			Horse tail extract (13.65mg/kg/injection)		
		3 injection	6 injection	9 injection	3 injection	6 injection	9 injection
		1338.82 (±79.33)	1421.88 (±93.12)	1265.58 (±75.36)	1177.29 (±108.37)	1226.96 (±96.18)	1394.73 (±110.17)
Control	962.72 (±32.75) ●●●	**	***	**	*	*	**
sham	1466.13 (±80.30)	ns	ns	ns	●	●	ns

Data are presented as mean (±SEM). Statistical analyses were performed using one-way ANOVA, followed by Dunnett test. ●P<0.05; ●●●P<0.001 compared with the control group, *P<0.05; **P<0.01; ***P<0.001 compare with the sham group. (ns) no significant differences.

Discussion

After injury the production of reactive oxygen species may increase leading to tissue damage via several different molecular pathways (McTigue and Tripathi, 2008). Radicals can cause damage to cardinal cellular components such as lipids, proteins, and nucleic acids (e.g., DNA), leading to subsequent cell death by modes of necrosis or apoptosis. The damage can become more widespread due to weakened cellular antioxidant defense systems. Flavonoids, a naturally occurring plant substance and currently available for the treatment of acute CNS injuries (Gilgun Sherki et al., 2002) is known as an antioxidant and free radical scavenger. Thus some of the protective effect of horse tail may be due to flovonoid. To our knowledge the present study is the first documentation in which the probable neuroprotective effect of inorganic silica (SM) were compared by horse tail extract.

The results obtained from the present study indicate that intra peritoneal injection of HT and SM at the time of injury and afterwards may prevent or delay the onset of neuronal loss in the spinal cord. Statistical analyses of data indicate that, in comparison with sham and all experimental groups, the numerical density of motoneurons in controls is significantly reduced while, except the HT treated subgroups who received 3 and 6 injections, there is no remarkable difference between experimental groups and shams. Similarly, there was no clear difference between the HT and SM treated groups.

It is obvious that damage to the nervous system does not stop immediately after the initial injury, but continues in the hours following trauma, a process called secondary injury (Gilgun Sherki et al., 2002). Reduction in the endogenous antioxidant defense system due to environmental and genetic factors may contribute to oxidative stress evolution. Antioxidants of varying chemical structures have been investigated as therapeutic agents in the treatment of acute CNS injury. The secondary injuries can be the result of a number of auto destructive phenomena such as neutrophilic infiltration (Genovese et al., 2005; Tonai et al., 2001). The resistance of CNS to regeneration might be related to the restriction in the numbers of macrophages recruited and activated by the injured CNS (Lazarov Spiegler et al., 1996) but the activated neutrophils may be implicated in the worsening of nerve injury and release of oxygen radicals which is toxic to the cell membrane component and free radical induced lipid peroxidation (Bagdatoglu *et al.*,2002; Marin *et al.*,1998; Konat and Wiggins, 1985). Between three different species of Equisetaceae; *E. arvense*, *E. ramosissimum* and *E. telmateia*, it has been shown that the free radical scavenging activity of the *E. telmateia* is higher than the other two and Electron Spin Resonance signal of DMPOOH radical adducts in the presence of *E. telmateia* phosphate buffer extract is reduced to 98.9% (Stajner et al., 2006). In the case of inorganic silica it has been reported that intra peritoneal injection of silica dust at the time of a compression injury to the spinal cord produces a delay of one to two days in the onset of secondary functional loss below the level of injury (Blight, 1994). The results obtained from the present research are in consistent with the above observations.

In conclusion, in the case of SM treated animals it seems that silica is the active agent but whether silica is the only constituent of HT which exerts HT neuroprotective effects is not certain. By applying a silica chelator it is possible to draw out the silica from the HT and then test if silica is the active agent. Also in vitro evaluation of silica anti-oxidant property and in vivo evaluation of motor function and measuring nerve conduction velocity could be performed to confirm the therapeutic benefits of silica in future studies.

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References

Bagdatoglu C., Saray A., Surucu H. S., Ozturk H. and Tamer L. (2002). Effect of trapidil in ischemia/reperfusion injury of peripheral nerves. Neurosurgery, 51: 212-9.

Behnam Rasouli M., Nikravesh M. R., Mahdavi Shahri, N. and Tehranipour M. (2000). Post operative time effects after sciatic nerve crush on the number of alpha motoneurons, using a stereological counting method (dissector). Iranian Biomedical J., 4: 45-49.

Blight A. R. (1994). Effects of silica on the outcome from experimental spinal cord injury: implication of macrophages in secondary tissue damage. Neuroscience, 60: 263-273.

Crouch M. F., Heydon K., Granaut S. M., Milburn P. J. and Hendry L. A. (1994). Retrograde axonal transport

of the alpha subunit of the GTP binding protein GZ in mouse sciatic nerve: a potential pathway for signal transduction in neurons. Eur. J. Neurosci. 6: 626-631.

Cruz Orive I. M. (1987). Particle number can be estimated using a dissector of unknown thickness. J. Microsc. 145: 121-142.

Dos Santos J. G., Blanco M. M., Do Monte F. H. M., Russi M., Lanziotti V. M. N. B., Leal L. K. A. M. and Cunha G. M. (2005). Sedative and anticonvulsant effects of hydroalcoholic extract of Equisetum arvense. Fitoterapia, 76: 508-513.

Dos Santos J. G., Do Monte F. H. M., Blanco M. M., Do Nascimento Bispo Lanziotti V. M., Maia F. D. and Leal L. K. (2005). Cognitive enhancement in aged rats after chronic administration of Equisetum arvense L. with demonstrated antioxidant properties in vitro. Pharmacol Biochem Behav, 8: 593-600.

Do Monte F. H., Dos Santos J. G. Jr., Russi M., Lanziotti V. M., Leal L. K., Cunha G. M. (2004). Antinociceptive and anti inflammatory properties of the hydroalcoholic extract of stems from Equisetum arvense L. in mice. Pharmacology Research 49: 239-243.

Genovese T., Mazzon E., Rossi A., Paola R., Cannavo G., Muia C., Crisafulli C., Bramanti P., Sautebin L. and Cuzzocrea S. (2005). Involvement of 5-lipoxygenase in spinal cord injury. Journal of Neuroimmunology, 166: 55-64.

Gilgun Sherki Y., Rosenbaum Z., Melamed E. and Offen D. (2002). Antioxidant Therapy in Acute Central Nervous System Injury: Current State. Pharmacological review, 54: 271-284.

Gundersen H. J. G. and Jensen E. B. (1987). The efficiency of systematic sampling in stereology and its prediction. J. Microsc., 147: 229-263.

Gundersen H. J. G. (1986). Stereolgy of arbitrary particles. J. Microsc., 143: 3-45.

Konat G. W. and Wiggins R. C. (1985). Effects of reactive oxygen species on myelin membrane proteins. J. Neurochem., 45: 1113-1118.

Lazarov-Spiegler O., Solomon A. S., Zeev-Brann, A. B., Hirschberg D. L., Lavie V. and Schwartz M. (1996). Transplantation of activated macrophages overcomes central nervous system regrowth failure. The FASEB Journal, 10: 1296-302.

McTigue D. M. and Tripathi R. B. (2008). The life, death, and replacement of oligodendrocytes in the adult CNS. J. Neurochem., 107: 1–19.

Marin P. C., Im M. J., Girotto J. A., Borschel G. and Bickel K. D. (1998). Effects of hydroxyethyl starch bound deferoxamine on ischemia/reperfusion injury in chronic nerve compression. J. Reconstr Microsurg., 14: 485-90.

Seaborn C. D. and Nielsen F. (2002). Dietary silicon and arginine affect mineral element composition of rat femur and vertebra. Biol. Trac. Elem. Res., 89: 239-250.

Seaborn C. D. and Nielsen F. H. (2002). Silicon deprivation decreases collagen formation in wounds and bones and ornitine transaminase enzyme activity in liver. Biol. Trac. Elem. Res., 89: 251-262.

Seniuk N. A. (1992). Neurotrophic factors: role in peripheral neuron survival and axonal repair. J. Reconstr. Microsurg., 8: 399-404.

Sterio D. C. (1984). The unbiased estimation of number and sizes of arbitrary particles using the dissector. J Microsc., 134: 127-136.

Stajner D., Popovic B. M., Canadanovic Brunet J. and Boza P. (2006). Free radical scavenging activity of three Equisetum species from Fruska gora mountain. Fitoterapia, 77: 601-604.

Tonai T., Shiba K. I., Taketani Y., Ohmoto Y., Murata K., Muraguchi M., Ohsaki H., Takeda E. and Nishisho T. (2001). A neutrophil elastase inhibitor (ONO-5046) reduces neurologic damage after spinal cord injury in rats. Journal of Neurochemistry, 78: 1064-1072.

Uzun E., Sariyar G., Adsersen A., Karakoc B., Otuk G., Oktayoglu E. and Pirildar S. (2004). Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. J. Ethnopharmacol, 95: 287-296.