Antioxidative and anti-inflammatory effects of *cichorium intybus* L. Seed extract in ischemia/reperfusion injury model of rat spinal cord

Neda Ghaffari,[a] Gholamreza Hassanzadeh,[a] Azin Nowrouzi,[b] Morteza Gholaminejhad,[a] Tahmineh Mokhtari,[c] Rahimeh Seifali,[a] Ibrahim Mohammed,[a] and Mohammad Akbari[a*]

[a]Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[b]Department of Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[c]Nervous System Stem Cells Research Center, Semnan University of Medical Sciences, Semnan, Iran.
*Correspondence to Mohammad Akbari (email: akbarimo@tums.ac.ir).
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Objectives The antioxidant and anti-inflammatory effects of aqueous extract of chicory seed (CSE, *Cichorium intybus* L. seed) following spinal cord ischemia/reperfusion (SCI/R) injury in rat model were evaluated.
Methods In this study 36 male Wistar rats were randomly divided to six groups: Control (Co), Sham (Sh), CSE, SCI/R, CSE + SCI/R (7 days pretreatment with CSE group + inducing I/R injury), SCI/R + CSE (induced I/R injury group +3 days treatment with CSE). SCI/R injury was induced by creating a longitudinal incision on the midline of abdominal region and clamping the aorta just below renal artery for 30 min. After 3 days, SC was removed and used for evaluation of antioxidant enzymes [including superoxide dismutase (SOD) and catalase (CAT)], oxidative stress markers [malondialdehyde (MDA)], inflammatory factors (IL-1β, IL18 and TNF-α) and histopathological changes. Before sacrificing the animals, the motional score was assessed.
Results Our results demonstrated that, in the SCI/R group, the mean levels of SOD, and CAT were significantly decreased ($P < 0.05$), while the mean level of MDA was significantly increased ($P < 0.05$) in comparison to Co and Sh groups. Also, the mean levels of SOD and CAT in the treatment group were higher than the SCI/R group ($P < 0.05$), while, the mean MDA content in the treatment group was significantly less than the SCI/R group ($P < 0.05$). In addition, comparison between SCI/R and treatment groups demonstrated a significant decrease in tissue damage in the treatment group.
Conclusion Our study demonstrated that, the neuroprotective effects of aqueous extract of *Ci. intybus* L. seed on SCI/R injury in rat by antioxidative and anti-inflammatory activities. Additionally, comparison of treatment and pretreatment groups show that the pretreatment usage of the extract is more effective than the treatment group.
Keywords *cichorium intybus* L., spinal cord ischemia/reperfusion, inflammation, oxidative stress

Introduction

Spinal cord (SC) injury is one of the most important complications in the health field and it causes different disorders for the patient.[1] Ischemia/reperfusion (I/R) injury is a type of SC injury which is common in aortic surgeries.[2] Patients with this difficulty suffer from paraplegia or paraparesis. Spinal cord I/R (SCI/R) injury has two steps: the first one is due to acute ischemia and the second one because of neuronal cell death and injuries following reperfusion. Inflammation and oxidative stress are two consequences which follows this damage.[3,4]

After the SCI/R injury, the level of inflammatory cells subsequently elevated, the secretion of inflammatory cytokines increased and these cytokines lead to inflammation.[4,5] Interleukin-1 beta (IL-1β), tumor necrosis factor-α (TNF-α) and IL18 are important types of pro-inflammatory factors and they have an important role in inflammation of central nervous system (CNS).[6,7] These cytokines cause production of other cytokines and expression of endothelial leukocyte adhesion which finally results in damage of endothelial cell and spinal cord ischemia.[8–11] Another important result of SCI/R injury is the oxidative stress and it happens due to increase in level of destruction of adenines which are precursors of reactive oxygen species (ROS), one of the oxidative stress factors.[12] Free radicals exist in different types of diseases such as cancer, atherosclerosis and neurodegenerative diseases.13 The main parameters of oxidative stress are malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD). The principal target of oxygen free radicals is unsaturated fatty acids of the cell membrane which leads to impaired cell function. One of the final products of these reactions is MDA which is a lipid peroxidation marker.[1,14]

Many studies have shown that herbal agents have some neuroprotective characteristics and Chicory (*Cichorium intybus* L.) is one of them with anti-inflammatory, anti-cancer and neuroprotective effects.[15–17] Chicory seed (CS) consists of different types of flavonoids. Flavonoids are polyphenolic compounds with antioxidant, anti-inflammatory and anti-cancer effects.[18]

Based on the characteristics of Chicory seed, this experimental study was designed to show the effects of aqueous extract of CS on the levels of inflammatory factors and antioxidant enzymes following SCI/R injury.

Materials and Methods

Animals

In this study, we used 36 male Wistar rats weighing up to 250–300 g. Housing condition includes a room temperature of 20–22°C, a 12-h light–dark cycle and free access to food and water. Animal consideration was performed according to the

guidelines of the Iranian Animal Ethics Committee, Tehran University of Medical Sciences, Tehran, Iran.

Aqueous Extract Preparation

After clearing the Chicory seeds, the seeds were powdered by an electric mill. Each 200 g of Chicory seeds powder was soaked in 1 L of distilled water and heated for 20 min in a boiling water bath for providing a 20% (w/v) solution. After the solution was reached to room temperature by means of Whatman No 1 filter paper, the solution was filtered and then lyophilized at the Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, and then stored at −20°C.[19]

Animal Groups

Animals were randomly divided into six groups (n = 6) as described below:

1. Control (Co) group intact animals without any interference.
2. Sham (Sh) group animals undergone surgical procedure without I/R induction (without clamping aorta) and normal saline (NS) was administered intraperitoneally (IP) for 3 days.
3. CS extract (CSE) group aqueous CSE was administered IP for 3 days in intact animals.
4. SCI/R group SCI/R injury was induced and NS was administered IP for 3 days.
5. CSE + SCI/R group the animals were pretreated with CSE for 7 days IP and subsequently, SCI/R injury was induced.
6. SCI/R + CSE group: SCI/R injury was induced and then CSE was administered IP for 3 days.

Surgical Procedure

The animals were anesthetized by ketamine (80 mg/kg) and xylazine (15 mg/kg). For accessing to abdominal aorta, we created a longitudinal incision on the midline of abdominal region and after removing the viscera, the aorta and inferior vena cava were observed in the posterior part of the abdomen. In the next step, we clamped the aorta by clips for 30 min just below the division of renal arteries. During surgery, the body temperature was stable and using ringer serum the organs stay hydrated. After removing clips, fascia and skin were sutured separately. During consciousness after surgery the animals were assessed neurologically. After 3 days the rats were anesthetized and spinal cord were removed for more analysis.

Assessment of Oxidative Stress

After 3 days, the rats were anesthetized and after laminectomy, the spinal cord species at lumbar segment just below renal artery were removed and frozen in nitrogen gas and then kept in −80°C refrigerators for determining the following biochemical parameters. SOD (cat # ZB-SOD48), CAT (cat # ZB-CAT48) and MDA (cat # ZB-MDA48) testing kits (Zellbio, Deutschland, Germany) were used for determining oxidative stress after SCI/R injury according to the manufacturer protocols.

Histopathological Study

For preparing histological slides, animals were anesthetized by IP injection according to protocol that mentioned before and then perfused with normal saline after 3 days. Then they were received 0.01M phosphate buffered saline (PBS) followed by 4% paraformaldehyde (400 ml/rat) for fixation. After removing spinal cord from vertebral column the samples were kept in the mentioned fixator and then embedded in paraffin for preparing transverse sections. The paraffin sections with the thickness of 5 μm was mounted on poly-l-lysine-coated slides for H&E staining. Several slides were prepared from each group and then the slides were considered by means of the light microscopy (Olympus, Tokyo, Japan) and histological images were captured and the neurons were assessed.

Statistical Analysis

Data were analyzed by using IBM SPSS, version 22. Results were demonstrated as mean ± S.E.M. The data were compared statistically by one-way ANOVA with post-hoc of Tukey's test. The significant level was set at $P < 0.05$.

Results

Effects of CSE on Antioxidant Parameters in Rats with SCI/R Injury

There was a significant reduction in the levels of CAT and SOD in SCI/R and CI/R + CSE groups compared with Co, Sh and CSE groups ($P < 0.05$, Figs. 1a and 1b). As well, there was a significant increase in the level of CAT and SOD in CSE + SCI/R and SCI/R + CSE groups compared with SCI/R group ($P < 0.05$, Figs. 1a and 1b). According to Fig. 1, CSE could increase significantly the level of MDA in SCI/R and SCI/R + CSE groups compared with Co, Sh and CSE groups ($P < 0.05$, Fig. 1c). As well, there was a significant decrease in the level of MDA of SCI/R + CSE group compared with SCI/R group ($P < 0.05$, Fig. 1c).

Effects of CSE on Pro-inflammatory Cytokines in Rats with SCI/R Injury

There was a significant increase in the levels of TNF-α, IL-1β and IL18 in SCI/R and CI/R + CSE groups compared with Co, Sh and CSE groups ($P < 0.05$, Figs. 1a–1c). There was a significant decrease in the level of TNF-α, IL-1β and IL18 in CSE + SCI/R and CI/R + CSE groups compared with SCI/R group ($P < 0.05$, Figs. 2a–2c).

Effects of CSE on Histopathological Changes in Spinal Cord Tissue of Rats with SCI/R Injury

The shrunken and eosinophilic neurons (loss the Nissl bodies) of anterior horn were increased in SCI/R group compared with Co group and vacuolations in the neuropil of the grey matter were recorded. Additionally, density of neurons decreased in SCI/R group (Figs. 3a and 3b). In treated groups of CSE + SCI/R and CI/R + CSE these type of pathogenesis was reduced compared with SCI/R group (Fig. 3b).

Discussion

In this study, we used CSE in a two ways of 7 days pretreatment and 3 days treatment to reduce the inflammation and oxidative stress in spinal cord tissue induced ischemia/reperfusion injury. We used SCI/R model of rat to induce the pathogenesis. According to the results, MDA and proinflammatory factors (including IL-1β, IL18 and TNF-α) increased and SOD and CAT decreased. As well, the number of shrunken and eosinophilic neurons increased. SCI/R which occurs during aortic surgeries may lead to sensory disorders such as total or partial

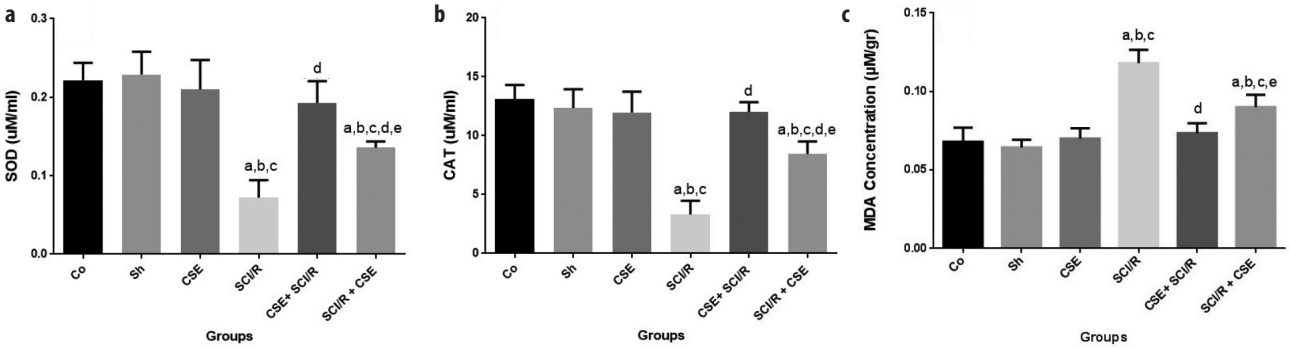

Fig. 1 **Effects of CSE on antioxidant parameters in rats with SCI/R injury. (a) $P < 0.05$ compared to Co group. (b) $P < 0.05$ compared to Sh group. (c) $P < 0.05$ compared to CSE group. (d) $P < 0.05$ compared to SCI/R group. (e) $P < 0.05$ compared to CSE + SCI/R group. Co: Control group, Sh: Sham group, CSE: aqueous extract of chicory seed group, SCI/R: spinal cord ischemia group, and CSE + SCI/R: pretreated with CSE and SCI/R injury induced group: SCI/R + CSE: SCI/R injury induced group treated with CSE.**

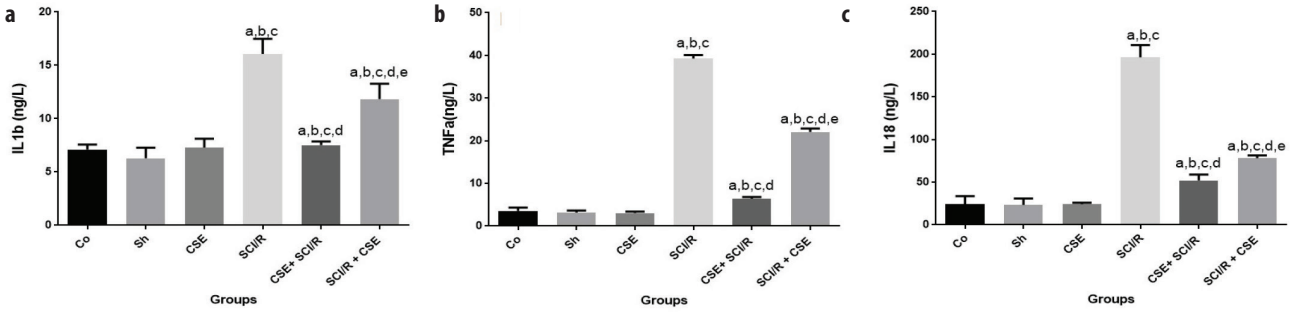

Fig. 2 **Effects of CSE on pro-inflammatory cytokines in rats with SCI/R injury. (a) $P < 0.05$ compared to Co group. (b) $P < 0.05$ compared to Sh group, (c) $P < 0.05$ compared to CSE group, (d) $P < 0.05$ compared to SCI/R group, (e) $P < 0.05$ compared to CSE + SCI/R group. Co: Control group, Sh: Sham group, CSE: aqueous extract of chicory seed group, SCI/R: spinal cord ischemia group, CSE + SCI/R: pretreated with CSE and SCI/R injury induced group, SCI/R + CSE: SCI/R injury induced group treated with CSE.**

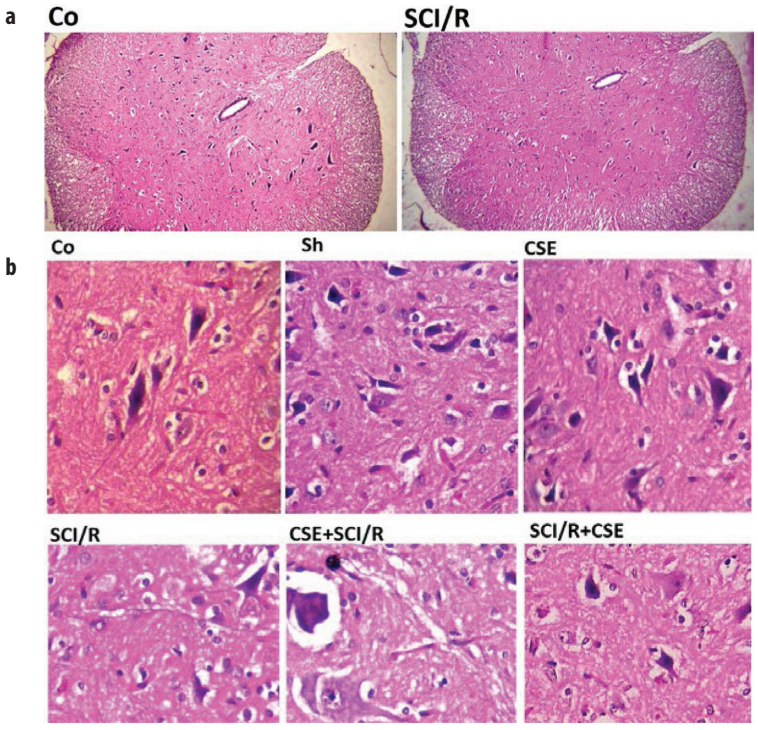

Fig. 3 **Effects of CSE on histopathological changes in spinal cord tissue of rats with SCI/R injury. (a) Comparing the histological changes of SCI/R with control group: increased shrunken and eosinophilic neurons (arrows) and vacuolations in the neuropil of the grey matter, as well as decreased the density of neurons compared to Co group (H&E staining, ×40), (b) comparing the histopathological changes in different groups. Increased shrunken and eosinophilic neurons (arrows) and vacuolations in the neuropil of the grey matter, as well as decreased the density of neurons compared to Co, Sh and CSE groups and decreased these pathological changes in CSE + SCI/R and SCI/R + CSE groups compared to SCI/R group (H&E staining, ×400).**

paralysis and causes different complications for patients.[21] I/R has been defined as an inadequacy of blood supply to the spinal cord, which is exacerbated by reperfusion and causes neurological injuries.[22] The pathogenesis and mechanism of SCI/R injury damage has always been a substantial clinical research subject. Oxidative stress, inflammation, lipid peroxidation are the most important mechanisms which occur after SCI/R,[23,24] which is in conformity with the results of this study. The first step of the SCI/R injury is followed by a secondary damage. After SCI/R injury, the expression of cytokines and adhesion molecules will increase significantly.[3] IL-1β, TNF-α and IL18 are important types of pro-inflammatory factors and they play an important role in inflammation of CNS.[5] In fact in this type of injury, neutrophils by producing inflammatory mediator such as neutrophil elastase and oxygen free radicals have an important role in endothelial cell damage which causes SCI/R injury.[6,25–27] Different evidences have shown that, free radicals are one of the main cause of I/R injury in the CNS. It has been proven that, ischemia leads to increase in level of lipid peroxidation reactions and ROS, which causes secondary neural tissue damage.[28] Lipid peroxidation is a mechanism which causes damage to cellular membranes that results to cell death and production of free radicals, MDA is one of the most important factor between these free radicals.[29,30] SOD and CAT are antioxidative factors which have been considered in SCI/R injury model.[31–33] In fact, CAT and SOD are antioxidant factors that delete ROS from cells.[34] So, the usage of antioxidants, free radical scavengers may be rational in cerebral I/R injury.[35,36] After all, the internal antioxidant mechanisms of the body during SCI/R injury are not adequate and this is the main reason for using different kinds of drugs for reducing the effects of SCI/R injury. According to Fan et al.[37] the level of inflammatory factors increase after SCI/R injury, and also this study demonstrated that, the level of inflammatory factors including IL-1β, TNF-α and IL18 were increased in damage group and also the mean level of SOD, CAT decreased while the MDA factor increased in comparison to Sham group. Our results confirmed that, the stress oxidative level and inflammatory response will increase as consequences of SCI/R injury which may lead to neuronal dysfunction. Yuksel et al.[21] demonstrated that, I/R injury leads to loss and degeneration of motor neurons which have dark and shrunken cytoplasm in histological slides. This result also is in conformity with our study.

The SC repairing is one of the most important problems in health field. Until now there is no any specific medication for treatment of SCI/R injury. Clinical and medical research is being conducted to develop effective treatment interventions for SCI/R injury. Much clinical and basic medical research was performed to develop effective therapeutic interventions for SC/IR injury. Finding a treatment for SCI/R injury is one of the most challenging subjects in neuroscience. In this study, we used CSE to reduce the pathogenesis of SCI/R injury. Our findings confirmed that, SCE can reduce the oxidative stress and inflammation in SC tissue and rescue the eosinophilic and shrunken neurons in this tissue. Treatment with CSE decreased the level of inflammatory factors and MDA also increased the mean level of anti-oxidant enzymes. Additionally, pretreatment with CSE was more effective in SCI/R injury prevention than treatment with this agent.

Recently, attention to plant products that can limit free radicals have been increased for better therapeutic control of I/R injury.[38] *Cichorium intybus*, that is known as "Chicory" is used as herbal medicine from North Africa to South Asia for years. Different parts of *C. intybus* are used for purifying blood and liver disorders in Iran.[39] CS contains various flavonoids.[18] Flavonoids are a group of polyphenolic compounds with antioxidant, anti-inflammatory and anti-cancer properties that helps keep the body from getting disturbed.[40] Different evidence showed that, plant polyphenols causes protection against neurodegenerative changes after brain ischemia reperfusion injury.[41] According to Sadeghi et al.[42] study, Chicory contains flavonoid compounds with antioxidant effects which plays an important role in the treatment of cardiovascular disease. Hassan et al.[43] concluded that, Chicory using its antioxidant role, was used as a natural agent to reduce oxidative stress and liver damage caused by nitrosamine compounds. Different studies demonstrated that, Chicory has therapeutic role for reducing oxidative stress.

Conclusion

In this study, we considered the effect of SCE on SCI/R injury model. We concluded that, 3 days after SCI/R injury induction SOD and CAT were significantly increased in the treatment group compared with damage group, while the level of MDA was decreased. Also, the level of inflammatory factors after using this extract was decreased in treatment group compared with damage group. Also, comparison of treatment and pretreatment groups show that, the pretreatment usage of the extract is more effective than treatment group in fact the level of antioxidant enzymes (including SOD and CAT) in pretreatment group is more than treatment group, also the level of oxidative stress markers (MDA) and inflammatory factors (IL-1β, IL18 and TNF-α) in pretreatment group is less than treatment group.

Conflict of Interest

None. ■

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