Eucommia ulmoides extract alleviated spinal cord injury in rats by inhibiting endoplasmic reticulum stress and oxidative stress

Hongjuan Cheng[1], Haoyu Dong[2], Jie Wei[2], Guopeng You[1]*

[1]Department of Health Management, Changzhi Medical College, Changzhi City, Shanxi Province, China; [2]Department of Endocrinology, Heping Hospital Affiliated to Changzhi Medical College, Changzhi City, Shanxi Province, China

***Corresponding Author:** Guopeng You, Department of Health Management, Changzhi Medical College, No. 161, Jiefang East Street, Luzhou District, Changzhi City, Shanxi Province, China. Email: gp_you22412@163.com

Received: 12 April 2022; Accepted: 29 August 2022; Published: 22 September 2022

ORIGINAL ARTICLE

Abstract

Spinal cord injury (SCI) is the major cause of severe disability worldwide, which leads to neuron death, neuronal degeneration, and functional changes in the spinal cord. Eucommia ulmoides extract (EUE) is composed of multiple iridoids and phenols and possesses anti-oxidative and anti-inflammatory effects in various pathologies. This study aims to evaluate the effects of EUE on SCI and the underlying mechanisms. Male adult Sprague-Dawley rats (250–280 g) were applied to mimic SCI in vivo. Western blot and ELISA kits assessed the expressions of apoptosis, oxidative stress, and endoplasmic reticulum stress (ER stress)-relevant proteins. The apoptosis rate of neurons in spinal cord specimens was measured by TUNEL assay. Finally, our data indicated that EUE inhibited SCI-induced apoptosis, oxidative stress, and ER stress through the suppression of mitogen-activated protein kinase (MAPK) pathway. Our data manifest EUE as a potential therapeutic target in SCI.

Keywords: endoplasmic reticulum stress; eucommia ulmoides extract (EUE); MAPK pathway; oxidative stress; spinal cord injury

Introduction

Spinal cord injury (SCI) is a leading cause of death and disability worldwide, which is induced by primary and secondary neural injury, and generates motor and sensory dysfunctions (Abbasi, 2022). Various mechanisms have been revealed to contribute to the pathological process of SCI, such as oxidative stress, cytotoxicity, excitotoxicity, and inflammation (Anjum *et al.*, 2020; Camilloni, *et al.*, 2021). As the pathophysiology underlying SCI is extremely intractable and complex, still there is a lack of preventive and treatment measures for SCI. Emerging studies indicate that multiple organelles dysfunctions are involved in SCI, of which the important one is endoplasmic reticulum (ER) stress (Chen, *et al.*, 2019). ER is the center of protein synthesis, folding and structural maturation in cells, and once ER stress is triggered, protein misfolding leads to cell death in SCI (Zhou *et al.*, 2020). Therefore, inhibition of oxidative stress and ER stress plays a key role in treating SCI.

Traditional Chinese medicine are used in the treatment of SCI for thousands of years, and has attracted lot of attention in the world (Lu *et al.*, 2020). Eucommia ulmoides Oliver is widely applied to nourish the liver and kidney, prevent miscarriage, and strengthen muscles and bones, and the leaf of *Eucommia ulmoides* has been functional food in China for thousands of years (Li *et al.*, 2021). With

ISSN 1757-837X online, DOI 10.15586/qas.v14i4.1109

the development of modern pharmacology, Eucommia ulmoides Oliver indicates anti-neuroinflammatory, anti-oxidative, and anti-hypertension effects in neurons (Fan *et al.*, 2020; Kwon *et al.*, 2014; Zhou *et al.*, 2020). Eucommia ulmoides extract (EUE) is composed of multiple iridoids and phenols, such as geniposidic acid, geniposide, and pyrogallol (Ishimitsu, *et al.*, 2021). Previous studies have shown that EUE possessed the neuroprotective effects in central. In the tail suspension test of Kasabach-Merritt (KM) mice, EUE caused antidepressant effect by promoting axon and dendrite growth, release of serotonin, and expression of synapsin I (Wu *et al.*, 2016). EUE could alleviate the dopaminergic neuron degeneration and neurological deficits in Parkinson's disease (PD) mice by suppressing p38/JNK-Fosl2 axis (Fan *et al.*, 2020). What's more, EUE inhibited the H2O2-induced effects, such as production of ROS, release of cytochrome C, and phosphorylation of JNK, p38, ERK1/2, and PI3K/Akt in SH-SY5Y cells (Kwon *et al.*, 2012).

MAPK signaling pathway is reported to contribute to various stresses in cell survival. In the C5 hemi-contusion injury rats, inhibition of MAPK pathway attenuated SCI-induced oxidative stress and neuron apoptosis, performing the decrease of ROS and suppression of NLRP3-inflammasome (Liu *et al.*, 2020). Furthermore, the activation of MAPKs results in ER stress in multiple diseases, and inhibition of MAPK pathway could relieve ER stress (Luan, *et al.*, 2021). However, whether inhibition of MAPK pathway could suppress ER stress in SCI is still unknown.

We therefore proposed to assess the effects of EUE on oxidative stress and ER stress in SCI rats. These studies were implemented to reveal the potential targets of anacardic acid and novel therapeutic strategy for SCI.

Materials and Methods

SCI model

All animal experiments were approved by the Experimental Animals Ethics Committee of Changzhi Medical College for the use of animals and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines. Male adult Sprague-Dawley rats (250–280 g) were obtained from Charles river Laboratories. The animal experiments followed the guidelines for the care and use of laboratory animals and contained five groups: (1) sham, (2) SCI, (3) SCI + 0.25 g/kg EUE, (4) SCI + 0.5 g/kg EUE, and (5) SCI + 1 g/kg EUE.

SD rats were anesthetized with 1% pentobarbital sodium. Because of performing the laminectomy at T8, dura was exposed for the following operations. Then, the moderate contusion injury was induced by releasing a 10 g weight from 2.5 cm onto the exposed dura but without destroying the dura. In the course of procedure, rats were maintained on the heating pad to keep their body temperature stable.

Neurological deficit assessment

The neurological deficits in SCI rats were assessed by Basso-Beattie-Bresnahan (BBB) locomotion scale according to previous studies (Chen *et al.*, 2018). The lower BBB score represents severer neurological deficits on a scale of 0–21.

HE and Nissl staining

To evaluate the tissue injury and cell death, the spinal cord specimens were fixed by formaldehyde and embedded by paraffin. HE staining kits (Boster Biotech, Wuhan, China) and Nissl staining solution (Boster Biotech, Wuhan, China) were used according to the guidelines.

Chemicals and antibodies

EUE was purchased from Sinuote Biotech (Sinuote, Xi'an, China). Anti-GRP-78, anti-XBP-1, anti-ATF-4, anti-ATF-6, anti-CHOP, anti-BAX, anti-Bcl-2, anti-cleaved-caspase-3, anti-p-P38, anti-P38, anti-p-ERK1/2, anti-ERK1/2, anti-p-JNK, anti-JNK, and anti-β-actin were obtained from Abcam (UK), CST (USA) and Boster Biological Technology (China).

Western blot

Western blot was applied to assess the expressions of GRP-78 (1:500 dilution), XBP-1 (1:1000 dilution), ATF-4 (1:500 dilution), ATF-6 (1:500 dilution), CHOP (1:500 dilution), BAX (1:500 dilution), Bcl-2 (1:500 dilution), cleaved-caspase-3 (1:500 dilution), p-P38 (1:500 dilution), P38 (1:1000 dilution), p-ERK1/2 (1:500 dilution), ERK1/2 (1:1000 dilution), p-JNK (1:300 dilution), JNK (1:1000 dilution), and β-actin (1:3000 dilution) in spinal cord specimens. First, spinal cord specimens were lysed by RIPA buffer (Beyotime, Hangzhou, China) with protease and phosphatase inhibitors. Then, the protein concentrations of lysates were measured by BCA Protein Assay kit (Beyotime, Hangzhou, China). Different molecular weight proteins were separated and transferred onto polyvinylidene fluoride membranes, and were incubated with corresponding primary antibodies for 16 h at 4°C. Finally, the blots were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Beyotime,

Jiangsu, China), and analyzed by Luminata Creseendo Western HRP substrate via Molecular Imager ChemiDoc XRS+ System (Bio-Rad, Philadelphia, PA).

Assays of SOD, MDA, GSH, and ROS

Enzyme-linked immunosorbent assay (ELISA) kits were used to assess the levels of Superoxide dismutase (SOD), Malondialdehyde (MDA), and glutathione, r-glutamyl cysteingl + glycine (GSH, Boster Biological Technology, Wuhan, China). Reactive oxygen species (ROS) assay kit (Beyotime, Nanjing, China) was used to measure the level of ROS. All experiments were carried out according to the guidelines.

Statistical analysis

Statistical analysis was analyzed by SPSS software, and performed as unpaired two-tailed Student's t test. Data were displayed as mean ± standard error of the mean (S.E.M.), and $P < 0.05$ was considered as significant differences.

Results

Eucommia ulmoides extract relieved SCI-induced neurological deficits

To evaluate the effects of EUE on SCI, BBB locomotion scale, HE staining, and Nissl staining were assessed. As shown in Figure 1A, SCI leads to significant decrease of BBB score and the serial concentration of EUE (0.5, 1 g/kg) revealed great improvement in BBB scores. Consistently, results of HE staining and Nissl staining (Figure 1B–C) indicated that the serial concentration of EUE (0.25, 0.5, 1 g/kg) could reduce SCI-induced changes in ultrastructure of specimens and cell apoptosis, suggesting the potential neuroprotective effect of EUE.

Eucommia ulmoides extract attenuated oxidative stress in SCI rats

To explore the effects of EUE on oxidative stress in SCI, the levels of SOD, MDA, GSH, and ROS were assessed. As shown in Figure 2A and B, the levels of SOD and GSH were remarkably decreased in SCI rats, and the levels of MDA and ROS were significantly increased in SCI rats. Compared with SCI group, intervention of EUE reversed SCI-induced changes in SOD, MDA, GSH, and ROS in a dosage-dependent manner (Figure 2A and B). These data demonstrated that EUE could suppress oxidative stress in SCI rats.

Eucommia ulmoides extract restrained endoplasmic reticulum stress in SCI rats

We detected the expressions of GRP-78, XBP-1, ATF-4, ATF-6, and CHOP to confirm the effects of EUE on ER stress in SCI. As shown in Figure 3, SCI activated ER stress manifesting the enormous up-regulation of GRP-78, XBP-1, ATF-4, ATF-6, and CHOP, which were restored with the intervention of EUE. These data suggested that EUE could inhibit ER stress in SCI rats.

Eucommia ulmoides extract inhibited neurons apoptosis in SCI rats

Apoptosis is the major type of neuron's death in SCI. To further investigate the effect of EUE on apoptosis in SCI rats, TUNEL assay and the protein expressions of Bax, Bcl-2, and cleaved caspase-3 were assessed. As revealed in Figure 4A, SCI induced the significant increase in neuron apoptosis, and the intervention of EUE attenuated apoptosis in a dosage-dependent manner. Additionally, the intervention of EUE alleviated SCI-induced up-regulation of Bax and cleaved-caspase-3, and the down-regulation of Bcl-2. These results revealed that EUE could resist SCI-induced neurons apoptosis.

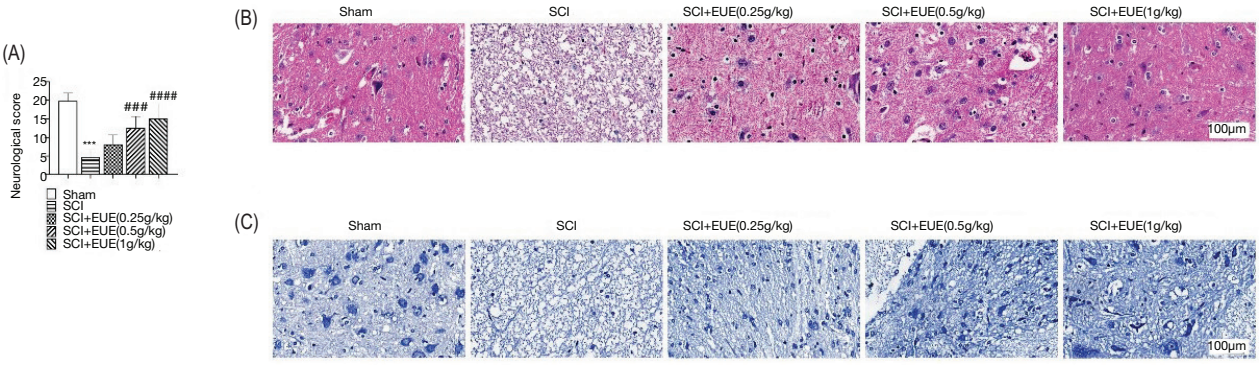

Figure 1. Effect of Eucommia ulmoides extract on neurological deficits in SCI rats. (A) Neurological score. (B) HE staining. (C) Nissl staining. Data were expressed as means ± S.E.M. n = 5 per group. ***$P < 0.001$ versus Sham, ###$P < 0.001$ versus SCI.

Eucommia ulmoides extract suppressed MAPK pathway in SCI rats

To quantify the effect of EUE on MAPK pathway in SCI rats, the expressions of p-P38, P38, p-ERK1/2, ERK1/2, p-JNK, and JNK were measured by western blot. As shown in Figure 5, the expressions of p-P38, p-PERK1/2, and p-JNK were up-regulated in SCI rats, revealing that MAPK pathway was activated in SCI rats, and the intervention of EUE suppressed the up-regulation of p-P38, p-PERK1/2, and p-JNK in a dosage-dependent manner.

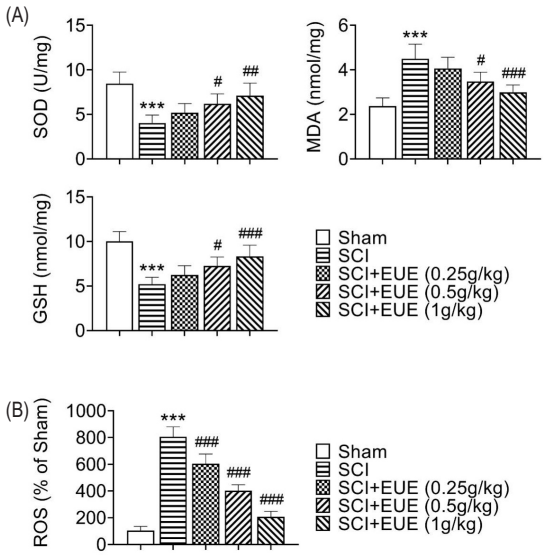

Figure 2. Effect of Eucommia ulmoides extract on the levels of oxidative stress-relevant proteins in SCI rats. (A) Expression of SOD, MDA, and GSH. (B) Level of ROS. Data were expressed as means ± S.E.M. n = 5 per group. ***P < 0.001 versus Sham, #P <0.05, ##P < 0.01, ###P < 0.001 versus SCI.

Discussion

SCI is the major cause of severe disability worldwide, leading to neuron death, neuronal degeneration, and functional changes in spinal cord (Thuret, *et al.*, 2006). Secondary damage results in biochemical changes and neuronal death (Liu *et al.*, 2015). Therefore, attenuating secondary damage is the potential therapeutic strategies in SCI. In the current study, we discovered that the neuroprotective effect of EUE on SCI rats and uncovered the underlying mechanisms. The results displayed that SCI led to neurological deficits, apoptosis, oxidative stress and ER stress, accompanied with the activation of MAPK pathway, and the intervention of EUE alleviated the changes. Thus, our data is the first research to indicate the neuroprotective effect of EUE on SCI-induced damage through inhibiting MAPK pathway.

MAPK pathway plays an essential role in determining neuronal fate in SCI (Liu *et al.*, 2015; Liu *et al.*, 2021). Phosphorylation of MAPK molecules, such as P38, ERK1/2, and JNK were associated with various stress responses in SCI (Liu *et al.*, 2020; Yang *et al.*, 2021). Oxidative stress is the major cause of cell death in multiple diseases, such as apoptosis, necrosis, and programmed cell death. As previous studies have shown, ROS production and activation of MDA, as well as the reduction of GSH and SOD, represent the activation of

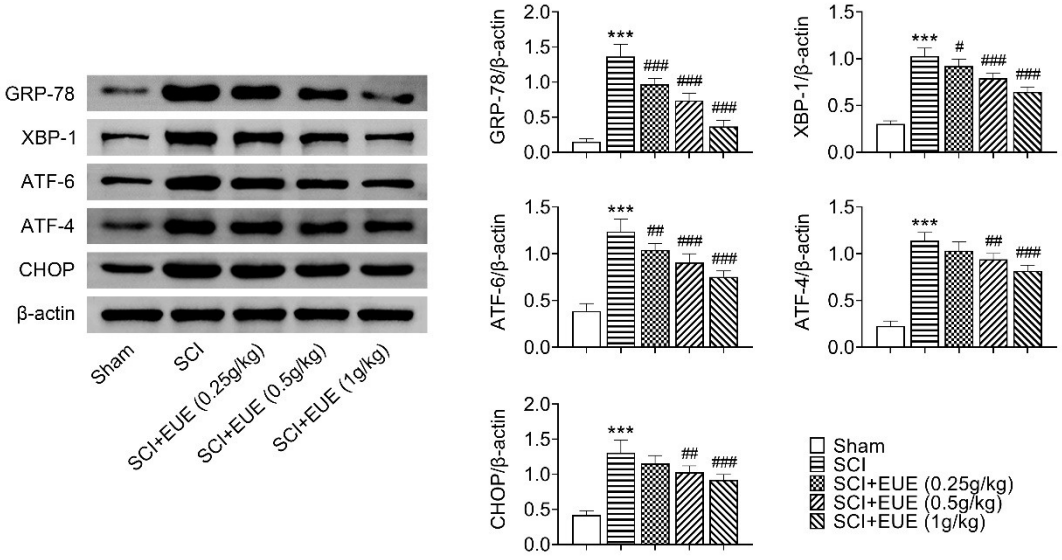

Figure 3. Effect of Eucommia ulmoides extract on the levels of ER stress-relevant proteins in SCI rats. Left: representative images of Western blot results. Right: optical density for the protein blot of GRP-78, XBP-1, ATF-6, ATF-4, CHOP, and β-actin. Data were expressed as means ± S.E.M. n = 5 per group. ***P < 0.01 versus Sham, #P < 0.05, ##P < 0.01, ###P < 0.001 versus SCI.

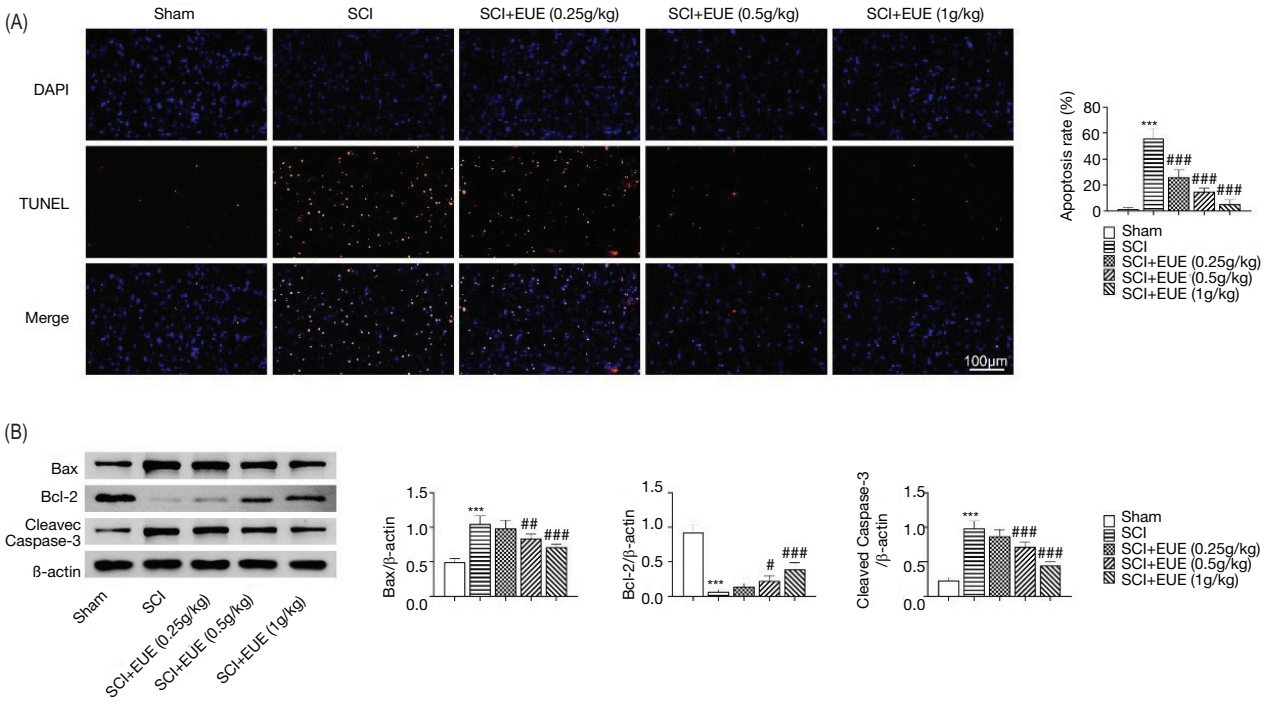

Figure 4. Effect of Eucommia ulmoides extract on apoptosis-relevant proteins in SCI rats. (A) TUNEL assay. (B) Expression of Bax, Bcl-2, cleaved-caspase-3, and β-actin. Data were expressed as means ± S.E.M. n = 5 per group. ***P < 0.001 versus Sham, #P < 0.05, ##P < 0.01, ###P < 0.001 versus SCI.

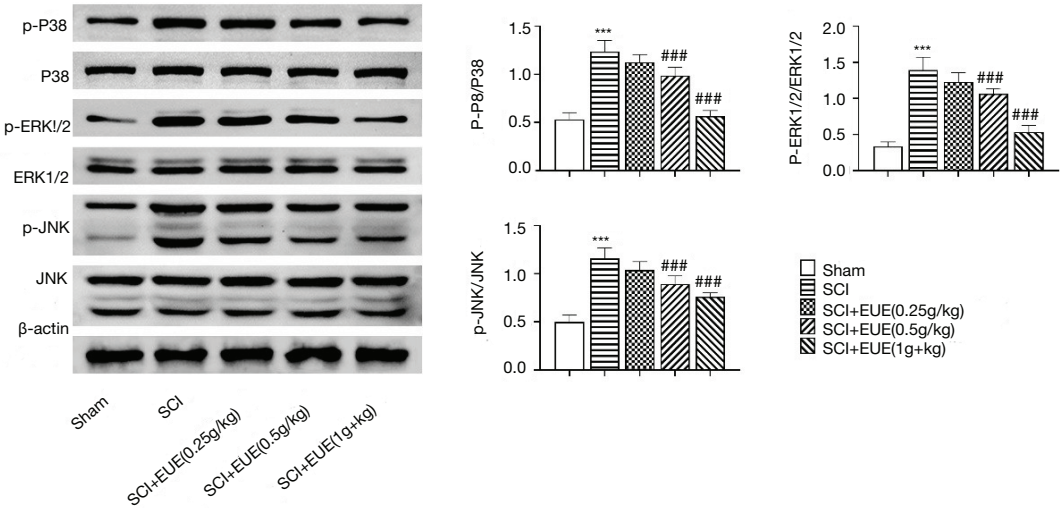

Figure 5. Effect of Eucommia ulmoides extract on apoptosis-relevant proteins in SCI rats. Left: representative images of Western blot results. Right: optical density for the protein blot of p-P38 against P38, p-ERK1/2 against ERK1/2, and p-JNK against JNK. Data were expressed as means ± S.E.M. n = 5 per group. ***P < 0.001 versus Sham, #P < 0.05, ##P < 0.01, ###P < 0.001 versus SCI.

oxidative stress (Luan, *et al.*, 2021). Activation of MAPK pathway promoted oxidative stress in a variety of pathologies. Our results indicated that EUE could significantly inhibit the phosphorylation of MAPK molecules, and suppress oxidative stress in SCI rats as a consequence.

ER stress is the primary cause of secondary injury after SCI (Zhou *et al.*, 2020). MAPK pathway serves as a coordinate activator of ER stress in various pathologies (Hotamisligil & Davis, 2016). Meanwhile, the relationship between ER stress and MAPK pathway in SCI is still unknown. It has been reported that inhibition of MAPK pathway performs as a critical mediator for ER homeostasis (Zhang *et al.*, 2020). Our data manifested that both ER stress and activation of MAPK pathway participated in SCI, presenting as the remarkably increased expressions

of GRP-78, XBP-1, ATF-6, ATF-4, and CHOP, and the obviously up-regulated phosphorylation of P38, ERK1/2, and JNK. As previously described, EUE possessed neuroprotective effect on SCI through inhibition of oxidative stress. Our further study revealed that EUE could suppress the up-regulation of GRP-78, XBP-1, ATF-6, ATF-4, and CHOP, the underlying mechanisms might be associated with the inhibition of MAPK pathway.

Apoptosis in spinal cord specimens is common under various stress, and leads to organizational structure and dysfunction in SCI. Bcl2 family is the well-known apoptosis-related family which determines cellular fate in the physiological condition of stress (Ren *et al.*, 2019). Our data manifested that apoptotic mediator Bax and cleaved-caspase-3 were up-regulated, and anti-apoptotic mediator Bcl2 was significantly down-regulated in SCI rats. Meanwhile, the intervention of EUE could attenuate SCI-induced apoptosis.

In summary, this study indicated that EUE could alleviate SCI in rats for the first time. The intervention of EUE promoted the neuron survival in spinal cord specimens through the suppression of oxidative stress and apoptosis, presenting as the inhibitor of the protein levels of apoptosis mediators Bax, cleaved-caspase3, ROS, and the oxidative stress cytokine MDA, including the enhancement of the expressions of anti-apoptotic protein and antioxidant indices such as Bcl-2, SOD, and GSH. Further studies demonstrated that EUE suppressed ER stress and activation of MAPK pathway. Therefore, EUE possesses the neuroprotective effect against SCI in rats through inhibiting MAPK pathway, and provides a novel therapeutic target for SCI.

References

Abbasi, J. Patients With Complete Paralysis Walk With Spinal Cord Implant. JAMA 2022;327:909. https://doi.org/10.1001/jama.2022.2565

Anjum, A., Yazid, M. D., Fauzi Daud, M., Idris, J., Ng, A. M. H., Selvi Naicker, A., et al. Spinal Cord Injury: Pathophysiology, Multimolecular Interactions, and Underlying Recovery Mechanisms. Int J Mol Sci. 2020;21. https://doi.org/10.3390/ijms21207533

Camilloni, A., Nati, G., Maggiolini, P., Romanelli, A., Latina, R. Chronic non-cancer pain in primary care: an Italian cross-sectional study. Signa Vitae 2021;7:54–62.

Chen, S., Ye, J., Chen, X., Shi, J., Wu, W., Lin, W., et al. Valproic acid attenuates traumatic spinal cord injury-induced inflammation via STAT1 and NF-kappaB pathway dependent of HDAC3. J Neuroinflammation. 2018;15:150. https://doi.org/10.1186/s12974-018-1193-6

Chen, Z., Guo, H., Lu, Z., Sun, K., Jin, Q. Hyperglycemia aggravates spinal cord injury through endoplasmic reticulum stress mediated neuronal apoptosis, gliosis and activation. Biomed Pharmacother. 2019;112:108672. https://doi.org/10.1016/j.biopha.2019.108672

Fan, S., Yin, Q., Li, D., Ma, J., Li, L., Chai, S., et al. Anti-neuroinflammatory effects of Eucommia ulmoides Oliv. In a Parkinson's mouse model through the regulation of p38/JNK-Fosl2 gene expression. J Ethnopharmacol. 2020;260: 113016. https://doi.org/10.1016/j.jep.2020.113016

Hotamisligil, G. S., Davis, R. J. Cell Signaling and Stress Responses. Cold Spring Harb Perspect Biol. 2016;8. https://doi.org/10.1101/cshperspect.a006072

Ishimitsu, A., Tojo, A., Satonaka, H., Ishimitsu, T. Eucommia ulmoides (Tochu) and its extract geniposidic acid reduced blood pressure and improved renal hemodynamics. Biomed Pharmacother. 2021;141:111901. https://doi.org/10.1016/j.biopha.2021.111901

Kwon, S. H., Kim, M. J., Ma, S. X., You, I. J., Hwang, J. Y., Oh, J. H., et al. Eucommia ulmoides Oliv. Bark. protects against hydrogen peroxide-induced neuronal cell death in SH-SY5Y cells. J Ethnopharmacol. 2012;142:337–45. https://doi.org/10.1016/j.jep.2012.04.010

Kwon, S. H., Ma, S. X., Hong, S. I., Kim, S. Y., Lee, S. Y., Jang, C. G. Eucommia ulmoides Oliv. bark. attenuates 6-hydroxydopamine-induced neuronal cell death through inhibition of oxidative stress in SH-SY5Y cells. J Ethnopharmacol. 2014;152:173–82. https://doi.org/10.1016/j.jep.2013.12.048

Li, A., Yuan, J. F., Gong, Q., Zhang, N., Chen, L. Y., Luo, Y. Y., et al. Effects of Eucommia ulmoides extract against renal injury caused by long-term high purine diets in rats. Food Funct. 2021;12:5607–20. https://doi.org/10.1039/d0fo02802a

Liu, S., Sarkar, C., Dinizo, M., Faden, A. I., Koh, E. Y., Lipinski, M. M., et al. Disrupted autophagy after spinal cord injury is associated with ER stress and neuronal cell death. Cell Death Dis. 2015;6:e1582. https://doi.org/10.1038/cddis.2014.527

Liu, Z., Yao, X., Jiang, W., Li, W., Zhu, S., Liao, C., et al. Advanced oxidation protein products induce microglia-mediated neuroinflammation via MAPKs-NF-kappaB signaling pathway and pyroptosis after secondary spinal cord injury. J Neuroinflammation. 2020;17:90. https://doi.org/10.1186/s12974-020-01751-2

Liu, Z., Yao, X., Sun, B., Jiang, W., Liao, C., Dai, X., et al. Pretreatment with kaempferol attenuates microglia-mediate neuroinflammation by inhibiting MAPKs-NF-kappaB signaling pathway and pyroptosis after secondary spinal cord injury. Free Radic Biol Med. 202;168:142–54. https://doi.org/10.1016/j.freeradbiomed.2021.03.037

Lu, Y., Yang, J., Wang, X., Ma, Z., Li, S., Liu, Z., et al. Research progress in use of traditional Chinese medicine for treatment of spinal cord injury. Biomed Pharmacother. 2020;127:110136. https://doi.org/10.1016/j.biopha.2020.110136

Luan, Y., Luan, Y., Feng, Q., Chen, X., Ren, K. D., Yang, Y. Emerging Role of Mitophagy in the Heart: Therapeutic Potentials to Modulate Mitophagy in Cardiac Diseases. Oxid Med Cell Longev. 2021:3259963. https://doi.org/10.1155/2021/3259963

Luan, Y., Ren, K. D., Luan, Y., Chen, X., Yang, Y. Mitochondrial Dynamics: Pathogenesis and Therapeutic Targets of Vascular Diseases. Front Cardiovasc Med. 2021;8:770574. https://doi.org/10.3389/fcvm.2021.770574

Ren, K. D., Liu, W. N., Tian, J., Zhang, Y. Y., Peng, J. J., Zhang, D., et al. Mitochondrial E3 ubiquitin ligase 1 promotes brain injury by disturbing mitochondrial dynamics in a rat model of ischemic stroke. Eur J Pharmacol. 2019;861:172617. https://doi.org/10.1016/j.ejphar.2019.172617

Thuret, S., Moon, L. D., Gage, F. H. Therapeutic interventions after spinal cord injury. Nat Rev Neurosci 2006;7:628–43. https://doi.org/10.1038/nrn1955

Wu, J., Chen, H., Li, H., Tang, Y., Yang, L., Cao, S., et al. Antidepressant Potential of Chlorogenic Acid-Enriched Extract from Eucommia ulmoides Oliver Bark with Neuron Protection and Promotion of Serotonin Release through Enhancing Synapsin I Expression. Molecules. 2016;21:260. https://doi.org/10.3390/molecules21030260

Yang, Y., Luan, Y., Feng, Q., Chen, X., Qin, B., Ren, K. D., et al. Epigenetics and Beyond: Targeting Histone Methylation to Treat Type 2 Diabetes Mellitus. Front Pharmacol. 2021;12:807413. https://doi.org/10.3389/fphar.2021.807413

Zhang, J., Wang, L., Xie, W., Hu, S., Zhou, H., Zhu, P., et al. Melatonin attenuates ER stress and mitochondrial damage in septic cardiomyopathy: A new mechanism involving BAP31 upregulation and MAPK-ERK pathway. J Cell Physiol. 2020;235:2847–56. https://doi.org/10.1002/jcp.29190

Zhou, K., Zheng, Z., Li, Y., Han, W., Zhang, J., Mao, Y., et al. TFE3, a potential therapeutic target for Spinal Cord Injury via augmenting autophagy flux and alleviating ER stress. Theranostics. 2020;10:9280–302. https://doi.org/10.7150/thno.46566

Zhou, Z., Hou, J., Mo, Y., Ren, M., Yang, G., Qu, Z., et al. Geniposidic acid ameliorates spatial learning and memory deficits and alleviates neuroinflammation via inhibiting HMGB-1 and downregulating TLR4/2 signaling pathway in APP/PS1 mice. Eur J Pharmacol. 2020;869:172857. https://doi.org/10.1016/j.ejphar.2019.172857