Huangqi Guizhi Wuwu Decoction Can Prevent and Treat Oxaliplatin Induced Neuropathic Pain by TNFα/IL-1β/IL-6/MAPK/NF-kB Pathway

Mingzhu Li
Cancer Hospital of Liaoning Province
Zheng Li
Cancer Hospital of Liaoning Province
Xiande Ma
Liaoning University of Traditional Chinese Medicine Shenyang Campus: Liaoning University of Traditional Chinese Medicine
Shengbo Jin
Liaoning University of Traditional Chinese Medicine Shenyang Campus: Liaoning University of Traditional Chinese Medicine
Yang Cao
Cancer Hospital of Liaoning Province
Xuebing Wang
Cancer Hospital of Liaoning Province
Jian Zhao
Cancer Hospital of Liaoning Province
Jianbo Wang
Liaoning University of Traditional Chinese Medicine Shenyang Campus: Liaoning University of Traditional Chinese Medicine
Jian Xu (xujian@cancerhosp-ln-cmu.com)
Cancer Hospital of Liaoning Province

Research

Keywords: Huangqi Guizhi Wuwu Decoction, Oxaliplatin, CINP, MAPK/NF-kB Pathway

Posted Date: January 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-138591/v1

Abstract

Background: The mechanism of Huangqi Guizhi Wuwu Decoction in the prevention and treatment of oxaliplatin-induced neuropathic pain was unclear. Therefore, we explored the effect and mechanism of Huangqi Guizhi Wuwu Decoction in chemotherapy-induced neuropathic pain (CINP).

Methods: Bodyweight and related behavioral testing of the rat model were utilized to explore the effect of Huangqi Guizhi Wuwu Decoction in CINP. ELISA was used to detect the levels of TNF-α, IL-1β, IL-6 inflammatory factors in the serum of chronic CINP rats induced by oxaliplatin. Immunohistochemistry and western blot analysis were applied to detect the expression of MAPK pathway related-proteins ERK1/2, p38, JNK, and the expression of downstream essential proteins c-Fos, CREB, and NF-κB.

Results: By bodyweight and related behavioral testing of the rat model, we found that Huangqi Guizhi Wuwu Decoction can improve the slow weight gain of oxaliplatin-induced chronic CINP model rats, effectively prevent and treat oxaliplatin-induced regular CIPN rat model of hyperalgesia, and oppress the mechanical pain threshold, cold pain threshold, and heat pain threshold decreased. By ELISA, immunohistochemistry, and western blot analysis, we found that Huangqi Guizhi Wuwu Decoction can down-regulate the levels of TNF-α, IL-1β, IL-6 inflammatory factors in the serum of chronic CINP rats induced by oxaliplatin, then suppressed the expression of MAPK pathway related-proteins ERK1/2, p38 and JNK. After that, the expression of downstream essential proteins c-Fos, CREB, and Nf-κB also decreased.

Conclusion: In conclusion, we found that Huangqi Guizhi Wuwu Decoction can combat nerve cell injury, reduce pain sensitization, and prevent and repair the damage of nerve cells in oxaliplatin CINP model rats by TNFα/IL-1β/IL-6/MAPK/NF-kB pathway.

Background

In the past few years, the incidence rate and mortality rate of malignant tumors in China have been on the rise, threatening people's health [1]. Cancer pain treatment has always been an essential part of cancer treatment, especially chemotherapy-induced neuropathic pain (CINP). CINP is a kind of intractable pain [2], and the prevalence varied from drug to drug, with a reported incidence ranging from 19–85% [3]. Oxaliplatin, as the first-line chemotherapy drug in treating gastrointestinal cancer, the incidence of oxaliplatin-induced CINP was about 76%-90% [4–6]. The clinical manifestations were rapid onset of neuropathy, aggravated by cold, and developed into chronic neuropathy after several treatment cycles [7–8]. It hinders the continuity of tumor treatment and disease control rate and seriously affects patients' quality of life, which is an urgent clinical problem to be solved.

Huangqi Guizhi Wuwu Decoction came from a traditional Chinese medicine book *Jin Kui Yao Lue*. Huangqi Guizhi Wuwu Decoction's main treatment effect was *Xue Bi*, a proper term of traditional Chinese medicine for these clinical manifestations of neuropathic pain, such as pain and numbness sensory disturbance, cold limbs, and aggravation of cold [9–11]. The meta-analysis of Huangqi Guizhi Wuwu

Decoction shows that it has a good clinical effect in preventing oxaliplatin-induced peripheral nerve injury compared with conventional western medicine [12–15]. However, the mechanism of how Huangqi Guizhi Wuwu Decoction affects CINP was still unclear.

Previous studies found that the mechanism of oxaliplatin-induced chronic neuropathic pain and Western medicine treatment mechanism showed the possible correlation of the MAPK signaling pathway [16–18]. Cluster analysis of network pharmacology of Huangqi Guizhi Wuwu Decoction also indicated that the MAPK signaling pathway was the most closely related to the treatment of neuropathic pain [13]. Therefore, in this research, we established an acknowledged rat model of chronic CINP induced by oxaliplatin, and compared the effects of Huangqi Guizhi Wuwu Decoction oral and topical use on hyperalgesia and neurocyte injury in rats, and partly explained that Huangqi Guizhi Wuwu decoction could prevent and repair CINP induced by oxaliplatin by regulating MAPK signaling pathway. The mechanism study of nerve cell injury in rats can provide a theoretical basis for the prevention and treatment of oxaliplatin-induced CINP, lay a scientific and favorable objective basis for clinical application, and offer new ideas for the development and application of modern Chinese medicine.

Methods And Materials

Rat model grouping and building

Forty-five female SD rats were randomly divided into five groups. Regular control group (**A group**), Model group (oxaliplatin chemotherapy-induced neuropathic pain chronic model, **B group**), Western prevention and treatment group (oxaliplatin-induced pain model + duloxetine gavage, **C group**), Traditional Chinese medicine gavage prevention and treatment group (oxaliplatin-induced pain model + Huangqi Guizhi Wuwu Decoction gavage, **D group**), and Traditional Chinese medicine is socking prevention and treatment group (oxaliplatin-induced pain model + Huangqi Guizhi Wuwu Decoction socking, **E group**).

Xiao *et al.* built the model of oxaliplatin chemotherapy-induced neuropathic pain [19]. The dosage of oxaliplatin was 2 mg/kg/d. On days 1, 2, 3, 4, and 5, oxaliplatin was intraperitoneally injected to construct a chronic neuropathic pain model. When these rats showed hyperalgesia symptoms such as unwillingness to move forward, lifting, licking, even retreating, and mechanical pain threshold, cold and hot pain threshold decreased. The model was considered successful.

The ratio of Huangqi Guizhi Wuwu Decoction follows the synopsis of J*in Kui Yao Lue*, a book of golden prescription of traditional Chinese medicine. The component of Huangqi Guizhi Wuwu Decoction was Huangqi 9g, Guizhi 9g, Peony 9g, Ginger 18g, Jujube 10g. Add these to water and bring to a boil.

The Chinese medicine soaking and the immersing method was as follows: After depilating rats' limbs, the rats were fixed with a particular bubble immersing device. The knee joints, elbow joints, and tail of rats were soaked in 35 ℃ Chinese medicine, and the mouth was exposed to prevent choking water and licking drugs. After washing, the rats were immersed in warm water, and their fur was dried.

Bodyweight and related behavioral testing

According to a previous study, bodyweight, mechanical withdrawal threshold, cold pain threshold, and thermal pain threshold were evaluated [20].

Elisa

The serums of five group rats were gained, and the level of TNFα (SCA133Ra), IL-1β (SEA563Ra), and IL-6 (SEA079Ra) were detected by Elisa kit following the related protocols.

Immunohistochemistry (IHC) analysis

L4-L5 dorsal root ganglions of five groups' rat spinal cord was gained and fixed in 4% formaldehyde. Then these dorsal root ganglions were embedded in paraffin. Then, these paraffin specimens were sliced into a 6-μm section, deparaffinized by xylene and rehydrated by a graded ethanol series.

These sections were then stained with hematoxylin and eosin to explore the change of pathological morphology of L4-L5 spinal dorsal root in these rats.

To explore the expression of ERK1/2, p38, JNK, C-Fos, CREB, and NF-kB, these specimens were incubated with a primary antibody against ERK1/2 (1:50; abcam, ab54230), p38 (1:250; abcam, ab170099), JNK (1:1000; abcam, ab208035), C-Fos (1:800, abcam, ab208942) CREB (1:400; abcam, ab32515) NF-kB (1:200; abcam, ab16502) at 4°C overnight. Then, these specimens were incubated with a secondary antibody (Gene Tech Co. Ltd., Shanghai, China) for 60 min and incubated with DAB kit (Gene Tech Co. Ltd.) for 10 min. The evaluation of ERK1/2, p38, and JNK expression was according to previous studies [21].

Western blotting

L4-L5 dorsal root ganglions of five groups' rat spinal cord was gained and then lysed in RIPA lysis buffer. The tissue lysates (40 μg/lane) were separated via SDS-polyacrylamide gel electrophoresis on 10% gels, and were transferred to PVDF membranes. After blocked by 5% fat-free dry milk in TBST for 1 hour, these membranes were incubated with anti-human ERK1/2 antibody (1:300; abcam, ab17942), an anti-human p38 antibody (1:2000; abcam, ab170099), an anti-human JNK antibody (1:2000; abcam, ab208035), an anti-human C-Fos antibody (1:2000, abcam, ab190289), an anti-human CREB antibody (1:2000, abcam, ab32515), an anti-human NF-kB antibody (1:500, abcam, ab19870), and an anti-human GAPDH antibody (1:3000, abcam, ab8245) overnight at 4°C. The second day, these PVDF membranes were incubated with a goat anti-rabbit IgG (Zhong Shan Jin Qiao Co. Ltd.) for one hour after immersed with TBS three times, and immunoreactive bands were visualized by an enhanced chemiluminescent reagent.

Statistical analysis

For the experimental data of rats in these five groups, SPSS 22.0 software was used for statistical analysis. Each group's data were expressed as mean ± standard deviation (x ± s) and were analyzed by

an independent sample t-test. $P < 0.05$ was thought statistically significant.

Results

The effect of Huangqi Guizhi Wuwu Decotion on body weight of oxaliplatin CINP model rats.

With the application of oxaliplatin, compared with A group rats, the velocity of bodyweight increasing of B, C, and D group rats was significantly slow from day 6 ($P < 0.05$). For D group rats, the weight increasing of rats slowed down until the 12th day. For B and C group rats, the weight increasing of rats slowed down until the 15th day. However, for E group rats, there was no significant difference in the value growing than the A group of rats ($P > 0.05$). The above results showed that Huangqi Guizhi Wuwu Decotion might prevent slow weight gain, especially in E group rats (Fig. 1).

The effect of Huangqi Guizhi Wuwu Decotion on mechanical withdrawal threshold of oxaliplatin CINP model rats.

With the application of oxaliplatin, from the 9_{th} day, group B rats' mechanical withdrawal threshold decreased gradually and lasted until the 15_{th} day. Group B rats' mechanical withdrawal threshold has significantly reduced than that in group A rats on the 9_{th} day, 12_{th} day, and 15_{th} day ($P < 0.05$).

On the 12_{th} day and 15_{th} day, compared with group B rats, group C and group D can significantly prevent the decrease of mechanical withdrawal threshold ($P < 0.05$). However, group E can more effectively control the reduction of the mechanical withdrawal threshold. There was no decrease on 3th day, 6th day, 12th day and 15th day even compared with group A rats ($P > 0.05$). Their results were shown in Fig. 2.

The effect of Huangqi Guizhi Wuwu Decotion on the cold pain threshold of oxaliplatin CINP model rats.

With oxaliplatin, group B rats' cold pain threshold decreased gradually and lasted until the 15_{th} day. The mean pain threshold of group B rats has significantly reduced than that in group A rats on the 6_{th} day, 9_{th} day, 12_{th} day, and 15_{th} day ($P < 0.05$). On the 6_{th} day, 12_{th} day, and 15_{th} day, compared with group B rats, group C, and group D can significantly prevent the decrease of cold pain threshold ($P < 0.05$). Group E can more effectively control the reduction of cold pain threshold. There was no decrease on 3_{th} day, 6_{th} day, and 15_{th} day compared with group A rats ($P > 0.05$), and group E was more effective than group C on the 12_{th} day and 15_{th} day ($P < 0.05$). Their results were shown in Fig. 3.

The effect of Huangqi Guizhi Wuwu Decotion on the thermal pain threshold of oxaliplatin CINP model rats.

With oxaliplatin, group B rats' thermal pain threshold decreased gradually and lasted until the 15_{th} day. Group B rats' thermal pain threshold has significantly reduced than that in group A rats on 3_{rd} day, 6_{th} day, 9_{th} day, 12_{th} day, and 15_{th} day ($P < 0.05$). On the 3_{rd} day and 9th day, group C can significantly prevent the decrease of thermal pain threshold ($P < 0.05$) compared with group B rats. On the 12th day,

group D can dramatically avoid the reduction of thermal pain threshold ($P < 0.05$) compared with group B rats. Group E can more effectively control the decrease in the thermal pain threshold. There was no decrease on the 9_{th} day, 12_{th} day, and 15_{th} day than group A rats ($P > 0.05$). Their results were shown in Fig. 4.

The effect of Huangqi Guizhi Wuwu Decotion on the morphology of nerve cells of L4-L5 dorsal root ganglions in oxaliplatin CINP model rats

By HE staining, we observed that the spinal cord's dorsal root ganglion cells were arranged regularly with regular cell spacing and standard nucleus size in group A rats. However, in group B rats, the cells of the dorsal root ganglion of the spinal cord were arranged disorderly. The distance between cells increased typically, the nucleus became smaller, and the suspected apoptotic cells could be seen, which was quite different from the normal nerve cells. For group C rats, the arrangement of dorsal root ganglion cells in the spinal cord was generally regular, with increased cell spacing and slightly reduced nucleus. The dorsal root ganglion of the spinal cord of group D rats was arranged regularly. The cell spacing was increased, the nucleus was decreased somewhat, and a small number of cell apoptosis were observed. There was no significant difference between group C rats. However, in group E rats, the rat spinal cord's dorsal root ganglion cells were arranged regularly with normal cell spacing and normal nuclear size. There was no significant difference compared with normal nerve cells. These results were shown in Fig. 5.

The effect of Huangqi Guizhi Wuwu Decotion on the level of TNFα, IL-1β, and IL-6 in the serum of oxaliplatin CINP model rats

Compared with group A rats, the level of TNFα was higher in group B-E rats. The difference was significant ($P < 0.05$). Compared with group B rats, the level of TNFα was lower in group C-E rats. The difference was significant ($P < 0.05$). Compared with group C rats, there was no significant difference in group D and E rats for the level of TNFα. However, compared with group D rats, the level of TNFα was lower in group E rats. The difference was significant ($P < 0.05$).

Compared with group A rats, the level of IL-1β was higher in group B-E rats. The difference was significant ($P < 0.05$). Compared with group B rats, the level of IL-1β was lower in group C-E rats. The difference was significant ($P < 0.05$). Compared with group C rats, there was no significant difference in group D and E rats for the level of IL-1β. However, compared with group D rats, the level of IL-1β was lower in group E rats. The difference was significant ($P < 0.05$).

Compared with group A rats, the level of IL-6 was higher in group B-E rats. The difference was significant ($P < 0.05$). Compared with group B rats, the level of IL-6 was lower in group C-E rats. The difference was significant ($P < 0.05$). Compared with group C rats, the level of IL-6 was higher in group D rats ($P < 0.05$), and there was no significant difference in group E rats ($P > 0.05$). However, compared with group D rats, the level of IL-6 was lower in group E rats. The difference was significant ($P < 0.05$). These results were shown in Fig. 6.

The effect of Huangqi Guizhi Wuwu Decotion on the expression of ERK1/2, p38, JNK, c-Fos, CREB and NF-kB in the L4-L5 dorsal root ganglions of oxaliplatin CINP model rats

Compared with group A rats, the expression of ERK1/2, p38, JNK, c-Fos, CREB, and NF-kB in the L4-L5 dorsal root ganglions of group B rats was significantly higher ($P < 0.05$, Fig. 7). Compared with group B, the expression of ERK1/2, p38, JNK, c-Fos, CREB, and NF-kB in the L4-L5 dorsal root ganglions of group C-E rats was also significantly lower ($P < 0.05$, Fig. 7). And group C and group E rats have the melancholiest expression of ERK1/2, p38, and c-Fos.

We also detected the expression of ERK1/2, p38, JNK, c-Fos, CREB, and NF-kB by IHC. The average optical density of ERK1/2, p38, JNK, c-Fos, CREB, and NF-kB expression was higher in group B rats than in group A rats. And the average optical density of ERK1/2, p38, JNK, c-Fos, CREB, and NF-kB expression was lower in group C-E rats than in group B rats (Fig. 8).

These results predicted that through lower the level of TNFα, IL-1β, and IL-6 in the serum of oxaliplatin CINP model rats, Huangqi Guizhi Wuwu Decotion might suppress the expression of MAPK signalings pathway family proteins such as ERK1/2, p38, and JNK to down-regulate the expression of downstream essential proteins, c-Fos, CREB and NF-kB, to combat nerve cell injury, reduce pain sensitization, and prevent and repair the harm of nerve cell in oxaliplatin CINP model rats.

Discussion

The target of oxaliplatin chronic neurotoxicity is spinal dorsal root ganglion. After entering the body, the drug first accumulated in the dorsal root ganglion of the spinal cord. Then, oxidative stress and mitochondrial damage were produced, which can result in apoptosis of nerve cells. After that, neurological dysfunction and neuropathic pain can occur [22–24]. This study found that Huangqi Guizhi Wuwu Decoction can improve the slow weight gain of oxaliplatin-induced chronic CINP model rats, effectively preventing and treating the oxaliplatin-induced regular CIPN rat model of hyperalgesia, and oppress the mechanical pain threshold, cold pain threshold, and heat pain threshold decreased. And the effect of Huangqi Guizhi Wuwu Decoction external immersing was better than that of Huangqi Guizhi Wuwu Decoction gavage. Furthermore, Huangqi Guizhi Wuwu Decoction can effectively antagonize the effect of oxaliplatin on the injury of spinal dorsal root ganglion cells in rats.

For the mechanism analysis, previous studies have found that TNFα, IL-1β, and IL-6 took part in the shape and maintain of neuropathic pain and can decrease neurons' excitability by MAPK/p38 pathway to alleviate mechanical hyperalgesia in rats [25–27]. MAPK/NF-κB was a critical signaling pathway involved in regulating the inflammation-related neural pathogenesis [28–29]. Li Tao and his colleagues also found that three members of MAPKs, ERK, JNK, and p38, were involved in the inflammation responses after acute brain injury [30]. Besides, NF-κB activation can contribute to pro-inflammation cytokines, and these cytokines can, in turn, activate NF-κB [31]. The positive feedback was believed to serve to amplify inflammatory signals. As for the relationships between MPAK and NF-kB, MAPK was a classical pathway that can initiate NF-kB activation [32]. And the activation of MAPK-NF-kB also promotes TNFα, IL-1β, and

IL-6 [33]. This also formed positive feedback. These findings predicted that TNFα/IL-1β/IL-6/MAPK/NF-kB played a vital role in the process of shaping and maintenance of neuropathic pain. In this study, we found that Huangqi Guizhi Wuwu Decoction can down-regulate the levels of TNF-α, IL-1β, IL-6 inflammatory factors in the serum of chronic CINP rats induced by oxaliplatin, then suppressed the expression of MAPK pathway related-proteins ERK1/2, p38 and JNK. After that, the expression of downstream essential proteins c-Fos, CREB, and Nf-κB also decreased. As a result, it combated nerve cell injury, reduced pain sensitization, and prevented and repaired nerve cell injury in oxaliplatin CINP model rats.

This study also has some limitations. We only analyzed the change of MAPK signal pathway in the treatment process of Huangqi Guizhi Wuwu Decoction on CINP. There must be other essential pathways that can regulate this process. Therefore, we will explore different critical paths by the high throughput sequencing method in the future and validate using our rats' model. We hope our research results can contribute to the development and application of modern Chinese medicine.

Conclusions

In conclusion, we found that Huangqi Guizhi Wuwu Decoction can combat nerve cell injury, reduce pain sensitization, and prevent and repair the damage of nerve cells in oxaliplatin CINP model rats by TNFα/IL-1β/IL-6/MAPK/NF-kB pathway. These findings predicted that Huangqi Guizhi Wuwu Decoction may effectively treat oxaliplatin CINP in clinical practice.

Abbreviations

CINP: Chemotherapy-induced neuropathic pain; IHC: Immunohistochemistry;

Declarations

Ethics approval and consent to participate

This protocol was approved by Institutional Review Board of Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute

Consent for publication

Not applicable

Availability of data and materials

The data and materials can be available from the corresponding author for reasonable reasons.

Competing Interests

The authors declare that they have no competing interests

Funding

This research was granted by the Natural Science Foundation of Liaoning Province Project (2020-ZLLH-50)

Authors' contributions

Mingzhu Li carried out the experiments and drafted the manuscript. Mingzhu Li, Jian Xu, Zheng Li, Xiande Ma, Shengbo Jin, Yang Cao, Xuebing Wang, Jian Zhao, Jianbo Wang and Jian Xu designed the study. Zheng Li performed the statistical analysis. Jian Xu conceived the study, and helped draft the manuscript.

Acknowledgements

Not applicable

References

[1] Zou XN, Zhao Ping. Analysis of cancer situation in China in the past 70 years. Chinese Journal of clinical oncology and rehabilitation, 2019;10:1153-61.

[2] Zheng WH, Yang HW. Research progress of chemotherapy-induced neuropathic pain. Cancer prevention and treatment research, 2010;9:1081-3.

[3] Fallon MT. Neuropathic pain in cancer. Br J Anaesth. 2013; 1:105-11.

[4] Pignata S, De Placido S, Biamonte R, Scambia G, Di Vagno G, Colucci G, et al. Residual neurotoxicity in ovarian cancer patients in clinical remission after first-line chemotherapy with carboplatin and paclitaxel: the Multicenter Italian Trial in Ovarian cancer (MITO-4) retrospective study. BMC Cancer. 2006;6:5.

[5] Kerckhove N, Collin A, Condé S, Chaleteix C, Pezet D, Balayssac D. Long-Term Effects, Pathophysiological Mechanisms, and Risk Factors of Chemotherapy-Induced Peripheral Neuropathies: A Comprehensive Literature Review. Front Pharmacol. 2017;8:86.

[6] Flatters SJL, Dougherty PM, Colvin LA. Clinical and preclinical perspectives on Chemotherapy-Induced Peripheral Neuropathy (CIPN): a narrative review. Br J Anaesth. 2017;4:737-49.

[7] Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol. 2004;22:23-30.

[8] Sakurai M, Egashira N, Kawashiri T, Yano T, Ikesue H, Oishi R. Oxaliplatin-induced neuropathy in the rat: involvement of oxalate in cold hyperalgesia but not mechanical allodynia. Pain. 2009;1:165-74.

[9] Cao Peng, Li Songlin, Huo Jiege. Treatment of oxaliplatin related peripheral neuropathy by blood arthralgia. Chinese Journal of basic medicine of traditional Chinese medicine, 2013;4: 430-1.

[10] Cao Peng, Huo JG. Treatment of chemotherapy-related peripheral neuropathy from blood arthralgia. Guangming traditional Chinese medicine, 2013;1:12-3, 15.

[11] Jiang Mei, Chen Lu, Ke XB. Discussion on the thinking of differentiation and treatment of oxaliplatin in neurologic differentiation of blood arthralgia theory. Chinese Journal of traditional Chinese medicine, 2012;9: 2135-7.

[12] Tian Jun, Yao XQ, Wu XY, Chen Che, Zhou Liang, Xu Zhe, et al. Systematic review and meta-analysis of Huangqi Guizhi Wuwu Decoction in the prevention and treatment of oxaliplatin peripheral nerve toxicity. Chinese Journal of experimental prescription, 2013;22:325-30.

[13] Chen Shanshan. Systematic review and meta-analysis of Huangqi Guizhi Wuwu Decoction in the prevention and treatment of oxaliplatin induced peripheral neurotoxicity [D]. Nanjing University of traditional Chinese medicine, 2018.

[14] Ma Jun. a multicenter, randomized, double-blind, controlled clinical study on the prevention and treatment of Oxaliplatin Neurotoxicity by modified Huangqi Guizhi Wuwu formula granules [D]. Nanjing University of traditional Chinese medicine, 2019.

[15] Huang ZB, Huang ZM, Chen GQ, Tao Yu, Huang ZY, Jin HL, et al. Clinical study on the prevention and treatment of oxaliplatin peripheral neurotoxicity with Huangqi Guizhi Wuwu Decoction. Shanghai Journal of traditional Chinese medicine, 2010;5:40-2.

[16] Nishimoto S, Kusakabe M, Nishida E. Requirement of the MEK5-ERK5 pathway for neural differentiation in Xenopus embryonic development. EMBO Rep. 2005; 6:1064-9.

[17] Yamamoto K, Chiba N, Chiba T, Kambe T, Abe K, Kawakami K, et al. Transient receptor potential ankyrin 1 that is induced in dorsal root ganglion neurons contributes to acute cold hypersensitivity after oxaliplatin administration. Mol Pain. 2015; 11:69.

[18] Huo JG, Hu Ying, Yang Jie, Cai XT, Hu CP, Wang XN, et al. Effect of Huangqi Guizhi Wuwu Decoction on peripheral nerve injury induced by chemotherapy. Journal of traditional Chinese medicine, 2012;23:2031-4.

[19] Xiao WH, Zheng H, Bennett GJ. Characterization of oxaliplatin-induced chronic painful peripheral neuropathy in the rat and comparison with the neuropathy induced by paclitaxel. Neuroscience. 2012; 203:194–206.

[20] Ling B, Authier N, Balayssac D, Eschalier A, Coudore F. Behavioral and pharmacological description of oxaliplatin induced painful neuropathy in rat. Pain, 2007; 128:225–34.

[21] Ashlin TG, Buckley ML, Salter RC, Johnson JL, Kwan AP, Ramji DP. The anti-atherogenic cytokine interleukin-33 inhibits the expression of a disintegrin and metalloproteinase with thrombospondin motifs-1, -4 and -5 in human macrophages: Requirement of extracellular signal-regulated kinase, c-Jun N-terminal kinase and phosphoinositide 3-kinase signaling pathways. Int J Biochem Cell Biol. 2014;100:113-23.

[22] Livni L, Lees JG, Barkl-Luke ME, Goldstein D, Moalem-Taylor G. Dorsal root ganglion explants derived from chemotherapy-treated mice have reduced neurite outgrowth in culture. Neurosci Lett. 2019;694:14-9.

[23] Kanat O, Ertas H, Caner B. Platinum-induced neurotoxicity: A review of possible mechanisms. World J Clin Oncol. 2017;4:329-35.

[24] Screnci D, McKeage MJ, Galettis P, Hambley TW, Palmer BD, Baguley BC. Relationships between hydrophobicity, reactivity, accumulation and peripheral nerve toxicity of a series of platinum drugs. Br J Cancer. 2000;4:966-72.

[25] Ramesh G, MacLean AG, Philipp MT. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. Mediators Inflamm. 2013; 2013:480739.

[26] Liang F, Liu M, Fu X, Zhou X, Chen P, Han F. Dexmedetomidine attenuates neuropathic pain in chronic constriction injury by suppressing NR2B, NF-κB, and iNOS activation. Saudi Pharm J. 2017;4:649-54.

[27] Huh Y, Ji RR, Chen G. Neuroinflammation, Bone Marrow Stem Cells, and Chronic Pain. Front Immunol. 2017; 8:1014.

[28] Chen X, Wu S, Chen C, Xie B, Fang Z, Hu W, et al. Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF-κB pathway following experimental traumatic brain injury. J Neuroinflammation. 2017;1:143.

[29] Chen T, Cao L, Dong W, Luo P, Liu W, Qu Y, et al. Protective effects of mGluR5 positive modulators against traumatic neuronal injury through PKC-dependent activation of MEK/ERK pathway. Neurochem Res. 2012;5:983-90.

[30] Tao L, Li D, Liu H, Jiang F, Xu Y, Cao Y, et al. Neuroprotective effects of metformin on traumatic brain injury in rats associated with NF-κB and MAPK signaling pathway. Brain Res Bull. 2018;140:154-161.

[31] Neurath MF, Pettersson S, Meyer zum Büschenfelde KH, Strober W. Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice. Nat Med. 1996;9:998-1004.

[32] Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A. Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. Int J Endocrinol. 2014;2014:674987.

[33] Ma J, Shi M, Zhang X, Liu X, Chen J, Zhang R, et al. GLP-1R agonists ameliorate peripheral nerve dysfunction and inflammation via p38 MAPK/NF-κB signaling pathways in streptozotocin-induced diabetic rats. Int J Mol Med. 2018;5:2977-85.

Figures

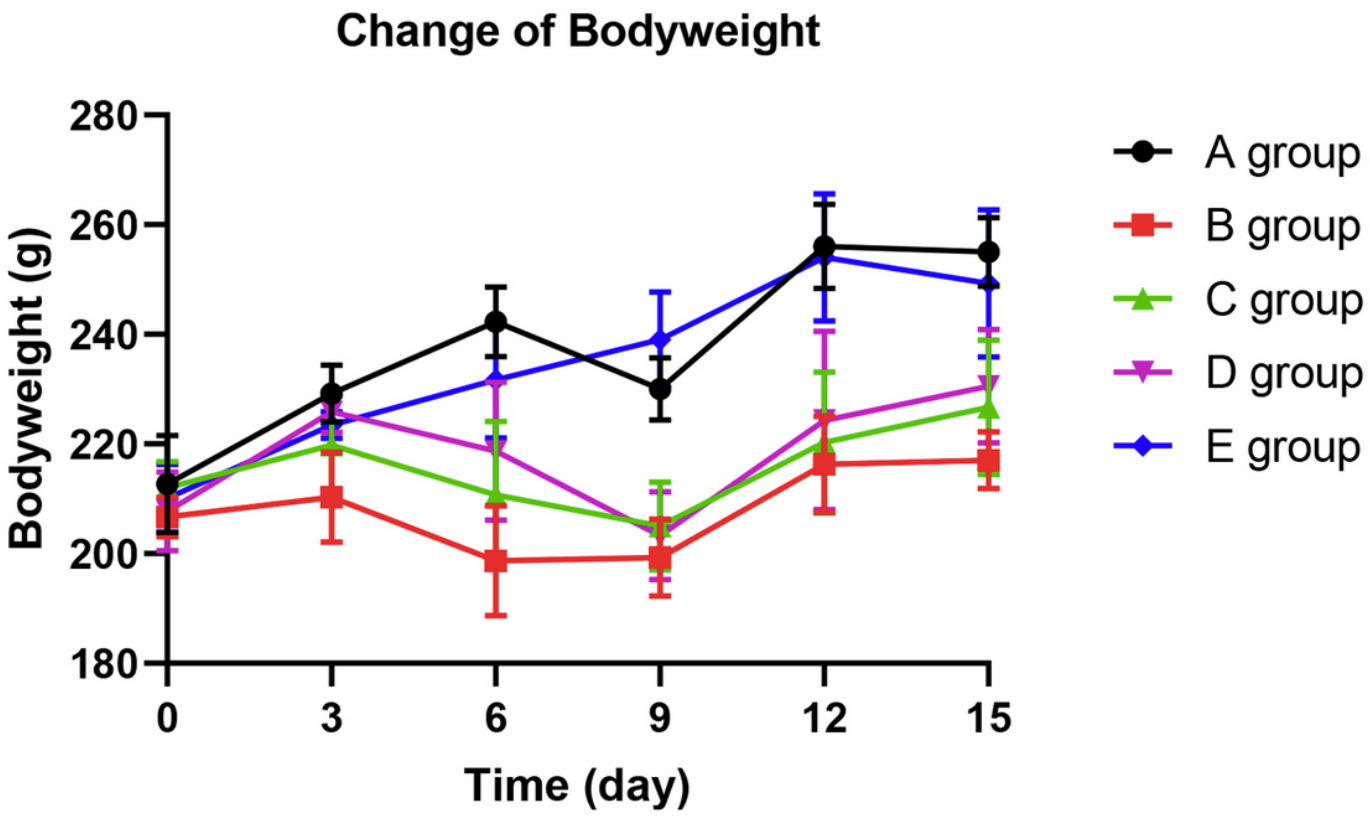

Figure 1

The effect of Huangqi Guizhi Wuwu Decotion on body weight of different rat group.

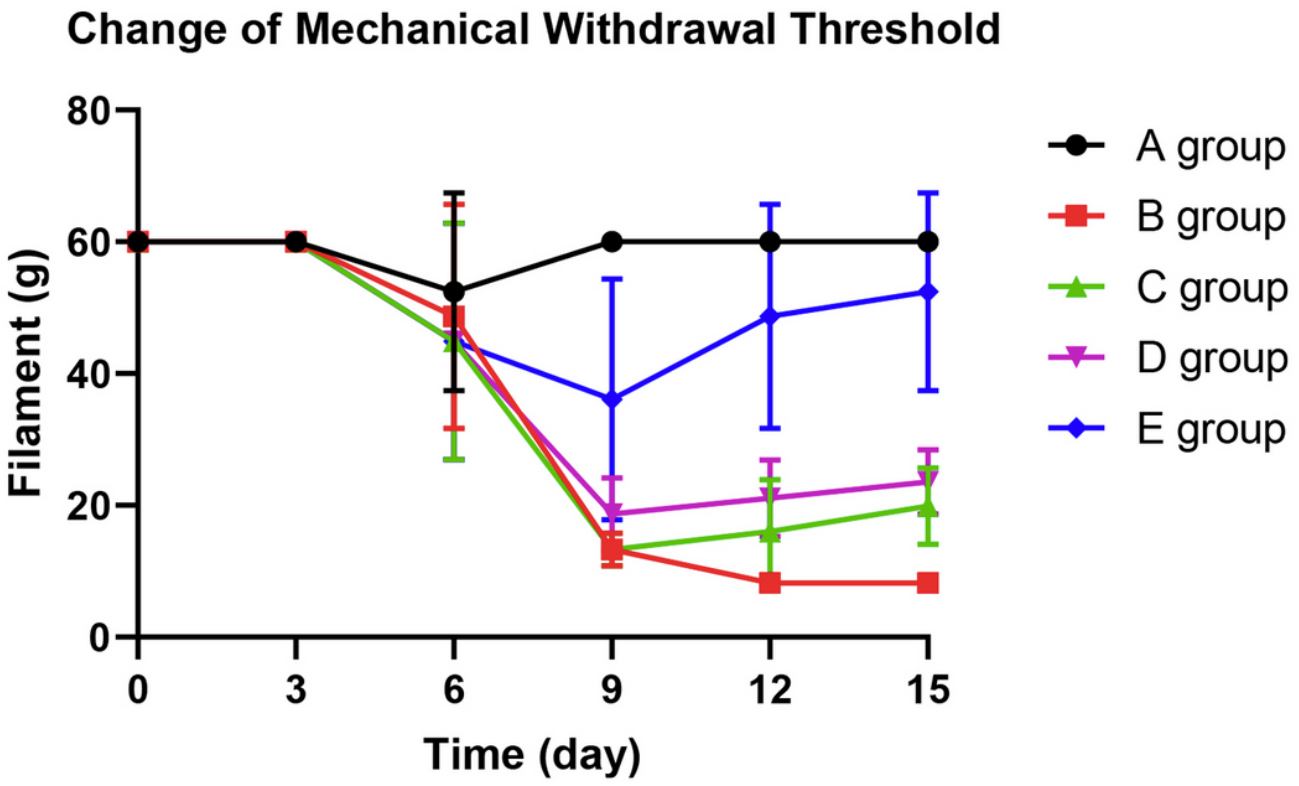

Figure 2

The effect of Huangqi Guizhi Wuwu Decotion on mechanical withdrawal threshold of different rat group.

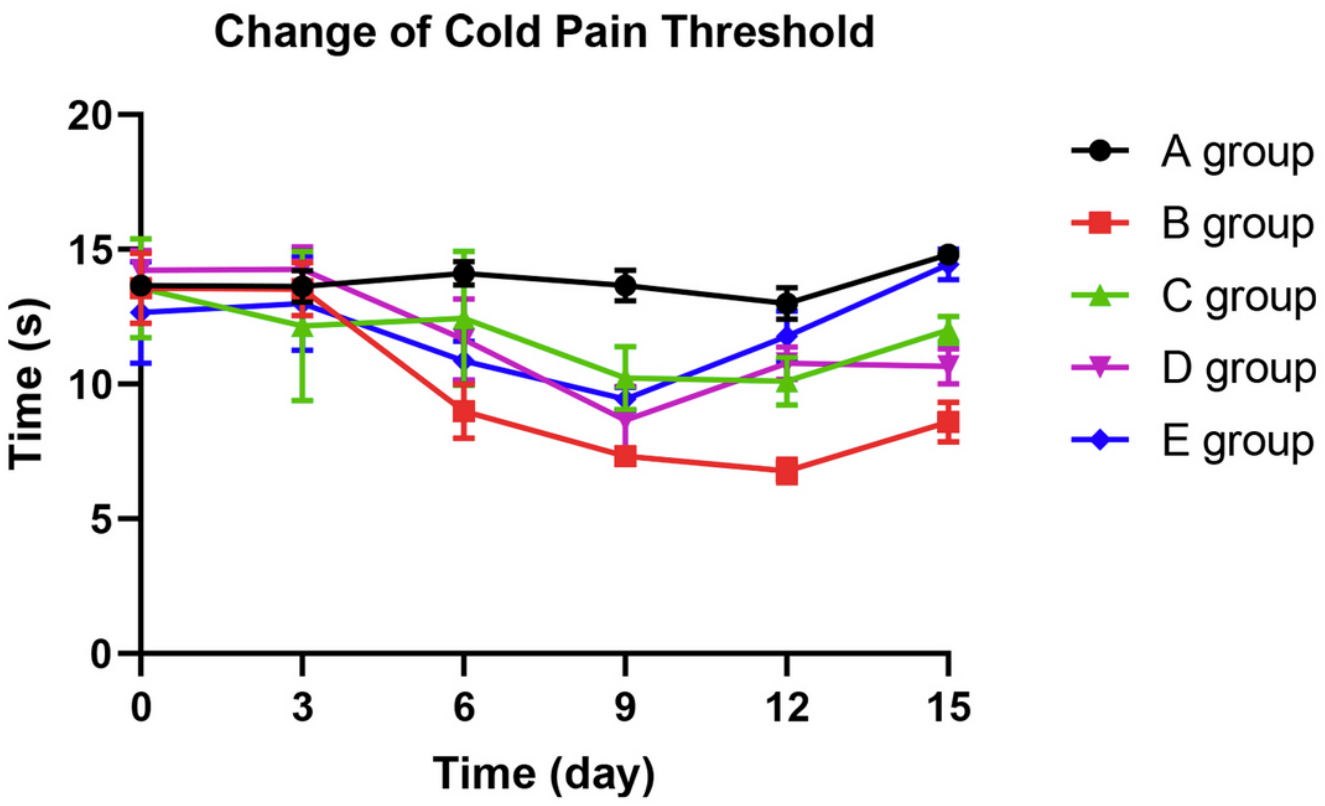

Figure 3

The effect of Huangqi Guizhi Wuwu Decotion on cold pain threshold of different rat group.

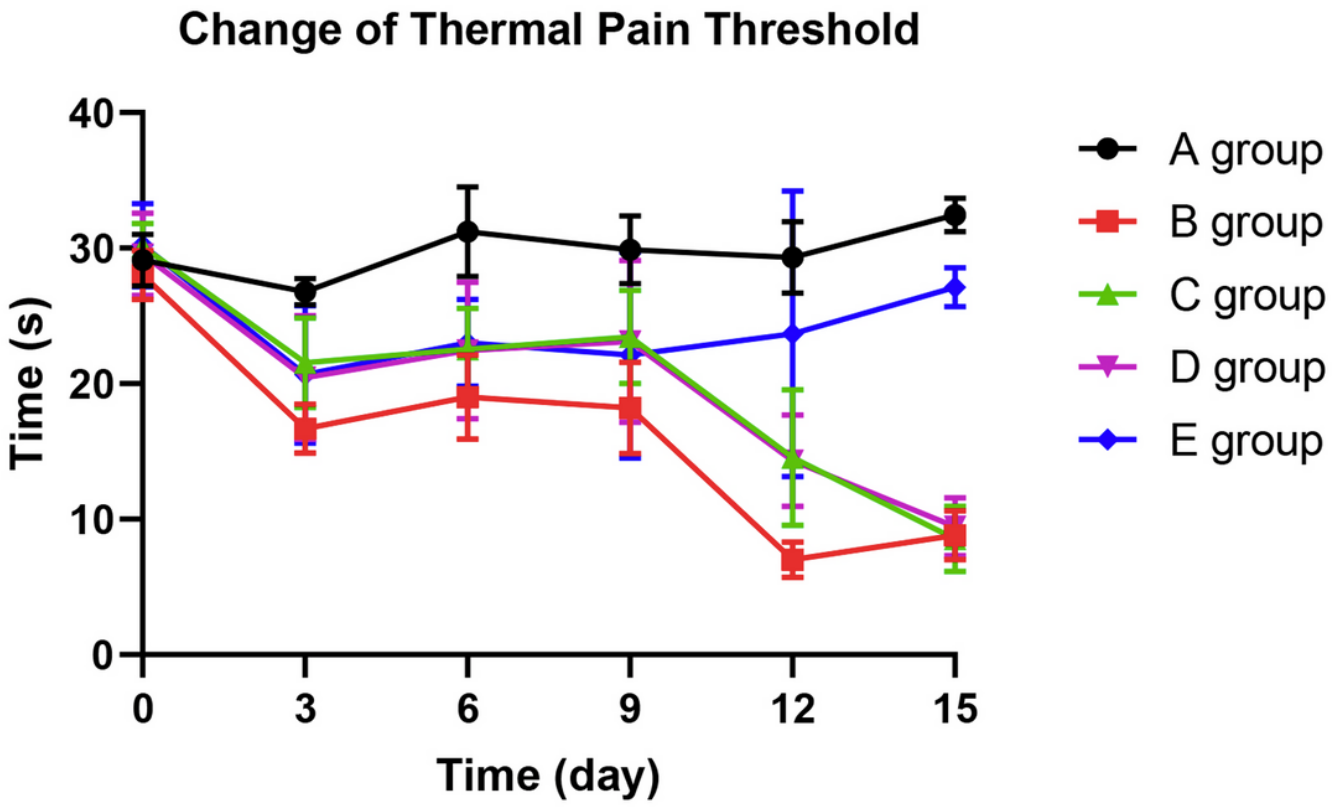

Figure 4

The effect of Huangqi Guizhi Wuwu Decotion on thermal pain threshold of different rat group.

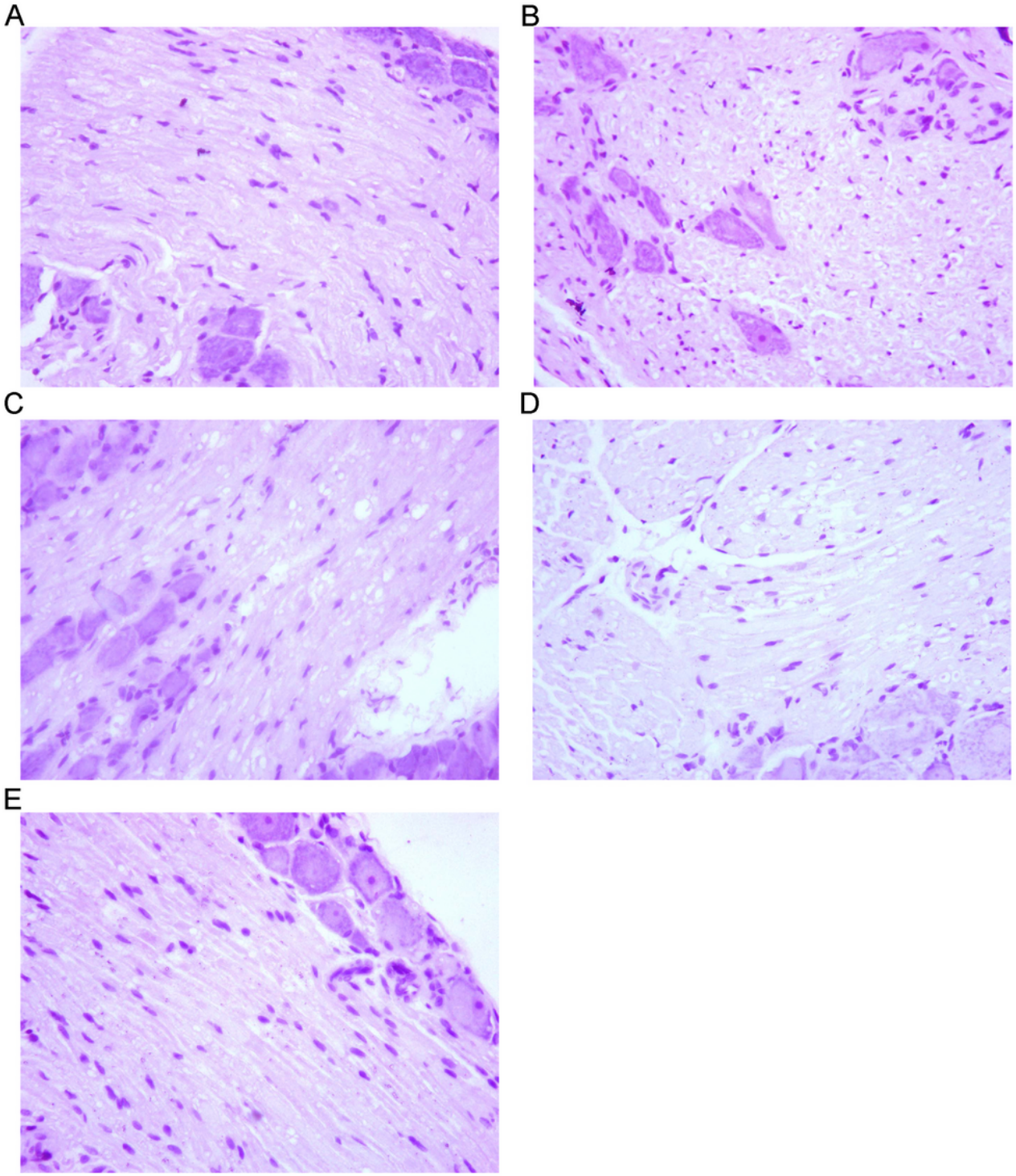

Figure 5

The effect of Huangqi Guizhi Wuwu Decotion on the morphology of nerve cells of L4-L5 dorsal root ganglions of different rat group. A-E: A group- E group.

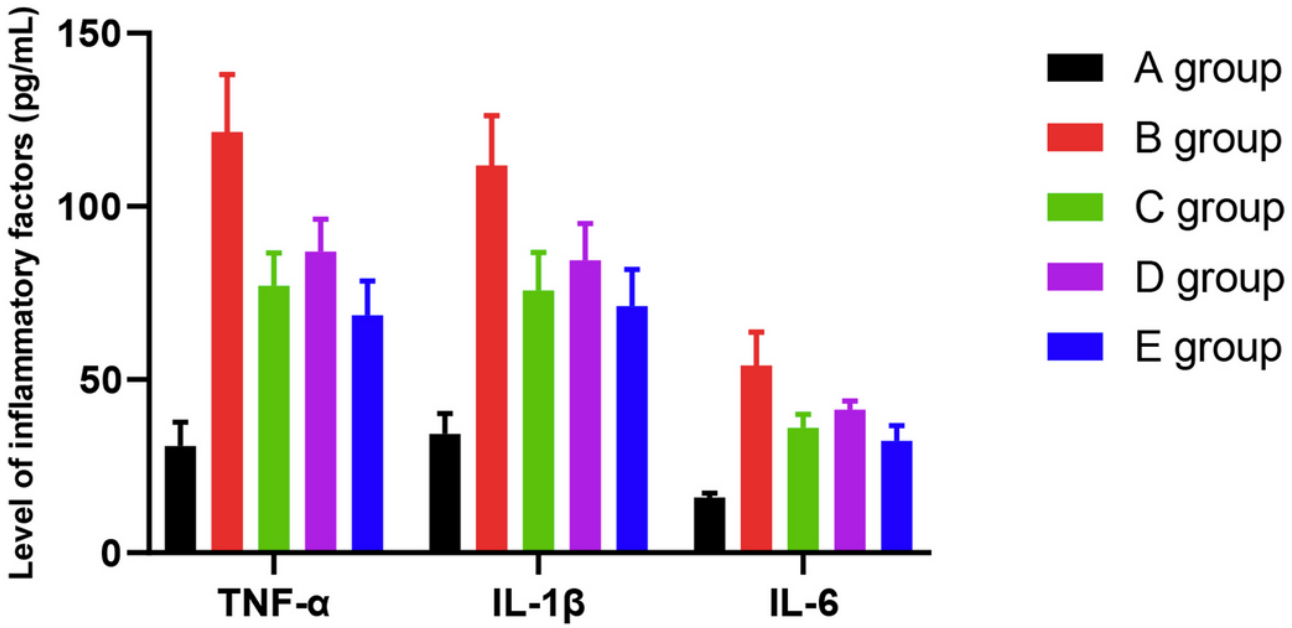

Figure 6

The effect of Huangqi Guizhi Wuwu Decotion on the level of TNFα, IL-1β, and IL-6 in the serum of different rat group.

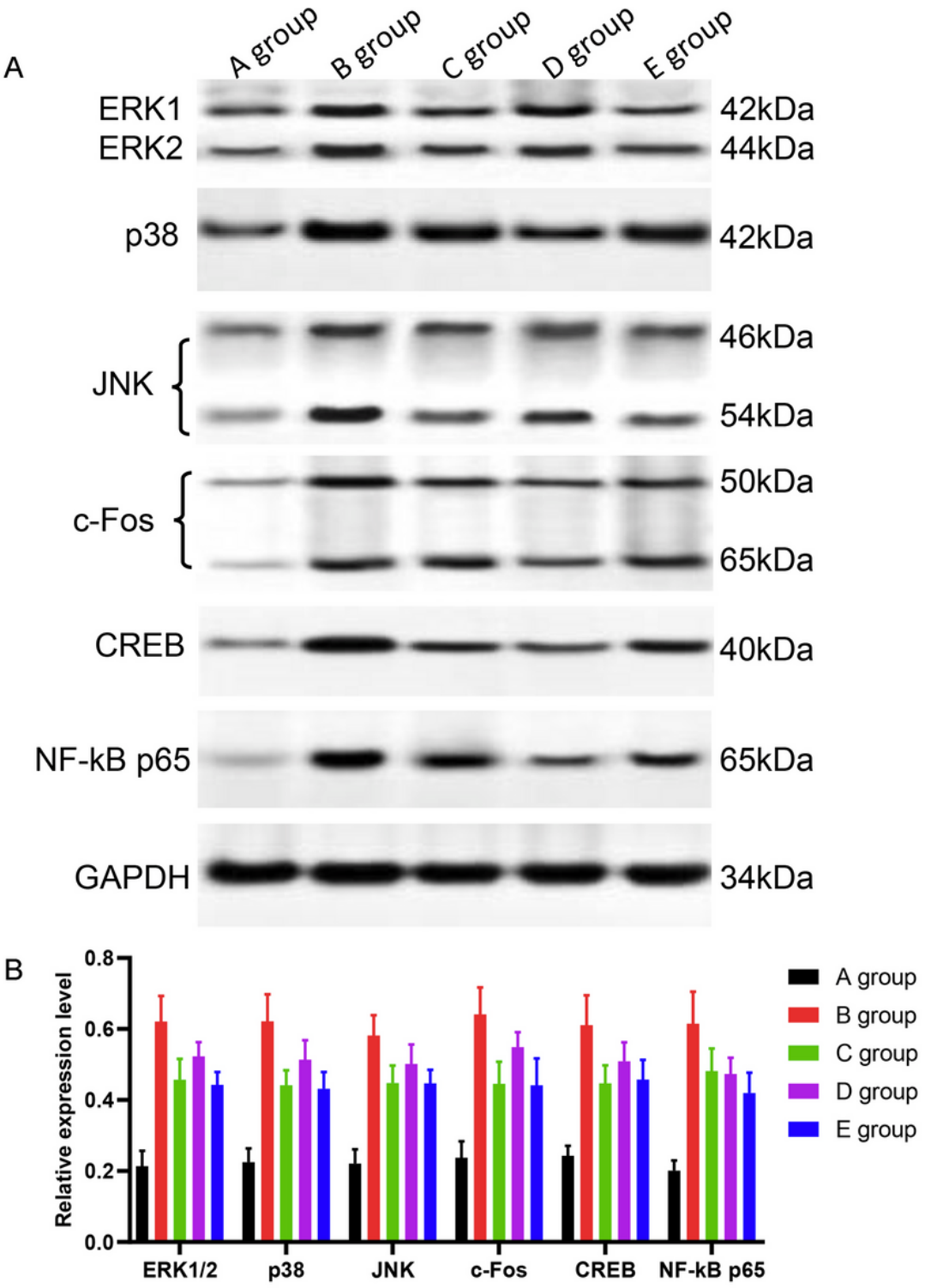

Figure 7

The effect of Huangqi Guizhi Wuwu Decotion on the expression of ERK1/2, p38, JNK, c-Fos, CREB and NF-kB in the L4-L5 dorsal root ganglions of different rat group detected by western blotting. A: The expression of ERK1/2, p38, JNK, c-Fos, CREB, NF-kB and GAPDH in different rat group. B: The relative expression level of ERK1/2, p38, JNK, c-Fos, CREB, and NF-kB compared to GAPDH in different rat group.

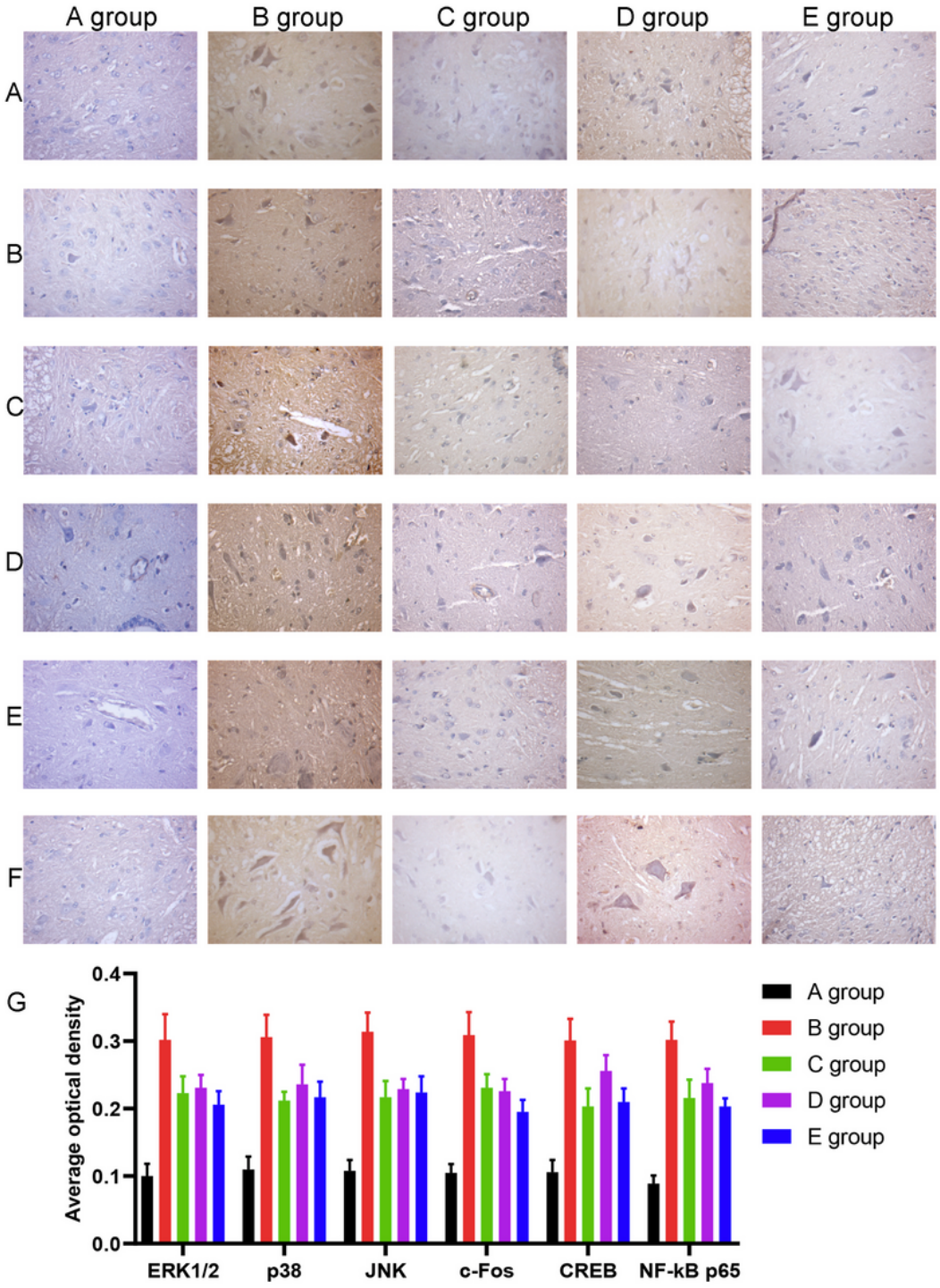

Figure 8

The effect of Huangqi Guizhi Wuwu Decotion on the expression of ERK1/2, p38, JNK, c-Fos, CREB and NF-kB in the L4-L5 dorsal root ganglions of different rat group detected by IHC. A-F: The typical pictures of ERK1/2, p38, JNK, c-Fos, CREB and NF-kB expression in the L4-L5 dorsal root ganglions of different rat group detected by IHC. G: The average optical density of ERK1/2, p38, JNK, c-Fos, CREB, and NF-kB expression in the L4-L5 dorsal root ganglions of different rat group.