



Research Article

Analgesic effects of Xing-Qi-Tong-Qiao Decoction (XQTQ) on paclitaxel-induced peripheral neuropathic pain (PIPNP) in rats: a metabolomic pathway analysis

Xiao-Qing Ma^{1,2}, Bao-Hong Wei^{1,2}, Shu-Man Hu¹, Wen-Zhe Yang^{1,2}, Le-Jia Hong¹, Lin Ju¹, Guan-Hua Du^{1,2} (✉), Xue Yang^{1,2} (✉)

¹ Marine Biomedical Research Institute of Qingdao, Qingdao 266071, China

² School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China

Received: 11 February 2025 / Revised: 11 March 2025 / Accepted: 14 March 2025

Abstract: Xing-Qi-Tong-Qiao Decoction (XQTQ) is a traditional Chinese prescription that embodies the homology of medicine and food. It is recognized for its effects in alleviating stasis, dispersing phlegm and nodules, activating blood circulation, relieving pain. However, its efficacy and mechanisms in alleviating paclitaxel-induced peripheral neuropathic pain (PIPNP) remain unclear. In this study, we investigate the analgesic effects of XQTQ and explore the potential mechanism using plasma metabolomics. The characteristic chromatogram of XQTQ was established using HPLC. A total of 36 SD rats were divided into 6 groups: control, model, positive drug, and three XQTQ combined groups. All rats, except those in control group, were induced with diseases features via intraperitoneal administration of paclitaxel. The pregabalin and XQTQ combined groups were administered orally once a day for 14 days. The paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) were measured. Rat plasma was collected for the detection of pain cytokines and the analysis of endogenous metabolite. Rats in the XQTQ combined groups exhibited a significant increase in PWT and PWL values, alongside a decrease in the levels of tumor necrosis factor- α (TNF- α , 14.82%), interleukin-6 (IL-6, 13.69%), interleukin-1 beta (IL-1 β , 19.34%), nerve growth factor (NGF, 11.40%), prostaglandin E2 (PGE 2, 12.74%), and 5-hydroxytryptamine (5-HT, 13.51%) compared to the model group ($P < 0.01$). Plasma metabolomics revealed 12 potential biomarkers associated with the model rats, which were significantly reversed by XQTQ. The main pathways involved included glycerophospholipid metabolism, linoleic acid (LA) metabolism, and alpha-linolenic acid (ALA) metabolism. XQTQ demonstrated significant analgesic effects and improved metabolism in the context of PIPNP. This research provides a reliable theoretical basis and novel research directions for clinical applications.

Keywords: paclitaxel-induced peripheral neuropathic pain (PIPNP); metabolic mechanism; pain cytokines; Xing-Qi-Tong-Qiao Decoction; UHPLC-MS/MS

Citation: Ma X. Q., Wei B. H., Hu S. M., et al. Analgesic effects of Xing-Qi-Tong-Qiao Decoction (XQTQ) on paclitaxel-induced peripheral neuropathic pain (PIPNP) in rats: a metabolomic pathway analysis. *Food & Medicine Homology*, 2027, 4: 9420147. <https://doi.org/10.26599/FMH.2027.9420147>

1 Introduction

Globally, the cancer burden is projected to reach 28.4 million cases by 2040, representing a 47% increase from 2020^[1]. It is estimated that 70% of cancer patients experience severe pain at some point during the course of their disease^[2]. Paclitaxel (PTX), which is widely used in clinic settings, is a first-line drug for the chemotherapy of digestive system tumor, lung cancer, and head and neck tumors. However, the use of PTX can lead to side effects, including pain, known as paclitaxel-induced peripheral neuropathic pain (PIPNP). The incidence of PIPNP is as high as 87%^[3,4]. Symptoms of sensory neuropathy typically present as paresthesia, numbness, and pain, significantly impacting the patient's quality of life. Currently, there are no effective therapeutic

drugs or health supplements in modern medicine for alleviating cancer-related pain, aside from opioids, which carry a risk of addiction. The three-step pain relief plan recommended by the World Health Organization is still in use; however, the effects of treatment are often unsatisfactory. Therefore, there is an urgent need to identify a safe and effective drug for the treatment of PIPNP.

The integration of traditional Chinese medicine (TCM) and Western medicine has been proposed as a promising therapeutic strategy for enhancing anti-neoplastic effects and alleviating associated side effects^[5,6]. Food and medicine homology is a significant aspect of TCM, possessing both health preventive and therapeutic effects. Its origins and historical development can be traced back to ancient texts such as the “*Huangdi Neijing*” and the

"*Dietetic Materia Medica*"^[7]. In contemporary society, as individuals seek to adopt healthier lifestyles, the concept of substances that are both medicinal and edible is receiving growing attention. Xing-Qi-Tong-Qiao Decoction (XQTQ) is a traditional Chinese prescription formulated by physicians based on the homology of medicine and food. The XQTQ formula incorporates Ginseng Radix et Rhizoma and Rosae Rugosae Flos as novel foods, while the other TCM ingredients are sourced from the catalog of medicinal and edible substances established by the National Health Commission of China. It is known for its effects in removing stasis, dispersing phlegm and nodules, activating blood circulation, relieving pain, soothing the liver and tranquilizing the mind. By integrating principles of modern formulation, it can be concluded that Eckloniae Thallus serves as the monarch herb, possessing effects that dissipate phlegm and nodules, diuretic properties, and swelling reduction. Rosae Rugosae Flos, Perillae Folium, Allii Macrostemonis Bulbus and Crataegifructus function as ministerial drugs that help ease the liver, relieve depression, activate Yang, dissipate nodules, and relieve pain. Additionally, Ostreae Concha, Poria, and Coicis Semen contribute to calming the heart and mind, as well as promoting water regulation and osmotic balance. The combination of these various herbs achieves effects such as activating blood circulation, dispersing nodules, relieving pain and tranquilizing the mind.

Metabolomics encompasses a set of powerful tools for detecting, identifying, and quantifying the endogenous metabolites involved in metabolic processes, thereby facilitating the interpretation of biological changes in the internal environment^[8]. It has become increasingly prevalent focused on exploring the mechanisms of diseases or treatments, as it offers comprehensive metabolic information about organisms^[9-11].

In our study, we utilized a PTX-induced rat model to evaluate the analgesic effects of XQTQ on behavioral tests and plasma cytokine levels. Additionally, we aimed to elucidate the mechanisms by which XQTQ alleviates PIPNP through the identification of potential endogenous metabolite biomarkers and the prediction of relevant pathways using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Consequently, this study provides a reliable theoretical basis for the clinical application of XQTQ.

2 Materials and methods

2.1 Medicinal materials

The medicinal slices, Ginseng Radix et Rhizom (*Panax ginseng* C.A.Mey.), Jujubae Fructus (*Ziziphus jujuba* Mill.), Eckloniae Thallus (*Ecklonia kurome* Okam.), Ostreae Concha (*Ostrea gigas* Thunberg), Poria (*Poria cocos* (Schw.)), Zingiberis Rhizome (*Zingiber officinale* Rosc.), Coicis Semen (*Coix lacryma-jobi* L. var. *mayuen* (Roman.) Stapf), Crataegifructus (*Crataegus pinnatifida* Bge.), Dioscoreae Rhizoma (*Dioscorea opposita* Thunb.), Chaenomelis Fructus (*Chaenomeles speciosa* (Sweet) Nakai), Cinnamomi Cortex (*Cinnamomum cassia* Presl), Cannabis Fructus (*Cannabis sativa* L.), Allii Macrostemonis Bulbus (*Allium chinense* G. Don), Perillae Folium (*Perilla frutescent* (L.) Britt), Citri Fructus (*Citrus medica* L.), Rosae Rugosae Flos (*Rosa rugosa* Thunb.). All medicinal slices were purchased in Bozhou City, China. Voucher specimens were deposited at the laboratory of the marine TCM platform, Marine Biomedical Research Institute of Qingdao (Qingdao, China).

2.2 Reagents

Guanosine (L/N: 111977-202202), protocatechuic acid (L/N: 110809-202207), ellagic acid (L/N: 111959-201903), scutellarin (L/N: 110842-202010), rosmarinic acid (L/N: 111871-202408), 6-gingerol (L/N: 111833-202007) were purchased from China Institute for Food and Drug Control. PTX (2 mg/kg, Jiangsu Hengrui Pharmaceutical Co., Ltd., Jiangsu, China), and pregabalin were purchased from Pfizer Manufacturing Deutschland GmbH (Freiburg, USA). Rat ELISA kit tumor necrosis factor α (TNF- α , L/N: 23100139H), interleukin 6 (IL-6, L/N: 23100141H), interleukin 1 β (IL-1 β , L/N: 23100172H), nerve growth factor (NGF, L/N: 23100169H), prostaglandin E2 (PGE 2, L/N: 23100152H), 5-hydroxytryptamine (5-HT, L/N: 23100102H). All these kits were purchased from FANKEW (Shanghai, China).

2.3 Preparation of XQTQ

The preparation of XQTQ was decocted following the traditional method. In brief, mixed medicinal slices (Ginseng Radix et Rhizom, Jujubae Fructus, Eckloniae Thallus, Ostreae Concha, Poria, Zingiberis rhizoma, Coicis Semen, Crataegifructus, Dioscoreae Rhizoma, Chaenomelis Fructus, Cinnamomi Cortex, Cannabis Fructus, Allii Macrostemonis Bulbus, Perillae Folium, Citri Fructus, Rosae Rugosae Flos, 3:6:6:30:6:3:6:3:6:9:1:6:5:5:5:3) were soaked in 10 times its weight of water for 20 min, then decocted at 100 °C for 1h and repeated once. The combined water extracts were immediately centrifuged at 4,000 r/min for 10 min and then concentrated followed by freeze-drying to obtain a powder for use. The extracted dried powder (1 g) was equivalent to 9.68 g of material herbs.

2.4 HPLC analysis

The XQTQ extract (2.12 g) was dissolved in 10 mL of Milli-Q water and then extracted three times with 10 mL of water saturated *n*-butanol on each occasion. The combined *n*-butanol extracts were collected and evaporated to dryness. The residue was dissolved in 5 mL of ethanol. Standard stock solutions of guanosine, protocatechuic acid, ellagic acid, scutellarin, rosmarinic acid, 6-gingerol (each approximately 1 mg/mL) were separately prepared in 70% methanol and stored at 4 °C. A mixed reference standard solution was prepared by combining and diluting the 6 standard stock solutions with 70% methanol to achieve a concentration of approximately 0.10 mg/mL for each compound. The solution was filtered through a 0.45 μ m PTFE filter prior to HPLC analysis. All samples were similarly filtered through a 0.45 μ m PTFE filter before HPLC analysis.

The profile of XQTQ was analyzed using a Thermo Fisher Ultimate 3000 RSLC instrument equipped with a diode array detector. The chromatography conditions were as follows: ZORBAX SB-C₁₈ column (250 mm $\times$ 4.6 mm, 5 μ m) maintained at 30 °C. A gradient elution using solvent A (Milli-Q water contains 0.1% phosphoric acid) and solvent B (acetonitrile) was applied as follows: 0–40 min, 2%–14% B; 40–60 min, 14%–19% B; 60–90 min, 19%–25% B; 90–100 min, 25%–30% B; 100–110 min, 30%–36% B; 110–140 min, 36%–37% B. The flow rate was 1 mL/min, the injection volume was 10 μ L and the detector wavelengths were 280 and 210 nm.

2.5 Model establishment and grouping

Male Sprague-Dawley rats weighing between 160–200 g were purchased from Ji'nan Pengyue experimental animal breeding Co., Ltd. (Ji'nan, Shangdong, China). All rats were housed under

automatically controlled environmental conditions, maintaining a 12 h light-dark cycle with free access to food and water. After 1 week of acclimatization, the rats were randomly divided into 6 groups according to body weight: control group (Control, $n = 6$), model group (Model, $n = 6$), pregabalin group (30 mg/kg, $n = 6$), low dose of XQTQ group (0.53 g/kg, $n = 6$), medium dose of XQTQ group (1.06 g/kg, $n = 6$), and high dose of XQTQ group (2.12 g/kg, $n = 6$).

Animals in the model, pregabalin, and XQTQ combined groups were intraperitoneally (i.p.) injected with PTX (2 mg/kg) on days 1, 3, 5, and 7. Starting from day 1, animals in the pregabalin and XQTQ combined groups were administered oral pregabalin at a dosage of 30 mg/kg once daily for 14 days, while the XQTQ combined groups were administered orally XQTQ, simultaneously. The control and model groups received an equivalent volume of saline or water. Animal procedures were approved by the Institutional Animal Care and Use Committee of the Yantai University (YTU23WT-P11).

2.6 Behavioral test

Paw withdrawal threshold (PWT) was measured before, during, and after PTX treatment (on days 1, 3, 5, 7, and 14) by an experimenter blinded to treatment groups. Rats were placed in elevated Plexiglas chambers upon a wire mesh floor and allowed to acclimate for 20 min before measuring the PWT. PWT was tested using calibrated von Frey filaments according to the “up and down” method^[12]. First, an intermediate filament (2.0 g) was applied perpendicularly to the toes of the hind-paw surface. If a positive response was observed, the next weaker filament (1.4 g) was used. Conversely, if a negative response was elicited, the next stronger filament (4.0 g) was employed. If a positive response occurs at 2.0 g for the first time, it is recorded as “X”, and a total of 6 trials are counted from 2.0 g. If a negative response occurs at 2.0 g for the first time, it is recorded as “O”, and a total of 5 times are counted.

Paw withdrawal latency (PWL) to noxious heat stimuli was measured using an the IITC Hot Plate Analgesia test before, during, and after PTX treatment (on days 2, 4, 6, 8, and 15). The rat was placed in a Plexiglas chamber on a glass plate above a light box. A radiant heat stimulus was applied by directing a beam of light through a hole in the light box onto the heel of each hind paw via the glass plate. The light beam was turned off when the rat lifted its foot, allowing for the measurement of PWL, defined as the time between when the light beam hit the foot and when the foot was lifted. Each trial was conducted 5 times at 5 min intervals with a cut-off time of 20 s imposed to prevent tissue injury.

2.7 Sample collection and preparation

After fasting for 12 h at the end of the experiment, the blood was collected in the anticoagulant tube through the rat abdominal aorta and centrifuged at 3,000 r/min for 15 min at 4 °C. The supernatant plasma was separated, transferred into test tubes, and stored at -70 °C for blood biochemical analysis and metabolomics analysis. Plasma TNF- α , IL-6, IL-1 β , NGF, PGE 2, and 5-HT were detected using ELISA kits according to the instructions. The absorbance of the solution was measured with a microplate reader (LabSystems Multiskan MS, Finland) at 450 nm.

2.8 Plasma metabolomics analysis

2.8.1 Metabolomics sample pre-treatment

Plasma samples are typically treated with methanol and

acetonitrile to precipitate proteins. Acetonitrile is frequently employed to dissolve samples and is well-suited for subsequent liquid chromatography analysis. In this experiment, we utilized methanol for protein precipitation and acetonitrile for sample dissolution. A plasma sample of 200 μ L was combined with 800 μ L of methanol and vortexed for 30 s. After centrifugation at 12,000 r/min for 15 min (Centrifuge 5424 R, Eppendorf AG, Germany) at 4 °C, the supernatant (800 μ L) was dried using a nitrogen stream and subsequently redissolved in 100 μ L of acetonitrile, followed by vortexing for 1 min. The supernatant was obtained for analysis after centrifugation at 12,000 r/min for 15 min at 4 °C. The same amount of each sample was taken and mixed well as a quality control sample for repeated injection during sample analysis to detect the stability of the instrument.

2.8.2 UPLC-MS/MS conditions

Chromatographic experiments were performed on a column of Acquity UPLC HSS T3 (100 mm $\times$ 2.1 mm, 1.8 μ m) using a Thermo UltiMate 3000 UPLC system (Thermo Fisher Scientific, USA). The column temperature was set at 35 °C with an injection volume of 5 μ L, and the mobile phase consisted of solvent A (0.05% formic acid in water) and solvent B (0.05% formic acid in acetonitrile) at a flow rate of 0.2 mL/min. The gradient elution procedure was as follows: 0–2 min, 95%–90% A; 2–4 min, 90%–60% A; 4–7 min, 60%–35% A; 7–12 min, 35%–10% A; 12–14 min, 10%–5% A; 14–16 min, 5% A.

Mass spectrometry was performed on a Q Exactive Orbitrap (Thermo Fisher Scientific, USA). The instrument was operated using a HESI source in positive and negative mode. The sheath gas volume flow was set at 40 psi, and the auxiliary gas volume flow was 10 psi. The spray voltage was 3.8 kV. The capillary temperature was 350 °C, and the auxiliary gas temperature was 350 °C. The quality scanning range was m/z 100–1,500, and the detection resolution was 7,000. The secondary mass spectrometry adopted high energy collision-induced dissociation with 20, 30, and 40 eV ionization energy and 17,500 resolutions.

2.8.3 Data analysis strategy

Raw data were uploaded to Progenesis QI Version 2.0 software (Waters, USA) for baseline filtering, peak identification, peak alignment, and normalization to obtain a two-dimensional data matrix composed of retention time, m/z , and ionic intensity of metabolite. The processed data matrix was imported into Simca 13.0 for multivariate statistical analysis. Firstly, unsupervised principal component analysis (PCA) was used to observe the sample separation and eliminate abnormal samples. The supervised orthogonal partial least squares discrimination analysis (OPLS-DA) was conducted. OPLS-DA was also used to predict the components with variable importance in the project (VIP) greater than 1 between control and model groups, and the components with VIP > 1 and $P < 0.05$ of t -test were screened and putatively identified through the database (KEGG, Human Metabolome Database (HMDB), LIPID MAPS Structure Database (LMSD), etc.). The screened potential biomarkers were introduced into the metabolic pathway analysis online platform (<https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml>) for seeking metabolic pathways related to PTX rats. Based on this, the regulatory effect of XQTQ on PTX rats was evaluated.

2.9 Statistical analysis

The experimental data were expressed with standard deviation. The difference between groups was compared using One-way

ANOVA with the Bonferroni method, and $P < 0.05$ was considered a significant difference.

3 Results and discussion

3.1 HPLC characteristic chromatogram of XQTQ for quality control

A method of the characteristic chromatogram was established of XQTQ, a total of 8 common peaks were characterized, and 6 chemical components were identified as guanosine (peak 1), protocatechuic acid (peak 2), ellagic acid (peak 4), scutellarin (peak 6), rosmarinic acid (peak 7), 6-gingerol (peak 8) were determined by reference substances using HPLC analysis (Fig. 1). The method is accurate and reproducible, and suitable for evaluating the quality of XQTQ.

3.2 Effects of XQTQ on PWT, PWL and the level of plasma cytokines

Compared with the control group, the value of PWT decreased significantly in the model group ($P < 0.01$), which proved the successful establishment of the model. Compared with the model group, pregabalin could slightly alleviate PWT decrease, statistical differences had been shown in the 14 days only. XQTQ combined with pregabalin alleviated PWT decrease in varying degrees. Statistical differences between the model and high dose of XQTQ combined group had been shown in the 1, 3, 5, 7, 14 days, medium and low doses had been shown in the 3, 7, 14 days and 1, 5, 14 days separately (Fig. 2). The effects of high, medium, and low doses were dose-dependent at 3 and 14 days.

Compared with the control group, the value of PWL decreased significantly in the model group ($P < 0.05$), which proved the successful establishment of the model. Compared with the model group, pregabalin could significantly increase the value of PWL in the 8 days ($P < 0.05$), the high dose of XQTQ combined group could significantly increase the value of PWL in the 2, 8 days ($P < 0.05$).

Compared with the control group, the model group exhibited significantly higher expression in the level of 5-HT, IL-1 β , IL-6, PGE 2, NGF, and TNF- α ($P < 0.01$) (Fig. 3). Compared with the model group, the pregabalin and different doses of XQTQ combined group can significantly decrease the level of TNF- α

(14.82%), IL-6 (13.69%), IL-1 β (19.34%), NGF (11.40%), PGE 2 (12.74%), and 5-HT (13.51%) ($P < 0.01$). XQTQ combined group reduced more than pregabalin in the level of IL-6, NGF, and TNF- α .

3.3 Metabonomics analysis

3.3.1 Identification of potential biomarkers in PTX rats and the metabolic pathway analysis

OPLS-DA was applied to explore potential biomarkers in model rats compared with control rats. As shown in Figs. 4A, B, the metabolites in the control and model groups were located in two different communities, which hinted the PTX rats model was established successfully. R^2Y was used to assess fitness and Q^2 was used to indicate the prediction ability of the model. The parameters were $R^2Y = 0.993$ and $Q^2 = 0.932$ in the positive ion mode, $R^2Y = 0.998$ and $Q^2 = 0.872$ in the negative ion mode, suggesting that the model group had apparent changes compared with the control group in the level of endogenous metabolites.

Twelve differential metabolites in plasma between PTX rats and normal rats were obtained and identified by Progenesis QI software ($VIP > 1$, $P < 0.05$), as well as KEGG, HMDB, and LMSD. According to KEGG (Table 1), these potential biomarkers were related to 7 pathways of glycerophospholipid metabolism, Linoleic acid metabolism (LA metabolism), alpha-Linolenic acid metabolism (ALA metabolism), Glycosylphosphatidylinositol-anchor biosynthesis (GPI-anchor biosynthesis), pentose and glucuronate interconversions, arachidonic acid metabolism and pyrimidine metabolism (Fig. 5).

3.3.2 Screening of potential biomarkers for XQTQ alleviates neuropathic pain effects

The plasma metabolite data from the high dose XQTQ combined group were used to investigate its mechanism in alleviating cancer pain. PCA manifested PTX induced obvious metabolic disturbances (Fig. 6). The PCA plots demonstrated clear discrimination between control and model groups, indicating that the model was successfully duplicated and our analytical method. The results of OPLS-DA indicated that different groups clustered in different areas. The pregabalin and XQTQ combined groups were located between the control and model groups and had a visual movement trend from the model to the control, indicating

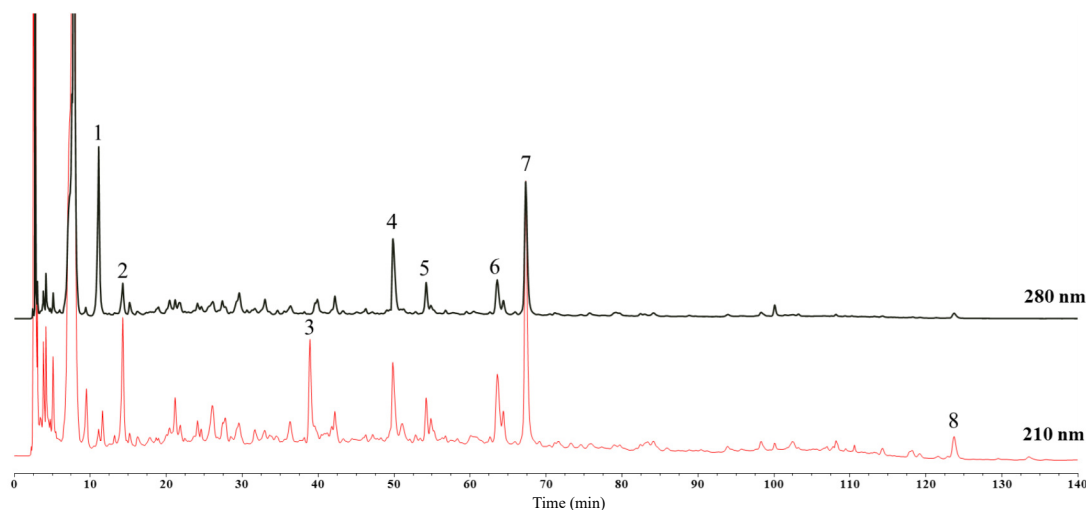


Figure 1 Chemical profile of XQTQ extract. 1. Guanosine, 2. protocatechuic acid, 4. ellagic acid, 6. scutellarin, 7. rosmarinic acid, 8. 6-gingerol.

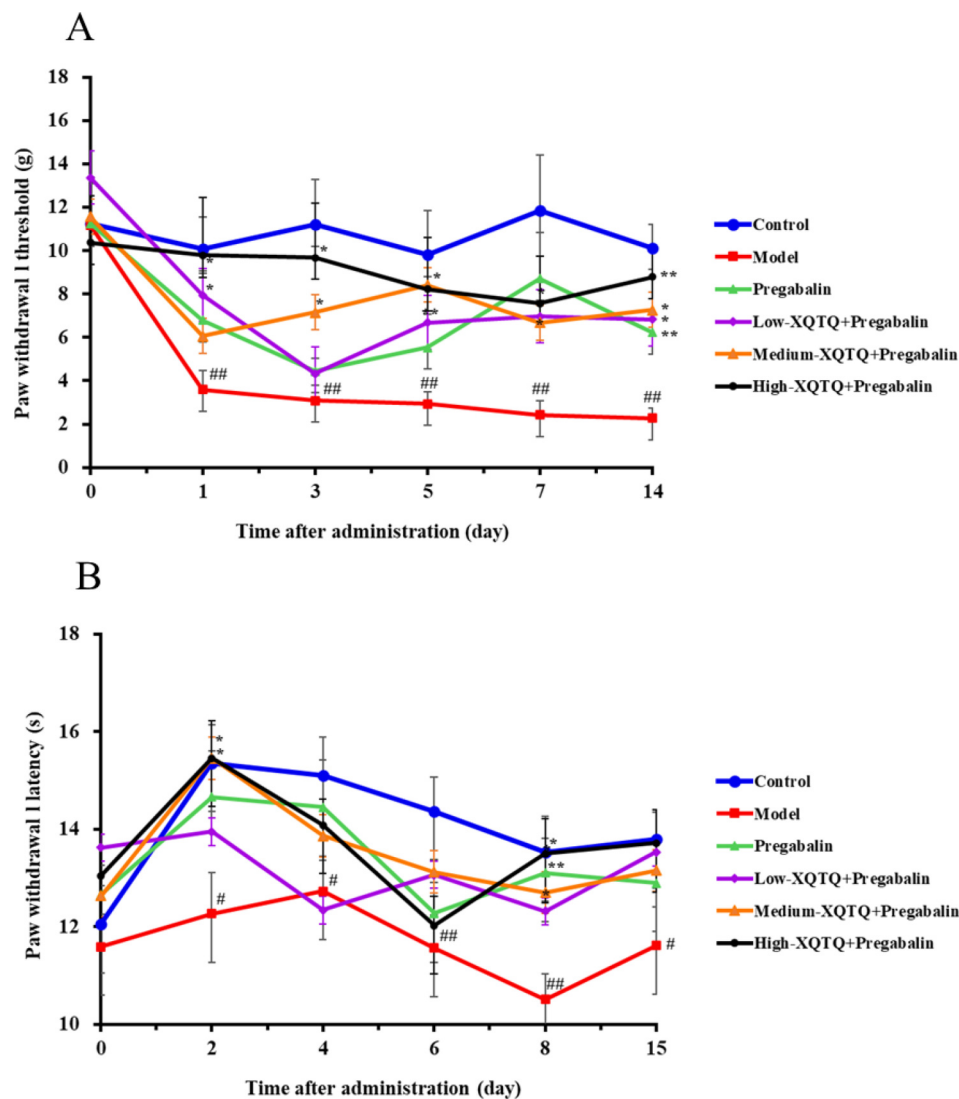


Figure 2 Behavioral test by measuring PWT and PWL in each time point. (A) PWT, (B) PWL. ^{*} $P < 0.05$ and ^{**} $P < 0.01$ when compared with the control group, ^{*} $P < 0.05$ and ^{**} $P < 0.01$ when compared to the model group. The same below.

that both groups were effective. XQTQ combined was closer to the control group than pregabalin use only on PTX rats (Figs. 4C, D), indicating that integrated therapy is more effective than pregabalin alone. As shown in Table 1, 10 metabolites were down-regulated in PTX, whereas the other 2 metabolites were up-regulated. XQTQ treatment rebalanced the alteration of metabolites which were disturbed by PTX. The expression of 12 disturbed metabolites appeared to be rebalanced.

PIPNP is a common clinical symptom in patients undergoing treatment with PTX. Due to the unclear mechanisms involved, effective agents for prevention or treatment are scarce. In this study, the analgesic effect of XQTQ was comprehensively evaluated in PIPNP rats. It was observed that high dose XQTQ combined group exhibited a significant increase in the PWT values on days 1, 3, 5, 7, and 14 days, indicating a strong analgesic effect. Clinical reports suggest that the combination of TCM with Western medicine yields significant therapeutic benefits^[13,14]. The PWT values in the high and medium dose of XQTQ group combined with pregabalin were higher than those in the pregabalin only group on days 3, 5, and 14. Additionally, the PWL for the high dose XQTQ group combined with pregabalin was greater than that of the pregabalin only group on days 2 and 8.

These results suggest that the combination of XQTQ with conventional Western medicine may provide enhanced therapeutic effects on PIPNP.

PGE 2^[15], 5-HT^[16,17], NGF^[18] are well-known pain mediators that play a pivotal role in development and maintenance of neuropathic pain. Moreover, an increasing body of evidence indicates that inflammation influences every facet of tumor development and progression^[19], IL-1 β , IL-6, and TNF- α are significant proinflammatory cytokines. Additionally, pathways that activate cytokines, such as TNF- α , IL-1 β , and monocyte chemoattractant protein-1 (MCP-1), are implicated in PIPNP as well^[20]. PGE 2, 5-HT, NGF, IL-1 β , IL-6, and TNF- α play crucial roles in the development of pain and hyperalgesia. In this study, the levels of these cytokines significantly rise in the model group, and the results support the notion that cytokines are implicated in the pathophysiology of PIPNP. After administration, the levels of these cytokines dramatically reduced to near-normal levels, indicating that the treatment group exhibited a significant regulatory effect on PIPNP. Compared to the pregabalin group, the XQTQ combined group exhibited a tendency to decrease the levels of IL-6, NGF, and TNF- α . However, there is no significant difference between the pregabalin and XQTQ combined group,

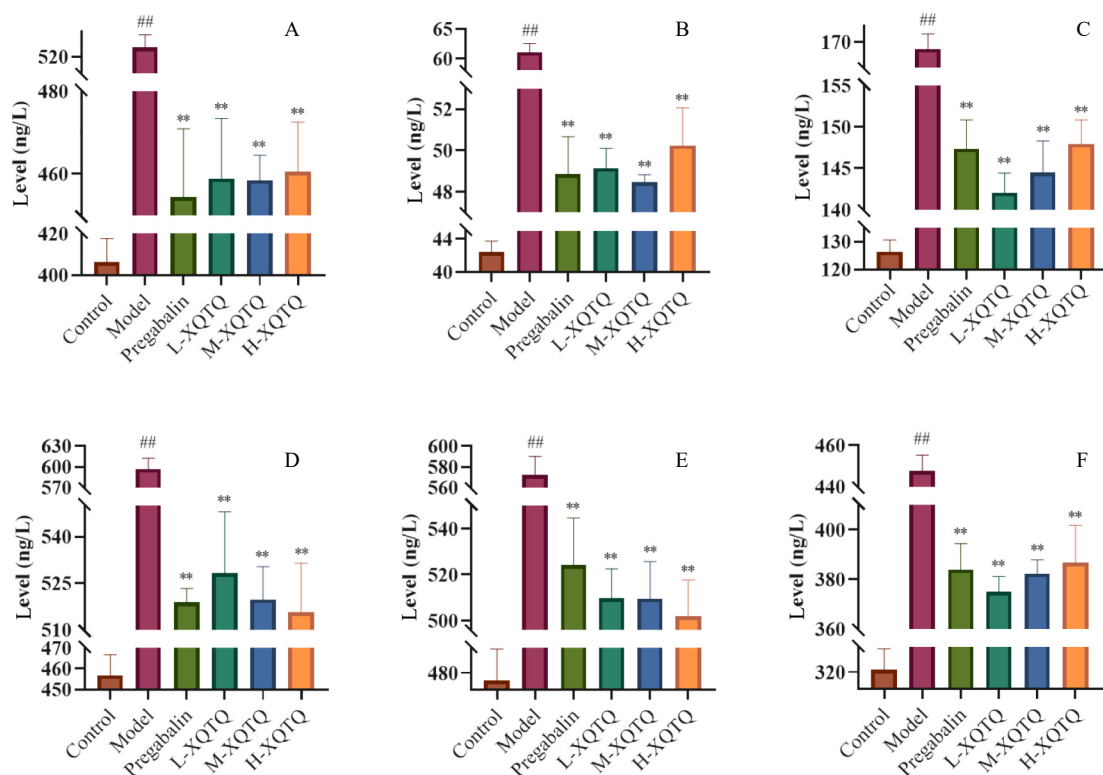


Figure 3 Effects of XQTQ on the levels of (A) 5-HT, (B) IL-1 β , (C) IL-6, (D) PGE 2 and (E) NGF, (F) TNF- α .

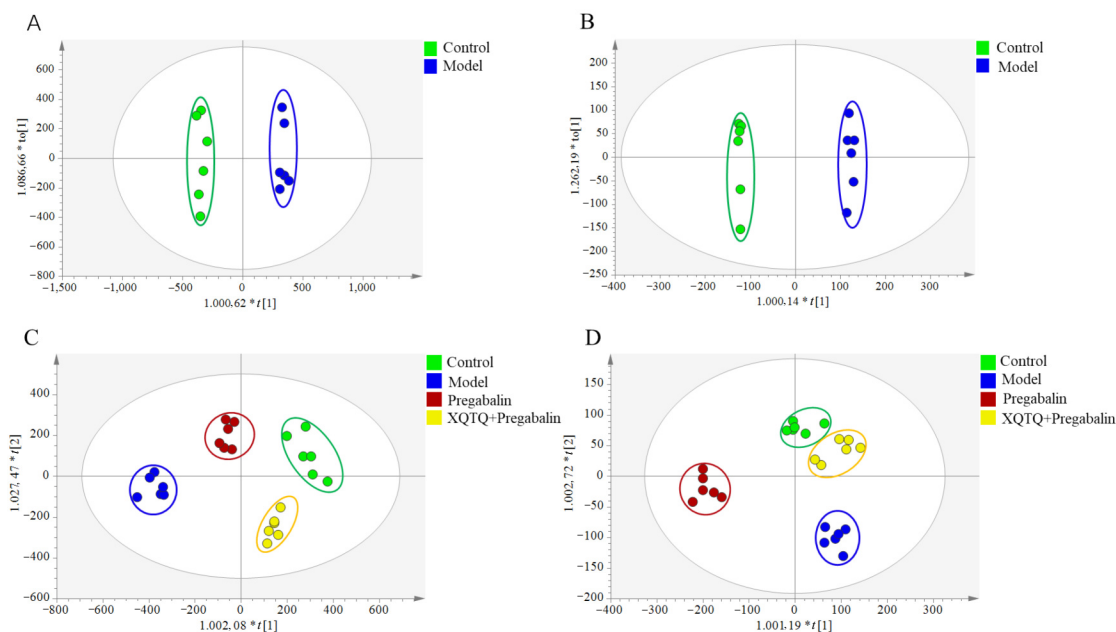


Figure 4 OPLS-DA scores of plasma samples of control group, model group, pregabalin and XQTQ combined group. (A) Control vs. Model in positive ion mode; (B) Control vs. Model in negative ion mode; (C) Control vs. Model vs. Pregabalin vs. XQTQ+pregabalin in positive ion mode; (D) Control vs. Model vs. Pregabalin vs. XQTQ+pregabalin in negative ion mode.

suggesting that the combination maintained a stable, lower ultimate level after treatment with pregabalin.

Meanwhile, a simple, reproducible, stable and accurate characteristic chromatogram had been modified to reflect the multi-components characteristics of the compound formula, resulting in the identification of 6 chemical components. Protocatechuic acid exhibited neuroprotective effects by exerting antioxidant properties and promoting the proliferation, induction,

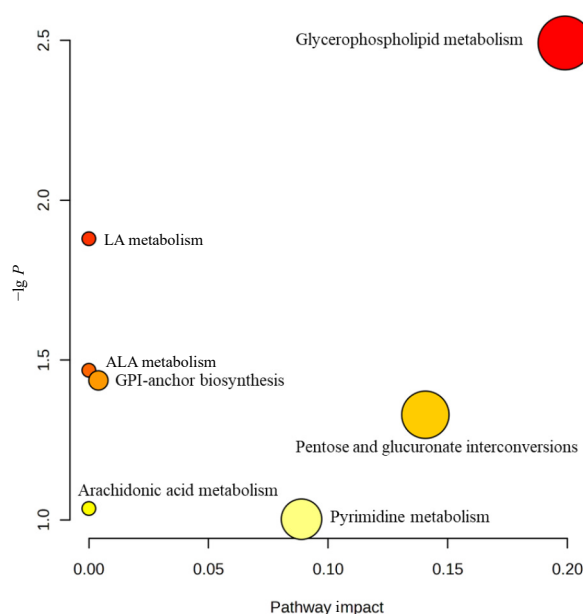
and differentiation of neural stem cells *in vitro*^[21,22]. Protocatechuic acid can also inhibit the secretion of inflammatory cytokines, including TNF- α , IL-1 β and IL-6, which have therapeutic effects on most inflammatory diseases^[23]. Ellagic acid primarily exhibits pharmacological actions such as anti-inflammatory effects^[24], anti-tumor properties, protection of the myocardium and nervous system^[25], and immune regulation. Scutellarin demonstrated a significant analgesic effect^[26]. Rosmarinic acid is a polyphenolic

Table 1 Identification and change trend of potential biomarkers

Analyst	HMDB ID	Retention time (min)	Formula	Observed m/z	Error (ppm)	Adduction	Model vs. Control	PH vs. Model	VIP	KEGG
Phosphatidylethanolamine (PE) (20:4(5Z,8Z,11Z,14Z)/P-18:1(11Z))	HMDB0009413	16.62	$C_{43}H_{76}NO_7P$	788.498	-1.519,18	$[M+K]^+$	↓	↑	1.82	C00350
Vitamin D ₂ 3-glucuronide	HMDB0010344	14.59	$C_{34}H_{52}O_7$	573.381	4.348,05	$[M+H]^+$	↓	↑	1.82	NA
Phosphatidylcholine (PC) (18:4(6Z,9Z,12Z,15Z)/24:1(15Z))	HMDB0008257	14.19	$C_{50}H_{90}NO_8P$	902.601	-2.827,48	$[M+K]^+$	↑	↓	1.42	C00157
Glycochenodeoxycholic acid 3-glucuronide	HMDB0002579	6.89	$C_{32}H_{51}NO_{11}$	626.351	-3.462,1	$[M+H]^+$	↓	↑	1.07	C03033
PC(14:0/20:4(5Z,8Z,11Z,14Z))	HMDB0007883	14.64	$C_{42}H_{76}NO_8P$	771.568	4.049,277	$[M+NH_4]^+$	↓	↑	1.07	C00157
Traumatin	HMDB0037326	7.26	$C_{12}H_{20}O_3$	257.139	-0.775,67	$[M+FA-H]^+$	↓	↑	1.91	C16309
3-Hydroxydodecanedioic acid	HMDB0000413	7.29	$C_{12}H_{22}O_5$	246.146	-1.630,97	$[M-H_2O-H]^-$, $[M-H]^-$	↓	↑	1.77	NA
3-Hydroxytetradecanedioic acid	HMDB0000394	9.28	$C_{14}H_{26}O_5$	273.171	-0.521,77	$[M-H]^-$	↓	↑	1.66	NA
Asparaginyl-Threonine	HMDB0028741	6.63	$C_8H_{15}N_3O_5$	465.197	4.824,5	$[2M-H]^-$	↑	↓	1.37	NA
6-Keto-prostaglandin F1a	HMDB0002886	12.50	$C_{20}H_{34}O_6$	369.228	-0.894,89	$[M-H]^-$	↓	↑	1.23	C05961
5,6-Dihydrouridine	HMDB0000497	7.33	$C_9H_{14}N_2O_6$	291.082	-3.854,16	$[M+FA-H]^-$	↓	↑	1.2	NA
5-Thymidylic acid	HMDB0001227	7.33	$C_{10}H_{15}N_2O_8P$	303.038	-1.421,62	$[M-H_2O-H]^-$	↓	↑	1.1	C00364

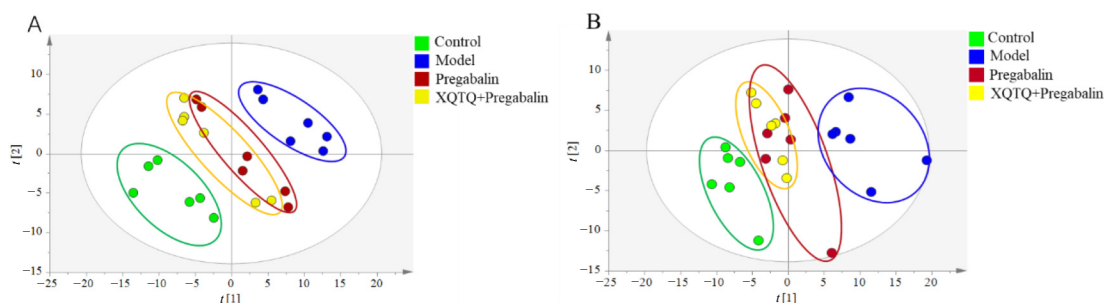
Note: The levels of potential biomarkers were labeled with (↓) down-regulated and (↑) up-regulated.

compound known for its significant anti-inflammatory^[27], antitumor^[28] and neuroprotective effects^[29]. These ingredients can serve as a core representative compound for further pharmacological and mechanism studies.

**Figure 5** Metabolic pathway analysis of PTX rats.

Metabolomics has been utilized as an important technique for elucidating the pharmacological mechanisms of TCM formulae under the framework of systematic theory^[30,31]. Indeed, there has been an increasing number of studies on TCM applying metabolomics^[32-34]. In our plasma metabolomics study, XQTQ treatment rebalanced the alteration of most metabolites that were disturbed by PTX. The expression of 12 disturbed metabolites appeared to be rebalanced. As shown in the pathway analysis results (Fig. 5), glycerophospholipid metabolism, LA metabolism, ALA metabolism, GPI-anchor biosynthesis were the significantly altered metabolic pathway.

Glycerophospholipid, LA and ALA metabolism were the most significantly altered metabolic pathway. Glycerophospholipids and their metabolites play a significant role in the pathogenesis of neuropathic pain^[35,36]. LA and ALA are involved in the regulation of inflammation and the nervous system^[37,38]. In our investigation of the metabolic profiles of PIPNP rats, we observed significant changes in the levels of specific metabolites. Three metabolites exhibited significant decreases: PE(20:4(5Z,8Z,11Z,14Z)/P-18:1(11Z)), PC(14:0/20:4(5Z,8Z,11Z,14Z)), and traumatin. In contrast, we observed an increase in the metabolite PC(18:4(6Z,9Z,12Z,15Z)/24:1(15Z)) in PIPNP rats. Importantly, treatment with XQTQ exhibited a significant capacity to reverse the levels of these metabolites, suggesting a potential therapeutic role for XQTQ in addressing metabolic dysregulation associated with PIPNP. Phosphatidylcholine and phosphatidyl ethanolamine serve multiple physiological functions, including increasing blood flow

**Figure 6** PCA scores of plasma samples of control group, model group, pregabalin and XQTQ combined group. (A) Control vs. Model vs. Pregabalin vs. XQTQ+pregabalin in positive ion mode; (B) Control vs. Model vs. Pregabalin vs. XQTQ+pregabalin in negative ion mode.

and permeability, as well as reducing blood viscosity. XQTQ has the effects of reducing blood viscosity, promoting bold circulation and removing blood stasis, which are closely related to the regulation of these metabolic pathways. Arachidonic acid metabolism is well-known for its association with inflammatory processes^[39]. Additionally, 6-keto-prostaglandin F1a plays a critical role in inhibiting platelet aggregation^[40]. XQTQ can significantly reverse the level of 6-keto-prostaglandin F1a, indicating that XQTQ had a beneficial regulatory effect on arachidonic acid metabolism. In addition, the metabolic pathway of GPI-anchored protein biosynthesis was affected, indicating that PTX-induced neuropathic pain can disorder GPI-anchored protein biosynthesis and remodeling.

In these metabolic pathways, the levels of biomarkers were markedly higher or lower in the model group compared to the control group. With XQTQ treatment, the levels of biomarkers approached near-normal levels, indicating that XQTQ could reverse these changes. Studies have reported metabolic disturbances in the pathways of glycerophospholipid metabolism, LA metabolism, ALA metabolism, GPI-anchor biosynthesis metabolism, and arachidonic acid metabolism in PIPNP^[41,42], which was consistent with the results of this experiment.

On the whole, XQTQ exhibited effective analgesic effects in rats with PIPNP. The underlying mechanism may be related to improvement in glycerophospholipid, LA metabolism, ALA metabolism. There is no doubt that this represents a potential link, and further research is warranted to explore the underlying molecular mechanisms in the future. Additionally, there is a limitation in our study. The potential biomarkers were putatively identified using various commercial databases, necessitating further experimental verification.

4 Conclusions

This study demonstrated that the combination of XQTQ and pregabalin significantly alleviates PIPNP and modulates pain and inflammatory cytokines. The underlying mechanism of XQTQ may be associated with improvements in glycerophospholipid metabolism, LA metabolism, ALA metabolism. These findings enhance our understanding of the specific mechanisms by which XQTQ exerts its effects in PIPNP rats and provide an experimental basis for its clinical application.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

Acknowledgements

This work was supported by the grants from Key R&D Program (Major Innovation Project, 2021CXGC010510) of Shandong Province.

References

- [1] Sung, H., Ferlay, J., Siegel, R. L., et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, **2021**, 71: 209–249. <https://doi.org/10.3322/caac.21660>
- [2] Turabi A, Plunkett A. R. The application of genomic and molecular data in the treatment of chronic cancer pain. *Journal of Surgical Oncology*, **2012**, 105: 494–501. <https://doi.org/10.1002/jso.21707>
- [3] Lee, J., Swain, S. M. Peripheral neuropathy induced by microtubule-stabilizing agents. *Journal of Clinical Oncology*, **2006**, 24: 1633–1642. <https://doi.org/10.1200/jco.2005.04.0543>
- [4] Velasco-González, R., Coffeen, U. Neurophysiopathological aspects of paclitaxel-induced peripheral neuropathy. *Neurotoxicity Research*, **2022**, 40: 1673–1689. <https://doi.org/10.1007/s12640-022-00582-8>
- [5] Ye, H. N., Liu, X. Y., Qin, B. L. Research progress of integrated traditional Chinese and Western medicine in the treatment of advanced gastric cancer. *World Journal of Gastrointestinal Oncology*, **2023**, 15: 69–75. <https://doi.org/10.4251/wjgo.v15.i1.69>
- [6] Sun, X., Lu, Y., Zhu, H., et al. Effects of psychological intervention on perioperative quality of life and serum PSA and FPSA levels of patients with prostate cancer treated with integrated traditional Chinese and Western medicine. *Evidence-based Complementary and Alternative Medicine*, **2021**: 9286905. <https://doi.org/10.1155/2021/9286905>
- [7] Li, J. J., Yang, G., Shen, Y. L. Research and discussion in the application and development of medicine and food homology plants. *Modern Food*, **2024**, 30: 30–35, 42. <https://doi.org/10.16736/j.cnki.cn41-1434/ts.2024.19.008>
- [8] Nicholson, J. K., Lindon, J. C. Metabonomics. *Nature*, **2008**, 455: 1054–1056. <https://doi.org/10.1038/4551054a>
- [9] Chen, C., Wu, H., Fu, X., et al. A UPLC-QTOF/MS-based hepatic tissue metabolomics approach deciphers the mechanism of Huachansu tablets-based intervention against hepatocellular carcinoma. *Journal of Pharmaceutical and Biomedical Analysis*, **2024**, 239: 115875. <https://doi.org/10.1016/j.jpba.2023.115875>
- [10] Wang, P., Wang, Q., Yang, B., et al. The progress of metabolomics study in traditional Chinese medicine research. *American Journal of Chinese Medicine*, **2015**, 43: 1281–1310. <https://doi.org/10.1142/s0192415x15500731>
- [11] Fang, Z., Gonzalez, F. J. LC-MS-based metabolomics: an update. *Archives of Toxicology*, **2014**, 88: 1491–1502. <https://doi.org/10.1007/s00204-014-1234-6>
- [12] Chaplan, S. R., Bach, F. W., Pogrel, J. W., et al. Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods*, **1994**, 53: 55–63. [https://doi.org/10.1016/0165-0270\(94\)90144-9](https://doi.org/10.1016/0165-0270(94)90144-9)
- [13] Huang, R. Y., Fu, J. Q., Wu, H., et al. Clinical effect of integrated traditional Chinese and Western medicine on cancer pain in patients with advanced malignant tumor. *China Modern Medicine*, **2022**, 29: 103–105.
- [14] Yang, X., Yuan, X., Tian, Y., et al. Study on the analgesic effects of icaritin in combination with morphine on bone cancer pain in mice. *Chinese Journal of Pain Medicine*, **2024**, 30: 28–37. <https://doi.org/10.3969/j.issn.1006-9852.2024.01.006>
- [15] Ma, W., St-Jacques, B. Signalling transduction events involved in agonist-induced PGE 2/EP 4 receptor externalization in cultured rat dorsal root ganglion neurons. *European Journal of Pain*, **2018**, 22: 845–861. <https://doi.org/10.1002/ejp.1172>
- [16] Nakamori, H., Naitou, K., Sano, Y., et al. Exogenous serotonin regulates colorectal motility via the 5-HT 2 and 5-HT 3 receptors in the spinal cord of rats. *Neurogastroenterology and Motility*, **2018**, 30: e13183. <https://doi.org/10.1111/nmo.13183>
- [17] Cortes-Altamirano, J. L., Olmos-Hernandez, A., Jaime, H. B., et al. 5-HT1, 5-HT2, 5-HT3 and 5-HT7 receptors and their role in the modulation of pain response in the central nervous system. *Current Neuropharmacology*, **2018**, 16: 210–221. <https://doi.org/10.2174/1570159x15666170911121027>
- [18] Su, S., Liu, Q., Jiang, M. Study on the relationship between the cerebrospinal fluid, serum pain substance related indexes and lumbar disc protrusion. *Journal of Clinical and Experimental Medicine*, **2016**, 15: 232–234. <https://doi.org/10.3969/j.issn.1671-4695.2016.03.011>
- [19] Grivennikov, S. I., Greten, F. R., Karin, M. Immunity, inflammation, and cancer. *Cell*, **2010**, 140: 883–899. <https://doi.org/10.1016/j.cell.2010.01.025>
- [20] Al-Mazidi, S., Alotaibi, M., Nedjadi, T., et al. Blocking of cytokines signalling attenuates evoked and spontaneous neuropathic pain

- behaviours in the paclitaxel rat model of chemotherapy-induced neuropathy. *European Journal of Pain*, **2018**, 22: 810–821. <https://doi.org/10.1002/ejp.1169>
- [21] Guan, S., Bao, Y. M., Jiang, B., et al. Protective effect of protocatechuic acid from *Alpinia oxyphylla* on hydrogen peroxide-induced oxidative PC12 cell death. *European Journal of Pharmacology*, **2006**, 538: 73–79. <https://doi.org/10.1016/j.ejphar.2006.03.065>
- [22] Guan, S., Liu, T. Q., Ge, D., et al. Studies on the effects of protocatechuic acid on cell proliferation and apoptosis in cultured neural stem/progenitor cells. *Chinese Pharmacological Bulletin*, **2009**, 25: 448–452.
- [23] Wu, H. Q., Wang, J., Zhao, Q., et al. Protocatechuic acid inhibits proliferation, migration and inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes. *Artificial Cells, Nanomedicine, and Biotechnology*, **2020**, 48: 969–976. <https://doi.org/10.1080/21691401.2020.1776307>
- [24] Bhattacharjee, A., Kulkarni, V. H., Chakraborty, M., et al. Ellagic acid restored lead-induced nephrotoxicity by anti-inflammatory, anti apoptotic and free radical scavenging activities. *Heliyon*, **2021**, 7: e05921. <https://doi.org/10.1016/j.heliyon.2021.e05921>
- [25] Wei, Y. Z., Zhu, G. F., Zheng, C. Q., et al. Ellagic acid protects dopamine neurons from rotenone-induced neurotoxicity via activation of Nrf2 signalling. *Journal of Cellular and Molecular Medicine*, **2020**, 24: 9446–9456. <https://doi.org/10.1111/jcmm.15616>
- [26] Liu, C., Liu, H., Zhang, F., et al. Scutellarin mitigates cancer-induced bone pain by suppressing CaMKII/CREB pathway in rat models. *Current Topics in Nutraceutical Research*, **2019**, 3: 17. <https://doi.org/10.37290/ctnr2641-452x.17:249-253>
- [27] Colica, C., Di Renzo, L., Aiello, V., et al. Rosmarinic acid as potential anti-inflammatory agent. *Reviews on Recent Clinical Trials*, **2018**, 13: 240–242. <https://doi.org/10.2174/157488711304180911095818>
- [28] Cao, W., Mo, K., Wei, S., et al. Effects of rosmarinic acid on immunoregulatory activity and hepatocellular carcinoma cell apoptosis in H22 tumor-bearing mice. *Korean Journal of Physiology & Pharmacology*, **2019**, 23: 501–508. <https://doi.org/10.4196/kjpp.2019.23.6.501>
- [29] Ren, P., Jiang, H., Li, R., et al. Rosmarinic acid inhibits 6-OHDA-induced neurotoxicity by anti-oxidation in MES23.5 cells. *Journal of Molecular Neuroscience*, **2009**, 39: 220–225. <https://doi.org/10.1007/s12031-009-9182-y>
- [30] Li, S., Zhang, B., Zhang, N. Network target for screening synergistic drug combinations with application to traditional Chinese medicine. *BMC Systems Biology*, **2011**, 5: 1–13. <https://doi.org/10.1186/1752-0509-5-s1-s10>
- [31] Shao, L. I., Zhang, B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chinese Journal of Natural Medicines*, **2013**, 11: 110–120. <https://doi.org/10.3724/sp.j.1009.2013.00110>
- [32] Wang, X., Cui, D., Dai, X., et al. HuangQin decoction attenuates CPT-11-induced gastrointestinal toxicity by regulating bile acids metabolism homeostasis. *Frontiers in Pharmacology*, **2017**, 8: 156. <https://doi.org/10.3389/fphar.2017.00156>
- [33] Ye, X., Wang, X., Wang, Y., et al. A urine and serum metabolomics study of gastroesophageal reflux disease in TCM syndrome differentiation using UPLC-Q-TOF/MS. *Journal of Pharmaceutical and Biomedical Analysis*, **2021**, 206: 114369. <https://doi.org/10.1016/j.jpba.2021.114369>
- [34] Li, J., Zhou, Z., Liu, D., et al. Therapeutic effects of Zhuling Jianpi capsule on experimental ulcerative colitis and characterization of its chemical constituents and metabolomics using UHPLC-Q-TOF-MS. *Heliyon*, **2023**, 9: 16553. <https://doi.org/10.1016/j.heliyon.2023.e16553>
- [35] Farooqui, A. A., Horrocks, L. A., Farooqui, T. Modulation of inflammation in brain: a matter of fat. *Journal of Neurochemistry*, **2007**, 101: 577–599. <https://doi.org/10.1111/j.1471-4159.2006.04371.x>
- [36] Piomelli, D., Sasso, O. Peripheral gating of pain signals by endogenous lipid mediators. *Nature Neuroscience*, **2014**, 17: 164–174. <https://doi.org/10.1038/nn.3612>
- [37] Stark, A. H., Crawford, M. A., Reifen, R. Update on alpha-linolenic acid. *Nutrition Reviews*, **2008**, 66: 326–332. <https://doi.org/10.1111/j.1753-4887.2008.00040.x>
- [38] Boyd, J. T., Lococo, P. M., Furr, A. R., et al. Elevated dietary ω -6 polyunsaturated fatty acids induce reversible peripheral nerve dysfunction that exacerbates comorbid pain conditions. *Nature Metabolism*, **2021**, 3: 762–773. <https://doi.org/10.1038/s42255-021-00410-x>
- [39] Kuehl Jr, F. A., Egan, R. W. Prostaglandins, arachidonic acid, and inflammation. *Science*, **1980**, 210: 978–984. <https://doi.org/10.1126/science.6254151>
- [40] Zhou, A. F., Cao, J. G., Zhou, Y., et al. Effect of Xieganbushen prescription on the content of thromboxane B₂ 6-keto-prostaglandin in F1a in plasm in prostatitis infertility rats prostate. *Chinese Archives of Traditional Chinese Medicine*, **2007**, 25: 1551–1552.
- [41] Wu, F., Xu, W., Deng, B., et al. Wen-Luo-Tong decoction attenuates paclitaxel-induced peripheral neuropathy by regulating linoleic acid and glycerophospholipid metabolism pathways. *Frontiers in Pharmacology*, **2018**, 9: 956. <https://doi.org/10.13193/j.archtcm.2007.08.16.zhouaf.061>
- [42] Feng, L. M., Chen, Y., Xu, D. Q., et al. An integrated strategy for discovering effective components of Shaoyao Gancan decoction for treating neuropathic pain by the combination of partial least-squares regression and multi-index comprehensive method. *Journal of Ethnopharmacology*, **2020**, 260: 113050. <https://doi.org/10.1016/j.jep.2020.113050>