The Therapeutic Effects of HexueTongbi to Combat the Oxaliplatin-Induced Peripheral Neuropathy Using Network Analysis in Rats

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Abstract

Background: Chemotherapy-induced peripheral neuropathy (CIPN) is common in pateints undergoing chemotherapy. Hexuetongbi (HXTB), a TCM, could treat CIPN. Objective: This study investigates HXTB in treating CIPN and the underlying mechanism in an oxaliplatin-induced rat model (model). Methods: The rat model was developed by intraperitoneal injection of oxaliplatin for four weeks. The HXTB was investigated on the behavior of rats. Network analysis, TCMSP, and GeneCards were used to identify CIPN targets and HXTB therapeutic entities. HXTB and CIPN molecular pathways were analyzed using GO enrichment and KEGG. H&E staining assessed dorsal root ganglion neuron morphology. qRT-PCR and Western Blot evaluated mRNA and protein levels. Results: The model group had significantly higher frequency of CPWR and lower MWT. HXTB reduced CPWR and increased MWT. H&E staining demonstrated abnormal neuron morphology, confirming model development. HXTB neurons remained normal. Skin, liver, kidney, and heart function were preserved. Network analysis identified 19 active HXTB constituents and 35 CIPN targets. Among the 35 targets, the PI3K/Akt signaling pathway was the main pathway identified. PI3K, Akt1, Akt2, and Bcl-2 mRNA and protein levels were up-regulated. Conclusion: HXTB can ameliorate CIPN by regulating the PI3K/Akt and Bcl-2 pathways to inhibit apoptosis of damaged dorsal ganglion neurons.

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using GO enrichment and KEGG. H&E staining assessed dorsal root ganglion neuron morphology. qRT-PCR and Western Blot evaluated mRNA and protein levels.

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Keywords: Oxaliplatin; CIPN; TCM; Dorsal root ganglion; Apoptosis

INTRODUCTION

Chemotherapy-induced peripheral neuropathy (CIPN) is the most common non-hematological and devastating side effect in patients using anticancer drugs containing platinum compounds, tubulin inhibitors, and glutamic acid derivatives (1). Most CIPN patients first experience pain and other sensory abnormalities in areas dominated by the longest nerve fibers, resulting in the phrase "stocking-glove" neuropathy (2). Despite numerous investigations on the mechanisms of CIPN, there is no approved treatment for preventing or treating CIPN.

According to studies, oxaliplatin is one of the chemotherapeutic entities with the highest incidence, accounting for 92 percent of all grades of CIPN (1, 3). A meta-analysis of randomized controlled trials and cohort studies showed that around half of all patients develop CIPN during treatment (4). Oxaliplatin is a platinum-based chemotherapeutic agent extensively used as a standard treatment for colorectal, gastric, and pancreatic cancers, typically in combination with fluorouracil, irinotecan, and capecitabine; however, it frequently causes severe peripheral neuropathy (1, 5). Oxaliplatin damages the dorsal root ganglion and causes acute and chronic neuropathy. Acute neuropathy, such as cold sensory disturbance in the limbs and perioral region, is usually brief and reversible, appearing within a few hours to a few days of oxaliplatin treatment (1, 5). Previous studies have suggested that voltage-gated ion channels and transient receptor potential channels are involved in oxaliplatin-induced acute neuropathy (6). The abnormal sodium ion channel activity on axonal cells' surface directly controls voltage-dependent sodium ion channel activity, prolonging the opening of Na+ channels and causing nerve overexcitation, leading to paresthesia and muscle tremors (7). However, the obvious mechanism of oxaliplatin-induced chronic neuropathy is still unknown. Some studies suggest that chronic neuropathy is caused by morphological changes in neurons, such as axonal degeneration and damage to neuronal cell bodies due to oxaliplatin accumulation in the dorsal root ganglia, which induces apoptosis (8-10).

The development of CIPN may result in prolonged infusion times, dose reduction, or premature cessation of chemotherapy which may negatively impact treatment efficacy and patient survival (11, 12). Several anticonvulsant drugs, such as cytoprotective agents, glutamine, calcium-magnesium mixture, vitamin E, and other drugs, have been explored for a long time to treat CIPN (13). These studies revealed that the response rate of patients receiving a calcium-magnesium mixture with the olinic acid, leucovorin, fluorouracil, and oxaliplatin (FOLFOX) regimen was significantly decreased (14). Furthermore, recent studies have shown that the calcium-magnesium combination does not substantially alleviate or prevent peripheral neuropathy induced by oxaliplatin (15, 16). Another meta-analysis study revealed that several promising protective drugs, including amifostine and glycinamide, lack clinical evidence to address CIPN (17). According to the American Society of Clinical Oncology (ASCO) guidelines for managing CIPN in cancer patients, duloxetine has been recommended to treat CIPN (18). Still, duloxetine has certain side effects.

There is currently no effective treatment for CIPN, which may be because the mechanism of nerve damage induced by oxaliplatin is not fully understood, and there is no appropriate genetic target to determine this

side effect (11). More research is required to identify accurate genetic markers and effective drugs to address this side effect. Interestingly, the ASCO guidelines also indicate that TCM'Kampo' may be exploited as a potential therapeutic option for the prevention and treatment of CIPN (19); however, there is currently inadequate published data to support this. TCMcompounds such as the HXTB formula/recipe comprise five herbs: Chuanwu, Asarum, Chuanmutong, Guizhi, and Angelica (20). Clincally, we observed that external washing with this formulation significantly affects the treatment of CIPN; however, its pharmacological mechanism is still unclear. To address this issue, we used network analysis, pharmacodynamics, and mechanistic approaches to investigate the mechanism of HXTB Formula in treating CIPN and the underlying molecular mechanism that could influence the therapy.

MATERIALS AND METHODS

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies (21).

Materials

HXTB formula consists of five types of TCM; Chuanwu (10 g), Guizhi (10 g), Asarum (10 g), Chuanmutong (10 g), and 10 grams Angelica (10 g) provided by the Chinese Pharmacy of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (Kangmei Pharmaceutical, batch numbers: 19020117, 190403, 190401, 190701, G5219921). The liquid extracts were prepared from the five types of TCM in the laboratory of Guangzhou University of Chinese Medicine. The extract concentration was determined to be 30g/L. Oxaliplatin (batch number: 190201) was obtained from Hainan Jinrui Pharmaceutical Co., Ltd (Hainan, China). 50 mg of oxaliplatin was added to 250 mL of 5% glucose solution, resulting in a final concentration of 0.2 mg/mL. 4% paraformaldehyde, H&E staining kit, paraffin, natural gum mounting media, and xylene (Product number: E672002-0500, A530011-0500, A606115-0250, E607318 -0200, E675007-0100) were obtained from Shanghai Sangon Bioengineering Co., Ltd. (Shanghai, China). Real-Time PCR Kit (Cat. No.: RR820A) was purchased from Takara Bio Inc. (Nojihigashi Kusatsu, Shiga Japan). Realtime-RT PCR primers (Table 1) were designed and synthesized by Sangon Bioengineering Co., Ltd. (Shanghai, China).; BCA Protein Content Detection Kit (Cat. No. : P0010) was obtained from Beyotime Biotechnology (Shanghai, China). Antibodies like anti-GAPDH Rabbit mAb (Cat. No.: AF0911, Affinity Biosciences LTD), Goat Anti-Mouse IgG (Cat. No.: BA1050, Boster Biological Technology co. Ltd), Goat Anti-Rabbit IgG (Cat. No. BA1054, Boster Biological Technology Co. Ltd), Anti-Bcl-2 Antibody (Cat. No. ab196495, Abcam), Anti-PI3 Kinase p110 beta Antibody [EPR5515(2)] (Abcam) Anti-AKT (Cat. No. ab151549, Abcam), and phospho S473 Antibody [EP2109Y] (Cat. No. ab81283, Abcam) were purchased.

SPF-grade SD male rats, weighing 200±20g (from the Guangdong Provincial Laboratory Animal Center, license number: SCXK (Guangdong) 2018-0002, experimental animal batch number: No.44007200068466), were obtained from Guangzhou Pharmaceutical University (experimental unit license number: SYXK (Guangdong) 2017-0125). The animals were housed in a calm, clean environment with an air filtering system at 20-25°C and 40-60% relative humidity. Animals had free access to food and water, and the animal laboratory maintained a 12-hour light/dark cycle.

2.2. Animal model development and study design

The Animal Ethics Committee approved all experimental animal protocols of Guangzhou University of TCM and complied with the National Institutes of Health Guidelines for the Use and Care of Laboratory Animals (published by the National Institutes of Health, Edition: 85-23, revised in 1996) . 30 SPF SD male rats were divided into three groups, 10 rats in each group, namely: Control group (group C), Model group (group M), and HXTB Recipe bath group (group H). Group C was intraperitoneally injected with 2 ml of 5% glucose solution. Rats in groups M and H were intraperitoneally injected with 2 ml of prepared oxaliplatin solution twice a week for a total of 4 weeks, respectively, on the 1^{st}, 2^{nd}, 8^{th}, 9^{th}, 15^{th}, 16^{th}, 22^{nd}, and 23^{rd} days of injection) to establish a standard oxaliplatin-induced peripheral neuropathy rat model (22).

For the cold stimuli-induced persistent pain test, group H was given an HXTB Recipe bath and placed in a

plastic box with a lid so that their feet were immersed in 2L of a HXTB solution (concentration of 17g/L) at controlled temperature of 38 . The animals were exposed to -42 daily for 30 min each time. Animals were given an HXTB bath for six days, rest for 1 day as a course of therapy (7 days as a course of treatment), and continuous treatment for three courses. Every day, animals in groups C and M were allowed to immerse their feet in the same volume of freshwater as Group H, and the conditions were the same. All groups were examined for general conditions and toxic reactions throughout the administration. The mechanical stimulation of the paw withdrawal threshold test and the test of persistent pain induced by cold stimulation were conducted weekly. After three weeks, the rats were anesthetized with 2% sodium pentobarbital, the abdomen was dissected, and the abdominal aortic blood was collected, followed by the removal of the L4-6 segment dorsal root ganglia for future experiments.

2.4. Toxicity evaluation

The main toxicity detection experiments included skin irritation tests, sensitization tests, acute toxicity tests, and chronic toxicity tests, and variations in rat body weight were closely monitored. Blood was collected for myocardial enzyme, liver and renal functions.

2.5. Mechanical stimulus withdrawal threshold assay

Mechanical stimulus withdrawal threshold (MWT) assay is used for behavior testing. MWT was carried out to measure mechanical nociception to evaluate an animal's ability to detect a noxious stimulus. The soles of the rats were stimulated using an electrical Von Frey pain meter, which recorded the stimulation's intensity. Before modeling (day 0), before administration, and on days 7, 14, 21, and 28 during administration, the rats were acclimatized in transparent acrylic cages with metal mesh chassis. Each bilateral hind foot was measured five times with a more than the 6-second delay between measurements. The hind foot was measured both before and after the probe was triggered.The likelihood of positive reactions was calculated after immediate foot withdrawal; twitching, foot licking, and other reflexes were excluded from the scope of the foot withdrawal (number of positive reactions/10 100%) (23).

2.6. Cold stimulation-induced persistent pain

According to Jasmin et al.,. The detection was carried out on the 7^{th}, 14^{th}, 21^{st}, and 28^{th} days before modeling and on the day 0 before administration (24). The rats were placed on a cold plate with a surface temperature of (4+-1) degC, and a transparent acrylic cage was placed around the plate's periphery. Before the test, the rats were given 5 minutes to acclimate to their environment. The cumulative number of foot withdrawal, twitching, and foot licking of the rat within 5 minutes, as well as the foot lift induced by the rat's normal activities, are excluded from the foot withdrawal.

2.7. Histopathological assessment

For pathological assessment, the L4-6 dorsal root ganglia were removed, fixed, dehydrated, paraffin-embedded, and sliced 5 m thick along the longitudinal axis. The histomorphological changes were observed under a microscope after standard HE staining. In tissue samples, typical pathological alterations such as inflammation, necrosis, degeneration, hyperplasia, and fibrosis were observed.

2.8. Network analysis

2.8.1 Active ingredients and target genes of each TCM ingredient in the HXTB

The TCMSP was used to search for all the chemical components of the five TCM in the HXTB recipe. The screening criteria were specified as bioavailability (OB) [?] 30% and drug-like properties (DL) [?] 0.18 (25), and the active compounds that met the conditions were collected. TCMSP obtained the target genes by screening the active components.

2.8.2 Disease target and construction of TCM regulatory network

The Genecard database (https://www.genecards.org/) contains information on known target genes for the therapy of CIPN. The acquired genes were compared to the screened target genes of active ingredients to

establish an intersection between the HXTB recipe and CIPN therapeutic interventions. The "disease-TCM-active ingredient-target" linkage/intersection was then visualized using Cytoscape v3.7.0 software.

2.8.3 Construction of protein interaction network

The screened targets were assessed online by the protein-protein interaction network (PPI) of the target gene on the String website (https://string-db.org/cgi/input.pl), and the level of confidence determined the information of each protein interaction. The score > 0.4 is selected as the set value, and the protein targets with the highest correlation in the network are screened based on each target gene's "node connection degree" (degree).

2.8.4 GO enrichment and KEGG pathway analysis of targets

GO enrichment analysis and KEGG pathway were carried out using the R software "ClusterProfiler." The findings were organized in descending order of P value, and the analysis results were visualized and transformed into bubble charts.

2.9. Quantitative Real-time PCR

Total RNA was extracted from tissues using triazole, and reverse transcription was performed using PrimeScript RT Master Mix (TaKaRa, Otsu, Japan) and subjected to SYBR Green quantitative real-time PCR using PCR Master Mix (Life Technologies) following the manufacturers' instructions. q-PCR were performed on an ABI 7500 real-time PCR system (Applied Biosystems, Waltham, MA, USA). The specific primer sequences are described in Table 1. Quantitative Real-time (qRT) PCR was utilized to measure the target genes' mRNA expression level. In brief, two L4-6 DRG tissues were harvested from each rat, and Triazole was added. Then, the tissues were disrupted with a high-speed mechanical homogenizer. The homogenates were centrifuged for 15 min at room temperature. The supernatant was mixed with 500 uL of isopropanol and centrifuged for 10 minutes. Then, the supernatant was discarded, and 75% ethanol was added, followed by centrifugation (5 minutes). The process was repeated once, and then samples were slightly dried at 4degC. Then, 30 uL of RNase-free water was added to each sample, and the RNA concentration was determined by a spectrophotometer. Reverse transcriptase (RT-PCR kit) was used to reverse-transcribe RNA to produce cDNA from the RNA template. The primers at a concentration of 4 μm were added to the corresponding PCR reaction solution to form a total reaction volume of 25 μl and start the Real-Time PCR reaction. The Real-Time PCR amplification and dissolution curves were checked, and the relative expression was calculated using the 2^{-Ct} method. The qPCR data were analyzed using SPSS software. The levels of expression were normalized to the actin levels.

2.10. Western Blot

Two L4-6 dorsal root ganglion tissues were harvested from each rat, mixed with RIPA lysis solution, and the tissues were disrupted with a high-speed mechanical homogenizer to obtain the supernatant. Then, the protein concentration was determined using the BCA protein kit. The protein solution was added to SDS-PAGE gel for electrophoretic separation. The separated proteins were transferred onto PVDF membranes (Millipore, USA). The membranes were blocked in 5% skimmed milk and were placed in 3 ml anti-PDX1 primary antibodies (1:1000 Bcl-2, 1:1000 Bcl-2, 1:1000PI3K, 1:1000Akt, 1: 10000 GAPDH) at 4 °C with gentle shaking, overnight. After washing with TBS, the secondary HRP conjugated antibody was incubated for 2 h at room temperature. Chemiluminescent images of immunostained bands on the membranes were recorded on X-ray films using the enhanced chemiluminescence (ECL, FUDE, FD8020, China) system according to the manufacturer's instructions.The gray values were scanned and examined with ImageJ utilizing a professional image analysis system.

2.11 Statistical analysis

The experiments were performed in triplicate. All results were plotted as the MEAN ± SEM for each experimental group's number of participants (n). Statistical analysis was performed using SPSS version 20

statistical software package. One-way analysis of variance (ANOVA) was used to check the significance of the results. Statistical significance was defined as a probability value, p value of 0.05.

3. RESULTS

3.1 General observations and animal behaviors

During this study, skin on the feet and other parts of the rats exposed to HXTB formula showed no abnormality. The abnormal activity in rats due to peripheral neuropathy induced by oxaliplatin was observed. Erythema, rash, edema, breakage, and skin color were also normal. The body weight of each group of rats was determined (Figure 1A). On day 49^{th}, the multiple comparison analysis revealed (Table 2) that the body weight of the rats in the normal control group was identical to that of the rats in the model control group and the HXTB recipe bath group. There was no significant difference between the model control group and the HXTB recipe bath group ($P>0.05$). Blood analysis revealed normal liver, kidney, and myocardial functions. No liver, renal, or cardiac damage was observed.

The Mechanical Withdrawal Threshold (MWT) and Cold-stimulated Paw Withdrawal Reflex (CPWR) were mainly observed in behavioral experiments. Based on the findings of multiple comparisons (Figure 1B), both the model group and the HXTB recipe bath group showed increased MWT during the model development compared to the control group (0-28 days). The cumulative MWT in the model group was decreased significantly for touch and hyperalgesia. By the end of the 28th day (Table 3), compared to the model and HXTB groups, the MWT value of the control group was significantly increased ($P <0.05$). From the first week (35th day) after administration, the MWT of the HXTB recipe bath group increased gradually. On day 49^{th} day (Table 4), MWT was comparable to that of the control group ($P=0.659>0.05$). However, the model control group recovered slowly following the discontinuation of oxaliplatin administration. The model group's MWT significantly differed from the other two groups after the trial ($P<0.05$).

Multiple comparisons revealed that the CPWR in the control group, the model control group, and the HXTB recipe bath group gradually increased during the model's induction (Figure 1C) (0-28 days). At the end of the oxaliplatin treatment during model development on the 28th day, there was an increase in CPWR that was significantly different from the control group (Table 5) ($P<0.05$). The CPWR of the HXTB recipe bath group, on the other hand, gradually decreased on the 35th day after administration. By the end of the experiment on the 49th day (Table 6), the HXTB recipe bath group exhibited similar results as the control group ($P=0.737> 0.05$), which was significantly different from the model control group ($P=0.0050$ $P<0.05$). The model group could not recover after discontinuing oxaliplatin, and there was no significant reduction in CPWR, which was significantly different from the other two groups at the end of the experiment ($P<0.05$).

3.2 The effect of HXTB formula on ratL4-6 dorsal root ganglion

H&E staining was performed to examine the effect of HXTB on the L4-6 dorsal root ganglia. As demonstrated in Figure 2a and Figure 2d, the neurons in the control and HXTB bath groups were numerous and distributed evenly, with conspicuous nucleocytoplasmic demarcation, obvious nucleoli, and no apparent inflammation. However, in the model group, neuronal cell bodies were more swollen (Fig. 2b). Under higher magnification (Fig. 2c), we can observe the gap between the cells (black arrows), and some neuron cell bodies and nuclei disappeared as a result of cytoplasmic lysis represented by red arrows. Also, satellite cells were reduced around neuronal cell bodies (yellow arrows), and an increase in nerve fiber demyelination was observed.

3.3 Screening results of active ingredients and target genes of HXTB formula

The TCMSP database yielded 665 chemical constituents, including 12 types of AR, 192 types of ARER, 18 types of CAC, 220 types of CR, and 223 types of ADBEH. There are 3 types of active ingredients AR, 8 types of ARER, 3 types of CAC, 7 types of CR, 10 types of ADBEH, including 3 types of common ingredients, for a total of 27 types of active ingredients (Table 7). According to the 27 active ingredients evaluated, 289 targets were acquired from the TCMSP database. After eliminating the common targets, 141 new targets remained.

3.4 Prediction of the effect of HXTB Recipe on CIPN

The GeneCard database yielded a total of 375 target genes associated with CIPN. The acquired genes were compared with the screened target genes of chemical constituents to determine the intersection (Figure 3A). For the therapy of CIPN, HXTB yielded a total of 35 targets. 8 active ingredients that could not correspond to the common targets were eliminated by comparison. The obtained 35 common targets and 19 active ingredients of TCM were processed through Cytoscape v3.7.0, and the "disease-traditional Chinese medicine-active ingredients-target" network diagram was constructed (Figure 3B). The blue nodes represent active ingredients, the yellow nodes represent common illness and drug targets, and the grey lines represent the interaction between active ingredients and disease targets. As illustrated in the figure, each active ingredient correlates to many targets. Each target gene corresponds to several active ingredients, reflecting the complexity of HXTB Recipe's mechanism of action in treating CIPN. Then, using the String website, 35 protein targets were assessed by the PPI of target genes, a PPI network was formed (Figure 3C), and the top 20 protein targets with the highest correlation in the network were screened (see Figure 3D).

3.5 GO enrichment and KEGG for molecular mechanism analysis

The GO enrichment analysis's findings are revealed in Figure 4A. Numerous enriched genes were found in the biological process (BP) analysis, such as those that respond to lipopolysaccharide, molecules of bacterial origin, steroid hormones, etc. There were many enriched genes in the analysis of cellular components (CC), including membrane raft, membrane microdomain, membrane region, and others. Similarly, numerous enriched genes were identified using molecular function (MF) analysis, such as those involved in binding tumor necrosis factor receptor superfamily members, steroids, and cytokine receptors. Based on the findings of a KEGG pathway enrichment analysis, the pathways that demonstrate the highest levels of correlation and enrichment are primarily the AGE-RAGE signaling pathway, the TNF signaling pathway, the C-type lectin receptor signaling pathway, and apoptosis (Figure 4B). After conducting additional study on the pathway maps of the abovementioned pathways and considering the most crucial targets in the protein interaction network, the three pathways of PI3K-Akt, JNK/MAPK, and COX-2 (PTGS2) may be most closely associated with HXTB Recipe's potential to treat CIPN.

3.6 The effect of HXTB on the mRNA and protein expression levels

From the findings, the mRNA expression of Akt1 and Akt3 was significantly decresed in the model group in comparison of the control and HXTB recipe bath group (Figure 5A). There was significant differences observed in the PI3K index among all groups ($P<0.05$). There was no significant difference ($P>0.05$) observed between the control group and the HXTB bath group (Table 8). Akt3, MAPK8, and PTGS2 were upregulated, but there was no difference between the HXTB bath group and the control group ($P>0.05$). Increased PI3K, Akt1, and Akt3 can inhibit cell apoptosis. The mRNA expression of Bcl-2 determines whether nerve cells are affected by the inhibitory apoptosis pathway. The expression levels of Bcl-2 in the three groups were all significantly different ($P<0.05$), as shown in Figure 5B, Table 9. The protein expression levels of PI3K, Akt (phospho S473), and Bcl-2 were significantly different between the HXTB recipe bath group and the model group ($P <0.05$).

4. Discussion

CIPN is a common side effect of platinum-containing chemotherapeutics such as oxaliplatin. Oxaliplatin can cause neuronal cell damage by directly affecting several genes and pathways, such as the Bcl-2 family on the apoptosis pathway, and activating the expression of p38, ERK1/2, and JNK/Sapk in the MAPK signaling pathway, resulting in neuronal apoptosis (26). The dorsal root ganglion is the predominant site of action for platinum-induced neurotoxicity (27). Platinum drugs can inhibit rRNA synthesis in neurons' nuclei, thus reducing the ability of protein synthesis, resulting in abnormal organelles and morphological changes in neurons (28).

Previously, TCM was employed for treating CIPN (29). Also, topical treatment with Tong-Luo-San-Jie alleviated and significantly restored PWL and MWT (30). Similarly, herein, similar to previous findings, we

revealed using HXTB, tactile sensation and hyperalgesia were gradually improved and cumulative MWT was increased and CPWR were decreased significantly in oxaliplatin-induced peripheral neuropathy (Figure 1B-C).

Accumulating platinum compounds such as oxaliplatin in the dorsal root ganglia of the peripheral nervous system is believed to be the main mechanism of OXIPN, causing apoptosis and, eventually neuronal damage (22). Similarly, herein, the H&E sections of the rat dorsal root ganglion were examined, and it was observed that the dorsal root ganglion cells of the oxaliplatin rat model, without the intervention of the HXTB recipe, were significantly damaged (22). Previously, Danggui Sini decoction (TCM) protected against neurotoxicity by oxaliplatin in rats by suppressing inflammatory lesions and improving ultra-microstructures (31). Herein, the morphology of neurons in the dorsal root ganglia of rats treated with the HXTB recipe tended to be normal, with no obvious cell damage (Figure 2).

HXTB contains herbs like; Chuanwu, Asarum, Chuanmutong, Guizhi, and Angelica (20). Previously, the neoconitine in Chuanwu showed the anti-nociceptive effect on the reticular nucleus of giant cells in rats. Also, benzoylneaconitine in aconitum showed a dose-dependent anti-nociceptive effect on neurons (32, 33), and diterpene alkaloids exhibited analgesic properties by modulating opioid receptors (34). Asarum contains methyl eugenol, which has been reported anesthetic, anticonvulsant, and analgesic component (35-37). Chuanmutong lignans display anti-neuritic activity, thus, inhibiting the release of inflammatory factors NO and TNF in the inflammatory response of BV-2 cells induced by lipopolysaccharide (37). Similarly, the extract of cinnamon twigs can reduce neuro-inflammation by downregulating the TLR4/MyD88 signaling pathway and decreasing the release of NO, IL-6, IL-1, and TNF (37, 38). Angelica dahurica's furanocoumarin can regulate the TRPV1 channel, significantly inhibits the neurotoxicity of rats caused by formalin, and inhibits pain (39). Additionally, it can eradicate ROS by activating the PI3K/Akt/NF- pathway, reducing the damage caused by oxidative stress and exert cytoprotective effects (40-42). These five TCM of the HXTB formula can reduce nerve cell damage, offer additional analgesia, and have anti-inflammatory properties.

The TCMSP database is more than just a repository of data. It includes tools for visualizing and analyzing TCM findings at the network level. A systematic and multi-target drug discovery approach could generate new candidates with improved therapeutic features (43). The TCMSP was developed using the framework of systems pharmacology for TCM to identify active ingredients in HXTB. We found the active ingredients of HXTB using TCMSP. 27 active ingredients were evaluated, and 289 targets were determined from the TCMSP database for each ingredient (Table 8). The disease-related target genes can be identified using the GeneCards (44). The GeneCard database yielded 375 targets, and HXTB had 35 CIPN-associated targets (Figure 3A). Cytoscape is a user-friendly and open bioinformatics platform with exceptional performance in both virtualization and manipulation (45). The disease-TCM-active ingredients-target network diagram was constructed with Cytoscape for the active ingredients, targets, and ingredient-target interactions (Figure 3B). The STRING database is used to collect, store, and integrate all publicly available sources of information on protein-protein interactions and replenish these with computational predictions (46). 35 protein targets were assessed, the PPI network was formed (Figure 3C), and the top 20 protein targets with the highest correlation in the network were screened (Figure 3D). GO, and KEGG pathway enrichment analyses were used to determine the molecular mechanisms and therapeutic effects HXTB (47). Several enriched genes involved in binding tumor necrosis factor receptor superfamily members, steroids, lipopolysaccharides and cytokine receptors were determined, etc. Following identifying these components that can protect nerve cells, we identified three pathways such as PI3K-Akt, JNK/MAPK, and COX-2 (PTGS2), most likely to affect nerve cells by HXTB recipe (Figure 4B). The mRNA expression levels of PI3K/Akt, MAPK8, and PTGS2 were determined at the molecular level. Previously, resveratrol prevented the CIPN by up-regulating PI3K/Akt signaling pathway (48). Herein, the mRNA expression levels of PI3K, Akt1, and Akt3 were significantly up-regulated, while MAPK8 and PTGS2 were unaffected (Figure 5A-B). The protein expression levels of PI3K and Akt (phospho S473) were significantly increased, conformed to the mRNA expression trend (Figure C-D). The high expression levels PI3K, Akt1, and Akt3. Akt3, MAPK8, and PTGS2 inhibit cell apoptosis (49). Also, the PI3K/Akt pathway regulates cell survival, apoptosis, and differentiation and has a vital role in biological processes by regulating the Bcl-2 apoptosis-related family proteins. Several studies have

demonstrated that activating the PI3K/Akt pathway can protect nerve cells (50-54) and reduce oxidative damage to nerve cells (54, 55). The findings suggest that the HXTB could inhibit neuronal cell apoptosis in the dorsal root ganglia via the PI3K/Akt pathway, thus ameliorating improving oxaliplatin-induced peripheral neuropathy, restoring normal function of dorsal ganglion.

5. CONCLUSION

According to the findings of this study, the HXTB recipe can significantly improve oxaliplatin-induced peripheral neuropathy in rats, reduce the phenomenon of touch and hyperalgesia, and improve as well as reduce the damage caused by oxaliplatin to peripheral neurons.The inhibition of neuronal apoptosis mediated by the PI3K/Akt pathway and its downstream Bcl-2 protein demonstrates that Bcl-2 apoptosis family proteins are significant factors affecting peripheral nerve damage caused by oxaliplatin, which can be further investigated in the future. Also, the PI3K/Akt and Bcl-2 pathways will enable us to understand better oxaliplatin-induced nerve damage. HXTB formula yielded high expression levels of high expression levels PI3K, Akt1, and Akt3. Akt3 at mRNA and mRNA levels, demonstrating the anti-apoptotic effects on the neurons. HXTB is TCM in origin and can play a great role in treating CIPN. Further research on the treatment mechanism of HTXB components will help create new therapeutic approaches for treating CIPN which will help reduce the pain and improve the patient's quality of life.

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6. Conflict of Interest

The authors declare no conflicts of interest in this work.

REFERENCES

1. Argyriou AA, Bruna J, Marmiroli P, Cavaletti G. Chemotherapy-induced peripheral neurotoxicity (CIPN): an update. Critical reviews in oncology/hematology. 2012;82(1):51-77.

2. Persson AK, Hoeijmakers JGJ, Estacion M, Black JA, Waxman SG. Sodium Channels, Mitochondria, and Axonal Degeneration in Peripheral Neuropathy. Trends in molecular medicine. 2016;22(5):377-90.

3. Staff NP, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: A current review. Ann Neurol. 2017;81(6):772-81.

4. Arnold M, Rutherford MJ, Bardot A, Ferlay J, Andersson TM, Myklebust T, et al. Progress in cancer survival, mortality, and incidence in seven high-income countries 1995-2014 (ICBP SURVMARK-2): a population-based study. The Lancet Oncology. 2019;20(11):1493-505.

5. Wilson RH, Lehky T, Thomas RR, Quinn MG, Floeter MK, Grem JL. Acute oxaliplatin-induced peripheral nerve hyperexcitability. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(7):1767-74.

6. Sittl R, Lampert A, Huth T, Schuy ET, Link AS, Fleckenstein J, et al. Anticancer drug oxaliplatin induces acute cooling-aggravated neuropathy via sodium channel subtype Na(V)1.6-resurgent and persistent current. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(17):6704-9.

7. Argyriou AA. Updates on Oxaliplatin-Induced Peripheral Neurotoxicity (OXAIPN). Toxics. 2015;3(2):187-97.

8. Jamieson SM, Liu J, Connor B, McKeage MJ. Oxaliplatin causes selective atrophy of a subpopulation of dorsal root ganglion neurons without inducing cell loss. Cancer Chemother Pharmacol. 2005;56(4):391-9.

9. Tsutsumi K, Yamashita Y, Ushio S, Kawashiri T, Kaname T, Fujita S, et al. Oxaliplatin induces hypomyelination and reduced neuregulin 1 expression in the rat sciatic nerve. Neuroscience research. 2014;80:86-90.

10. Argyriou AA, Polychronopoulos P, Iconomou G, Chroni E, Kalofonos HP. A review on oxaliplatin-induced peripheral nerve damage. Cancer treatment reviews. 2008;34(4):368-77.

11. Colvin LA. Chemotherapy-induced peripheral neuropathy: where are we now? Pain. 2019;160 Suppl 1(Suppl 1):S1-s10.

12. Grammatico S, Cesini L, Petrucci MT. Managing treatment-related peripheral neuropathy in patients with multiple myeloma. Blood and lymphatic cancer : targets and therapy. 2016;6:37-47.

13. Hu LY, Mi WL, Wu GC, Wang YQ, Mao-Ying QL. Prevention and Treatment for Chemotherapy-Induced Peripheral Neuropathy: Therapies Based on CIPN Mechanisms. Current neuropharmacology. 2019;17(2):184-96.

14. Hochster HS, Grothey A, Childs BH. Use of calcium and magnesium salts to reduce oxaliplatin-related neurotoxicity. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007;25(25):4028-9.

15. Gamelin L, Boisdron-Celle M, Morel A, Poirier AL, Berger V, Gamelin E, et al. Oxaliplatin-related neurotoxicity: interest of calcium-magnesium infusion and no impact on its efficacy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(7):1188-9; author reply 9-90.

16. Loprinzi CL, Qin R, Dakhil SR, Fehrenbacher L, Flynn KA, Atherton P, et al. Phase III randomized, placebo-controlled, double-blind study of intravenous calcium and magnesium to prevent oxaliplatin-induced sensory neurotoxicity (N08CB/Alliance). Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(10):997-1005.

17. Albers JW, Chaudhry V, Cavaletti G, Donehower RC. Interventions for preventing neuropathy caused by cisplatin and related compounds. The Cochrane database of systematic reviews. 2014(3):Cd005228.

18. Hershman DL, Lacchetti C, Loprinzi CL. Prevention and Management of Chemotherapy-Induced Peripheral Neuropathy in Survivors of Adult Cancers: American Society of Clinical Oncology Clinical Practice Guideline Summary. Journal of oncology practice. 2014;10(6):e421-e4.

19. Cascella M, Muzio MR. Potential application of the Kampo medicine goshajinkigan for prevention of chemotherapy-induced peripheral neuropathy. Journal of integrative medicine. 2017;15(2):77-87.

20. Weili T, Ying W. Clinical observation on effect of Chinese herbal fumigation and washing with Xiwenjing Huoxue Tongluo Decoction on limb numbness after chemotherapy for malignant tumor. 2021;7(6):22-5.

21. Tveden-Nyborg P, Bergmann TK, Jessen N, Simonsen U, Lykkesfeldt J. BCPT policy for experimental and clinical studies. Basic & clinical pharmacology & toxicology. 2021;128(1):4-8.

22. Holmes J, Stanko J, Varchenko M, Ding H, Madden VJ, Bagnell CR, et al. Comparative neurotoxicity of oxaliplatin, cisplatin, and ormaplatin in a Wistar rat model. Toxicological sciences : an official journal of the Society of Toxicology. 1998;46(2):342-51.

23. Zheng FY, Xiao WH, Bennett GJ. The response of spinal microglia to chemotherapy-evoked painful peripheral neuropathies is distinct from that evoked by traumatic nerve injuries. Neuroscience. 2011;176:447-54.

24. Jasmin L, Kohan L, Franssen M, Janni G, Goff JR. The cold plate as a test of nociceptive behaviors: description and application to the study of chronic neuropathic and inflammatory pain models. Pain. 1998;75(2-3):367-82.

25. Liu H, Wang J, Zhou W, Wang Y, Yang L. Systems approaches and polypharmacology for drug discovery from herbal medicines: an example using licorice. Journal of ethnopharmacology. 2013;146(3):773-93.

26. Scuteri A, Galimberti A, Maggioni D, Ravasi M, Pasini S, Nicolini G, et al. Role of MAPKs in platinum-induced neuronal apoptosis. Neurotoxicology. 2009;30(2):312-9.

27. Jongen JL, Broijl A, Sonneveld P. Chemotherapy-induced peripheral neuropathies in hematological malignancies. Journal of neuro-oncology. 2015;121(2):229-37.

28. Yan F, Liu JJ, Ip V, Jamieson SM, McKeage MJ. Role of platinum DNA damage-induced transcriptional inhibition in chemotherapy-induced neuronal atrophy and peripheral neurotoxicity. Journal of neurochemistry. 2015;135(6):1099-112.

29. Li QY, Cai FH, Lu Y, Liu H, Wang X, Li FL, et al. External Treatment With Chinese Herbal Medicine for Chemotherapy-Induced Peripheral Neuropathy: A Systematic Review and Meta-Analysis. Frontiers in pharmacology. 2022;13:764473.

30. Wang J, Zhang R, Dong C, Jiao L, Xu L, Liu J, et al. Topical treatment with Tong-Luo-San-Jie gel alleviates bone cancer pain in rats. J Ethnopharmacol. 2012;143(3):905-13.

31. Ding R, Wang Y, Zhu J-P, Lu W-G, Wei G-L, Gu Z-C, et al. Danggui Sini decoction protects against oxaliplatin-induced peripheral neuropathy in rats. 2020;19(4):663-71.

32. Hikino H, Murayama M. Mechanism of the antinociceptive action of mesaconitine: participation of brain stem and lumbar enlargement. British journal of pharmacology. 1985;85(3):575-80.

33. Suzuki Y, Oyama T, Ishige A, Isono T, Asami A, Ikeda Y, et al. Antinociceptive mechanism of the aconitine alkaloids mesaconitine and benzoylmesaconine. Planta Med. 1994;60(5):391-4.

34. Nesterova YV, Povet'yeva TN, Suslov NI, Zyuz'kov GN, Pushkarskii SV, Aksinenko SG, et al. Analgesic activity of diterpene alkaloids from Aconitum baikalensis. Bulletin of experimental biology and medicine. 2014;157(4):488-91.

35. Sell AB, Carlini EA. Anesthetic action of methyleugenol and other eugenol derivatives. Pharmacology. 1976;14(4):367-77.

36. Sayyah M, Valizadeh J, Kamalinejad M. Anticonvulsant activity of the leaf essential oil of Laurus nobilis against pentylenetetrazole- and maximal electroshock-induced seizures. Phytomedicine : international journal of phytotherapy and phytopharmacology. 2002;9(3):212-6.

37. Yano S, Suzuki Y, Yuzurihara M, Kase Y, Takeda S, Watanabe S, et al. Antinociceptive effect of methyleugenol on formalin-induced hyperalgesia in mice. European journal of pharmacology. 2006;553(1-3):99-103.

38. Yang H, Cheng X, Yang YL, Wang YH, Du GH. Ramulus Cinnamomi extract attenuates neuroinflammatory responses via downregulating TLR4/MyD88 signaling pathway in BV2 cells. Neural regeneration research. 2017;12(11):1860-4.

39. Chen X, Sun W, Gianaris NG, Riley AM, Cummins TR, Fehrenbacher JC, et al. Furanocoumarins are a novel class of modulators for the transient receptor potential vanilloid type 1 (TRPV1) channel. The Journal of biological chemistry. 2014;289(14):9600-10.

40. Kang U, Han AR, So Y, Jin CH, Ryu SM, Lee D, et al. Furanocoumarins from the Roots of Angelica dahurica with Inhibitory Activity against Intracellular Reactive Oxygen Species Accumulation. Journal of natural products. 2019;82(9):2601-7.

41. Wang KS, Lv Y, Wang Z, Ma J, Mi C, Li X, et al. Imperatorin efficiently blocks TNF-α-mediated activation of ROS/PI3K/Akt/NF-ϰB pathway. Oncology reports. 2017;37(6):3397-404.

42. Lee MY, Lee JA, Seo CS, Ha H, Lee H, Son JK, et al. Anti-inflammatory activity of Angelica dahurica ethanolic extract on RAW264.7 cells via upregulation of heme oxygenase-1. Food and chemical toxicology : an

international journal published for the British Industrial Biological Research Association. 2011;49(5):1047-55.

43. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. Journal of cheminformatics. 2014;6:13.

44. Hong J, Ding J, Hong HH, Xu XW, Pan B, Ruan Y, et al. Identifying the Mechanism of Polygoni Cuspidati Rhizoma et Radix in Treating Acute Liver Failure Based on Network Pharmacology and Molecular Docking. Gastroenterology research and practice. 2022;2022:2021066.

45. Li M, Li D, Tang Y, Wu F, Wang J. CytoCluster: A Cytoscape Plugin for Cluster Analysis and Visualization of Biological Networks. International journal of molecular sciences. 2017;18(9).

46. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic acids research. 2019;47(D1):D607-d13.

47. Li Y, Wang L, Xu B, Zhao L, Li L, Xu K, et al. Based on Network Pharmacology Tools to Investigate the Molecular Mechanism of Cordyceps sinensis on the Treatment of Diabetic Nephropathy. Journal of diabetes research. 2021;2021:8891093.

48. Li X, Yang S, Wang L, Liu P, Zhao S, Li H, et al. Resveratrol inhibits paclitaxel-induced neuropathic pain by the activation of PI3K/Akt and SIRT1/PGC1α pathway. J Pain Res. 2019;12:879-90.

49. Li N, Yang F, Liu D-Y, Guo J-T, Ge N, Sun S-Y. Scoparone inhibits pancreatic cancer through PI3K/Akt signaling pathway. World J Gastrointest Oncol. 2021;13(9):1164-83.

50. Sisalli MJ, Secondo A, Esposito A, Valsecchi V, Savoia C, Di Renzo GF, et al. Endoplasmic reticulum refilling and mitochondrial calcium extrusion promoted in neurons by NCX1 and NCX3 in ischemic preconditioning are determinant for neuroprotection. Cell death and differentiation. 2014;21(7):1142-9.

51. Limbourg FP, Huang Z, Plumier JC, Simoncini T, Fujioka M, Tuckermann J, et al. Rapid nontranscriptional activation of endothelial nitric oxide synthase mediates increased cerebral blood flow and stroke protection by corticosteroids. The Journal of clinical investigation. 2002;110(11):1729-38.

52. Abbruzzese G, Morón-Oset J, Díaz-Castroverde S, García-Font N, Roncero C, López-Muñoz F, et al. Neuroprotection by Phytoestrogens in the Model of Deprivation and Resupply of Oxygen and Glucose In Vitro: The Contribution of Autophagy and Related Signaling Mechanisms. Antioxidants (Basel, Switzerland). 2020;9(6).

53. Zhao H, Mitchell S, Koumpa S, Cui YT, Lian Q, Hagberg H, et al. Heme Oxygenase-1 Mediates Neuroprotection Conferred by Argon in Combination with Hypothermia in Neonatal Hypoxia-Ischemia Brain Injury. Anesthesiology. 2016;125(1):180-92.

54. Zhou J, Zhang S, Zhao X, Wei T. Melatonin impairs NADPH oxidase assembly and decreases superoxide anion production in microglia exposed to amyloid-beta1-42. Journal of pineal research. 2008;45(2):157-65.

55. Hao T, Rockwell P. Signaling through the vascular endothelial growth factor receptor VEGFR-2 protects hippocampal neurons from mitochondrial dysfunction and oxidative stress. Free radical biology & medicine. 2013;63:421-31.

Table 1 Primer sequences

Primer Name	Forward Sequence	Reverse Sequence
PI3K	CTGGAGAGCTTGGAGGACGA	TCGCAAGAACCAGAATAAGAAGTG
Akt1	AATACCTGGTGTCGGTCTCA	TCGAGCTCATCCTAATGGAG
Akt2	GGCCCCGGTACTTCCTTC	TAGCCCGTATCCACTCTTCCCTCTC
Akt3	TGGCACCAGAGGTATTAGAAG	TATCAAGAGCCCTGAAAGCAA

Primer Name	Forward Sequence	Reverse Sequence
MAPK8	CCACCACCAAAGATCCCTGACAAG	TCATCTACAGCAGCCCAGAGGTC
PTGS2	TGTATCCCGCCCTGCTGGTG	CGTTGATGGTGGCTGTCTTGGTAG
Bcl-2	ACGGTGGTGGAGGAACTCTTCAG	TTCAGAGACAGCCAGGAGAAATCAAAC

Table 2 Multiple comparisons of the body weight of rats on the 49th day (the end of the experiment)

Group	**Mean Diff.**	**95.00% CI of diff.**	**95.00% CI of diff.**	**P Value**
Control vs. Model	98.27	41.8131	154.7297	0.002
Control vs. HXTB	99.37	42.9131	155.8297	0.002
Model vs. HXTB	1.100	-55.3583	57.5583	0.968

Table 3 Multiple comparisons of the thresholds of mechanically stimulated paw withdrawal reflex in rats on day 28 (end of modeling)

Group	**Mean Diff.**	**95.00% CI of diff.**	**95.00% CI of diff.**	**P Value**
Control vs. Model	15.4839	13.07214	17.89563	0.000
Control vs. HXTB	15.9974	13.58562	18.40912	0.000
Model vs. HXTB	0.5135	-1.89826	2.92523	0.675

Table 4 Multiple comparisons of the withdrawal reflex threshold of mechanical stimulation on the 49th day (the end of the experiment) in rats

Group	**Mean Diff.**	**95.00% CI of diff.**	**95.00% CI of diff.**	**P Value**
Control vs. Model	7.6225	5.02288	10.22212	0.000
Control vs. HXTB	0.5835	-2.01612	3.18312	0.659
Model vs. HXTB	-7.0390	-9.63862	-4.43938	0.000

Table 5 Multiple comparisons of cold-stimulated-induced withdrawal reflexes on day 28 (end of modeling) in rats

Group	**Mean Diff.**	**95.00% CI of diff.**	**95.00% CI of diff.**	**P Value**
Control vs. Model	-18.5714	-27.03091	-10.11195	0.000
Control vs. HXTB	-11.1429	-19.60234	-2.68338	0.013
Model vs. HXTB	7.4286	-1.03091	15.88805	0.082

Table 6 Multiple comparisons of cold-stimulated-induced withdrawal reflexes on day 49 (end of experiment) in rats

Group	**Mean Diff.**	**95.00% CI of diff.**	**95.00% CI of diff.**	**P Value**
Control vs. Model	-12.0000	-19.05150	-4.94850	0.002
Control vs. HXTB	-1.1429	-8.19436	5.90864	0.737

Group	Mean Diff.	95.00% CI of diff.	95.00% CI of diff.	P Value
Model vs. HXTB	10.8571	3.80564	17.90864	0.005

Table 7 Active Ingredients of HXTB Formula

ID	Name
MOL002086	1-[(5R,8R,9S,10S,12R,13S,14S,17S)-12-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydr
MOL002087	delta4,16-Androstadien-3-one
MOL000538	Hypaconitine
MOL012140	4,9-dimethoxy-1-vinyl-$b-carboline
MOL012141	Caribine
MOL001460	Cryptopin
MOL001558	Sesamin
MOL002501	[(1S)-3-[(E)-but-2-enyl]-2-methyl-4-oxo-1-cyclopent-2-enyl] (1R,3R)-3-[(E)-3-methoxy-2-methyl-3-oxoprop-1-e
MOL002962	(3S)-7-hydroxy-3-(2,3,4-trimethoxyphenyl)chroman-4-one
MOL000422	Kaempferol
MOL009849	ZINC05223929
MOL000358	beta-sitosterol
MOL000359	Sitosterol
MOL000449	Stigmasterol
MOL001736	(-)-taxifolin
MOL000492	(+)-catechin
MOL000073	ent-Epicatechin
MOL004576	Taxifolin
MOL011169	Peroxyergosterol
MOL001494	Mandenol
MOL002883	Ethyl oleate (NF)
MOL005802	propyleneglycol monoleate
MOL000953	CLR
MOL001506	Supraene
MOL001749	ZINC03860434
MOL003791	Linolein, 2-mono-
MOL007514	methyl icosa-11,14-dienoate

Table 8 Multiple comparison of mRNA expression of PI3K, Akt1, Akt2, Akt3, MAPK8, PTGS2, Bcl-2 in rat L4-6 dorsal root ganglion

Target	Group	Mean Diff.	95.00% CI of diff.	P
PI3K	Control vs. Model	-0.6389	-1.1152 to -0.1626	0.017
	Control vs. HXTB	-1.356	-1.8319 to -0.8793	0.000
	Model vs. HXTB	-0.7167	-1.1930 to -0.2404	0.010
Akt1	Control vs. Model	0.2314	0.0608 to 0.4020	0.016
	Control vs. HXTB	-0.0900	-0.2606 to 0.0806	0.244
	Model vs. HXTB	-0.3214	-0.4921 to -0.1509	0.004
Akt2	Control vs. Model	-0.1725	-0.2273 to -0.1177	0.000
	Control vs. HXTB	-0.2136	-0.2683 to -0.1588	0.000
	Model vs. HXTB	-0.0411	-0.0958 to 0.0137	0.116
Akt3	Control vs. Model	0.2821	0.1090 to 0.4552	0.007

Target	Group	Mean Diff.	95.00% CI of diff.	P
	Control vs. HXTB	-0.1178	-0.2909 to 0.0553	0.147
	Model vs. HXTB	-0.3999	-0.5730 to -0.2268	0.001
MAPK8	Control vs. Model	0.3315	0.2279 to 0.4352	0.000
	Control vs. HXTB	0.2404	0.1368 to 0.3441	0.001
	Model vs. HXTB	-0.0910	-0.1948 to 0.0126	0.075
PTGS2	Control vs. Model	0.4138	0.2006 to 0.6272	0.003
	Control vs. HXTB	0.2277	0.0145 to 0.4411	0.040
	Model vs. HXTB	-0.1861	-0.3994 to 0.0272	0.077
Bcl-2	Control vs. Model	0.7725	0.5371 to 1.0079	0.000
	Control vs. HXTB	0.3620	0.1266 to 0.5974	0.009
	Model vs. HXTB	-0.4105	-0.6459 to -0.1751	0.005

Table 9 Multiple comparison of PI3K, Akt (phospho S473) and Bcl-2 protein expression in rat L4-6 dorsal root ganglia

Target	Group	Mean Diff.	95.00% CI of diff.	P
PI3K	Control vs. Model	-0.1163	-0.5299 to 0.2974	0.517
	Control vs. HXTB	-0.5696	-0.9833 to -0.1559	0.015
	Model vs. HXTB	-0.4533	-0.8670 to -0.0396	0.036
Akt1(phospho S473)	Control vs. Model	0.1078	-0.1214 to 0.3371	0.293
	Control vs. HXTB	-0.3107	-0.5399 to -0.0815	0.016
	Model vs. HXTB	-0.4186	-0.6478 to -0.1893	0.004
Bcl-2	Control vs. Model	0.2364	-0.0279 to 0.5009	0.071
	Control vs. HXTB	-0.1948	-0.4592 to 0.0696	0.122
	Model vs. HXTB	-0.4312	-0.6957 to -0.1668	0.007

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