Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Synergistic and Toxicity-Attenuating Effects of *Periplaneta americana* Extract CII-3 Combined with Cisplatin on Lewis Lung Cancer-Bearing Mice

Qiaoling Yang[1+], Mili Shi[1+], Guangming Liu[1,2], Fang Peng[1], Xiaobo Liu[1], Meixian Guo[1]

[1]Department of Pharmacology, College Of Pharmacy, Dali University, [2]Yunnan Key Laboratory of Insect Biomedicine Research and Development, Dali, People's Republic Of China

[+]The first two authors contributed equally to this work

Submitted: 18-Nov-2019 Revised: 14-Feb-2020 Accepted: 06-May-2020 Published: 16-Feb-2021

ABSTRACT

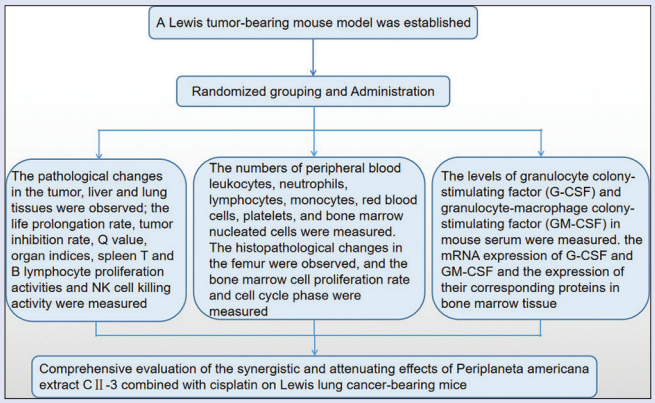
Objective: The objective was to study the synergistic and attenuating effects of CII-3 combined with cisplatin. **Materials and Methods:** A Lewis tumor-bearing mouse model was established. After 15 days of continuous administration of CII-3 and cisplatin, the pathological changes in the tumor, liver, lung, and femur tissues were observed; the life prolongation rate, tumor inhibition rate, Q value, organ indices, spleen T- and B-lymphocyte proliferation activities, NK cell killing activity, the bone marrow cell proliferation rate and cell cycle phase, and the number of peripheral blood cells and bone marrow nucleated cells were measured. The expression of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in mouse serum and bone marrow tissue was measured. **Results:** The combination of CII-3 and cisplatin could enhance the ability of cisplatin to inhibit tumor cell proliferation and protects the liver damage and femoral injury and could significantly increase the life prolongation rate; tumor inhibition rate of cisplatin; the liver, spleen, lung, and thymus indices; the T- and B-lymphocyte proliferation activity; the NK cell killing activity; and the number of peripheral blood cells and bone marrow nucleated cells. The combination of drugs can stimulate the transformation of bone marrow cells from S phase to G2/M phase, significantly increase the proliferation rate of bone marrow cells and the contents of G-CSF and GM-CSF in mouse serum, and downregulate the mRNA and protein levels of them in bone marrow tissue. **Conclusion:** These results suggest that CII-3 combined with cisplatin can significantly enhance the antitumor effect and reduce the toxicity and side effects of cisplatin in Lewis tumor-bearing mice.

Key words: Bone marrow suppression, *Periplaneta americana,* synergism and attenuation

SUMMARY

- *Periplaneta americana* extract CII-3 has the effect of synergism and toxicity reduction on tumor chemotherapy drugs.

Abbreviations used: RBCs: Red blood cells; WBCs: White blood cells; G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; CTX: Cyclophosphamide; GRA: Neutrophils; MON: Monocytes; PLT: Platelets; LYM: lymphocytes.



Correspondence:

Assoc Prof. Xiaobo Liu,
Department of Pharmacology,
College of Pharmacy, Dali University, Dali City,
Dali Bai Autonomous Prefecture, Yunnan Province,
China.
E-mail: yndlxb@126.com
Ms. Meixian Guo,
Laboratory Technician, Department of Pharmacology,
College of Pharmacy,
Dali University, Dali City, Dali Bai Autonomous
Prefecture, Yunnan Province, China.
E-mail: yndllyo@126.com
DOI: 10.4103/pm.pm_499_19

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Malignant tumors are one of the three major diseases in the world that harm human health and life. Together with surgery and radiotherapy, chemotherapy is one of the three major cancer treatment methods. In recent years, with the advent of highly effective anticancer drugs, chemotherapy has played an important role in the comprehensive treatment of malignant tumors. However, the antitumor activities of these powerful drugs can also cause different degrees of damage to normal tissues and often cause a series of *in vivo* adverse reactions.[1] Cisplatin is one of the most widely used chemotherapeutic drugs[2-5] and has been used to treat ovarian cancer, testicular cancer, uterine cancer, breast cancer, gastric cancer, brain cancer, head-and-neck cancer, lung cancer, and other solid cancers.[6-9] Although cisplatin has a strong anticancer effect, its use is limited by various side effects, such as neurotoxicity, nephrotoxicity, and ototoxicity, especially bone marrow

Cite this article as: Yang Q, Shi M, Liu G, Peng F, Liu X, Guo M. Synergistic and toxicity-attenuating effects of *Periplaneta americana* extract CII-3 combined with cisplatin on Lewis lung cancer-bearing mice. Phcog Mag 2020;16:817-29.

suppression.[10-13] Bone marrow suppression refers to the decrease in the activity of blood cell precursors in bone marrow. Red Blood cells (RBCs), White Blood Cells (WBCs), and platelets in the peripheral blood all originate from the proliferation and differentiation of hematopoietic stem cells in the bone marrow. As chemotherapeutic drugs target rapidly proliferating cells, hematopoietic stem cells are also severely affected, resulting in a decrease in the number of RBCs, WBCs, and platelets in the peripheral blood.[1] Moreover, bone marrow suppression often delays the time of chemotherapy, reduces the dosage of drugs, and even stops chemotherapy, which affects the anticancer effect. Therefore, it is very important to alleviate the serious adverse reactions caused by chemotherapeutic drugs in cancer patients and to find a safe and effective way to prevent and treat the toxic side effects of bone marrow suppression, which is also one of the hotspots of cancer clinical research in recent years. The effect of bone marrow suppression on hematopoietic stem cells is achieved through the influence of hematopoietic growth factors in the bone marrow hematopoietic microenvironment. In the course of cancer treatment, blood transfusion and a growth factor injection can accelerate hematopoietic recovery and improve bone marrow performance to a certain extent.[14] The common hematopoietic growth factors are granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF).[15] G-CSF and GM-CSF can positively regulate bone marrow hematopoiesis. Their specific mechanism is to enhance the proliferation, differentiation, and development of hematopoietic stem/progenitor cells into various types of hematopoietic cells. Moreover, G-CSF[16-17] and GM-CSF[18] are usually effective and safe when administered with a clinical supplementation of RBCs and platelets. Therefore, these two hematopoietic growth factors may be used to study the function to improve the hematopoietic system.

Traditional Chinese medicine, as an adjuvant therapy, has been used to alleviate the symptoms of advanced cancer.[19] Studies have shown that traditional Chinese medicine plays an important role in the whole process of cancer prevention and treatment, creating good conditions for chemotherapy, reducing side effects (such as bone marrow suppression), improving the quality of life of patients during chemotherapy, and reducing medical costs.[20-23] Therefore, the development and exploration of traditional Chinese medicine is of great significance to improve the efficacy of cancer chemotherapy.

Insects are the largest biological group in nature. There are more than twice as many medicinal insects as medicinal plants. Medicinal insects have a unique immune system, which provides a new resource for the development of natural medicines. *Periplaneta americana* is a winged cockroach, a subclass of Insecta and commonly known as a "cockroach." Its medicinal application began in Shennong's Herbal Classic. Modern pharmacological studies have shown that *P. americana* protects the stomach and liver, enhances immunity, promotes wound healing, and exerts antivirus and antitumor activities.[24-28] Our research team has studied the medicinal value of *P. americana* for more than 30 years. Our team has developed and produced a series of clinical medicines derived from *P. americana*, such as Kangfuxin oral liquid (Z51021834), Xinmailong injection (Z20060443), and Ganlong capsule (Z20050113) that have been approved by the China Food and Drug Administration. Using modern extraction and separation technologies and pharmacological research methods, we screened the antitumor activity component CII-3 from *P. americana*. The main components of CII-3 are small-molecule peptides. The Folin-phenol method stipulated by the national pharmacopeia is adopted to determine the total peptide content in CII-3 [Table 1]. The molecular weight of the main components of CII-3 was 62.17% from 12355Da ~ 612.6Da and 34.26% from 612.6Da ~ 181Da. According to our previous research, CII-3 combined with cyclophosphamide (CTX) can significantly enhance the antitumor effect and reduce the toxicity and side effects of CTX in H22 tumor-bearing mice and can improve the immune function and hematopoietic system function of the body. However, there is no systematic in-depth study on the synergistic and attenuating effects of CII-3 combined with chemotherapeutics in the treatment of lung cancer. Therefore, we planned to further explore the synergistic and toxicity-attenuating effects of *P. americana* extract CII-3 combined with cisplatin for the treatment of Lewis tumor-bearing mice; our experiments were based on the results of previous studies; we aimed to provide a scientific basis for the application of *P. americana* extract CII-3 in this field.

MATERIALS AND METHODS

Experimental cells and animals

The Lewis lung cancer mouse strain was purchased from Guangzhou Gino Co., Ltd. The Yac-1 cell line was preserved in our laboratory; 180 SPF C57BL/6 mice aged 6- to 8-week weeks (20 ± 2 g; half male and half female) were purchased from Hunan Slake Jingda Laboratory Animal Co., Ltd. All procedures were in accordance with the European Community Guidelines for Animal Experimentation. The animal experimentation studies were approved by the Ethics Committee on Animal Research in Dali University.

Preparation of reagents

First, 10 mg cisplatin (Qilu Pharmaceutics, Shandong, China) powder was mixed with 10 mL normal saline (NS) (Guizhou Tiandi Pharmaceutics, Guizhou, China) on an ultraclean workbench (Suzhou Antai Air Technology Co., Ltd., Jiangsu, China); this mixture was regarded as a high concentration mixture and was diluted to 1.5 mg, 3 mg, and 6 mg/kg with the same multiple of NS. Then, 0.25 g *P. americana* extract CII-3 (He Zhengchun Research Office of Dali University, Dali, China) was precisely weighed and dissolved in 50 mL of NS for a quick 100 mg/kg suspension of CII-3.

Cell culture and animal modeling

After routine resuscitation culture, Lewis cells were transferred to the third generation. When they grew to 80%–90% confluence, the cell suspension was prepared and counted. The cell density was 2×10^7 cells/mL, and 0.2 mL of the mixture was inoculated into the armpit of each C57BL/6 mouse for 15 days. The tumors were removed, and a certain concentration of cell suspension was prepared. A total of 0.2 mL of the cell suspension was inoculated into the right forearm armpit of each C57BL/6 mouse, and the cell suspension was subcultured once every 2 weeks until the third generation. Lewis lung cancer tumor-bearing mice were inoculated under the armpit for 15 days, and the mice were sacrificed by cervical dislocation. Under sterile conditions, the axillary tumor mass was removed and a 3×10^7 cells/mL cell suspension was prepared. A 0.2 mL cell suspension was inoculated under the right forearm armpit of each C57BL/6 mouse, and the Lewis tumor-bearing mouse solid tumor model was established.

Table 1: Total peptide content of CII-3

Batch number	20140828	20140829	20140830	Mean value
Total peptide content per capsule with excipients (mg/capsule)	62.44	62.47	62.08	62.33

Grouping and administration of Lewis tumor-bearing model

A total of 180 C57BL/6 mice (half male and half female) were fed for 3 days at a controlled temperature of 22°C–25°C and a humidity of 50%–80% to establish the Lewis tumor-bearing mouse solid tumor model. After the model was established, the mice were randomly divided into 9 groups with 20 mice in each group [Table 2]. Mice no. 1–10 in each group were used to observe the survival time to calculate the life prolongation rate and mice no. 11–20 were used to detect other indicators. The mice were given the drugs after the model had been established for 24 h. The blank group and the model group were given NS by gastric perfusion, while the combined group was given CII-3 by gastric perfusion and cisplatin by intraperitoneal injection half an hour later every day. The mice were weighed every other day for 15 days, and the time of death was recorded. After 15 days, the indexes were tested.

Synergistic and toxicity-attenuating effects of CII-3 combined with cisplatin in Lewis tumor-bearing mice

Tumor, lung, and liver histological examination

Twenty-four hours after the last administration, blood was collected from the eyeballs (picked eyeball to take blood). Then, all mice were sacrificed by cervical dislocation. The tumors, lungs, and livers were removed completely and fixed with a 10% formaldehyde solution (Shanghai Sailan Technology Trade Co., Ltd., Shanghai, China), dehydrated, and embedded in paraffin. H and E (H and E, Shanghai Sailan Technology Trade Co., Ltd., Shanghai, China) staining was performed to visualize the histological examination of tumors.

The survival time of the mice

The death time of the mice in each group was recorded, and the corresponding life prolongation rate[29] was calculated as follows:

$$life\ prolongation\ rate\% = \left(\frac{the\ mean\ living\ days\ of\ the\ mice\ in\ the\ treatment\ group}{the\ mean\ living\ days\ of\ the\ mice\ in\ the\ control\ group} - 1\right) \times 100$$

Determination of the tumor weight and tumor inhibition rate in mice

Twenty-four hours after the last administration, the subcutaneous tumors were stripped, weighed, and recorded, and the inhibition rate of each group was calculated. At the same time, the Q value was calculated according to Jin's formula[30] to evaluate the synergistic effect of the combined drugs:

$$tumor\ growth\ inhibition\ rate\ \% = \frac{(average\ tumor\ weight\ in\ control\ group - average\ tumor\ weight\ in\ administered\ group)}{average\ tumor\ weight\ in\ control\ group} \times 100$$

; $Q = \frac{E(AB)}{[EA + (1 - EA) \times EB]}$, where E (AB) is the combined tumor inhibition rate of the two drugs and EB is the tumor inhibition rate of cisplatin. Q = 0.85–1.15 represents the sum of the two drugs; Q > 1.15 represents the synergy of the two drugs; Q < 0.85 represents the antagonism of the two drugs.

Table 2: Grouping and administration of Lewis tumor-bearing mice

Groups	Route of administration	Dosage (mL/10 g/d)
Blank	Intragastric administration	0.2
Model	Intragastric administration	0.2
100 mg/kg CII-3	Intragastric administration	0.2
1.5 mg/kg cisplatin	Intraperitoneal injection	0.1
3 mg/kg cisplatin		
6 mg/kg cisplatin		
1.5 mg/kg cisplatin + 100 mg/kg CII -3	Intraperitoneal injection + Intragastric administration	0.1+0.2
3 mg/kg cisplatin + 100 mg/kg CII -3		
6 mg/kg cisplatin + 100 mg/kg CII -3		

Determination of the organ index in mice

After the mice were sacrificed, the liver, spleen, lung, and thymus gland were excised and weighed to calculate the organ index: $organ\ index(mg/g) = \frac{organ\ weight\ (mg)}{body\ weight\ (10\ g)}$ (10 g is the actual body weight of the mouse).

Changes in immune function in mice

Detection of the proliferative activity of T- and B-lymphocytes

The splenic cell suspension at a concentration of 2×10^6 cells/mL was prepared under sterile conditions 24 h after the final administration and was inoculated into the 96-well culture plate at a concentration of 200 μL/well. Then, 2 μL Con A (Sigma) was added to each well of the Con A group to a final concentration of 0.5 mg/mL, and 2 μL LPS medium was added to each stimulation well of the LPS (Sigma) group with a final concentration of 5 μg/mL; the control well did not have Con A or LPS; there were six duplicate wells each for the stimulation and control groups. An MTT (Beijing Solibao Technology Co., Ltd., Beijing, China) assay was used to detect cell proliferation activity and calculate the stimulation index (SI). $SI = \frac{OD\ value\ of\ stimulation\ well}{OD\ value\ of\ control\ well}$

Detection of killing activity of NK cells

The effector cells were prepared by adjusting the concentration of cell suspension with RPMI-1640 culture solution (Gibco) containing 10% fetal bovine serum (Gibco) to 2×10^6 cells/mL. The Yac-1 cells in the logarithmic growth phase were taken; the concentration was adjusted to 4×10^4 cells/mL to prepare the target cells so that the ratio of effector cells to target cells was 50:1. Effector cells and Yac-1 cells were added into 96-well plates with a final volume of 200 μL. At the same time, three repetitive wells were set up, including the effector cell control well, target cell control well, and blank control well. The killing activity of NK cells was detected by an MTT assay:

$$killing\ activity\ of\ NK\ cells\% = \frac{\left[1 - (OD\ value\ of\ effector - target\ cells - OD\ value\ of\ effector\ cells)\right]}{OD\ value\ of\ target\ cells} \times 100$$

Improvement in the bone marrow suppression activity of CII-3 combined with cisplatin in Lewis tumor-bearing mice

Changes in peripheral hemogram in mice

Twenty-four hours after the last administration, 20 mL orbital venous plexus blood was collected. The number of peripheral WBCs, neutrophils (GRA), monocytes (MON), platelets (PLT), lymphocytes

(LYM), and RBCs was measured by a whole blood cell analyzer (Nanjing Pulang Medical Equipment Co., Ltd., Jiangsu, China).

Detection of the number of nucleated cells in the bone marrow of mice

Twenty-four hours after the last administration, the mice were sacrificed by cervical dislocation, and both femurs were removed. The bone marrow cavity was rinsed with an RPMI-1640 culture solution and all bone marrow was rinsed into a centrifuge tube and the volume was set to 2 mL, the suspension of bone marrow nucleated cells was obtained, and the cells were counted under the microscope.

Femur histological examination

Femoral bones of the mice in each group were removed, decalcified with 10% concentrated nitric acid, fixed in a 10% formaldehyde solution, and then dehydrated. The bones were embedded in paraffin and sectioned at a 5-μm thickness for staining with H and E for histological examination of the bone marrow under a light microscope.

Detection of the proliferation activity of the bone marrow cells in mice

Twenty-four hours after the last administration, the mice were sacrificed by cervical dislocation, and both femurs were removed. The bone marrow cavity was rinsed with a 0.9% sodium chloride solution, and all bone marrow was rinsed into a centrifuge tube. The supernatant was centrifuged at a rotating speed of 1000 r/min for 5 min, and then, the supernatant was discarded. Then, 1 mL 1× erythrocyte lytic fluid (Beijing Solibao Technology Co., Ltd., Beijing, China) was added, lysed for 5 min, and centrifuged for 5 min at 1000 r/min; 1 mL cold PBS was added, mixed, and centrifuged for 5 min at 1000 r/min, and the supernatant was discarded. The process was repeated twice to make a single-cell suspension. The single-cell suspension was fixed with 70% cold ethanol for 48 h, washed twice with PBS, mixed with 100 μL PBS, and then dyed with 50 g/mL propidium iodide dye solution (Beyotime Institute of Biotechnology, Shanghai, China), and the reaction was conducted at 4°C in the dark for 30 min. The bone marrow cell cycle of each group of mice was detected by a BD FACSCalibur flow cytometer (Becton, Dickinson and Company, NJ, USA). The proliferation index (PI) of the bone marrow cells was calculated as follows:

$$PI = \frac{S + G2/M}{G0/G1 + S + G2/M} \times 100 .$$

Effects of CII-3 in improving bone marrow suppression in Lewis tumor-bearing mice treated with cisplatin

Cytokines levels of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the serum

Serum samples were collected from the sacrificed mice; the levels of G-CSF and GM-CSF in the serum were measured by enzyme-linked immunosorbent assay kits (Elabscience Biotechnology Co., Ltd., Hubei, China).

Analysis of the mRNA expressions of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the femurs

Total RNA was extracted from the femurs. Then, total RNA from each sample was reverse transcribed into cDNA using an RT SuperMix (Nanjing Nuowizan Biotechnology Co., Ltd., Nanjing, China), and the synthesized cDNA was used for real-time quantitative polymerase chain reaction (RT-qPCR) amplification using SYBR qPCR Master Mix. Furthermore, the nucleotide sequences of forward and reverse primers used for PCR are shown in Table 3. The cycling conditions were 95°C for 5 min, followed by 35 cycles of 95°C for 30 s and 72°C for 5 min. RT-qPCR analysis was performed with the CFX96™ Real-Time System.

Table 3: Primers used for reverse transcription-quantitative polymerase chain reaction

Genes	Primers (5′ to 3′)	Bases	Sequence length (bp)
β-actin			
Forward	GGCTGTATTCCCCTCCATCG	20	154
Reverse	CCAGTTGGTAACAATGCCATGT	22	154
G-CSF			
Forward	CGCATGAAGCTAATGGGTGAGT	22	87
Reverse	GACGGGTCTGAGGCACTTGTT	21	87
GM-CSF			
Forward	TTACTTTTCCTGGGCATTGTGG	22	114
Reverse	CAGGAGGTTCAGGGCTTCTTTG	22	114

G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor

Western blot analyses for the protein levels of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the femurs

Femur tissue was taken, and RIPA lytic fluid (Beijing Solibao Technology Co., Ltd., Beijing, China) was used to lyse the tissue. The supernatant was extracted, and the protein concentration was measured by a BCA kit (Beyotime Institute of Biotechnology, Shanghai, China). Protein samples of the same amount were taken (50 μg/well), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Beyotime Institute of Biotechnology, Shanghai, China) gel electrophoresis was used, and the proteins were transferred to a membrane. Tris-buffered saline with Tween 20 with 5% skim milk was used to block the membrane, the primary antibody was added, and the membrane was incubated at 4°C overnight. Then, the secondary antibody was added and incubated for 2 h, and the color was developed by an ECL luminescence solution (Millipore). Image-Pro Plus 6.0 analysis software was used to quantitatively analyze the gray values of the protein bands. The mean value was taken as the gray value and the relative content of the target protein = target protein density/β-actin density.

Statistical analysis

The results are expressed as the means ± standard deviations (SDs). Statistical differences were determined by one-way analyses of variance. $P < 0.05$ was considered statistically significant.

RESULTS

Synergistic and attenuating effects of CII-3 combined with cisplatin in Lewis tumor-bearing mice

Effects of CII-3 combined with cisplatin on tumor histopathology in mice

As shown in Figure 1, in the model group, tumor cell growth was vigorous, the cell number was large, and cells had a tight arrangement; in addition, the number of hemorrhagic and necrotic cells decreased. In the CII-3 group, most of the tumor cells had an incomplete morphology with large areas of necrosis and apoptosis and the number of tumor cells decreased. The tumor cells were ruptured and necrotic in the groups of cisplatin (1.5, 3, and 6 mg/kg), and there was a dose-dependent change. There was no significant difference between the low-dose group of cisplatin and the group of CII-3; CII-3 combined with cisplatin groups (1.5, 3, and 6 mg/kg) significantly inhibited the growth of tumor cells, decreased the number of cells, induced massive hemorrhage and necrosis, and showed a dose-dependent change. There was no

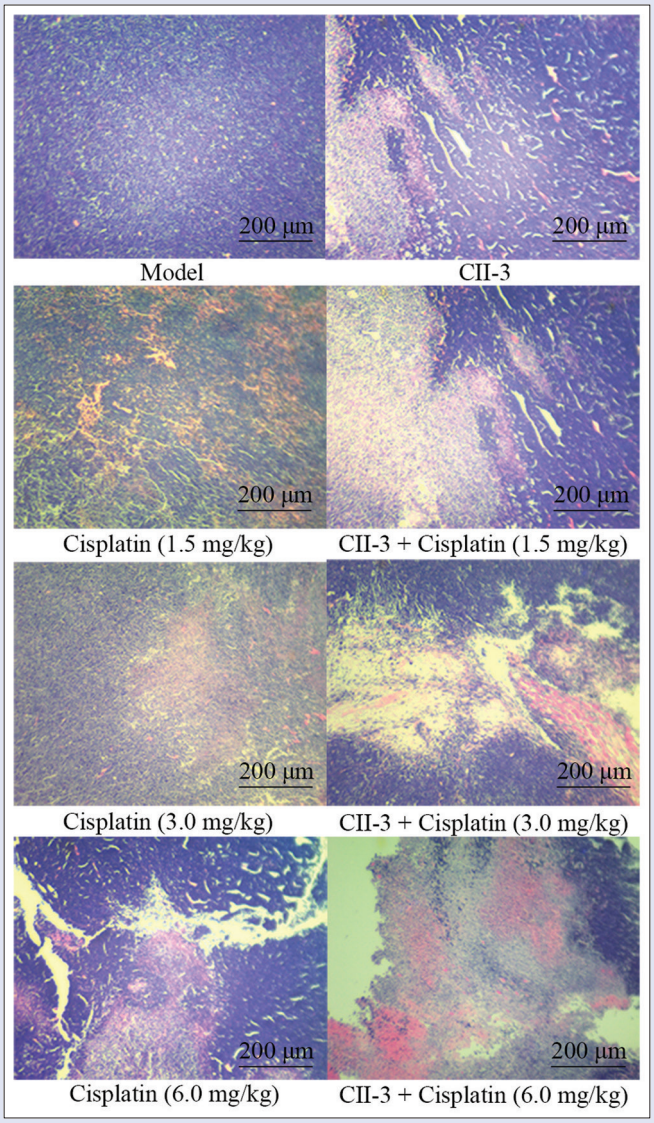


Figure 1: Effects of CII-3 combined with cisplatin on tumor tissue pathological slices of Lewis tumor-bearing mice

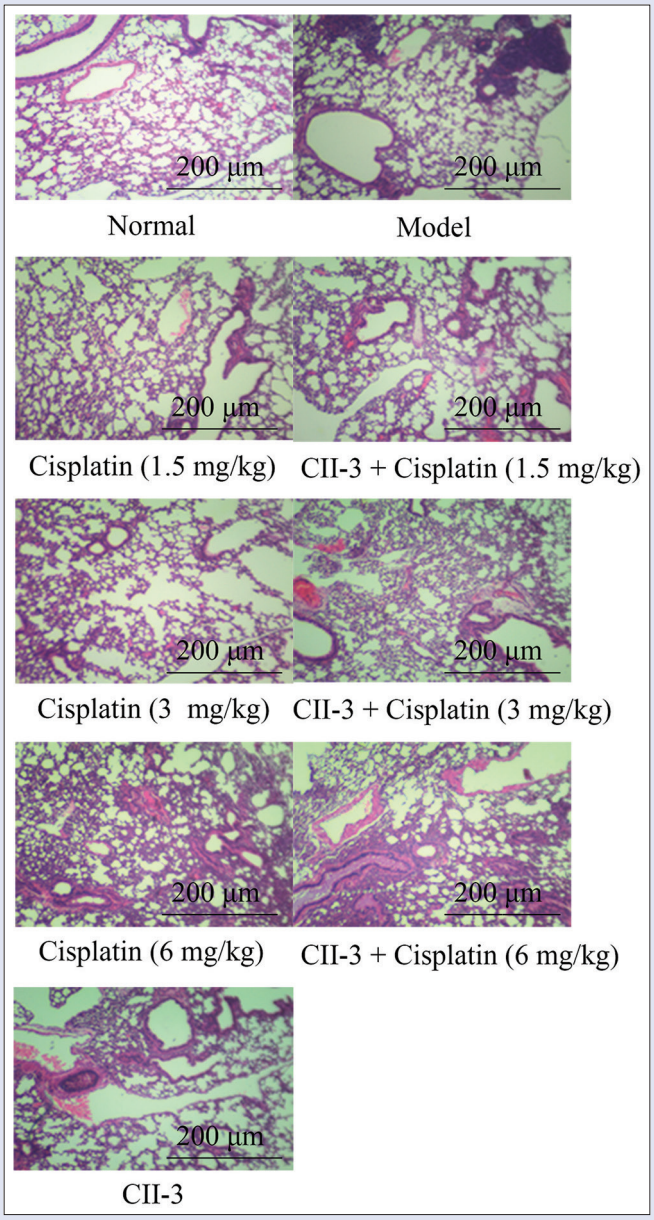


Figure 2: Effects of CII-3 combined with cisplatin on lung tissue pathological slices of Lewis tumor-bearing mice

significant difference between the low-dose combined drug group and the high-dose cisplatin group. These results suggest that CII-3 can enhance the inhibitory effect of cisplatin on the proliferation of cancer cells.

Effects of CII-3 combined with cisplatin on lung histopathology in mice

As shown in Figure 2, in the normal group, the lung tissue structure was clear and the morphology was relatively normal. Lung tissue lesions occurred in the model group, and some heterogeneous metastatic cancer cells were observed. Compared to those in the model group, the lung tissue lesions in the medication administration groups were significantly improved and no heterogeneous metastatic cancer cells were observed. These results suggest that CII-3 combined with cisplatin can inhibit the metastasis of cancer cells.

Effects of CII-3 combined with cisplatin on liver histopathology in mice

As shown in Figure 3, in the normal group, the liver cells were neatly arranged with a clear structure and a polygonal shape and no obvious inflammatory cells were observed. In the model group, the liver cells were disordered and the structure was not clear. Compared with that of mice in the model group, the liver tissue structure of mice in the cisplatin group was significantly loose, with irregular cell arrangement, abundant cytoplasm, empty vacuoles, and inflammatory cell infiltration. Compared with the cisplatin group, the CII-3 and cisplatin group had a liver injury that significantly improved and the inflammatory cell infiltration significantly decreased, as observed in the normal liver cable structure. These results show that CII-3 can improve cisplatin-induced liver injury.

Effects of CII-3 combined with cisplatin on survival time in mice

As shown in Table 4, the survival days and the life prolongation rates of the CII-3 combined with cisplatin group were prolonged compared with those of the model group ($P < 0.05$). CII-3 combined with cisplatin (3 and 6 mg/kg) improved the survival status of mice, and the life prolongation

Table 4: Effects of CII-3 combined with cisplatin on the survival time of Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	The survival days (d)	The life prolongation rate (%)
Model	-	13.6±1.1	-
Cisplatin (1.5 mg/kg)	1.5	17.2±0.8	26.5
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	22.0±1.6[a]	61.8
Cisplatin (3 mg/kg)	3	14.8±2.8	8.8
CII-3 + cisplatin (3 mg/kg)	3+100	26.3±4.0[a,b]	92.6
cisplatin (6 mg/kg)	6	15.9±1.9	16.2
CII -3 + cisplatin (6 mg/kg)	6+100	24.2±1.9[a,b]	77.9
CII -3	100	17.0±4.7	25.0

Compared with the model group, [a]$P<0.05$; compared the combination regimen with the same dose of cisplatin groups, [b]$P<0.01$

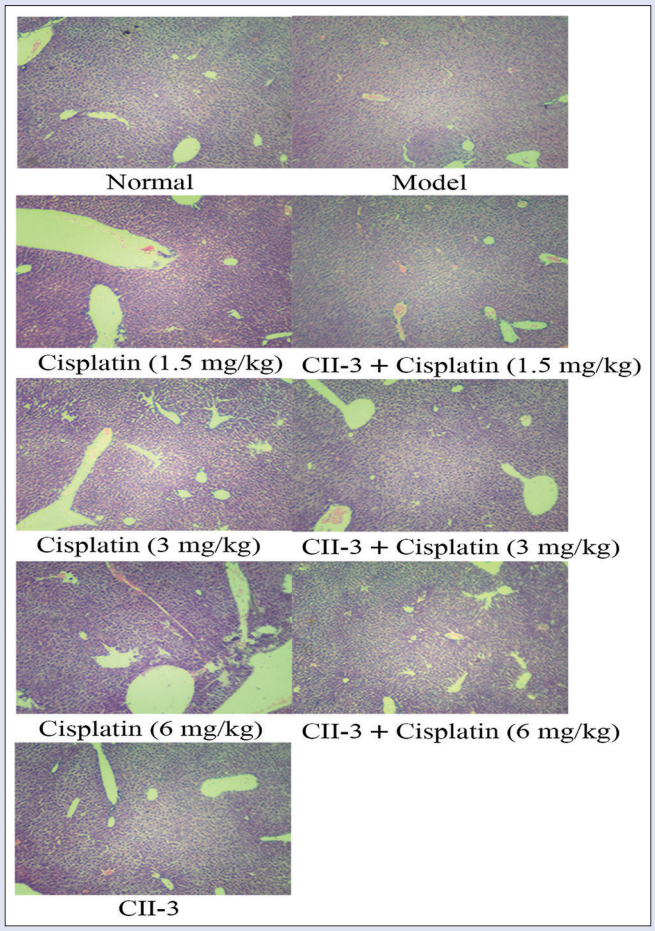


Figure 3: Effects of CII-3 combined with cisplatin on liver tissue pathological slices of Lewis tumor-bearing mice

rates of the different doses were 92.6% and 77.9%, respectively. There was a significant difference between the CII-3 combined with cisplatin group and the model group ($P < 0.01$). There was no significant difference between the CII-3 combined with cisplatin (1.5 mg/kg) group and the cisplatin group with the same dose, but the survival rate of mice in this group was 61.8% and the survival days were also higher than those in the single cisplatin group. The results showed that CII-3 combined with cisplatin could increase the life prolongation rate of mice.

Determination of the tumor weight and tumor inhibition rate in mice

As shown in Table 5, the growth of transplanted tumors in mice in each drug treatment group was slower than that in the model group and the weight of the transplanted tumors in each group was less than that in the model group ($P < 0.05$). Compared with the tumor weight in the cisplatin group, the tumor weights in the CII-3 combined with cisplatin (3 and 6 mg/kg) groups were significantly different ($P < 0.01$); the tumor inhibition rate in the CII-3 combined with cisplatin (1.5 mg/kg) group was 35.78% compared with that in the cisplatin group, and the rate was not significantly different; however, the tumor weight was lower in this combination group than in the cisplatin group with the same dose and CII-3 group. In addition, the Q values of each combined dose group were 0.91, 1.27, and 1.20 ($Q > 1.15$), respectively. These results suggest that CII-3 combined with cisplatin has synergistic antitumor effects.

Determination of the organ index in mice

As shown in Table 6, compared with those of the model group, the liver, spleen, lung, and thymus indices of the CII-3 group were not significantly different, while the liver, spleen, lung, and thymus indices of the cisplatin groups were significantly decreased ($P < 0.05$); compared with the indices of the cisplatin group, the liver, spleen, lung, and thymus indices of the CII-3 combined with cisplatin groups were significantly increased ($P < 0.05$). These results indicate that CII-3 can reduce the toxicity of cisplatin on organs, improve the organ atrophy caused by cisplatin in mice bearing tumors, and promote the growth of organs in mice bearing tumors.

Detection of the proliferation activity of T- and B-lymphocytes

As shown in Table 7, compared with that in the normal group, the T-cell proliferation activity in the other groups was significantly decreased ($P < 0.05$ or $P < 0.01$); however, this decrease was not observed in the CII-3 combined with cisplatin groups (1.5 and 3 mg/kg); compared with the T-cell activity in the model group, the T-cell activity was increased in all groups and there was a significant difference between the CII-3 combined with cisplatin groups (3 and 6 mg/kg) and the cisplatin group with the same dose ($P < 0.05$ or $P < 0.01$). For the B-cell proliferation activity, compared with the normal group, the cisplatin groups experienced a significant decrease in the B-cell activity ($P < 0.05$ or $P < 0.01$); compared with that in the model group, the B-cell activity of the CII-3 combined with cisplatin group was increased and the B-cell activity of the CII-3 combined with cisplatin group (3 mg/kg) was significantly higher than that of the cisplatin group at the same dose ($P < 0.01$). The results show that CII-3 could increase the activity of spleen lymphocytes and alleviate the toxicity and side effects caused by cisplatin in mice bearing tumors.

Detection of the killing activity of NK cells

As shown in Table 8, compared with that in the normal group, the activity of NK cells in the other groups was significantly decreased ($P < 0.01$). Compared with that in the model group, the NK cell activity in the CII-3 combined with cisplatin groups (3 and 6 mg/kg) was increased and there was a significant difference between the CII-3 combined with cisplatin group (6 mg/kg) and the cisplatin group with the same dose ($P < 0.05$). The results show that CII-3 could

Table 5: Effects of CII-3 combined with cisplatin on tumor weight, tumor inhibition rate, and *Q* value in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	Tumor weight (g)	Tumor inhibition rate (%)	*Q*
Model	-	2.04±0.22	-	-
Cisplatin (1.5 mg/kg)	1.5	1.64±0.06[a]	20.09	-
CII-3+cisplatin (1.5 mg/kg)	1.5+100	1.31±0.15[a]	35.78	0.91
Cisplatin (3 mg/kg)	3	1.10±0.28[a]	46.08	-
CII-3+cisplatin (3 mg/kg)	3+100	0.51±0.25[a,b]	75.49	1.27
Cisplatin (6 mg/kg)	6	0.77±0.14[a]	62.25	-
CII-3+cisplatin (6 mg/kg)	6+100	0.28±0.12[a,b]	86.27	1.20
CII-3	100	1.56±0.43[a]	24.02	-

Compared with the model group, [a]*P*<0.05; the combination regimen compared with the cisplatin groups with the same dose, [b]*P*<0.01. *Q*=0.85-1.15 represents the sum of the two drugs; *Q*>1.15 represents the synergy of the two drugs; *Q*<0.85 represents the antagonism of the two drugs

Table 6: Effects of CII-3 combined with cisplatin on the organ index in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	Liver index (mg/10 g)	Spleen index (mg/10 g)	Lung index (mg/10 g)	Thymus index (mg/10 g)
Normal	-	5.37±0.53	0.34±0.05	0.66±0.03	0.44±0.62[d]
Model	-	5.27±1.04	0.51±0.13	0.69±0.06	0.05±0.02[b]
Cisplatin (1.5 mg/kg)	1.5	4.37±1.31	0.29±0.09	0.63±0.01	0.03±0.01[b]
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	6.06±0.83	0.62±0.46[a,e]	0.78±0.08[a,e]	0.11±0.06[b,e]
Cisplatin (3 mg/kg)	3	5.05±0.20	0.26±0.05[c]	0.70±0.10	0.02±0.002[b]
CII-3 + cisplatin (3 mg/kg)	3+100	6.19±0.62[e]	0.42±0.12[e]	0.84±0.05[a,c,e]	0.03±0.03[b]
Cisplatin (6 mg/kg)	6	4.51±0.85	0.21±0.04[c]	0.68±0.11	0.04±0.05[b]
CII-3 + cisplatin (6 mg/kg)	6+100	5.39±0.55[e]	0.24±0.04[c]	0.80±0.11[a,c,e]	0.04±0.01[b]
CII-3	100	5.36±0.69	0.53±0.15	0.62±0.08	0.10±0.04[b]

Compared with the normal group, [a]*P*<0.05, [b]*P*<0.01; compared with the model group, [c]*P*<0.05, [d]*P*<0.01; compared the combination regimen with the same dose of cisplatin groups, [e]*P*<0.05.

Table 7: Effects of CII-3 combined with cisplatin on T- and B-lymphocyte proliferation in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	T-lymphocyte proliferation activity (SI)	B-lymphocyte proliferation activity (SI)
Normal	-	1.48±0.12[d]	2.24±0.03[c]
Model	-	0.84±0.06[b]	1.78±0.10[a]
Cisplatin (1.5 mg/kg)	1.5	0.96±0.09[a,d]	1.89±0.20[a]
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	1.12±0.11[d]	2.42±0.10
Cisplatin (3 mg/kg)	3	1.02±0.06[b]	1.32±0.36[b,d]
CII-3 + cisplatin (3 mg/kg)	3+100	1.44±0.06[f]	2.08±0.20[f]
Cisplatin (6 mg/kg)	6	1.27±0.05[b]	0.97±0.12[b,d]
CII-3 + cisplatin (6 mg/kg)	6+100	1.76±0.31[b,c,e]	1.07±0.19[b,d]
CII-3	100	1.25±0.05[a,c]	2.12±0.05

Compared with the normal group, [a]*P*<0.05, [b]*P*<0.01; compared with the model group, [c]*P*<0.05, [d]*P*<0.01; compared the combination regimen with the same dose of cisplatin groups, [e]*P*<0.05, [f]*P*<0.01

Table 8: Effects of CII-3 combined with cisplatin on NK cell activity in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	Killing activity of NK cells
Normal	-	88.68±5.67[c]
Model	-	64.59±3.45[a]
Cisplatin (1.5 mg/kg)	1.5	64.67±4.55[a]
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	61.99±2.17[a]
Cisplatin (3 mg/kg)	3	57.75±2.31[a]
CII-3 + cisplatin (3 mg/kg)	3+100	69.67±8.12[a,b]
Cisplatin (6 mg/kg)	6	54.78±1.89[a]
CII-3 + cisplatin (6 mg/kg)	6+100	65.24±5.12[a,d]
CII-3	100	53.66±6.18[a,b]

Compared with the normal group, [a]*P*<0.01; compared with the model group [b]*P*<0.05, [c]*P*<0.01; the combination regimen compared with the cisplatin groups with the same dose, [d]*P*<0.05

increase the activity of NK cells induced by cisplatin and enhance their killing power and immune response.

Improvement of the bone marrow suppression of CII-3 combined with cisplatin in Lewis tumor-bearing mice

Effects of CII-3 combined with cisplatin on peripheral blood cells

As shown in Tables 9 and 10, compared with the number in the model group, the number of peripheral WBCs, neutrophils, lymphocytes, monocytes, and erythrocytes in the cisplatin group was decreased; the number of peripheral WBCs, neutrophils, lymphocytes, and monocytes in the cisplatin groups (3 and 6 mg/kg) was significantly decreased compared with the number in the model group ($P < 0.05$ or $P < 0.01$). Compared with the number in the cisplatin group with the same dose, the number of peripheral WBCs, neutrophils, lymphocytes, monocytes, RBCs, and platelets in the combination regimen group was significantly increased. The number of peripheral WBCs, neutrophils, and lymphocytes in the combination regimen group was increased significantly ($P < 0.01$) compared with those in the cisplatin groups (1.5 and 3 mg/kg), and the number of peripheral mononuclear cells in the combination regimen

Table 9: Effects of CII-3 combined with cisplatin on peripheral blood cells in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	WBC (10^9 cells/L)	GRA (10^9 cells/L)	LYM (10^9 cells/L)
Normal	-	2.79±0.79[d]	3.23±1.65[d]	0.87±0.39
Model	-	11.81±3.45[b]	10.67±1.67[b]	1.23±0.93
Cisplatin (1.5 mg/kg)	1.5	9.17±3.19[a]	7.55±3.31[a]	2.19±0.72[a]
CII 3 + cisplatin (1.5 mg/kg)	1.5+100	13.75±7.51[b,f]	9.52±2.98[b]	3.43±0.83[b,d,f]
Cisplatin (3 mg/kg)	3	4.83±1.88[a,c]	4.52±2.46[c]	2.91±0.91[b,c]
CII-3 + cisplatin (3 mg/kg)	3+100	9.06±3.63[a,f]	7.77±2.06[a,f]	3.44±1.75[b,c]
Cisplatin (6 mg/kg)	6	5.76±0.86[c]	1.07±0.72[d]	1.39±0.62
CII-3 + cisplatin (6 mg/kg)	6+100	8.83±1.82[a,e]	4.08±0.98[d,e]	1.53±0.75
CII-3	100	9.46±1.98[a]	5.53±1.59[c]	2.36±0.99[a,c]

Compared with the normal group. [a]P<0.05, [b]P<0.01; compared with the model group, [c]P<0.05, [d]P<0.01; the combination regimen compared with the cisplatin groups with the same dose, [e]P<0.05, [f]P<0.01

Table 10: Effects of CII-3 combined with cisplatin on peripheral blood cells in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	MON (10^9 cells/L)	RBC (10^{12} cells/L)	PLT (10^9 cells/L)
Normal	-	0.17±0.09	9.29±0.76[d]	444±72.02
Model	-	0.35±0.18	4.84±1.49[b]	325.50±130.46
Cisplatin (1.5 mg/kg)	1.5	0.18±0.07[c]	4.82±0.72[b]	509.00±237.20[c]
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	0.27±0.12	5.97±1.31[b]	486.60±66.83[c]
Cisplatin (3 mg/kg)	3	0.27±0.24[a]	4.71±0.97[b]	399.00±59.24
CII-3 + cisplatin (3 mg/kg)	3+100	0.26±0.06	5.39±0.58[b]	481.20±64.61
Cisplatin (6 mg/kg)	6	0.11±0.03[d]	4.11±0.23[b]	412.00±36.19
CII-3 + cisplatin (6 mg/kg)	6+100	0.33±0.12[f]	5.66±1.19[b,e]	530.40±98.19[c]
CII-3	100	0.49±0.18[a]	5.55±0.42[b]	573.00±120.73[c]

Compared with the normal group, [a]P<0.05, [b]P<0.01; compared with the model group, [c]P<0.05, [d]P<0.01; the combination regimen compared with the cisplatin groups with the same dose, [e]P<0.05, [f]P<0.01

Table 11: Effects of CII-3 combined with cisplatin on bone marrow nucleated cells in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	Bone marrow nucleated cells (10^6 cells/mL)
Normal	-	14.72±0.95
Model	-	13.04±4.03
Cisplatin (1.5 mg/kg)	1.5	10.26±0.91[a,b]
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	12.03±1.39
Cisplatin (3 mg/kg)	3	6.47±1.23[a,c]
CII-3 + cisplatin (3 mg/kg)	3+100	11.28±0.95[a,e]
Cisplatin (6 mg/kg)	6	3.51±0.97[a,c]
CII-3 + cisplatin (6 mg/kg)	6+100	5.99±0.29[a,c,d]
CII-3	100	10.12±0.45[a,b]

Compared with the normal group, [a]P<0.01; compared with the model group, [b]P<0.05, [c]P<0.01; the combination regimen compared with the cisplatin groups with the same dose, [d]P<0.05, [e]P<0.01

(6 mg/kg) was increased significantly ($P < 0.01$) compared with that in the cisplatin group. These results suggest that CII-3 can alleviate the toxicity of cisplatin on peripheral hemograms and can improve the immunity of mice bearing tumors treated with chemotherapy.

Effects of CII-3 combined with cisplatin on bone marrow nucleated cells

As shown in Table 11, the number of bone marrow nucleated cells was significantly decreased in the combination groups and cisplatin groups compared with the number in the normal group (P<0.01). Compared with the number in the model group, the number of bone marrow nucleated cells in the cisplatin groups was significantly decreased ($P < 0.05$ or $P < 0.01$). Compared with that in the cisplatin groups, the number of bone marrow nucleated cells in the CII-3 combined with cisplatin (3 and 6 mg/kg) groups was significantly increased ($P < 0.05$ or $P < 0.01$). The results show that CII-3 could significantly improve the reduction in nucleated cells in bone marrow caused by cisplatin.

Effects of CII-3 combined with cisplatin on femur histopathology in mice

As shown in Figure 4, in the normal group, the femoral tissue structure was clear and orderly, no RBC hemorrhage or necrosis was observed, and no fat cells or vacuoles were formed. In the model group, the femoral tissue structure was fuzzy and disordered, most of the RBCs were incomplete in morphology, and large areas of necrosis and apoptosis occurred. Compared with the model group, the cisplatin groups had a femur tissue structure that was not orderly and there were still RBC rupture and necrosis. The number of RBCs was significantly reduced and changed in a dose-dependent manner. Compared with the cisplatin groups, the CII-3 combined with the cisplatin groups showed an obvious improvement. These results suggest that CII-3 combined with cisplatin can improve femoral injury in tumor-bearing mice.

Effects of CII-3 combined with cisplatin on the proliferation activity of bone marrow cells in mice

As shown in Table 12, compared with the rate in the normal group, the proliferation rate of bone marrow cells in each medication administration group was reduced and the difference was statistically significant ($P < 0.05$ or $P < 0.01$). Compared with that in the model group, the proliferation rate of bone marrow cells in the cisplatin groups was significantly reduced ($P < 0.05$ or $P < 0.01$) and this reduction was dose dependent. Compared with that in the cisplatin groups, the bone marrow cell proliferation rate in the CII-3 combined with cisplatin groups was significantly increased ($P < 0.05$). These results suggest that CII-3 can alleviate the decrease in bone marrow nucleated cells induced by cisplatin.

Effects of CII-3 combined with cisplatin on the bone marrow cell cycle in mice

As shown in Figure 5, compared with that in the normal group, the ratio of G1 phase cells in the model group was decreased, the ratio of S phase cells was increased, and the ratio of G2/M phase cells was decreased;

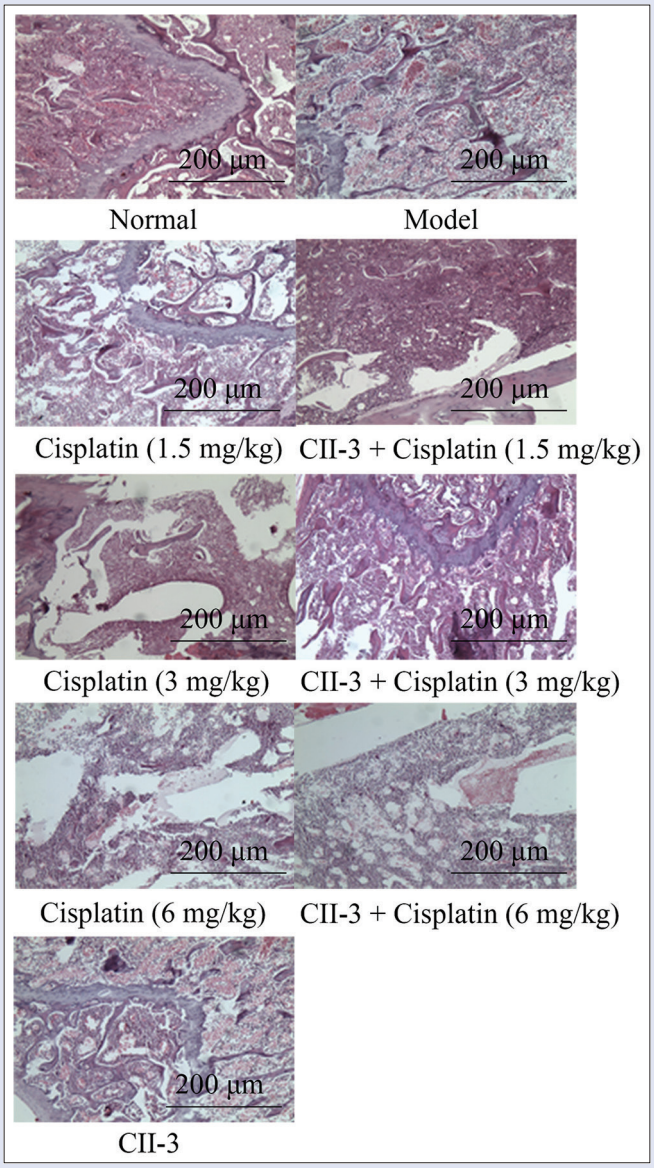


Figure 4: Effects of CII-3 combined with cisplatin on femur tissue pathological slices of Lewis tumor-bearing mice

these results indicate that the bone marrow cells were blocked in S phase and the PI was decreased, resulting in the decreased proliferation rate of bone marrow cells. Compared with that in the model group, the ratio of G1 phase cells was increased in the cisplatin groups and the combination group, the ratio of S phase cells was decreased, and the ratio of G2/M phase cells was increased. Compared with those in the cisplatin groups, the proportion of G1 phase cells was increased, the proportion of S phase cells was decreased, and the proportion of G2/M phase cells was increased in the CII-3 combined with cisplatin groups; in addition, the cell cycle ratios of each cycle were closer to that of the normal group. These results indicate that CII-3 can stimulate the transformation of bone marrow cells from S phase to G2/M phase, accelerate the process of the cell reproductive cycle, increase the cell PI, and thus, increase the proliferation rate of bone marrow cells.

Table 12: Effects of CII-3 combined with cisplatin on the proliferation activity of bone marrow cells in Lewis tumor-bearing mice (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	Proliferation index (%)
Normal	-	34.68±1.26
Model	-	31.68±2.13
Cisplatin (1.5 mg/kg)	1.5	22.89±1.98[b,c]
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	29.22±1.78[a,e]
Cisplatin (3 mg/kg)	3	19.57±3.10[b,d]
CII-3 + cisplatin (3 mg/kg)	3+100	25.31±2.56[a,c,e]
Cisplatin (6 mg/kg)	6	12.11±1.67[b,d]
CII-3 + cisplatin (6 mg/kg)	6+100	17.73±0.98[b,d,e]
CII-3	100	26.95±2.66[a]

Compared with the normal group, [a]$P<0.01$; compared with the model group, [b]$P<0.05$, [c]$P<0.01$; the combination regimen compared with the cisplatin groups with the same dose, [d]$P<0.05$, [e]$P<0.01$

Effects of CII-3 in improving bone marrow suppression in Lewis tumor-bearing mice treated with cisplatin

Effects of CII-3 combined with cisplatin on hematopoiesis-related cytokines

As shown in Table 13, compared to those in the normal group, the serum levels of G-CSF and GM-CSF in each medication administration group were significantly decreased ($P < 0.05$ or $P < 0.01$); compared with those in the model group, the levels of G-CSF and GM-CSF in the cisplatin groups were significantly decreased ($P < 0.05$ or $P < 0.01$). Compared with the cisplatin groups, the CII-3 combined with cisplatin (1.5 and 6 mg/kg) groups had significantly increased levels of G-CSF ($P < 0.05$ or $P < 0.01$) and the serum levels of GM-CSF in the CII-3 combined with cisplatin (1.5 and 3 mg/kg) groups were increased significantly ($P < 0.05$), indicating that CII-3 could increase the serum levels of G-CSF and GM-CSF in mice.

Effects of CII-3 combined with cisplatin on the mRNA levels of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor

As shown in Figure 6, compared to the model group, the cisplatin group had significantly decreased mRNA levels of G-CSF and GM-CSF ($P < 0.01$). Compared with that in the corresponding cisplatin group, the G-CSF level in the combined drug groups (1.5 and 3 mg/kg) was increased significantly ($P < 0.01$) and the GM-CSF level in the combined drug groups (1.5, 3, and 6 mg/kg) was increased significantly ($P < 0.05$). These results suggest that CII-3 could ameliorate the decrease in G-CSF and GM-CSF in bone marrow tissue induced by cisplatin.

Effects of CII-3 combined with cisplatin on the protein levels of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor

As shown in Figures 7 and 8, compared with those of the mice in the model group, the protein levels of G-CSF and GM-CSF in the bone marrow tissues of the mice in each medication administration group were significantly decreased ($P < 0.01$), indicating that cisplatin could significantly reduce the protein levels of G-CSF and GM-CSF in bone marrow tissue. The protein levels of G-CSF and GM-CSF in the bone

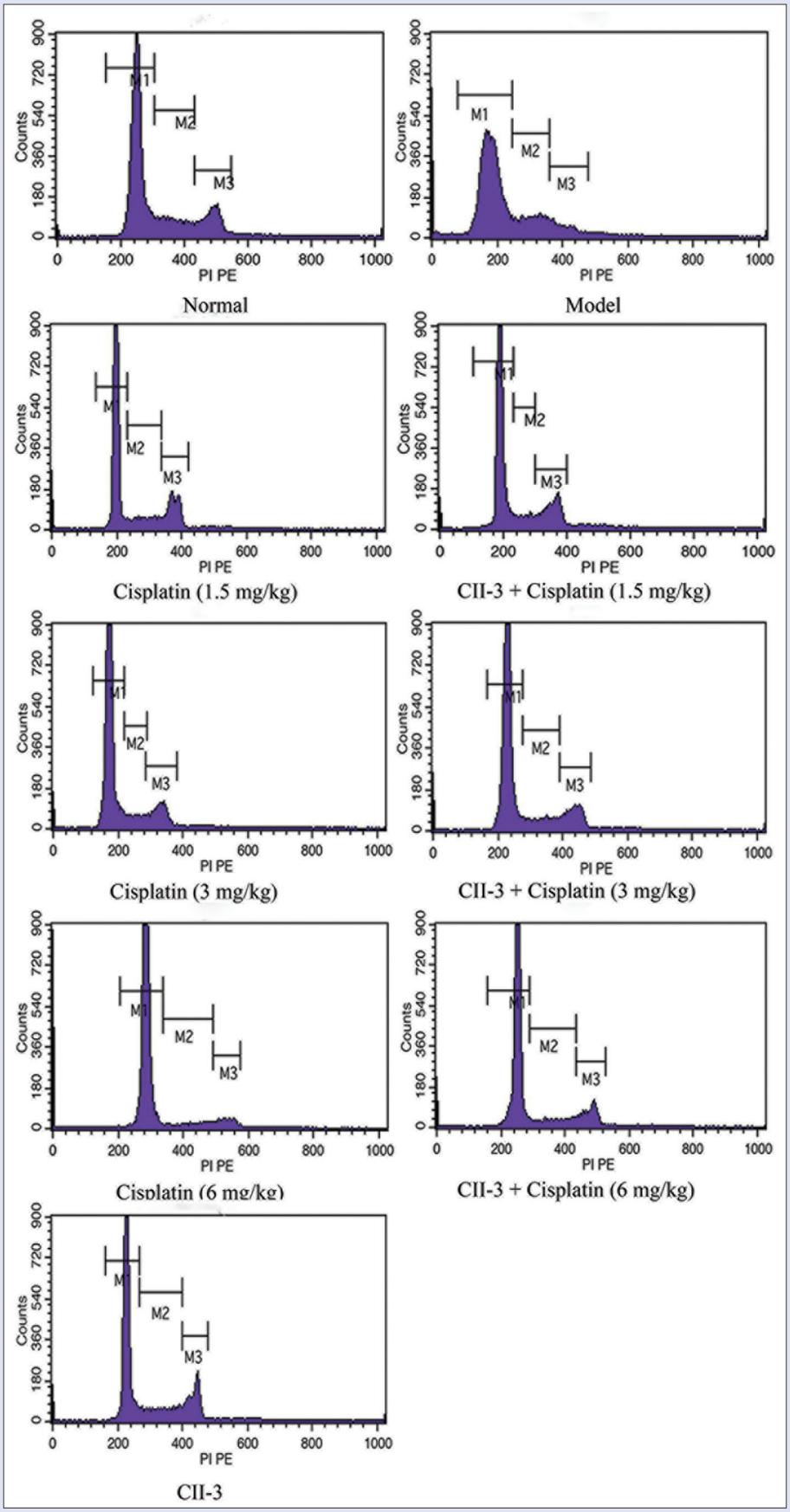


Figure 5: Effects of CII-3 combined with cisplatin on the bone marrow cell cycle in Lewis tumor-bearing mice

Table 13: Effects of CII-3 combined with cisplatin on hematopoiesis-related cytokines (means±standard deviations, *n*=10)

Groups	Dosage (mg/kg)	G-CSF (Pg/mL)	GM-CSF (Pg/mL)
Normal	-	3784.74±533.65[c]	127.28±21.51[d]
Model	-	2966.78±332.15[a]	69.97±4.97[a]
Cisplatin (1.5 mg/kg)	1.5	1838.16±167.10[b,d]	48.81±5.24[b,c]
CII-3 + cisplatin (1.5 mg/kg)	1.5+100	2472.06±297.42[b,c,e]	97.14±3.38[a,e]
Cisplatin (3 mg/kg)	3	2079.93±404.44[b,c]	20.80±5.61[b,d]
CII-3 + cisplatin (3 mg/kg)	3+100	2126.96±416.99[b,c]	59.91±3.08[a,e]
Cisplatin (6 mg/kg)	6	1011.44±268.99[b,d]	23.79±1.36[b,c]
CII-3 + cisplatin (6 mg/kg)	6+100	1942.21±200.81[b,c,f]	34.97±1.50[b,c]
CII-3	100	3147.29±896.23	96.44±12.64[a]

Compared with the normal group, [a]P<0.05, [b]P<0.01; compared with the model group, [c]P<0.05, [d]P<0.01; the combination regimen compared with the cisplatin groups with the same dose, [e]P<0.05, [f]P<0.01. G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor

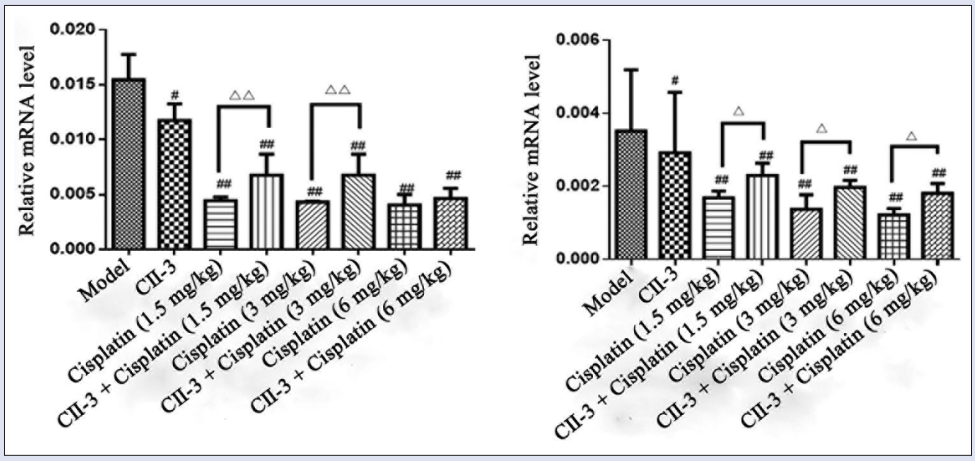


Figure 6: Effects of CII-3 combined with cisplatin on the mRNA levels of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. The data are expressed as the means ± standard deviations (n = 10). Compared with the model group, #P < 0.05, ##P < 0.01; the combination regimen compared with cisplatin groups with the same dose, ΔP < 0.05, ΔΔP < 0.01

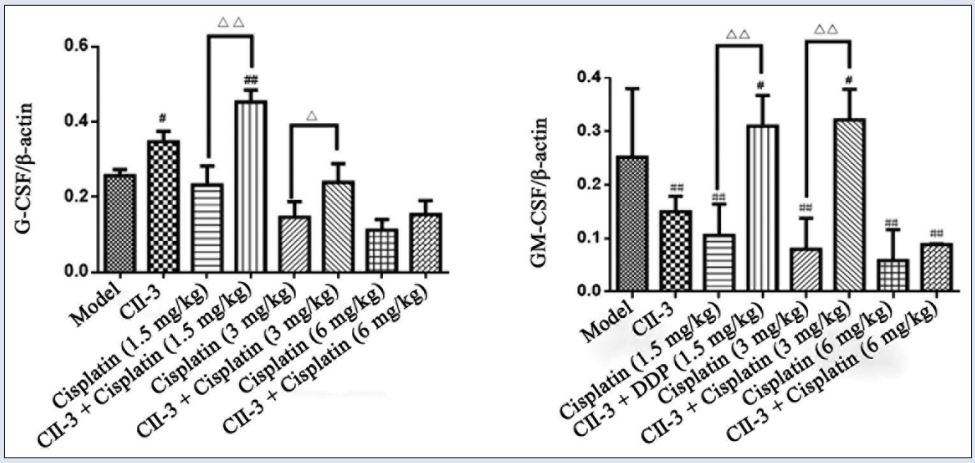


Figure 7: Effects of CII-3 combined with cisplatin on the protein levels of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. The data are expressed as the means ± standard deviations (n = 10). Compared with the model group, #P < 0.05, ##P < 0.01; the combination regimen compared with the cisplatin groups with the same dose, ΔP < 0.05, ΔΔP < 0.01

marrow tissues of mice in the CII-3 combined with cisplatin (1.5 and 3 mg/kg) groups were significantly higher than those in the cisplatin groups (P < 0.05 or P < 0.01). These results suggest that CII-3 can increase the downregulation of the protein levels of G-CSF and GM-CSF in bone marrow tissue induced by cisplatin.

DISCUSSION

Currently, chemotherapy is a routine method for the treatment of malignant tumors in the clinic due to its definite curative effect. However, chemotherapy usually targets rapidly dividing cells to destroy cancer

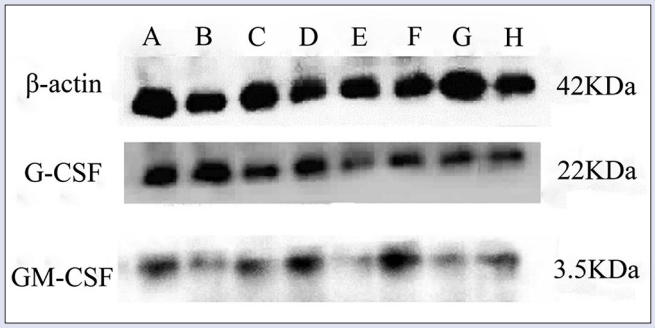


Figure 8: Effects of CII-3 combined with cisplatin on the protein levels of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. (a) Model group; (b) CII-3 group; (c) cisplatin group (1.5 mg/kg); (d) CII-3 + cisplatin (1.5 mg/kg) group; (e) Cisplatin group (3 mg/kg); (f) CII-3 + cisplatin (3 mg/kg) group; (g) cisplatin group (6 mg/kg); (h) CII-3 + cisplatin (6 mg/kg) group

cells, leading to serious side effects. The occurrence of bone marrow suppression is one of the side effects.[31,32] To reduce the side effects and drug resistance of chemotherapy, an increasing number of studies have focused on the combination of drugs that make cancer cells sensitive to traditional chemotherapeutic drugs. The natural products of traditional Chinese medicine are usually safe and have a low toxicity. They are ideal chemosensitizers for cancer treatment.[33-35]

The main purpose of this study was to explore whether CII-3 can achieve synergistic and toxicity-attenuating effects in cancer chemotherapy. The results showed that CII-3 combined with cisplatin could enhance the inhibitory effect of cisplatin on the proliferation of tumor cells and could significantly increase the life prolongation rate and the tumor inhibition rate of cisplatin in mice; these effects indicated that CII-3 had a synergistic effect. At the same time, CII-3 combined with cisplatin can improve the liver injury induced by cisplatin; significantly alleviate the decrease in the liver, spleen, lung, and thymus indices induced by cisplatin; inhibit cancer cell metastasis; and significantly increase the T- and B-lymphocyte proliferation activity and the NK cell killing activity, indicating that CII-3 has the role of toxicity attenuation.

Bone marrow suppression is one of the main toxic and side effects of chemotherapy, and this suppression is also the main reason that patients cannot complete treatment on time. When the bone marrow system is inhibited, the related indicators of bone marrow hematopoiesis will also change. Clinical examination shows that the most objective and direct indicator is the decrease in the blood cells of each lineage. Bone marrow depression caused by most chemotherapeutic drugs usually occurs 1–3 weeks after chemotherapy.[36] Leukocyte decreases are the main cause, but platelet decreases may also occur and erythrocyte decreases are not obvious. The absolute value of neutrophils is more important than the total number of WBCs for granulocyte suppression, which is one of the bone marrow suppression indicators that need intervention. In addition, monocytes, lymphocytes, and bone marrow nucleated cells are also affected by bone marrow suppression. The results showed that the number of peripheral WBCs, neutrophils, lymphocytes, monocytes, RBCs, platelets, and bone marrow nucleated cells increased in the combined groups compared with the number in the cisplatin groups at the same dose, and this increase could improve the femoral injury in tumor-bearing mice. By detecting the bone marrow cell cycle, we found that CII-3 can stimulate the transformation of bone marrow cells from S phase to G2/M phase, accelerate the process of the cell reproductive cycle, increase the cell PI, and thus, increase the proliferation rate of bone marrow cells. These results suggest that CII-3 can alleviate bone marrow depression induced by cisplatin.

Bone marrow toxicity of chemotherapeutic drugs mainly manifests as hematological toxicity, such as the effect on hematopoietic stem cells, so the inhibition of bone marrow toxicity may be related to the activity of hematopoietic growth factors.[37] G-CSF and GM-CSF are two common hematopoietic factors. They have synergistic effects, which can directly stimulate stem cells to differentiate into myeloid and erythroid cells, and their contents can indirectly reflect the hematopoietic function of bone marrow. According to the results that CII-3 had the effect of improving bone marrow suppression in cisplatin-treated Lewis tumor-bearing mice, we hypothesize that the ability of CII-3 to improve bone marrow suppression is related to the bone marrow hematopoietic factors G-CSF and GM-CSF in the bone marrow hematopoietic microenvironment. To further elucidate the effects of CII-3 in improving bone marrow suppression, we measured the serum cytokines G-CSF and GM-CSF, which are related to hematopoiesis, as well as their mRNA and protein levels in bone marrow cells. The results showed that CII-3 could increase the levels of G-CSF and GM-CSF in the serum of mice and the mRNA and protein levels of G-CSF and GM-CSF in bone marrow tissues. These results indicate that CII-3 could promote bone marrow hematopoiesis by stimulating the production of the hematopoietic growth factors G-CSF and GM-CSF in the bone marrow hematopoietic microenvironment, thus improving bone marrow suppression.

CONCLUSION

CII-3 combined with cisplatin can enhance the antitumor effect of cisplatin; alleviate the toxicity and side effects of cisplatin on immune organs, liver injury, and bone marrow suppression; and improve the lymphocyte transformation function and NK cell killing power, suggesting that CII-3 has a synergistic and attenuating effect in cancer chemotherapy. For the main toxic side effect of bone marrow suppression, the improvement of CII-3 is related to the increase in G-CSF and GM-CSF in serum and the upregulation of the mRNA and protein levels of G-CSF and GM-CSF in bone marrow. These results provide a basis for the adjuvant application of CII-3, an antitumor active component of *P. americana*, in cancer chemotherapy. The mechanisms of the synergism and toxicity attenuation abilities of CII-3 need to be elucidated further.

Financial support and sponsorship

This work was supported by the basic research program of local universities in Yunnan province (2017FH001-096), Scientific Research Fund Project of Yunnan Education Department in 2019 (2019Y0263).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Yu C, Jiang Z, Hou A, Mu Y, Liu W, Tan S. Shen-Cao granules formulated based on traditional Chinese medicine alleviates bone marrow suppression caused by platinum-based anticancer reagents. Medicine (Baltimore) 2017;96:e6818.
2. Siddik ZH. Cisplatin: Mode of cytotoxic action and molecular basis of resistance. Oncogene 2003;22:7265-79.
3. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 2005;4:307-20.
4. Cepeda V, Fuertes MA, Castilla J, Alonso C, Quevedo C, Pérez JM. Biochemical mechanisms of cisplatin cytotoxicity. Anticancer Agents Med Chem 2007;7:3-18.
5. Einhorn LH. Curing metastatic testicular cancer. Proc Natl Acad Sci U S A 2002;99:4592-5.
6. Armstrong DK, Bookman MA, McGuire W, Bristow RE, Schilder JM, Gynecologic Oncology Group. A phase I study of paclitaxel, topotecan, cisplatin and Filgrastim in patients with

newly diagnosed advanced ovarian epithelial malignancies: A Gynecologic Oncology Group study. Gynecol Oncol 2007;105:667-71.

7. Ridwelski K, Gebauer T, Fahlke J, Kröning H, Kettner E, Meyer F, *et al.* Combination chemotherapy with docetaxel and cisplatin for locally advanced and metastatic gastric cancer. Ann Oncol 2001;12:47-51.
8. Cullen KJ, Yang Z, Schumaker L, Guo Z. Mitochondria as a critical target of the chemotheraputic agent cisplatin in head and neck cancer. J Bioenerg Biomembr 2007;39:43-50.
9. Niell HB, Herndon JE 2nd, Miller AA, Watson DM, Sandler AB, Kelly K, *et al.* Randomized phase III intergroup trial of etoposide and cisplatin with or without paclitaxel and granulocyte colony-stimulating factor in patients with extensive-stage small-cell lung cancer: Cancer and Leukemia Group B Trial 9732. J Clin Oncol 2005;23:3752-9.
10. Chaudhry V, Rowinsky EK, Sartorius SE, Donehower RC, Cornblath DR. Peripheral neuropathy from taxol and cisplatin combination chemotherapy: Clinical and electrophysiological studies. Ann Neurol 1994;35:304-11.
11. Pabla N, Dong G, Jiang M, Huang S, Kumar MV, Messing RO, *et al.* Inhibition of PKCδ reduces cisplatin-induced nephrotoxicity without blocking chemotherapeutic efficacy in mouse models of cancer. J Clin Invest 2011;121:2709-22.
12. Hatzopoulos S, Di Stefano M, Albertin A, Martini A. Evaluation of cisplatin ototoxicity in a rat animal model. Ann NY Acad Sci 1999;884:211-25.
13. Noach EJ, Ausema A, van Os R, Akkerman I, Koopal S, Weersing E, *et al.* Chemotherapy prior to autologous bone marrow transplantation impairs long-term engraftment in mice. Exp Hematol 2003;31:528-34.
14. Liang DC. The role of colony-stimulating factors and granulocyte transfusion in treatment options for neutropenia in children with cancer. Paediatr Drugs 2003;5:673-84.
15. Zhu Y, Wang L, Yang Z, Wang J, Li W, Zhou J, *et al.* Hematopoietic effects of pae oniflorin and albiflorin onradiotherapy-induced myelosuppression mice. Evid Based Complement Alternat Med 2016;2016:5789381.
16. Yerushalmi R, Goldvaser H, Sulkes A, Ben-Aharon I, Hendler D, Neiman V, *et al.* Adjuvant docetaxel and cyclophosphamide (DC) with prophylactic granulocyte colony-stimulating factor (GCSF) on days 8 and 12 in breast cancer patients: A retrospective analysis. Plos One 2014;9:1-5.
17. Manko J, Walter-Croneck A, Jawniak D, Grzasko N, Gorska-Kosicka M, Cioch M, *et al.* A clinical comparison of the efficacy and safety of biosimilar G-CSF and originator G-CSF in haematopoietic stem cell mobilization. Pharmacol Rep 2014;66:239-42.
18. Cohen JB, Bucur S, Winton EF, Sinha R, Heffner LT, King N, *et al.* Combination of GM-CSF with fludarabine-containing regimens in chronic lymphocytic leukemia and indolent non-Hodgkin lymphoma. Clin Lymphoma Myeloma Leuk 2015;15:514-8.
19. Ling Y. Traditional Chinese medicine in the treatment of symptoms in patients with advanced cancer. Ann Palliat Med 2013;2:141-52.
20. Lin H, Liu J, Zhang Y. Developments in cancer prevention and treatment using traditional Chinese medicine. Front Med 2011;5:127-33.
21. Qi F, Li A, Inagaki Y, Gao J, Li J, Kokudo N, *et al.* Chinese herbal medicines as adjuvant treatment during chemo-or radio-therapy for cancer. Biosci Trends 2010;4:297-307.
22. Qi F, Zhao L, Zhou A, Zhang B, Li A, Wang Z, *et al.* The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of only terminal stage of cancer. Biosci Trends 2015;9:16-34.
23. Zhang F, Kong LL, Zhang YY, Li SC. Evaluation of impact on healthrelated quality of life and cost effectiveness of traditional Chinese medicine: A systematic review of randomized clinical trials. J Altern Complement Med 2012;18:1108-20.
24. Srivastava D, Gaur SN, Arora N, Singh BP. Clinico-immunological changes post-immunotherapy with *Periplaneta americana*. Eur J Clin Invest 2011;41:879-88.
25. Zhang H, Wei L, Zhang Z, Liu S, Zhao G, Zhang J, *et al.* Protective effect of *Periplaneta americana* extract on intestinal mucosal barrier function in patients with sepsis. J Tradit Chin Med 2013;33:70-3.
26. Huang YF, Li LJ, Gao SQ, Chu Y, Niu J, Geng FN, *et al.* Evidence based anti-osteoporosis effects of *Periplaneta americana* L on osteoblasts, osteoclasts, vascular endothelial cells and bone marrow derived mesenchymal stem cells. BMC Complement Altern Med 2017;17:413.
27. Shen Y, Sun J, Niu C, Yu D, Chen Z, Cong W, *et al.* Mechanistic evaluation of gastroprotective effects of Kangfuxin on ethanol-induced gastric ulcer in mice. Chem Biol Interact 2017;273:115-24.
28. Zhao Y, Yang A, Tu P, Hu Z. Anti-tumor effects of the American cockroach, *Periplaneta americana*. Chin Med 2017;12:26.
29. Dai Z, Liu X, Ji Z, Liu L, Kang H, Wang X, *et al.* The effect-enhancing and toxicity-reducing action of the extract of herba *Scutellariae barbatae* for chemotherapy in hepatoma H22 tumor-bearing mice. J Tradit Chin Med 2008;28:205-10.
30. Zhou YY, Wang HY, Tang ZG, Ma DL. Two new formulae for evaluating the effectiveness of drug combinations and the revision of Bürgi's and Jin's modified Bürgi's formulae. Acta Pharmacol Sin 1984;5:217-21.
31. Hassan MN, Waller EK. Treating chemotherapy-induced thrombocytopenia: Is it time for oncologists to use thrombopoietin agonists? Oncology (Williston Park) 2015;29:295-6.
32. Barreto JN, McCullough KB, Ice LL, Smith JA. Antineoplastic agents and the associated myelosuppressive effects: A review. J Pharm Pract 2014;27:440-6.
33. Banerjee S, Wang Z, Kong D, Sarkar FH. 3,3'-Diindolylmethane enhances chemosensitivity of multiple chemotherapeutic agents in pancreatic cancer. Cancer Res 2009;69:5592-600.
34. Fulda S, Debatin KM. Sensitization for tumor necrosis factor-related apoptosisinducing ligand-induced apoptosis by the chemopreventive agent resveratrol. Cancer Res 2004;64:337-46.
35. Li X, Zhu F, Jiang J, Sun C, Wang X, Shen M, *et al.* Synergistic antitumor activity of withaferin A combined with oxaliplatin triggers reactive oxygen species-mediated inactivation of the PI3K/AKT pathway in human pancreatic cancer cells. Cancer Lett 2015;357:219-30.
36. Zhang MX, Xiao JH, Hu YC. Complications and prevention of bone tumor chemotherapy. Chin J Orthop 2000;S1:85-6.
37. Liu FY, Sha QP, Wang WW. The effect and mechanism of supplementing qi and nourishing blood to inhibit bone marrow hematopoietic proliferation in mice. J Chin Med 2019;37:2328-31.