Research Square

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In vivo anti-tumor effect of Atractylodes macrocephala Koidz and Zingiber prescriptions combined with paclitaxel in mice bearing ID8 ovarian carcinoma

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Abstract

Background

Paclitaxel is the first-line chemotherapy drug for ovarian carcinoma, and chemotherapy resistance to paclitaxel is closely related to the recurrence and poor prognosis of ovarian carcinoma. Our previous in vitro experiments demonstrate that Atractylenolide-I and 6-shogaol can be used as natural antagonists to target and block the TLR4 /MyD88-mediated paclitaxel resistance pathway.

Purpose

The majority of traditional herbal preparations are water-based prescriptionss, which results in a low yield of pharmacologically active lipid-soluble components. This preparation follows the decoction method of classic prescriptions (half decoction in wine and water), and maked the *Atractylodes macrocephala* Koidz and Ginger alcohol /aqueous extracts.

Methods

The effective contents of Atractylenolide-I and 6-shogaol in This preparation were determined by HPLC with main active components.

Results

This preparation were effective in inhibiting the growth of paclitaxel-treated tumors, accompanied by increased apoptosis and improved survival. In combination with paclitaxel, *Atractylodes macrocephala* Koidz and Zingiber alcohol /aqueous extracts had better anticancer efficacy than Atractylodes-I and 6-shogaol. Furthermore, no hepatic or kidney toxicity could be observed.

Conclusion

Atractylodes macrocephala Koidz and Zingiber alcohol /aqueous extracts may provide a clinically useful natural remedy for the treatment of cancer with active components that promote paclitaxel sensitization.

1. Introduction

70% of Ovarian cancer (OC) patients relapse within 2 years, and the 5-year survival rate is only 40–50% [1]. Chemo-resistance has been identified as one of the main reasons for recurrence, short survival, and poor prognosis of OC. Paclitaxel is effective as a first-line drug for OC, but drug resistance may ensue because of paclitaxel's multi factor, multi-channel and multi-level interactions [2]. While its primary

mechanism involves cell cycle arrest, paclitaxel is also a pathogen associated molecular pattern (PAMP) molecule that can be recognized by TLR4, a type I transmembrane protein that also recognizes a variety of ligands, including lipopolysaccharide from the cell wall of Gram-negative bacteria [3]. Engagement of the TLR4 /MD-2 heterodimer activates the MyD88-dependent signal transduction pathway to induce tumor cells to release a variety of factors or upregulate anti-apoptotic signals. MyD88 positive tumor cells can produce a large amount of TGF-β1 and other immunosuppressive factors via autocrine signaling, which contributes to the formation of a tumor immune microenvironment in which tumor cells escape attack by immune effector cells and continue to proliferate, infiltrate and metastasize [4]. Studies by our lab and the Mayo Clinic [5] have confirmed MyD88 positivity in about 70% of OC patients [6]. Therefore, differential expression of the TLR4 /MyD88 signaling pathway may promote the immune escape of tumor cells, prevent the apoptosis of tumor cells, and thereby lead to paclitaxel chemotherapy resistance [6].

Atractylodes macrocephala Koidz (AM), belonging to the Compositae, has been used for the treatment of inflammation, anti-tumor. AM contains several bioactive sesquiterpenoids such as Atractylodes-I (AO-I), Atractylodes-II (AO-II), and Atractylodes-III (AO-III) and polyacetylenes, and in vitro treatment of macrophages with AO-I compounds inhibited lipopolysaccharide induced TNF-a and iNOS expression [7]. Zingiber has various antioxidant, anti-inflammatory, antiemetic, anticancer and antimutagenic properties. Shogaols are important biomarkers used for the quality control of many Zingiber-containing products, due to their diverse biological activities [8]. In vivo and in vitro studies confirmed that Gingerols, especially 6-shogaol (6-S), induce apoptosis and autophagy, and inhibit metastasis [9, 10]. Our previous in vitro cell experiments confirmed, AO-I and 6-S can increase the sensitivity of paclitaxel to MyD88 positive OC cells, reduce the immunosuppressive effect of OC cells, improve the immune suppression state, and enhance the body's response to tumor cells [11–13]. Therefore, blocking the TLR4 /MyD88 signaling pathway to improve the sensitivity of paclitaxel chemotherapy is an important direction for research on paclitaxel chemotherapy sensitization.

Natural drugs provide a sustainable alternative for enhancement of chemotherapeutic efficacy. According to the characteristics and advantages of Traditional Chinese Medicine (TCM) in tumor prevention and treatment, the innovative application of natural products and ancient prescriptions provides a resource for further development. In this study, we performed in vivo experiments to investigate the growth environment of ovarian tumors and explore whether a classic prescription in Chen Ziming's "women's Daquan recipe" (Supplementary Material 2) can reduce paclitaxel chemo-resistance by antagonizing the paclitaxel resistance and immune escape mediated by the TLR4 /MyD88 signaling pathway. Our research on the active components of alcohol /aqueous extract from AM and Zingiber provides a theoretical basis for developing new drugs and therapies for ovarian carcinoma, a strategy for understanding the mechanism of AO-I and 6-S effects on paclitaxel chemo-resistance, and innovative ideas and future directions for the study of alcohol / aqueous extracts from AM and Zingiber.

2. Materials And Methods

2.1 Materials and reagents

AM and Ginger were provided and boiled by the Chinese Pharmacy of Sichuan Cancer Hospital in Chengdu, Sichuan Province. The morphology and chemical identification of the crude drugs were evaluated [14]. The AM and Ginger were roughly crushed, soaked 6 times in 60% ethanol for 4 hours, and then extracted by thermal reflux at 70°C for 2 hours. The procedure was repeated twice, and then the filtrates were combined, recovered in ethanol, and concentrated to produce AM and Zingiber alcohol /aqueous extracts with a relative density of about 1.25 in a vacuum concentrator at 60–70°C. The crude drugs and reference markers (6-S reference substance batch No: A0370, purchased from Chengdu mustbio-technology Co., Ltd.; and AO-I reference substance batch No: A0541, purchased from Chengdu mansite Biotechnology Co., Ltd.) were verified by HPLC (waters 2695 HPLC).

Paclitaxel, absolute ethanol and polyoxyethylene castor oil (provided by the Institute of cancer prevention and treatment center of Sichuan Cancer Hospital). Preparation of paclitaxel: paclitaxel stock solution, absolute ethanol, polyoxyethylene castor oil, and physiological saline, prepared into a 1 mg/ml concentration of paclitaxel dilution. Preparation of S liquid: 1:1 ratio of absolute ethanol and polyoxyethylene castor oil.

2.2 Molecular docking of AO-I, 6-S and paclitaxel to human MD-2 structural mode

Docking simulation of AO-I (CID: 5321018), 6-S (CID: 5281794) and paclitaxel (CID: 36314) were carried out with the program AutoDock4 (open source, Scripps Research Institute), and the crystal structure of human MD-2 was obtained from Protein Data Bank (2E56)

2.3 Animals

Adult female C57 mice (6–8 weeks years old, body weight 18 ± 2 g) were purchased from Sichuan Chengdu Da shuo Laboratory Animal Co., Ltd., quality certificate number SCXK (Chuan) 2020-030 (Chengdu, China). All animals were maintained at 22 ± 2°C, 60 ± 10% humidity, on a 12-h light/dark cycle (6:00–18:00). Standard laboratory diet and water were provided ad libitum. All experimental animal handling procedures were conducted in strict accordance with the "Guidelines for the Care and Use of Laboratory Animals" (Ministry of Science and Technology of China, 2006), the Experimental Animal Ethics Committee of Sichuan Cancer Institute, and the "Guidelines for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH) (NIH Publication No. 85 − 23, revised in 1996).

2.4 HPLC analysis of AM and Zingiber alcohol /aqueous extract

AO-I and 6-S control samples were stored at − 4°C as 1 mg/ml stock solutions in ethanol. For the standard curve, the solutions were diluted to 5, 10, 20, 50, 100 and 200 $\mu g \cdot ml^{-}$. HPLC analysis (Milford, MA) was performed using an X Bridge column (250×5 mm, 4.6 μm), with acetonitrile (a) as the mobile

phase and 0.2% phosphoric acid (b) for gradient elution. The gradient protocol (0 ~ 10 min, 5 ~ 5% a; 10 ~ 30 min, 5 ~ 30%A; 30 ~ 40 min, 30 ~ 80%A; 40 ~ 45 min, 80 ~ 5%A) was conducted with a flow rate of 1.0 ml·min^{-1}, a detection wavelength of 277 nm, a column temperature of 30°C, and an injection volume of 10 µl.

2.5 In vivo pharmacokinetic analysis of AM and Zingiber alcohol /aqueous extract

Eighteen adult female SD rats were given an equivalent dose of 0.5ml intragastric administration according to body surface area. Each time, they fasted without food or water 12 hours before the gavage. At 5, 10, 15, 30, and 45 minutes, as well as 1.5, 2, 3, 4, 8, 12, 24 and 36 hours after administration, plasma samples (50 µl) were collected in a 1.5 ml centrifuge tube from the undus venous plexus. Then, 10 µL external standard solution and 150 µL acetonitrile were added and the mixture was vortexed for 2 min, followed by high-speed centrifugation at 3000 rpm for 10 min. The supernatant was recovered for HPLC analysis and used to draw a drug concentration curve.

2.6 Construction of OC tumor-bearing mouse model

ID8 mouse EOC cells (provided by the Institute of cancer prevention and treatment center of Sichuan Cancer Hospital) have been confirmed to be MyD88 positive [15]. Female C57 mice aged 6–8 weeks were inoculated with ID8 cells subcutaneously in the left armpit (3×10^7/ml, 100 ul). When the tumor volume reached 120 mm^3, the mice were treated with paxlitaxel or extracts. TCM and paclitaxel group was given a equivalent dose by body surface area (3.25 g/kg, equivalent to 25 g/person, once daily) of AM and Zingiber alcohol / aqueous extract by gavage and 0.2 ml paclitaxel solution was injected intraperitoneally; AO-I and 6-S combine paclitaxel group was given the control substance by gavage and 0.2 ml paclitaxel solution by intraperitoneal injection; paclitaxel group was given 0.2 ml normal saline by gavage and 0.2 ml paclitaxel solution by intraperitoneal injection; control group was given 0.2 ml normal saline by gavage and 0.2 ml s solvent by intraperitoneal injection. TCM gavage was performed for twice a day and 21 consecutive days; and intraperitoneal injection was performed every 3 days for 7 consecutive days. The gavage was given 12-hour interval between each morning and evening gavage; intraperitoneal injection was given 5 hours after each morning gavage.

Tumor volumes were calculated using the prescriptions [($v = \pi ab^2/6$, a is the long diameter, b is the wide diameter)], and mice were collected as follows: (1) After the first administration and after the third administration paclitaxel, 3 mice were randomly selected from 18 mice in each group. The mice were sacrificed by anesthesia and cervical dislocation, and then the tumor tissues were collected for further analysis. (2) After administration of all drugs, the mice were sacrificed by cervical dislocation. Three mice were randomly selected from 18 mice in each group. The whole blood was collected for determination of plasma enzyme levels, and the tumor, liver and kidney tissues were collected for further analysis. (3) Six mice were randomly selected from 18 mice in each group. After the treatment, they were fed normally, and the time of death (or when the tumor volumes reached 1 cm^3) was calculated to determine the survival rate.

2.7 Immunohistochemical evaluation of the expression of target gene PCNA

Transplanted tumor tissues of mice were fixed in 10% formalin and encapsulated, and longitudinal sections of 5 mm thickness were prepared. The dewaxed slices were placed into a dyeing cylinder, and 3% methanol hydrogen peroxide was added at room temperature for 10 min. The slices were than washed 3 times in PBS (5 min per wash), immersed in 0.01 M citrate buffer (pH 6.0), and heated in a microwave oven to boiling. After 5 min, the process was repeated. The solution was cooled, and the slices were washed twice with PBS for 5 min per wash. Then goat serum blocking solution was added dropwise for 20 minutes at room temperature, followed by primary antibody (PCNA, rabbit polyclonal antibody, 1:600 (concentration), 10205-2-ap, protein), 4°C overnight; and then biotinylated secondary antibody (Goat anti rabbit working solution, sp-9001, Beijing Zhongshan Jinqiao biology Co, Ltd.) at 37°C for 30 min. The samples were washed 3 times with PBS (5 min per wash); and then the DAB color development kit was used for visualization. Hematoxylin was used for counterstaining, and then the samples were dehydrated, sealed with transparent and neutral gum, and photographed with an optical microscope (Olympus IX71, Olympus, Tokyo, Japan). Two researchers used the image pro plus 6.0 image analysis system to measure the integrated optical density and area of the collected images, and the average optical density of each image was calculated.

2.8 Measurement of the apoptosis rate of tumor cells

Mouse tumor tissues were fixed in 10% formalin, dehydrated, embedded and sliced. Conventional dewaxing was performed, and then the samples were treated with trypsin K working solution at 37°C for 25 min, rinsed with PBS 3 times, and then air dried. The TUNEL reagent(Lot number: 45197300, Roche Group, Switzerland) was prepared as a 1:9 mixture of No. 1 liquid + No. 2 liquid, and 50 μL of the mixture was overlaid onto the specimen, which was placed in a dark wet box at 37°C for 1 h. The samples were rinsed 3 times with PBS and air dried, and then 50 μL No. 3 solution (converter POD) was overlaid on the specimens, which were covered with a glass slide or sealing film and incubated in a dark wet box at 37°C for 30 min. The slides were again rinsed 3 times with PBS, and 50–100 μL DAB chromogenic agent were added at 25°C for 10 min before rinsing 3 times with PBS. Hematoxylin was added for counterstaining, and the samples were washed with tap water, dehydrated with alcohol, and sealed with xylene transparent, neutral gum. Finally, the stained slides were photographed with an optical microscope (Olympus IX71, Olympus, Tokyo, Japan). The integrated optical density and area of the collected images were measured by two researchers using the image pro plus 6.0 image analysis system to calculate the percentage of apoptosis.

2.9 Analysis of the tumor inhibition rate and survival time of mice

During the administration period, the feeding, activity and death of mice were observed every day, and the body weight was recorded every other day. From the first day after administration, the tumor volume was

measured with vernier calipers and Doppler ultrasound every 3 days. The tumor volume was calculated according to the prescriptions ($v = \pi AB^2/6$, where a is the long diameter and b is the wide diameter), and a tumor growth curve was drawn. At 72 hours after the end of the 21 days drug treatment period, the mice were sacrificed by cervical dislocation. four mice were randomly selected from the 18 mice per group. The tumors were excised, and tumor blocks were stripped away. The tumor inhibition rate was calculated according to the following prescriptions:

Tumor inhibition rate (%) = 100% × (tumor volume of blank group - tumor volume of experimental group) / tumor volume of blank group

2.10 Statistical analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using GraphPad-Prism software 8. The statistical significance of the data between groups was evaluated by Student's t-test (for two groups) or one-way ANOVA for multiple comparisons. P value < 0.05 was considered statistically significant. All experiments were repeated at least three times.

3. Results

3.1 AM and Zingiber alcohol /aqueous extract promotes apoptosis and reduces the proliferative activity of tumor cells

For increased understanding of the function of AO-I and 6-S, we performed molecular docking experiments (Fig. 1). AO-I and 6-S can be fitted into the hydrophobic pocket of human MD-2 that overlaps with the binding site of paclitaxel, with a more energetically favorable interaction for AO-I and 6-S, which suggest that they may competitively displace paclitaxel from MD-2 [16, 17]. These results are consistent with the possibility that AO-I and 6-S may function as antagonists that inhibit TLR4-mediated paclitaxel signaling and are supported by our previous findings that AO-I and 6-S are able to sensitize EOC cells to paclitaxel by blocking the MD-2-mediated TLR4 / MyD88-dependent signaling pathway. Therefore, we sought to determine whether AO-I and 6-S, as well as AM and Zingiber alcohol /aqueous extract, a Chinese herbal medicine that they are derived from, may have in vivo efficacy in the ID8 mouse OC tumor model. HPLC chromatograms results (Fig. 2) that the content of AO-I was 4.83mg/ml; The content of 6-s is 0.17mg/ml, and these compounds have half-lives of about 1–2 hours in mice (Table 1). Consequently, we opted to use multiple doses with evaluation of tumor apoptosis levels after each round of administration.

Table 1
Time-blood concentration relationship

compound	parameter	Means($\bar{x}$±SD)
AO-I	$T_{1/2}$(h)	1.81 ± 0.49
	T_{max}(h)	1.5 ± 0.41
	C_{max}(ug/ml)	0.82 ± 0.02
6-S	$T_{1/2}$(h)	1.93 ± 0.48
	T_{max}(h)	1.0 ± 0.55
	C_{max}(ug/ml)	0.32 ± 0.01

To evaluate effects on apoptosis of mouse tumors, we performed TUNEL assays (Table 2). After the first paclitaxel administration, the apoptosis percentage of AM and Zingiber alcohol / aqueous extract and paclitaxel group, AO-I and 6-S combine paclitaxel group, and paclitaxel group treated mice were significantly higher than the percentages in the no treatment control group mice, but there were no significant differences between the treated groups ($P = 0.081 > 0.05$; Fig. 3A-D). After the third administration paclitaxel, however, the apoptosis percentage was greater for AM and Zingiber alcohol / aqueous extract plus paclitaxel group, AO-I and 6-S plus paclitaxel group than for paclitaxel group ($P < 0.001$; Fig. 3E-H), suggesting that paclitaxel group displayed chemo-resistance.

Table 2
The percentage of apoptosis (%) in ID8 mouse ovarian cancer tissue

Groups	percentage of apoptosis Means($\bar{x}$±SD)	
	After the first dose of PTX	After the 3rd PTX dose
Natural remedy + PTX	23.72 ± 13.01	21.40 ± 8.857
AO-I + 6-S + PTX	24.15 ± 7.424	20.28 ± 7.443
PTX	22.16 ± 2.735	6.281 ± 2.410
Control	2.86 ± 1.39	2.145 ± 0.4883

As additional verification, we evaluated OC cell proliferation levels in the four treatment groups (Table 3). Consistent with the apoptosis results, all of the treatment groups had reduced proliferation rates after the first round of treatment ($P < 0.05$ for 3 dosing groups compared to the no treatment control group; Fig. 4I-L), but after three rounds of treatment, paclitaxel alone group was less effective than AM and Zingiber alcohol / aqueous extract plus paclitaxel group or AO-I and 6-S plus paclitaxel group ($P < 0.05$; Fig. 4M-P). These results suggest that AM and Zingiber alcohol / aqueous extract and AO-I and 6-S each are effective in reducing paclitaxel resistance after multiple rounds of administration.

Table 3
Cell proliferation (IOD) in ID8 mouse ovarian cancer tissue

Groups	Average Optical Density of Cell Proliferation Activity($\bar{x}$±SD)	
	After the first dose of PTX	After the 3rd PTX dose
Natural remedy + PTX	1.412 ± 0.1081	1.629 ± 0.9227
AO-I + 6-S + PTX	1.453 ± 0.1926	2.655 ± 1.963
PTX	1.399 ± 0.5812	3.498 ± 1.704
Control	5.620 ± 2.869	4.745 ± 1.379

3.2 AM and Zingiber alcohol /aqueous extract inhibits tumor growth in tumor bearing mice

To evaluate the effects of AM and Zingiber alcohol / aqueous extract on tumor growth, we excised transplanted tumors after growth in ID8 mice. The tumors were visibly smaller after combination treatment (Fig. 4A), which could also be observed by Doppler ultrasound prior to excision (Fig. 4B). We also used vernier calipers to establish a growth curve for the transplanted tumors, which showed that the tumors in the non-administration group grew exponentially, with the average tumor volume reaching 611.3 mm^3, while the tumors grew progressively slower in paclitaxel group (403.8 mm^3), AO-I and 6-S plus paclitaxel group (144.1 mm^3), AM and Zingiber alcohol / aqueous extract plus paclitaxel group (103.4 mm^3) (Fig. 4C). There was a significant difference among the groups ($P < 0.0001$), suggesting that AM and Zingiber alcohol /aqueous extract plus paclitaxel was more effective at slowing tumor growth than paclitaxel alone or AO-I and 6-S plus paclitaxel. Further analysis of survival rates (Fig. 4D) shows that the median mortality was 13 weeks for AM and Zingiber alcohol / aqueous extract plus paclitaxel group, 8 weeks for AO-I and 6-S plus paclitaxel group, 7 weeks for paclitaxel group, and 5 weeks for non-administration group, with significant statistical differences between the survival rates of each group ($P < 0.0001$). Thus, these results suggest that AM and Zingiber alcohol /aqueous extract functions in combination with paclitaxel to suppress OC tumor growth and improve survival, with superior efficacy to AO-I and 6-S.

3.3 Safety analysis of AM and Zingiber alcohol /aqueous extract

For additional verification, we performed staining assays for liver and kidney damage. The results show that there was no obvious tissue damage in the liver and kidney of mice in each group by HE staining (Supplementary Material 1A). We also evaluated levels of liver /kidney damage markers in serum, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN) and creatinine (Cr). ($P > 0.05$). No significant differences were observed among the groups ($P > 0.05$),

indicating that AO-I, 6-S and AM and Zingiber alcohol /aqueous extract had no obvious drug toxicity (Supplementary Material 1B, Table 4).

Table 4
ID8 mice plasma liver and kidney function index

Biochemical index	Groups				P
	Natural remedy + PTX	AO-I + 6-S +PTX	PTX	Control	
ALT(u/L)	24.28 ± 5.556	27.38 ± 5.127	24.20 ± 4.327	26.13 ± 4.457	0.759
AST(u/L)	115.0 ± 15.99	124.2 ± 29.52	133.4 ± 9.120	102.1 ± 14.26	0.165
Cr(umol/L)	7.575 ± 2.819	9.025 ± 1.810	9.725 ± 2.892	7.960 ± 1.536	0.536
BuN(mmol/L)	7.568 ± 1.170	7.733 ± 1.445	7.650 ± 0.9449	7.708 ± 1.210	0.997

4. Discussion

OC treatment with first-line paclitaxel and platinum chemotherapy is effective in reducing tumor growth [1]. We reasoned that the combination of AM and Zingiber may be an effective combination for use with paclitaxel and that their effects may extend to an in vivo mouse model. To evaluate the in vivo effect of this combination, we prepared extracts according to recommendations in Chen Ziming's "Complete Effective Prescriptions for Womers Diseases: Eight Treatises on Postpartum Nausea and Not Eating" and is taken orally according to the dosage of ancient prescriptions. Our results demonstrate that AM and Zingiber alcohol / aqueous extract decreased the cell proliferation rate and increased the apoptosis of paclitaxel-treated cells after a single round of administration. Furthermore, the protective effects could still be observed after three rounds of treatment, whereas paclitaxel alone lost efficacy, which suggests that AM and Zingiber alcohol /aqueous extract counteracts chemo-resistance. Consistent with this possibility, the tumors of mice treated with AM and Zingiber alcohol /aqueous extract and paclitaxel grew slower than tumors treated with paclitaxel alone. Furthermore, the survival was significantly prolonged after combination treatment. Based on our docking assays, the mechanism may involve the ability to bind TLR4, though additional studies will be needed to confirm this possibility.

Notably, the anticancer effect of AM and Zingiber alcohol / aqueous extract was more potent than that of AO-I and 6-S in paclitaxel-treated OC model mice. These results are consistent with the possibility that other components of the extract may contribute to its anti-cancer properties. One study compared the antitumor effects of three lactones of AM and found that AO-II had a better antitumor effect than AO-I and AO-III [18]. Additionally, AO-II can significantly inhibit the proliferation of Lovo cells and induce

apoptosis by activating PARP cleavage and Caspase-3 expression [19]. Furthermore, Gingerol and shogaol, the active ingredients of Zingiber, each have anti-tumor and anti-inflammatory effects [20].Therefore, it is likely that multiple components of both AM and Zingiber may contribute to the potent activity of the combination. To evaluate the safely of AM and Zingiber extract, we also examined behavior, appearance, and liver and kidney function after treatment, and no harmful effects were observed for the extract.

Platinum first-line chemotherapy drugs for OC carry a high risk of gastrointestinal discomfort, with more than 60% of chemotherapy patients vomiting and more than 70–90% having nausea. Severe nausea and vomiting is a primary cause for poor follow-up treatment compliance, and even significantly shortens the overall survival time [21, 22]. Various components of Zingiber can improve nausea and vomiting in chemotherapy patients. Particularly the Gingerol and shogaol class of compounds, interact with several pathways that are directly implicated in CINV in addition to pathways that could play secondary roles by exacerbating symptoms [23].

PBG is a 5-HT specific receptor agonist, which promotes the entry of 5-HT into human blood, stimulates the chemical sensing area of brain medulla oblongata, and causes nausea and vomiting. Studies have confirmed that Gingerol has an inhibitory effect on PBG induced vomiting in a dose-response relationship [24, 25]. Other research confirmed that 6-S exhibited antiemetic effect at least partially by acting on the 5-HT3 receptor ion-channel complex, probably via binding to a modulatory site instead of the serotonin binding site [26], Also can directly act on M3 choline receptor in gastrointestinal tract [27].

This prescription is mainly used to treat postpartum nausea and vomiting, TCM is based on the belief that if the spleen is suitable to rise, it will be healthy, and if the stomach is suitable to fall, it will be harmonious; if the rise and fall are unbalanced, the body will produce nausea. AM combined with Zingiber combine a rise and a fall to regulate qi, reduce nausea and stop vomiting. Therefore, AM and Zingiber prescription may improve the gastrointestinal reactions such as non-disease nausea and vomiting and improve the quality of life. Confirmed by clinical research that Gingerol can significantly improve the complete remission rate of CINV in acute and delayed stages, and effectively improve Chemotherapy patients' appetite and quality of life [28]. Our hope is that through the treatment model of integrated traditional Chinese and Western medicine, to bring better outcomes for OC patients.

5. Conclusions

Hence, the scientific and rational combination of AM and Zingiber extract and first-line chemotherapy for OC, supplemented by scientific and accurate in vivo research results, can prove that AM and Zingiber extract can increase the sensitivity of paclitaxel to OC. AM and Zingiber extract a kind of safe, cheap and effective chemo-sensitizing natural remedy and natural therapy, it can try to solve multi-target drug resistance problems such as cancer.

Abbreviations

AO-I
Atractylenolide-I
AM
Atractylodes macrocephala Koidz
EOC
Epithelial ovarian carcinoma
HPLC
High performance liquid chromatography
NACT-IDS
Neoadjuvant Chemotherapy-Interval debulking surgery
TCM
Traditional Chinese Medicine
6-S
6-shogaol

Declarations

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Authors' contributions

Guo-Nan Zhang and Jian-Ming Huang designed the study. Xin-Ru Li performed the experiments and wrote the manuscript. Guo-Nan Zhang, Jian-Ming Huang and Xin-Ru Li interpreted the results and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Availability of data and materials

The data used to support the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Ethics Committee of Sichuan Cancer Hospital Medical Center (Approval No.KY-2019-035-01), and the experimental procedures were conducted in accordance with the ethical standards of the Chinese Association of Accreditation of Laboratory Animal Care.

Consent for publication

Not applicable

Conflict of interest

The authors declare no potential conflict of interest

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Base, Initial Alignment, IAKF, Sequential Filter, Filter Divergence, Smartphone

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Figures

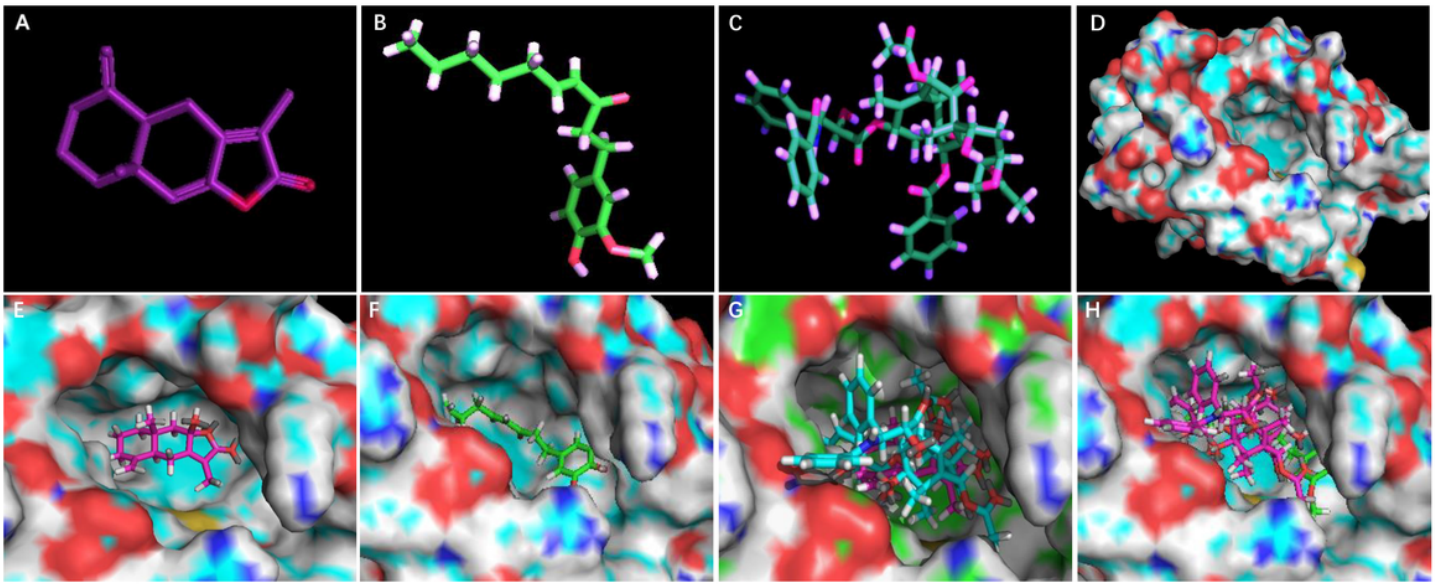

Figure 1

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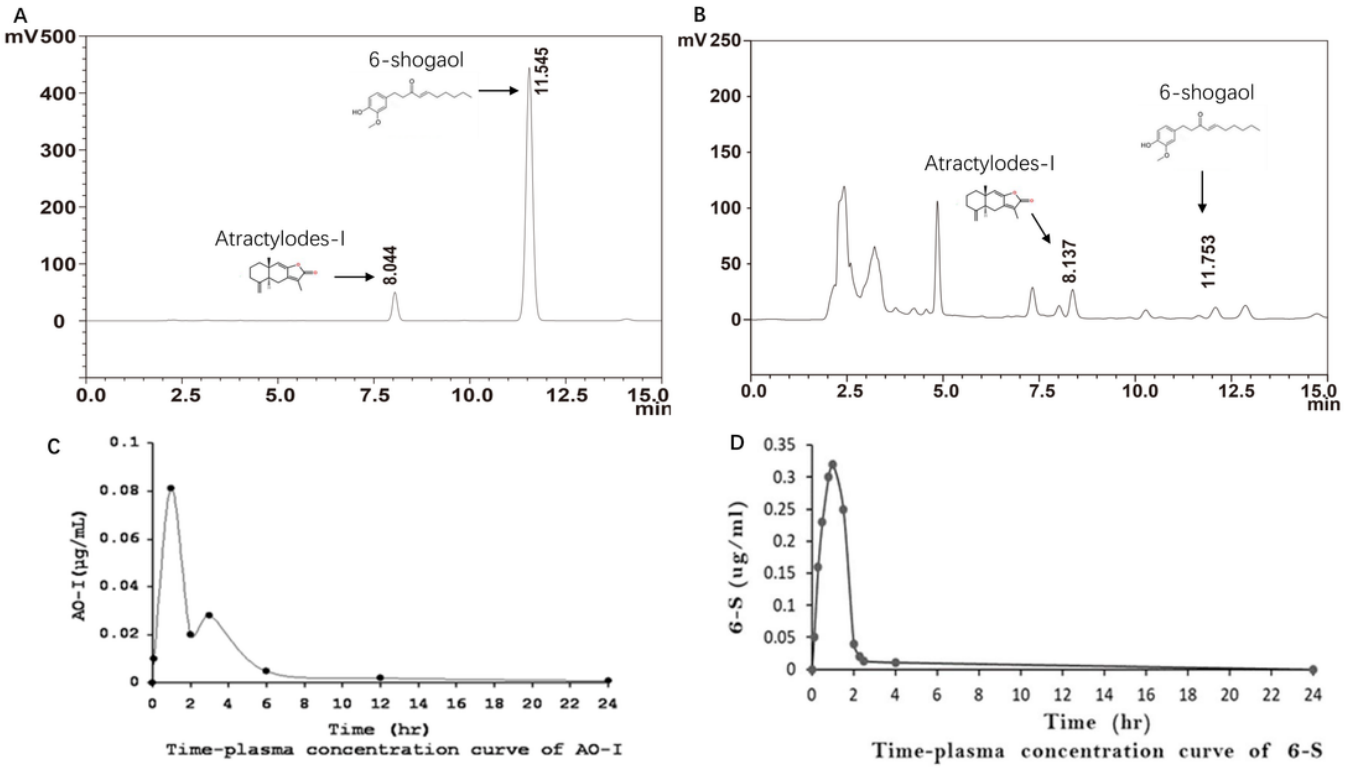

Figure 2

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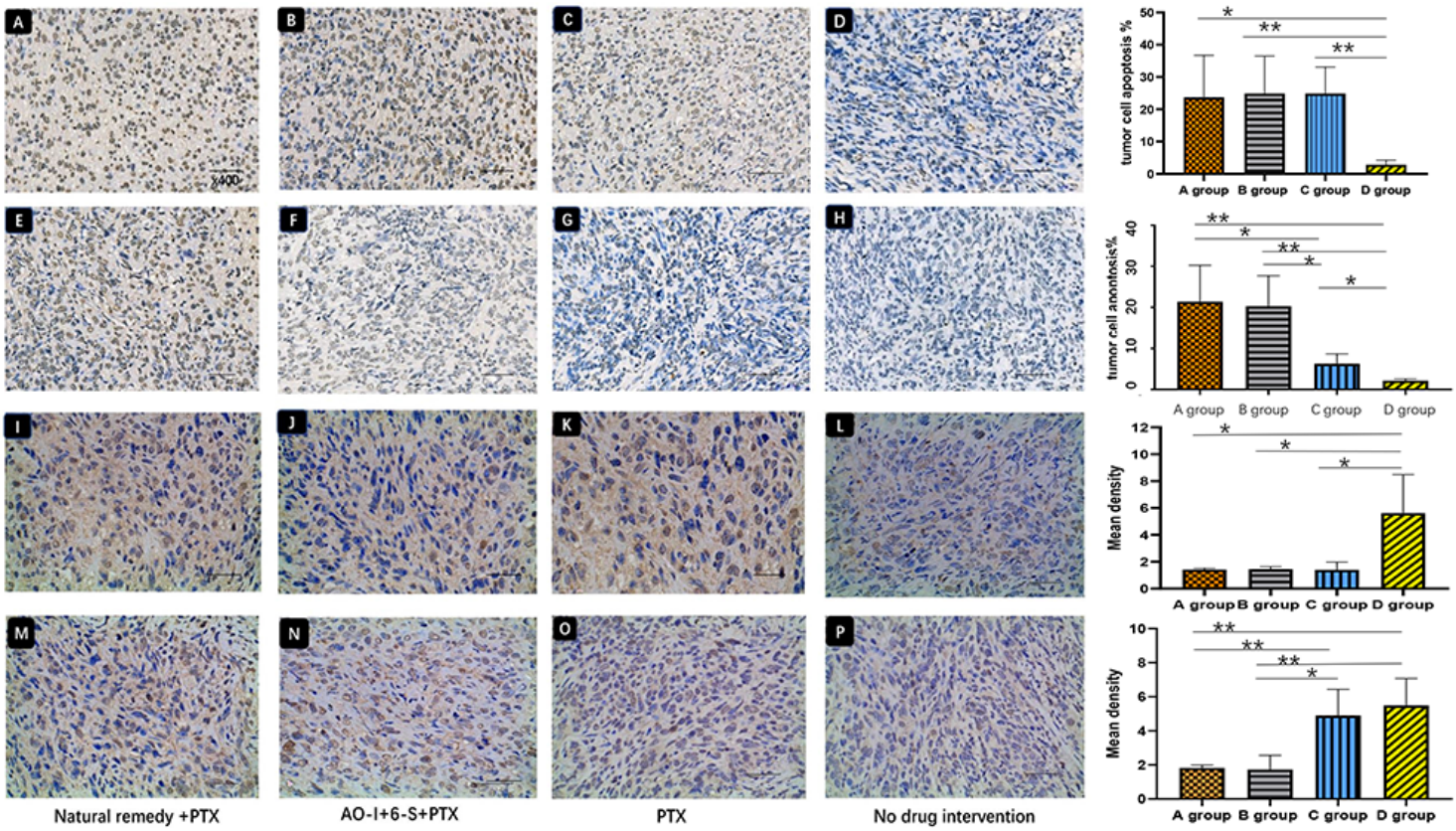

Figure 3

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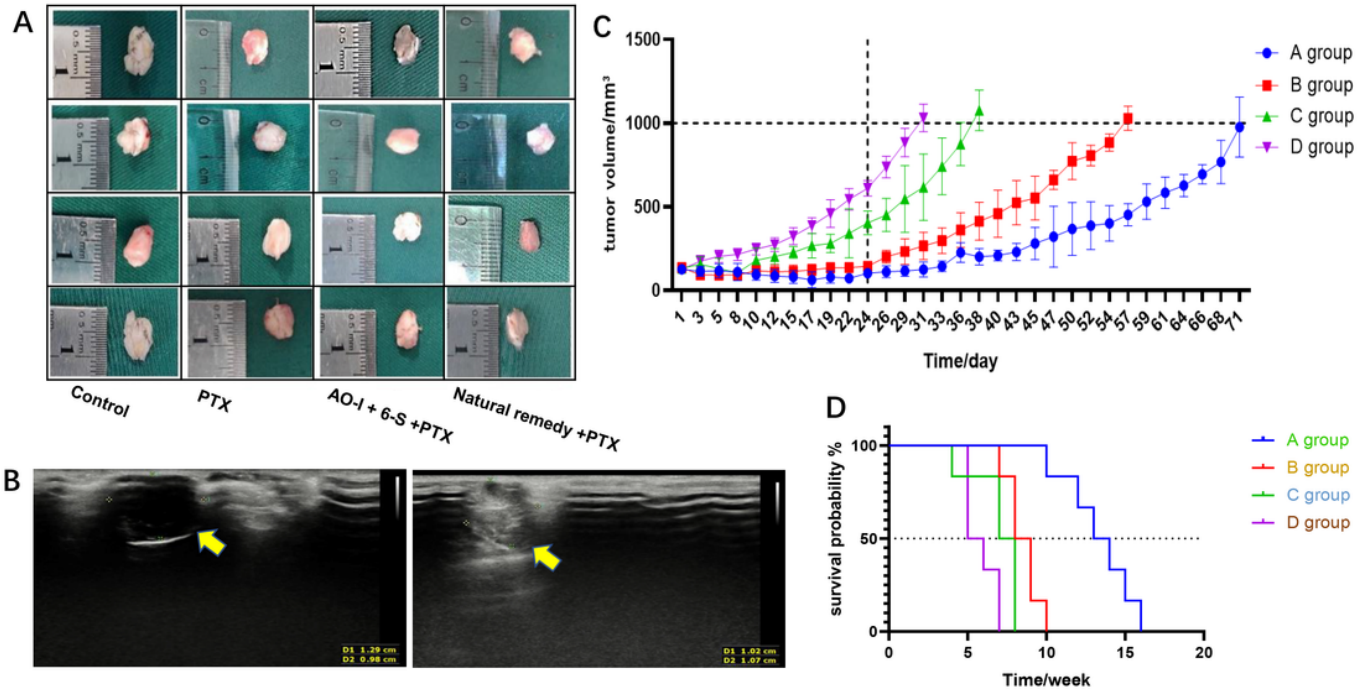

Figure 4

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