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Study on the mechanism of *Atractylodes macrocephala* extract regulating mitochondrial endoplasmic reticulum stress through PI3K/Akt signaling pathway to reverse epithelial-mesenchymal transition in uterine fibroids

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Abstract: **Background**: Uterine fibroids involve abnormal cell proliferation and fibrosis, with epithelial-mesenchymal transition (EMT) playing a key role. Mitochondrial-endoplasmic reticulum stress and related signaling pathways are implicated in this process, but the potential of natural extracts for modulation remains underexplored. **Objective**: This study aimed to investigate whether *Atractylodes macrocephala* extract can reverse EMT progression in uterine fibroids by regulating mitochondrial-endoplasmic reticulum stress via relevant signaling pathways. **Methods**: A mouse model of uterine fibroids was established and divided into normal, model, and *Atractylodes macrocephala* extract groups. Measurements included uterine weight, organ coefficient, cell proliferation, and apoptosis rate. Caspase-4 activity analysis, Western blotting, and immunofluorescence microscopy were used to assess protein and gene expression related to EMT, apoptosis, and signaling pathways. **Results**: The uterine fibroid model was successfully established. Treatment with *Atractylodes macrocephala* extract significantly inhibited uterine fibroid cell proliferation, promoted apoptosis, and reduced fibrosis. Mechanistically, the extract ameliorated EMT by effectively suppressing PI3K/Akt pathway activity. It concurrently exacerbated endoplasmic reticulum stress (indicated by increased Caspase-4 activity) to promote apoptosis while enhancing lysosome generation. **Conclusion**: *Atractylodes macrocephala* extract inhibits proliferation, promotes apoptosis, and reduces fibrosis in uterine fibroids by suppressing the PI3K/Akt pathway and enhancing endoplasmic reticulum stress. These findings provide a novel strategic basis for developing natural targeted therapies against uterine fibroids.

Keywords: *Atractylodes macrocephala* extract; Epithelial-mesenchymal transition; Mitochondrial endoplasmic reticulum; PI3K/Akt; Uterine fibroids

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INTRODUCTION

Uterine fibroids are mainly caused by abnormal proliferation of uterine smooth muscle cells and have a high incidence rate, especially in women of childbearing age (Vannuccini *et al*., 2024). The clinical manifestations of this disease are abnormal increase in menstrual volume and prolonged menstrual cycle. In severe cases, they even threaten women's fertility and overall quality of life (Ahmad *et al*., 2023). The pathological mechanism of uterine fibroids covers multiple levels, including genetic tendency, sex hormone fluctuations and epithelial-mesenchymal transition (EMT). EMT not only promotes tumor invasion and metastasis to a certain extent, but also may accelerate the growth process of uterine fibroids (Manfioletti and Fedele, 2023, Yang *et al*., 2022). In view of this, it is particularly important to actively explore efficient and safe treatment strategies. Rhizoma Atractylodis Macrocephalae is extracted from the rhizome of the traditional Chinese medicine *Atractylodes macrocephalae*. It is rich in polysaccharides with a molecular weight of 5.84 kDa. Tang X *et al*. (2024) demonstrated in cellular and animal experiments that Atractylodes macrocephala extract downregulates Interleukin-17A and tumor necrosis factor-alpha (TNF-α), exerting therapeutic effects on psoriasis-like lesions. The polysaccharides inhibit the PI3K/AKT/mTOR pathway, exerting anti-inflammatory effects, while also modulating immune function and potentially benefiting cardiovascular health (Luo *et al*., 2022). Studies have found that (Enserink and Chymkowitch, 2022) *Atractylodes macrocephala* extract can inhibit tumor cell proliferation and exert anti-tumor effects by reducing Cyclin-Dependent Kinases and Cyclin B1 levels, causing the cell cycle to stop at the G2/M phase. In addition, some studies have confirmed that *Atractylodes macrocephala* extract can promote the release of various pro-apoptotic factors such as Cyt c (Pessoa, 2022), Apoptosis-inducing factor (AIF) (Vadlamudi and Kang, 2022) and Endonuclease G (Endo G) (Choi *et al*., 2023) into the cytoplasm by acting on the PTP on the mitochondrial membrane, thereby inhibiting human colon cancer cells and accelerating their programmed cell death. In addition, *Atractylodes macrocephala* extract activates Cysteine-aspartic acid protease 9, promoting the expression of pro-apoptotic proteins and the release of apoptosis-inducing factors such as Smac/DIABLO, AIF

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and Endo G, further inducing tumor cell apoptosis (Zhang *et al*., 2022). In addition, *Atractylodes macrocephala* lactone compounds can not only restore cancer cells sensitivity to apoptotic signals by downregulating the expression of B-cell lymphoma 2 (Bcl-2), TNF-α, Vascular endothelial growth factor, etc., but also reduce the inflammatory response in the tumor microenvironment, reduce the blood supply and oxygen supply of tumor cells and thus inhibit the behavior of tumor cells (Liu *et al*., 2024). This study aims to explore whether *Atractylodes macrocephala* extract can inhibit the PI3K/Akt signaling pathway, aggravate endoplasmic reticulum stress-induced cell apoptosis and thus control the epithelial-mesenchymal transition in uterine fibroids.

PI3K/Akt signaling involves in EMT. Some studies have shown that (Shi *et al*., 2023) this pathway promotes Neural cadherin and Vimentin level by upregulating Snail family transcriptional repressor 1, Snail family transcriptional repressor 2 and Twist-related protein 1, which affects the EMT process of uterine fibroids to a certain extent. Yu, Y *et al*. and colleagues found (Yu *et al*., 2023) that *Atractylodes macrocephala* extract regulates PI3K/Akt signaling, thereby increasing PIP3 level, indirectly promoting the phosphorylation and activation of mTOR and Akt, thereby exerting an anti-tumor biological effect; at the same time, it can also regulate the expression of proteins such as Glycogen synthase kinase 3 beta and Forkhead box O, further exerting cell cycle regulation and apoptosis inhibition in tumor cells. In addition, modern pharmacological studies have shown (Chan *et al*., 2020) that *Atractylodes macrocephala* extract has anti-tumor and immune system regulation pharmacological effects, but whether it can have a certain inhibitory effect on the EMT process of uterine fibroids requires further experimental research and clinical verification to confirm.

According to the research report of Ma, F *et al*. (2024), mitochondrial endoplasmic reticulum stress regulates various cell functions. It activates PI3K/Akt signaling, thereby regulating the expression of cell adhesion molecules such as E-cadherin and integrins, as well as the rearrangement of the cytoskeleton, further promoting the transformation of uterine fibroid cells from epithelial phenotype to mesenchymal phenotype and may participate in onset and progression of uterine fibroids. Therefore, this study explores the specific mechanism by which *Atractylodes macrocephala* extract regulates the PI3K/Akt signaling pathway, thereby affecting mitochondrial endoplasmic reticulum stress and reversing EMT in uterine fibroids and further provides a basis for developing new therapies based on natural drugs.

MATERIALS AND METHODS

Experimental materials

30 female SD mice aged 4-6 weeks and weighing 16-25 grams were purchased from Kaixue Biotechnology LTD. The mice were kept in an environment of 22-25ºC and 35%-40% humidity and adapted for 1 week.

Atractylodes macrocephala extract was purchased from the National Institutes for Food and Drug Control (NIFDC, China), batch number: BST-2023-045.

Identification standard: High-performance liquid chromatography (HPLC) confirmed that the content of the main active components (atractylenolide I, II and III) was ≥98% (complying with the Chinese Pharmacopoeia 2020 edition). The specimen was archived at the Herbarium of Chongqing University of Traditional Chinese Medicine (voucher number: CQMU-HERB-2023-087). Major drugs and reagents used in this study are listed in Table 1.

Preparation method

100 g of *Atractylodes macrocephala* powder was reflux-extracted twice with 70% ethanol (v/v) at a solid-to-liquid ratio of 1:10 (2 h each time, 80ºC). The filtrates were combined and concentrated under reduced pressure until no alcohol odor remained. The resulting extract was then loaded onto an AB-8 macroporous resin column and impurities were removed by sequential elution with water and 30% ethanol. The 70% ethanol eluate was collected, concentrated and freeze-dried to obtain a yellowish-brown powder, which was stored at -80ºC for further use.

The experiment was approved by the Ethics Committee.

Methods

Modeling and grouping

Uterine fibroid models were constructed in the model group and the experimental group by intramuscular injection of estradiol benzoate (0.0005 mg/g, 3 times a week for 8 weeks) and intraperitoneal injection of progesterone (0.004 mg/g, 2 times a week for 4 weeks). HE staining revealed thickened muscle fibers, hyperplasia of uterine smooth muscle cells and connective tissue, along with distinct ultrastructural alterations including mitochondrial swelling, cristae disappearance, endoplasmic reticulum (ER) dilation and vacuolization, indicative of ER stress. These findings confirmed successful model establishment. Finally, 18 models were successfully established, including 9 in the model group and 9 in the *Atractylodes macrocephala* extract group, with a modeling success rate of 90%. After successful modeling, mice in the experimental group received subcutaneous injection of *Atractylodes macrocephala* extract (4 mg/g) for 15 days and normal and model group received saline control. One mice in model group died during intervention and 8 mice were finally involved in subsequent experiments.

Specimen collection

After the mice were fasted for 4 hours, blood was drawn from tail vein and centrifuged at 3000 rpm for 20 minutes to obtain supernatant which was stored at -80 degrees for subsequent research.

Biological sample collection

Within 2 hours after the last administration, the weight of the mice was measured and the eyeballs were removed for blood collection. The blood was centrifuged at 2500r/min for 15 minutes at 5-6℃ and the serum was separated and refrigerated for later use. The mice were then killed, the uterus was placed on ice and then stored in a low-temperature refrigerator for subsequent testing.

Primary cell culture

Tissue samples were washed 2-3 times with HBSS solution containing penicillin (1000U/ml) and streptomycin (1000μg/ml), minced, and digested with 8 mL of 0.4% collagenase solution at 37℃ for 2.5 hours. After digestion, add penicillin, streptomycin, concentrations of 100U/ml and 100μg/ml and 10% FBS DMEM-F12 culture medium to further terminate the digestion process and place the mixture in a constant temperature incubator for further culture. Differential adhesion technology is used to remove fibroblasts. After three rounds of purification, the non-adherent cells are identified as uterine fibroid cells, while uterine smooth muscle cells are cultured following identical procedures to ensure the cellular purity meets experimental standards.

Uterine weight and uterine coefficient detection

After measuring and recording the weight of each mouse, anesthetize it and kill it by decapitation. The uterine tissue was removed and weighed and the uterine coefficient was calculated (uterine weight/body weight × 1000).

Staining treatment

HE staining: The mouse uterine tissue was frozen in liquid nitrogen and fixed with formaldehyde before sectioning. It was first dewaxed twice with xylene, each time for 5 minutes and then hydrated with gradient alcohol for 3 minutes. After rinsing, it was stained with hematoxylin for 5 minutes, differentiated with 1% hydrochloric acid alcohol solution for 30 seconds and blued with 0.2% ammonia solution for 2 minutes. It was further stained with eosin for 10 minutes and after dehydration and sealing, it was carefully observed under an optical microscope. DAPI, Lysosome and Merge staining: For DAPI staining, dilute PBS to 1:5000, remove the culture medium, wash 3 times with PBS, fix with 3.7% formaldehyde for 10 minutes and stain after permeabilization with 0.2% Triton X-100 for 5 minutes; for Lysosome staining, add neutral red to the cells in the logarithmic phase and incubate until the lysosomes are completely taken up and then observe after washing with PBS; for Merge staining, add Merge dye after fixing the sample and washing with PBS and all the changes in the internal structure of the cells are observed under a fluorescence microscope.

Preparation steps for transmission electron microscopy

Uterine fibroid samples were obtained and small pieces of tissue with a size of 1×1×1mm were cut and immersed in glutaraldehyde solution (2.5%). After fixing for 4 hours at 4°C, 0.1M 4% glutaraldehyde solution with a pH of 7.4 was used. The tissue was rinsed three times with PBS for 15 minutes. After that, the sample tissue block was placed in 1% osmium acid solution, fixed for 2 hours and rinsed again with PBS for three times. Then, it was dehydrated with 50%~100% alcohol (15 minutes each) and acetone and 812 embedding agent were mixed in a ratio of 1:1. The tissue was infiltrated overnight. After polymerization at 60°C for 48 hours, sections (thickness 60~80) were prepared and their microstructures were observed by transmission electron microscopy.

Cell proliferation test

Smooth muscle cells and uterine fibroid cells were inoculated into 96-well plates (3000 cells per well). After incubation overnight, they were cultured in 1% O2 (hypoxia) and 21% O2 (control) environments respectively. Samples were collected at 6 hours, 12 hours, 24 hours and 48 hours and operated according to the standard SRB staining process.

Cell apoptosis test

Cells were seeded in 6-well plates (6×10^6 per well), then digested and fixed with trypsin, stained with Annexin V and nucleic acid dyes (Invitrogen) and the degree of apoptosis was analyzed by flow cytometry.

qPCR

RNA was isolated and PCR amplification was performed using cDNA as a template. The primer sequences of relevant target genes and GAPDH are shown in Table 2. The amplification process was performed using SYBR Green kit. The specific procedure was: pre-denaturation at 95°C 5 minutes and 40 cycles of amplification, including 15 seconds 95°C and 35 seconds 62°C. The level of mRNA was further determined using the 2-ΔΔCt method.

Caspase-4 activity analysis

In the experiment, cell samples treated with *Atractylodes macrocephala* extract and control cells cultured under standard conditions were collected. The activity level of Caspase-4 was determined using a kit from Beyotime (Haimen, China).

Western blot experiment

The cell samples (normal group, model group and *Atractylodes macrocephala* extract group) were washed twice and lysed followed separation on SDS-PAGE for western blot using antibodies against CRP78 (ab21685, 1:1000), CHOP (ab11419, 1:1000), Capase4 (ab32351, 1:1000), Bax (ab32503, 1:1000), Bcl-2 (ab141523, 1:1000), AKT (ab8805, 1:1000) and the secondary antibody (goat anti-rabbit 1:2000).

Immunofluorescence microscopy

Cells were fixed, treated with 0.3% Triton X-100 in PBS in an ice bath and then blocked with 1% BSA for 1 hour followed by addition of primary antibody (1:1000) and

then secondary antibody (1:2500). After 1 hour, cells were washed. Finally, the endoplasmic reticulum stress tracer (displayed as green fluorescence) and DAPI (displayed as blue fluorescence) were added and protected with anti-fading reagent for subsequent observation and preservation.

Statistical analysis

GraphPad Prism version 6 and ImageJ processed data. The measured data were described as mean ± standard deviation and analyzed by one-way ANOVA with Tukey post hoc test. $P<0.05$ indicates significance.

RESULTS

The mouse model of uterine fibroids was successfully constructed and the application of Atractylodes macrocephala extract can improve EMT and PI3K/AKT signaling is abnormal

The model was successfully constructed and the uterine fibroid cells of the model group mice were spindle-shaped, containing a large amount of actin in the cells and smooth muscle actin was evenly distributed in cytoplasm (Fig. 1A red) and nucleus was clear (Fig. 1B blue). HE staining showed irregular cell arrangement and disordered tissue structure. The structure of the uterine myometrium in the *Atractylodes macrocephala* extract group was improved, the cells were arranged more orderly (Fig. 1C) and the uterine weight and uterine coefficient were significantly reduced (Fig. 1D-1F). After treatment, the fibrous tissue and vascular structure were normal, without abnormal hyperplasia, inflammatory infiltration was reduced and the nuclear morphology was normal without atypia (Fig. 1G). The levels of PI3K and AKT in the *Atractylodes macrocephala* extract group decreased significantly (P value less than 0.05) and the expression of Caspase-3 protein was effectively inhibited. (Fig. 1H).

Atractylodes macrocephala extract improves mitochondrial apoptosis of uterine fibroid cells and aggravates endoplasmic reticulum stress apoptosis

The mice in the model group showed obvious endoplasmic reticulum stress and cell damage, endoplasmic reticulum dilation, mitochondrial swelling and cristae disappearance, vacuolization and partial cytoplasm dissolution (Fig. 2A). In contrast, after treatment with *Atractylodes macrocephala* extract, nuclear shrinkage improved, mitochondrial morphology was closer to normal, endoplasmic reticulum dilation and vacuolization were reduced and cell structure was improved. To verify the improvement effect of *Atractylodes macrocephala* extract, 8 model group mice were treated with the positive drug mifepristone. The cell structure of positive drug group was more normal, the endoplasmic reticulum expansion and vacuolization were significantly reduced and the mitochondrial morphology was close to normal, indicating that it had a significant effect in alleviating endoplasmic reticulum stress. Although *Atractylodes macrocephala* extract had a similar effect, the effect was weaker.

Further analysis revealed distinct responses to *Atractylodes macrocephala* extract in tervention between cell types. In uterine fibroid cells, the proliferation rate significantly decreased (Fig. 2B, $P>0.05$), while apoptosis rate and Caspase-4 activity markedly increased (Fig. 2C-2D, $P<0.05$). These effects mirrored those observed in the positive control (mifepristone) group. In contrast, smooth muscle cells showed no statistically significant changes in these parameters ($P > 0.05$). WB experiments showed that the expression of CHOP, Caspase-4, Bax and Bcl-2 proteins changed significantly after treatment with *Atractylodes macrocephala* extract (Fig. 2E), which was consistent with the change trend of cell apoptosis rate and Caspase-4 activity, while the change trend of PI3K/AKT pathway proteins was opposite, indicating that *Atractylodes macrocephala* extract enhanced the endoplasmic reticulum stress-induced cell apoptosis process by blocking the PI3K/AKT signaling pathway.

Atractylodes macrocephala extract can induce endoplasmic reticulum stress and lysosome generation in uterine fibroid cells and PI3K/AKT signaling is inhibited

After intervention with *Atractylodes macrocephala* extract, the expression of CRP78, CHOP, Caspase-4 and BAX mRNA (Fig. 3A-3D) and protein (Fig. 4A-4D) in uterine fibroid cells significantly increased with time and the expression of Bcl-2 mRNA and protein showed a decreasing trend (Fig. 3E-4E). The expression of AKT mRNA (Fig. 3F) and protein (Fig. 4F-4G) in PI3K/AKT pathway was inhibited and the degree of inhibition was stronger than that in positive drug group ($P<0.05$). The results showed that *Atractylodes macrocephala* extract induced ER stress and lysosome generation by inhibiting PI3K/AKT pathway.

Atractylodes macrocephala extract induces ER stress response by inhibiting PI3K/Akt signaling

To verify the inhibitory effect of *Atractylodes macrocephala* extract on the PI3K/AKT signaling pathway, 0.5 ng/ml IGF-1 was injected into mice in *Atractylodes macrocephala* extract group and found levels of AKT and PI3K proteins increased (Fig. 5D-5E), the inhibition of uterine fibroid cell proliferation was reversed and the longer the intervention time, the more significant the reversal (Fig. 5A-5B). The number of lysosomes decreased (Fig. 5C), the endoplasmic reticulum stress tracer decreased and the overlap of lysosome localization decreased.

DISCUSSION

Elevated TGF-β levels in uterine fibroids activate Smad proteins, promoting collagen and fibronectin expression to enhance the mesenchymal phenotype (Han *et al*., 2022). Chronic inflammation, mediated by Interleukin-6 (IL-6) and TNF-α, further promotes EMT through pathways such

as JAK/STAT, creating a favorable microenvironment for fibroid growth (Liu *et al*., 2021). Consistent with these mechanisms, our study successfully established a uterine fibroid model. In model group mice, fibroid cells exhibited a spindle-shaped morphology with abundant actin, and smooth muscle actin (α-SMA) was evenly distributed throughout the cytoplasm. These characteristics confirm the successful establishment of typical uterine fibroid tissue, providing a reliable pathological model for subsequent investigations.

Compared with model group, uteri treated with *Atractylodes macrocephala* extract exhibited improved myometrial tissue structure, characterized by more orderly cell arrangement, reduced fibrous tissue proliferation, and decreased inflammatory infiltration. These findings suggest that the extract may attenuate fibroid development by inhibiting fibrotic processes. Notably, although Caspase-3 expression was inhibited, no obvious abnormal proliferation or nuclear atypia was observed, indicating that *Atractylodes macrocephala* extract may regulate cell fate through non-canonical pathways. Further investigation into the effects of this extract on apoptosis and other forms of programmed cell death may reveal additional therapeutic mechanisms.

This study demonstrates that *Atractylodes macrocephala* extract can not only effectively promote the apoptosis of uterine fibroid cells, but also inhibit their proliferation and reduce cell damage to a certain extent. During this process, the expression of CHOP and Caspase-4 and apoptosis regulatory proteins such as Bax and Bcl-2 showed significant changes, which were consistent with the changes in cell apoptosis rate and Caspase-4 activity. Oxidative stress is one of the key factors affecting ER stress (Lu *et al*., 2020). Through accumulation of excessive reactive oxygen species (ROS), the normal protein folding function of the ER is disturbed, thereby further inducing stress response. As an antioxidant, *Atractylodes macrocephala* extract reduces intracellular oxidative stress and consequently mitigates oxidative stress-induced ER stress (Luo *et al*., 2022, Qiu *et al*., 2023). In this study, the expression of PI3K/AKT pathway-related proteins were opposite to the changes in cell apoptosis rate and Caspase-4 activity, indicating that *Atractylodes macrocephala* extract aggravated ER stress apoptosis by inhibiting PI3K/AKT signaling. Zhang, C *et al*. (2022) pointed out in a network pharmacology analysis that *Atractylodes macrocephala* extract can exert anti-tumor effects through multiple targets such as Protein kinase B alpha (Akt1) and IL-6 and inhibiting the AKT pathway can aggravate ER stress-induced cell apoptosis, consistent with our results. The effect of *Atractylodes macrocephala* extract on AKT1 may explain its mechanism of aggravating ER stress apoptosis, suggesting that AKT is a target for treating uterine fibroids. In this study, *Atractylodes macrocephala* extract increased the level of ER stress and triggered the apoptosis pathway by upregulating the expression of CRP78, CHOP, Caspase-4, etc. These changes were verified at both mRNA and protein levels, indicating that *Atractylodes macrocephala* extract can effectively induce ER stress response and also provides a new natural candidate for treating uterine fibroids and suggests the potential of AKT as a therapeutic target.

ER stress frequently accompanies increased lysosome generation to facilitate clearance of damaged organelles and proteins (Wang *et al*., 2022). ER stress induced by *Atractylodes macrocephala* extract may promote lysosome formation, enhance cell autophagy, clear damaged cell components and initiate apoptosis (Xie *et al*., 2016). In the experiment, after the injection of IGF-1, an agonist of PI3K/AKT signaling, the inhibited proliferation activity of uterine fibroid cells in the *Atractylodes macrocephala* extract group was significantly reversed. The analysis showed that *Atractylodes macrocephala* extract has good biological activity, can increase miR-34a expression, inhibit downstream PD-L1 mRNA and protein expression and can also inhibit tyrosinase. Yan, S. *et al*. (2020) confirmed that *Atractylodes macrocephala* serum can inhibit PI3K/Akt/mTOR pathway and reduce the levels of Beclin-1, LC3 and p-PI3K, thereby inhibiting the autophagy process, making cells more sensitive to apoptosis and reducing abnormal autophagy, which is crucial for reversing EMT in uterine fibroids. In addition, *Atractylodes macrocephala* extract can regulate the expression of serum motilin (Yan *et al*., 2020), substance P and ATP in the colon, improve vitamin B6 metabolism and have a significant effect on energy metabolism. Xu, W *et al*. (2020) used RNA-seq analysis and KEGG pathway experiments to show that *Atractylodes macrocephala* total polysaccharides can activate macrophages through NF-κB and Jak-STAT signaling pathways.

As a classical gynecological Chinese medicine, *Atractylodes macrocephala* can effectively inhibit NO, TNF-α, IL-6 and Monocyte chemoattractant protein-1 level by RAW 264.7 macrophages induced by LPS. Zhou, Y *et al*. (2019) also pointed out that *Atractylodes macrocephala* extract can inhibit inducible Inducible nitric oxide synthase protein level in a concentration-dependent manner, block the phosphorylation and degradation of inhibitory κBα and inhibit the nuclear translocation process of NF-κB p65 to a certain extent, as well as inhibit the activity of GSK 3β, ultimately reducing chronic inflammation in uterine fibroids, reducing the production of profibrotic factors and helping to reverse EMT. *Atractylodes macrocephala* extract protects intestinal function by antagonizing passive transport, while its component AMAP-2 stimulates RAW264.7 macrophages to release nitric oxide, enhancing immune function (Çelik and Çelik, 2022, Kuisma *et al*., 2022). While the mouse uterine fibroid model was successfully established, it may not fully recapitulate the complex pathological features and biological behaviors of human fibroids.

Table 1: Main drugs and reagents.

Drugs/Reagents	Purchased companies
Mouse anti-mouse E2 antibody	Invitrogen
Rabbit anti-mouse P antibody	Gibco
Mouse anti-human FSH antibody	Sigma
Mouse anti-mouse LH antibody	Sigma
ELISA kit and supporting reagents	Tiangen Biochemical Technology LTD
HE stain	Beijing Zhongshan Jinqiao Biotechnology LTD

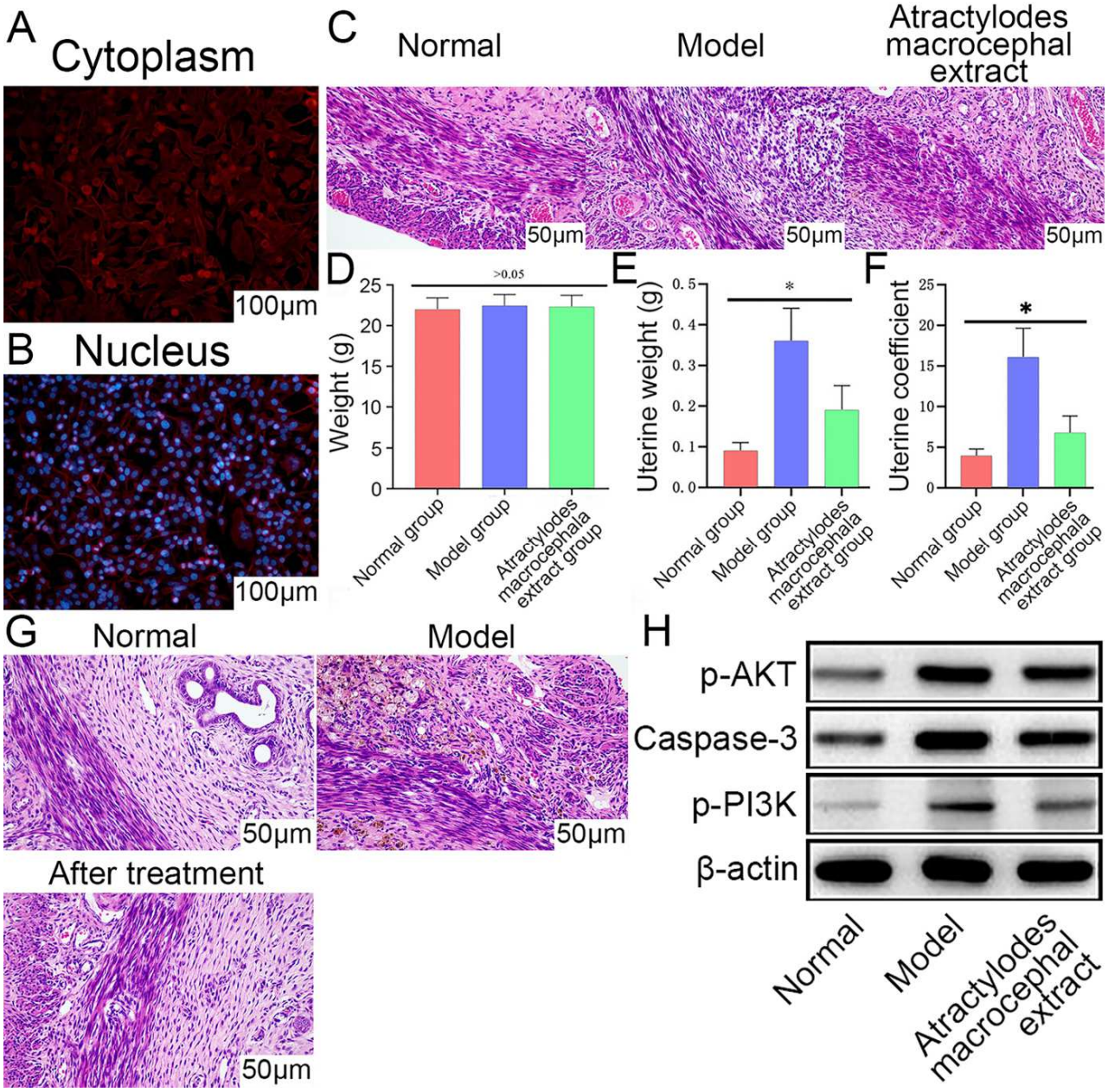

Fig. 1: The mouse model of uterine fibroids was successfully constructed, and the application of *Atractylodes macrocephala* extract can improve EMT, and the PI3K/AKT signaling pathway is abnormal in this process.
Note: (A): Cytoplasm; (B): Nucleus; (C): HE staining of normal group, model group, and *Atractylodes macrocephala* extract group cell arrangement; (D): Mouse body weight; (E): Mouse uterine weight; (F): Mouse uterine coefficient; (G): HE staining of *Atractylodes macrocephala* extract group after treatment, *Atractylodes macrocephala* extract group before treatment, and normal group cell arrangement; (H): *Atractylodes macrocephala* extract group, model group, and normal group protein strips. n=8，*P<0.05.

Table 2: PCR primer sequences.

Primer		Sequences
CRP78	Forward primer	5'-CCTAGCTGTGTCAGAATCTCCATCC -3'
	Reverse primer	5'- GTTTCAATGTCACCATCCAAGATCC -3'
CHOP	Forward primer	5'- AGCAAAGCTATAATCCCCCTCAG -3'
	Reverse primer	5'- GAAGGAGAAAGGCAATGACTCA -3'
Capase4	Forward primer	5'- TCACCTGCCTGCAAGGAATG -3'
	Reverse primer	5'- TGGCGTTGAAGAGCAGAAAGC -3'
Bax	Forward primer	5'- GGACGAACTGGACAGTAACATGG -3'
	Reverse primer	5'- GCAAAGTAGAAAAGGGCGACAAC -3'
Bcl-2	Forward primer	5'- ATCGCCCTGTGGATGACTGAG -3'
	Reverse primer	5'- CAGCCAGGAGAAATCAAACAGAGG -3'
AKT	Forward primer	5'- GCAGGATGTGGACCAACGTGAG -3'
	Reverse primer	5'- GCAGGCAGCGGATGATGAAGG -3'
GAPDH	Forward primer	5'- AAAGGGTCATCATCTCCGCC -3'
	Reverse primer	5'- AGTGATGGCATGGACTGTGG -3'

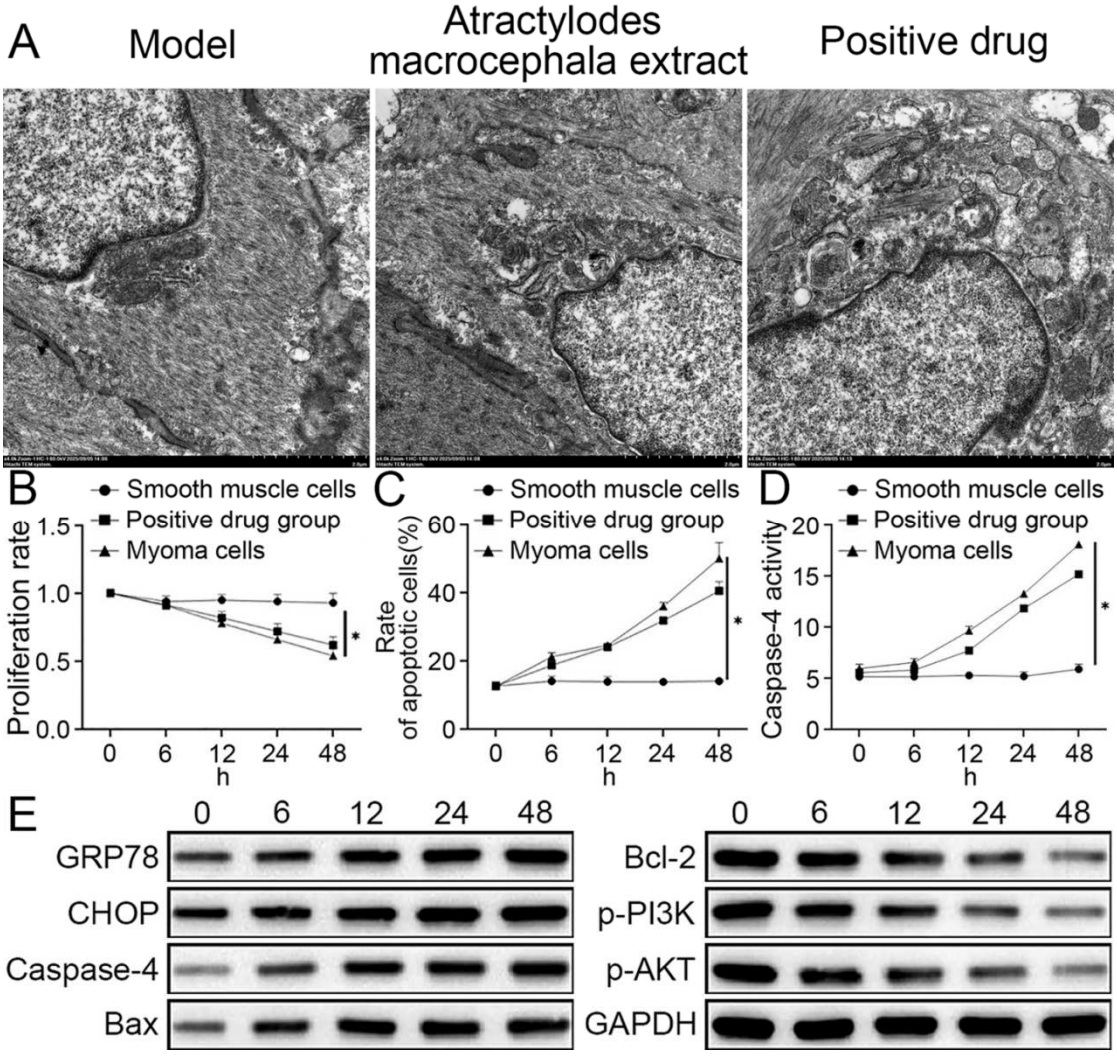

Fig. 2: *Atractylodes macrocephala* extract improves mitochondrial apoptosis of uterine fibroid cells and exacerbates endoplasmic reticulum stress apoptosis.

Note: (A): Tissue ultrastructure under electron microscope; (B): Cell proliferation rate; (C): Apoptotic cell ratio; (D): Protein Caspase-4 activity; (E): 0-48hr protein expression. n=8，*P<0.05.

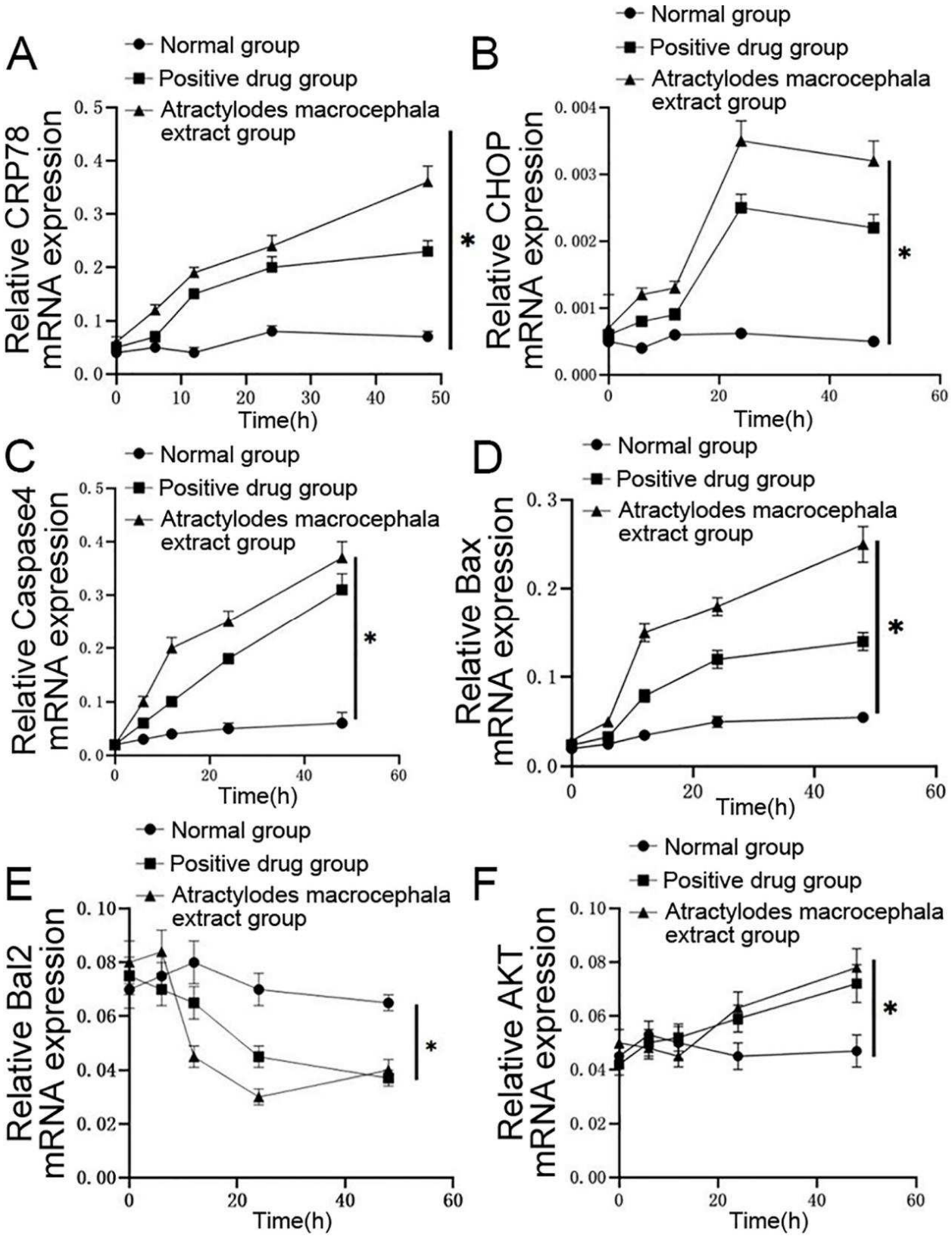

Fig. 3: Expression of key proteins in ER stress and PI3K/AKT pathway mRNA.
Notes: (A): CRP78 mRNA expression; (B): CHOP mRNA expression; (C): Capase4 mRNA expression; (D): Bax mRNA expression; (E): Bcl-2 mRNA expression; (F): AKT mRNA expression. n=8，*P<0.05.

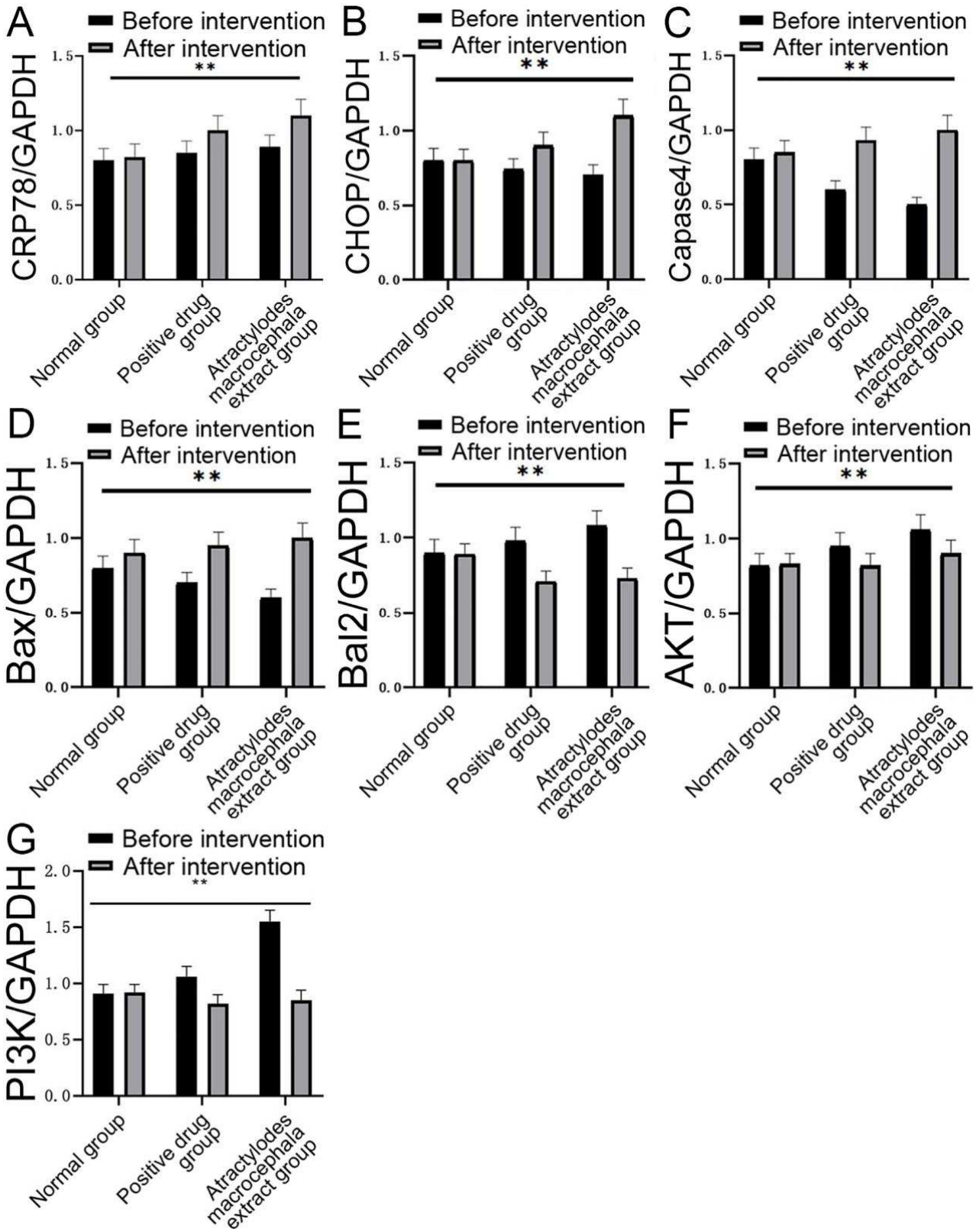

Fig. 4: Expression of key proteins in ER stress and PI3K/AKT pathway proteins.
Notes: (A): CRP78 protein level; (B): CHOP protein level; (C): Capase4 protein level; (D): Bax protein level; (E): Bal protein level; (F): AKT protein level; (G): PI3K protein level. n=8，**P<0.01.

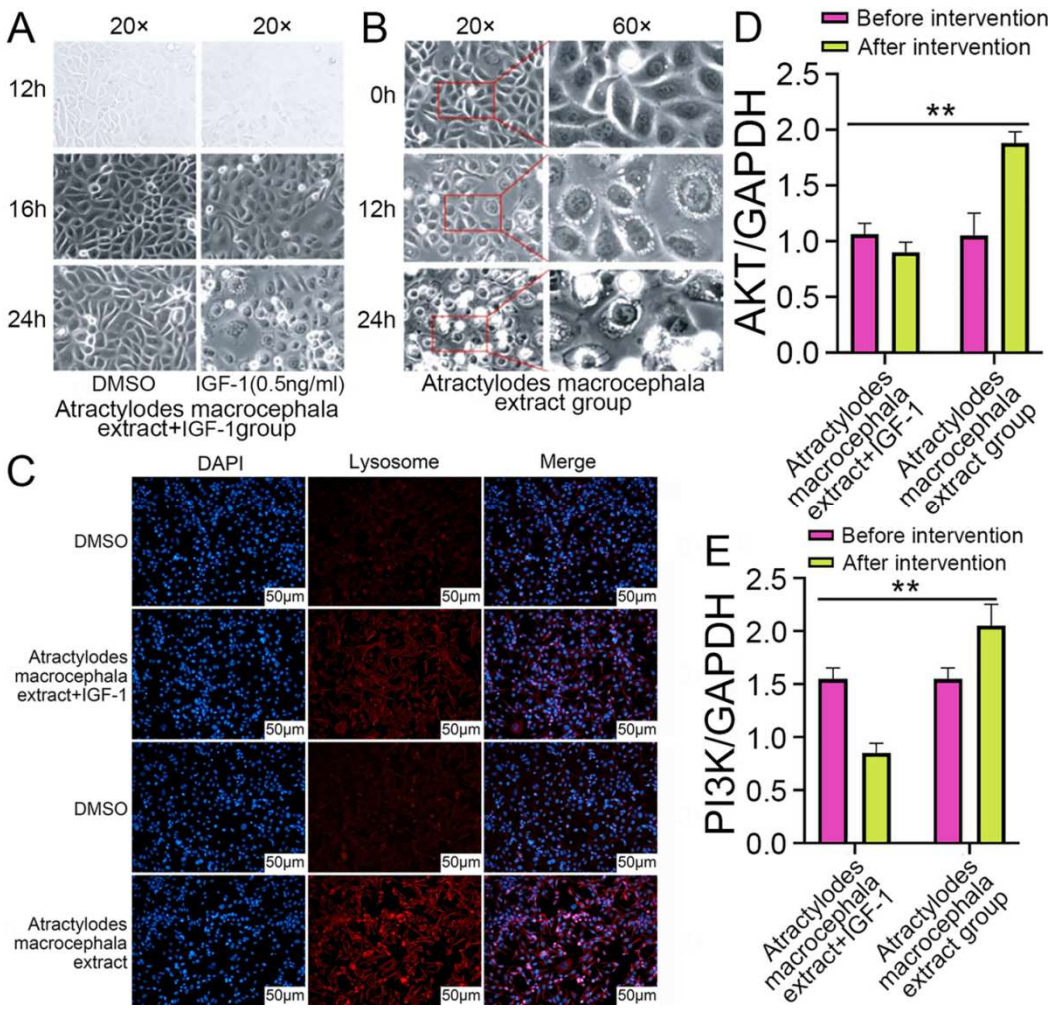

Fig. 5: The effect of *Atractylodes macrocephala* extract on ER stress is achieved by inhibiting the PI3K/Akt signaling pathway.

Note: (A): Cell morphological changes in the *Atractylodes macrocephala* extract + IGF-1 group at 12-24h; (B): Cell morphological changes in the *Atractylodes macrocephala* extract group at 0-24h; (C): Cell nuclear morphology detected by cell immunofluorescence experiment; (D): AKT protein expression level; (E): PI3K protein level. n=8，$^{**}P<0.01$.

Therefore, further verify whether these findings are applicable to clinical situations; future studies will design and implement randomized controlled clinical trials based on the current animal experimental results to evaluate the safety and efficacy of *Atractylodes macrocephala* extract in humans and determine the optimal dose range.

CONCLUSION

In summary, *Atractylodes macrocephala* extract has the potential to inhibit tumor cell proliferation, promote cell apoptosis and reduce fibrosis in the uterine fibroid model; it can also increase the apoptosis rate, reduce inflammatory response and oxidative stress by enhancing the expression of endoplasmic reticulum stress-related genes and reducing PI3K/AKT signaling, suggesting the possibility of *Atractylodes macrocephala* extract as an effective natural medicine for the treatment of uterine fibroids. However, in the present study, a positive control group was not included in all key experiments. Further studies should incorporate relevant controls to enhance the comparability and completeness of the results. Additionally, the validation experiments on the PI3K/AKT signaling pathway did not examine phosphorylated protein levels. Future investigations should include phosphorylation analysis to more comprehensively assess pathway activity.

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Authors' contributions

Ling Huang: Conceptualization, Methodology, Investigation, Data Curation, Writing – Original Draft. Tao Wang: Validation, Formal analysis, Visualization, Writing – Review & Editing.

Li Chen: Resources, Supervision, Project administration.
Lei Shi: Conceptualization, Funding acquisition, Supervision, Writing – Review & Editing.
All authors have read and agreed to the published version of the manuscript.

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Data availability statement
Data were available from the corresponding author upon reasonable request.

Ethical approval
This study was approved by the Ethics Committee of Jian gjin Central Hospital of Chongqing. (Approval No.: GXC20240802). All procedures were performed in accordance with relevant guidelines and regulations.

Conflicts of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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