

Induction Effect and Underlying Mechanism of Scutellarin on Apoptosis of Human Colorectal Cancer SW480 Cells and AOM/DSS Mouse

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Research

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Abstract

Background: This study was aimed to investigate the effect of Scutellarin on apoptosis of human colorectal cancer SW480 cells and azoxymethane (AOM)/dextran sulfate sodium (DSS) mouse, and clarify its mechanism.

Methods: 3-(4,5)-dimethylthiazolium (-z-y1)-3,5-di-phenyltetrazolium bromide (MTT) and cell migration assay were performed to detect the viability and proliferation of SW480 cells that treated with different concentrations of Scutellarin. Hoechst33342 Staining to determine apoptosis, detecting Caspase-3 and Caspase-9 activities after administration of different concentrations of Scutellarin. AOM/DSS mouse were administrated with Scutellarin; immunohistochemistry and western blot was employed to detect the effect on the expression of BCL2-Associated X (Bax) and B-cell lymphoma-2 (Bcl-2) proteins, and the message Ribonucleic Acid (mRNA) levels of Bax, Bcl-2, Caspase-3 and Caspase-9 were assessed with quantitative reverse transcription polymerase chain reaction (qRT-PCR). The data was analyzed by one-way analysis of variance (ANOVA) with SPSS19.0 software, expressed as mean \pm standard deviation ($\bar{x} \pm s$).

Results: Scutellarin could inhibit the proliferation of SW480 cells after treatment with different concentrations of Scutellarin, while the chromatin condensation and nucleus showed more intense blue fluorescence. Moreover, Scutellarin could significantly increase the activities of Caspase-3 and Caspase-9 as well as different concentrations of Scutellarin could significantly down-regulate the expression of apoptosis related protein Bcl-2 and up-regulate the expression of Bax protein. When compared with the solvent control group, the relative expression levels of the related gene Bcl-2 were down-regulated by different concentrations of Scutellarin, while the Bax, Caspase-3 and Caspase-9 genes were up-regulated.

Conclusion: Scutellarin can inhibit the in vitro activity of colon cancer SW480 cells, promote Bax expression, inhibit Bcl-2 expression, and up-regulate the activities of apoptotic enzymes Caspase-3 and Caspase-9 to induce apoptosis in vivo and in vitro, suggesting that it has a certain therapeutic effect on colon cancer.

1. Background

Colorectal cancer, as one of the heterogeneous diseases with high incidence and mortality^[1], usually occurs at the junction of the rectum and sigmoid colon^[2]. As one of the common malignant tumors of the digestive tract, it ranks third in the global incidence^[3]. In recent years, due to changes in dietary structure, colon cancer has become one of the most rapidly increasing incidences of malignant tumors in China, seriously threatening people's health and lives^[4]. The distant metastasis of colorectal cancer is the main reason for the low cure rate and high mortality of colorectal cancer patients^[5]. At present, the treatments for colorectal cancer mainly include chemotherapy and surgery. Studies have found that the overall survival rate of patients who refuse chemotherapy and surgery is significantly reduced^[6]. However, in these patients undergoing surgery and chemotherapy, postoperative recurrence^[7] and post-treatment

toxicity^[8] are facing a major challenge. Therefore, looking for low-toxic and high-efficiency anti-tumor drugs is currently a research hotspot in the medical community at home and abroad.

Scutellarin is a flavonoid, which is the main active ingredient of *Scutellaria barbata*. Scutellarin has a variety of pharmacological activities, such as anti-angiogenesis^[9], cardioprotection^[10], and anti-inflammatory^[11, 12], and it has the protective effect on nervous system in different model animals, especially in Alzheimer's disease model animals^[13]. In vivo, vitro and clinical trials, Scutellarin has shown good therapeutic effects on various diseases^[14]. Scutellarin can well protect ischemic cerebral injury, in vitro, it can increase the viability of oxygen/glucose-deprived astrocytes while in vivo, it can reduce the infarct size of local cerebral ischemia in rats and improve the neurological function^[15]. Besides Scutellarin has protective effect on the nervous system, it also has shown obviously therapeutic effect on cancer. After the melanoma cells were treated with Scutellarin, the migration, invasion and adhesion of melanoma cells were significantly inhibited. These results indicate that the inhibitory effect of Scutellarin on melanoma cells may be achieved by inhibiting the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway regulates numerous cellular processes such pathway^[16]. Moreover, Scutellarin can also inhibit A549 lung adenocarcinoma cells proliferation and promote apoptosis by up-regulating pro-apoptotic Bax protein and down-regulating anti-apoptotic Bcl-2 protein^[17].

Pro-apoptotic Bax protein and anti-apoptotic Bcl-2 protein belong to B-cell lymphoma 2 (Bcl-2) family proteins, Bcl-2 protein inhibits apoptosis by regulating the release of cytochrome c while oligomerization of Bax protein promotes apoptosis by changing the permeability of mitochondrial membrane^[18]. Apoptosis is a way of programmed cell death, studies have found that promoting apoptosis can inhibit the occurrence of cancer to a certain extent^[19]. Scutellarin can also inhibit the proliferation of cancer cells by promoting apoptosis. Treatment of human leukemia cells K562 with Scutellarin induced significant apoptosis, and inhibited the growth of K562 human leukemia cells by targeting rapidly accelerated fibrosarcoma (Raf)/ mitogen-activated protein kinase kinase (MEK) /extracellular regulated protein kinases (ERK) signal pathway^[20]. Moreover, Scutellarin can also enhance the apoptosis of ovarian cancer cells induced by cisplatin by increasing the ratio of Bax/Bcl-2^[21]. Furthermore, through in vitro MTT experiments, it was found that Scutellarin-cyclodextrin conjugates can significantly inhibit the proliferation of human colon cancer cell lines HCT116, SW480 and HT29, and has anti-tumor effects^[22]. Although Scutellarin can inhibit colorectal proliferation and induce apoptosis of intestinal cancer cells, the specific mechanism of action is unknown^[23].

Therefore, this experiment used an AOM/DSS induced colitis associated cancer (CAC) animal model in vivo, and human colon cancer cells SW480 in vitro as research objects. Combining to investigate the chemoprophylaxis effect of Scutellarin on inflammation-related colon cancer, and to determine the expression of apoptosis-related genes and proteins in the colorectal mucosa of mice.

2. Materials And Methods

Drugs and reagents

Scutellarin was obtained from Must Biotechnology Corporation (Chengdu, China) and was dissolved in dimethyl sulfoxide (DMSO). AOM and DSS were purchased from Sigma-Aldrich (American); fetal bovine serum (FBS) was obtained from QuaCell Biotechnology Corporation (Guangdong, China). MTT kit, BeyoFast™ SYBR Green qRT-PCR Mix and Apoptosis and Necrosis Assay Kit were acquired from Beyotime Biotechnology Corporation (Shanghai, China). And PrimeScript™ RT Reagent Kit was purchased from TaKaRa Biotechnology Corporation (Beijing, China), while BCA Protein Assay Kit from Multi Science Biotechnology Corporation (Shanghai, China).

Cell culture and passage

The SW480 cells were purchased from Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). When they were cultured to about 90%, trypsinized and centrifuged with moderate culture medium (Sigma-Aldrich, American) which contained 10% FBS and 1% penicillin-streptomycin, as well as discarded the supernatant. Next, the cells were resuspended in culture medium and added to three new culture bottles with new culture medium, and the morphology of the cells was observed under a microscope (Leica, Germany). Finally, the cells were cultured in an incubator (Thermo, American).

Cell viability assay

SW480 cells in logarithmic growth stage were spread on 96-well plates with a density of 5×10^3 cells in each well, 6 multiple wells in each group. Four groups were designed: 160 µg/ml, 80 µg/ml, 40 µg/ml and normal control group (0.2% DMSO) were established according to the preliminary experimental results. After the cells were attached to the well, the drugs were administered for 24 h and 48 h respectively. And the culture medium containing MTT was added to measure the absorbance value of each well at 570 nm on a preheated microplate reader (Thermo Scientific Varioskan Flash, Type 3001). Calculating the inhibition rate of cells in each well based on the measured absorbance data, cell inhibition rate (%) = (control group OD value - drug group OD value) / control group OD value \times 100%.

Effect of Scutellarin on the morphology of SW480 cells

SW480 cells at logarithmic growth stage were collected, digested and inoculated into a 96-well plate with 1×10^4 cells in each well. The cells were treated with Scutellarin 160 µg/ml, 80 µg/ml, 40 µg/ml and 0.2% DMSO for 48 h, and the cell morphology was observed under an inverted microscope.

Cell migration experiment

SW480 cells were seeded in a 24-well plate, each well was added 500 µl cells with a density of 1×10^6 cells/ml, and each group had 3 duplicates. Then observing cells until they adhered to the well and grew. When the cells grew to about 90%, using a 10 µl pipette tip to make a straight scratch, then washing the

dead cells in the 24-well plate with PBS, finally giving 500 ul different concentrations of Scutellarin. After 0 h, 12 h and 24 h, observing the scratch migration of each group.

Clone formation assay

SW480 cells were seeded in a 6-well plate, after the cells were stick to the well, culture medium of Scutellarin of different concentrations and the normal control group with 0.2%DMSO were added. Next, cells were cultured in the incubator for 7 days. Taking out the 6-well plates from the incubator, then washing cells twice with PBS, staining with crystal violet for 10 min, and the experimental results were photographed and recorded after washing with PBS again.

Hoechst 33342 dye staining

1×10^4 cells were inoculated into each well of the 96-well plate, cultured with Scutellarin containing medium for 24 h, next fixed with fixative solution for 10 min, and then added with Hoechst33342 working solution for staining at 37°C incubators for 5 min. Fluorescence microscope was used to detect and record the cells.

Apoptosis and necrosis assay

SW480 cells with a density of 1×10^6 were seeded in a 6-well plate and cultured for 24 h with different concentrations of Scutellarin. Adding 5 ul Hoechst and PI dye into the 6-well plate and mixing immediately. The samples were incubated at 4°C for 30 min, washed with PBS and photographed under a fluorescence microscope.

Activity detection of Caspase-3 and Caspase-9

SW480 cells were collected after 24 h of different concentrations of Scutellarin treatment, then added lysis buffer, lysed in ice bath for 15 min. After centrifugation at 4°C for 15 min, protein concentration was determined by Bradford method to achieve 1–3 mg/ml protein concentration. 40 ul test buffer was added into the reaction system, 50 ul test sample and 10 ul AC-devd-PNA (2 mM) was mixed and incubated at 37°C for 2 h, finally, detected on a microplate reader.

Animal handling

C57BL/6 male mice (n = 50) were purchased from Sichuan Provincial People's Hospital (Chengdu, China), and randomly divided into 5 groups according to body weight, 10 mice in each group. 5 groups were AOM + DSS model control group, 100 mg/kg Scutellarin group, 50 mg/kg Scutellarin group, 25 mg/kg Scutellarin group and blank control group, respectively. The first four groups were intraperitoneally injected with AOM 12.5 mg/kg^[24], while the blank control group was intraperitoneally injected with 0.9% NaCl solution, with the dose volume of 0.01 ml/kg. 1 week later, the first 4 groups were given 2.5% DSS solution free drinking for 1 week, followed by 2 weeks of pure water which repeated for 3 cycles. Starting

from week 2, the drug groups were intraperitoneally injected with different concentrations of Scutellarin every day until the end of three cycles. Weight, fecal status, and blood in the stool were recorded weekly.

Immunohistochemistry assay

The colorectal tissues of the mice in each group were paraffin-embedded and cut into 5 μm thin slices. Immunohistochemical staining: Bax, Bcl-2 monoclonal antibody (1:100, Proteintech Group, Inc.), incubated at 4°C overnight, then incubated with the secondary antibody at room temperature for 50 min. After the tissue slices were colored with DAB, they were re-stained with cymbalin, sealed with neutral gum, and finally observed and photographed under a microscope.

Western blot analysis

SW480 cells and colon tissues treated with Scutellarin of different concentrations were collected, centrifuged at 4°C for 10 min, then added with pyrolysis liquid. Finally, the total protein content of samples was determined by BCA method. Equal amount of protein was taken from each sample, and the same volume of sample loading buffer was added. After boiling for 5 min, each group of protein was separated by using 8% SDS-PAGE, subsequently transferred to a PVDF membrane. The PVDF membranes were blocked with 3% BSA for 1.5 h and probed with primary antibodies against GAPDH (1:5000, in 1% BSA, Multi Science Biotechnology Corporation), Bax and Bcl-2 (1:1000, in 1% BSA). After placing the membranes in a refrigerator overnight at 4°C, adding the secondary antibody (1:5000, in antibody diluent). Place them on a shaker at room temperature for 90 min, and proceeding with the enhanced chemiluminescence reagent (ECL) luminescence kit. Quantity one software was used to analyze, and the ratio of the optical density value of the target protein to the internal reference protein (GAPDH) was regarded as the experimental result for statistics.

Quantitative RT-PCR analysis

Total RNA was extracted from different groups of cells and tissues, then using NanoDrop 2000 micronucleic acid analyzer (Thermo, American) to detect the purity and concentration of total RNA. The PrimeScript RT Reagent Kit was used for reverse transcription of cDNA, next detecting its purity and concentration with NanoDrop 2000 micronucleic acid analyzer again. At last, BeyFast™ SYBR Green qRT-PCR Mix was used for quantitative fluorescence PCR reaction. qRT-PCR reaction condition is: predenaturation at 95°C for 2 min; 95°C 15 sec, 60°C 30 sec, 72 °C 30 sec, \times 40 cycles. PCR primers were designed based on nucleic acid sequences provided by GenBank database (Table 1).

Statistical analysis

The data was analyzed by one-way analysis of variance (ANOVA) with SPSS19.0 software, expressed as mean \pm standard deviation ($\bar{x} \pm s$).

3. Results

Scutellarin suppressed the proliferation of SW480

Compared with the normal control group, the growth of SW480 cells was significantly inhibited after different concentrations of Scutellarin were applied to SW480 cells for 24 h and 48 h respectively (Fig. 1a), which showing dose-dependent and time-dependent effects ($p < 0.01$). Compared with the normal group, the growth of SW480 cells was inhibited by different concentrations of Scutellarin in the clone formation experiment after 7 days (Fig. 1b) and the cell morphology was shown in Fig. 1c after treating with Scutellarin.

Scutellarin prevented the migration of SW480 cells

Compared with the normal control group, SW480 cells showed significantly lower mobility after being treated with Scutellarin. Among them, the mobility of the administration group was significantly lower than that of control group after treatment for 24 h, indicating that Scutellarin can effectively inhibit the two-dimensional migration of SW480 cells (Fig. 2).

Scutellarin accelerated the apoptosis of SW480 cells

Compared with the normal control group, Hoechst33342 staining normal group showed uniform blue light, light color, regular shape, with no shrinkage and fragmentation (Fig. 3a). However, the nuclei of most cells in the Scutellarin groups showed bright fluorescent staining, and more obvious pyknosis and fragmentation. According to the apoptosis and necrosis figure, the cells in the administration group appeared to red fluorescence and strong blue fluorescence, especially in the high-dose administration group. While the cells in the control group showed weak red fluorescence and weak blue fluorescence (Fig. 3b).

Scutellarin effected the expression of apoptotic protein in SW480 cells

Compared with the normal control group, the activity of apoptotic proteases caspase-9(Fig. 4a) and caspase-3(Fig. 4b) were increased by treating with different concentrations of Scutellarin. And the effect was proportional to the concentration, which showed that the higher the concentration, the better the effect ($P < 0.01$). The expression of bcl-2 protein (Fig. 4c) was decreased in the 40 ug/ml group ($P < 0.05$), but there was no significant difference in the Bax protein (Fig. 4d) ($P > 0.05$). As well, the expression of Scutellarin 80 ug/ml and 160 ug/ml could significantly decrease Bcl-2 and increase Bax protein expression ($P < 0.01$).

Effects of Scutellarin on apoptosis related genes in SW480 cells

The results showed that compared with the normal control group, there was no significant difference in Bax expression in the 40 ug/ml group, while Scutellarin 80 ug/ml and 160 ug/ml had obvious differences in caspase-3(Fig. 5a), Caspase-9(Fig. 5b), Bax(Fig. 5c) and Bcl-2(Fig. 5d) expression levels ($P < 0.05$ or $P < 0.01$), and showed a certain dose-effect relationship. It was suggested that Scutellarin may increase the

expression levels of Caspase3, Caspase9 and Bax, and reduce the expression levels of Bcl-2, thus promoting the apoptosis of SW480 cells.

Effects of Scutellarin on animal models of AOM/DSS

During the period of 2.5% DSS free drinking, the AOM/DSS model group mice were depressed, and their body hair was messy and dark, some mice even had loose stools and blood in the stool, while the mice with the intervention of Scutellarin was obviously better than that of the model group. After the end of the experiment, the intestinal mucosa of mice in each experimental group showed thickened to different degrees, and the induced tumors were mainly distributed in the intestinal segment within 4 ~ 6 cm from the anus. Look at the normal group, the colorectal mucosa of mice was intact and smooth, and no tumors were found. In terms of weight (Fig. 6a) and tumor (Fig. 6b), the mice in the AOM/DSS group was significantly reduced and the tumors were obvious ($P < 0.01$). Compared with the model group, the body weight of the 25 mg/kg, 50 mg/kg and 100 mg/kg of the scutellarin groups increased with the increase of the dose, while the number of tumors decreased ($P < 0.01$).

Effects of Scutellarin on apoptosis related proteins in animals

It can be seen from the figure that compared with the normal control group, the expression of Bcl-2 in the model control group was significantly increased ($p < 0.01$), while the expression of Bax was not different ($p > 0.05$). For Bcl-2 protein (Fig. 7b and c), the Scutellarin 25 mg/kg group showed no effect on the expression of Bcl-2 ($P > 0.05$). Scutellarin 50 mg/kg and Scutellarin 100 mg/kg group can conspicuously reduce the expression of Bcl-2 protein ($P < 0.01$). For Bax protein (Fig. 7a and c), the 25 mg/kg, 50 mg/kg, and 100 mg/kg Scutellarin groups could significantly increase the expression of Bax protein ($P < 0.01$). According to the immunohistochemistry assay (Fig. 7d), it was showed that the trend of Bax and Bcl-2 protein expression was the same with western blotting assay.

Effects of Scutellarin on apoptosis related genes in animals

The results showed that compared with the normal control group, there was no significant difference in Bax expression in the 40 ug/ml group, while Scutellarin 80 ug/ml and 160 ug/ml had obvious differences in Bax(Fig. 8a), Bcl-2(Fig. 8b), caspase-3(Fig. 8c) and Caspase-9(Fig. 8d) expression levels ($P < 0.05$ or $P < 0.01$), and there was a certain dose-effect relationship. It was suggested that Scutellarin may increase the expression levels of Bax, Caspase3 and Caspase9 and reduce the expression levels of Bcl-2, thus promoting the apoptosis of SW480 cells.

4. Discussion

Here, we found that Scutellarin had the ability to inhibit proliferation, migration and promote the apoptosis of SW480 in vivo. Additionally, in AOM/DSS model animals, we have verified that Scutellarin can reduce tumor formation to a certain extent. Furthermore, this study investigated the mechanism of

Scutellarin against colon cancer through western blot, immunohistochemistry and qRT-PCR technology, in order to provide a theoretical basis for the clinical treatment of colon cancer with Scutellarin.

Scutellarin is a flavonoid compound isolated from *Scutellaria barbata*, *Asarum* and other traditional Chinese medicine. It has various pharmacological effects such as improving immunity, anti-inflammatory, as well as anti-tumor^[25]. Tumor has become one of the main diseases that affect the health and livelihood of our citizens. The occurrence of tumors is related to the imbalance of cell proliferation and apoptosis. Therefore, inducing cell apoptosis and inhibiting proliferation have become vital research directions in cancer treatment^[26]. Previous studies have reported that Scutellarin has pharmacological effects against colon cancer^[27], lung cancer^[17], lymphoma and liver cancer^[28] by promoting cell apoptosis which makes Scutellarin has a good clinical application prospect. For instance, Scutellarin induced the apoptosis of hepatocarcinoma cells HepG2 by inhibiting antiapoptotic proteins BCL-XL and Mcl-1, the downstream targets of STAT3^[29]. Moreover, Scutellarin can also induce apoptosis of human Burkitt lymphoma Namalwa cells by activating the caspase pathway^[30]. In addition, Scutellarin promoted cell apoptosis by regulating the P53 pathway and down-regulating the expression of Bcl-2/Bax^[31].

There are two main ways of apoptosis: endogenous pathway and exogenous pathway^[32]. The exogenous pathway is a death receptor-mediated apoptosis pathway, which activates the FAS-related death domain and forms a death-inducing signal complex^[33]. While the endogenous pathway is an apoptosis pathway which is mediated by mitochondria. The Bcl-2 protein family is one of the most important regulators of apoptosis. Among them, Bcl-2 and Bax are the main anti-apoptotic and pro-apoptotic members, respectively. They regulate apoptosis by regulating the function of mitochondria^[34]. The release of cytochrome C (Cyt-C) is the key to the pathway of mitochondrial apoptosis. Cytochrome forms multimers after combining with the apoptotic protease activator. The Caspase9 precursor in the cytoplasm is recruited through the Caspase at the amino terminal of Apaf-1, in order to initiate the Caspase cascade reaction, and then activates downstream Caspase3, completes the cleavage of the substrate, as well as causes cell apoptosis^[35].

As far as we know, this study is the first to combine in vivo and in vitro experiments to prove that Scutellarin inhibits colon cancer through the mitochondrial apoptosis pathway. In vitro, through MTT and soft agarose cloning experiments, we observed that Scutellarin can significantly inhibit the proliferation of SW480 cells in a dose- and time-dependent manner. Previous research reported that inhibiting the proliferation of colon cancer cells can further promote cell apoptosis^[36]. Therefore, we observed apoptotic SW480 cells by Hoechst33342 staining. Further analysis showed that Scutellarin can reduce the expression of Bcl-2 mRNA and protein in tumor cells, increase the mRNA and protein expression of Bax, and increase the activity of Caspase-3 and Caspase-9 apoptotic proteases as well as the expression of mRNA levels. It shows that Scutellarin can induce colon cancer SW480 cell apoptosis through mitochondrial apoptosis pathway. Although Scutellarin has been reported in the literature that it can promote the apoptosis of human colon cancer cell HCT116 by regulating the P53 signaling pathway^[31],

and the regulation of caspase3, Bcl-2 and Bax is consistent with the results we have observed, its pro-apoptotic mechanism in animal models of colon cancer has not been demonstrated.

In vivo, we used AOM/DSS animal models to simulate colon cancer. The AOM/DSS model has simple carcinogenic methods, high tumor formation rate, and short cycle. It has become one of the commonly used enteritis-related colorectal cancer animal models. Nam SH et al. confirmed that tussilagone can treat colon cancer induced by AOM/DSS by regulating the apoptosis of colon tissue^[37]. In addition, some scholars have found that taurine can not only promote the apoptosis of human nasopharyngeal carcinoma cells, but also increase the expression of the apoptosis marker cleaved caspase-9 in mice induced by AOM/DSS^[38]. Furthermore, an extracellular polysaccharide (EPS1-1) of *Rhizopus nigricans* can also increase the apoptosis of colon cancer cells in AOM/DSS mice, up-regulate the expression of Bax and down-regulate the expression of Bcl-2^[39]. Which is consistent with the results observed in our experiment. In vivo, we have confirmed that Scutellarin has the effect of inducing colonic tissue apoptosis through western blot, qRT-PCR and immunohistochemistry.

5. Conclusion

The above results indicate that Scutellarin can inhibit human colon cancer SW480 cells activity in vitro and treat colon cancer induced by AOM/DSS by promoting the expression of Bax, inhibiting the expression of Bcl-2, and increasing the expression of the apoptotic proteases Caspase-3 and Caspase-9, which leading to apoptosis. It suggests that Scutellarin has a certain therapeutic effect on colorectal cancer, and lays the foundation for further research.

Abbreviations

AOM

Azoxymethane

DSS

Dextran sulfate sodium

mRNA

message Ribonucleic Acid

BAX

BCL2-Associated X

BCL-2

B-cell lymphoma-2

qRT-PCR

Quantitative reverse transcription polymerase chain reaction

ANOVA

One-way analysis of variance

MTT

3-(4,5)-dimethylthiazolium (-z-y1)-3,5-di- phenytetrazoliumromide

CAC
Colitis associated cancer
PI3K
Phosphoinositide 3-kinase
Akt
protein kinase B
mTOR
mammalian target of rapamycin
Raf
rapidly accelerated fibrosarcoma
MEK
mitogen-activated protein kinase kinase
ERK
extracellular regulated protein kinases
DMSO
Dimethyl sulfoxide
FBS
Fetal bovine serum
PAGE
Polyacrylamide gel electrophoresis
SDS
Sodium dodecyl sulfate
PVDF
Polyvinylidene Fluoride
PBS
Phosphate-buffered saline
ECL
Enhanced chemiluminescence reagent
DAB
Diaminobenzidine

Declarations

Ethics approval and consent to participate

The animal experiments were approved by the Animal Care and Use Committee of Chengdu University of Traditional Chinese Medicine.

Consent for publication

Not applicable.

Availability of data and materials

All the data and materials in this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

S.Z, and L.T carried out the experiments, data curation, software, and writing of the original draft preparation. XL.M and HB.X conceived and designed the research project. H.Z, Q.S. and L.C contributed to the data investigation and writing of the original draft. M.H, M.Z, H.Y, S.R and ML.L contributed to the conceptualization, methodology, investigation, project administration, data validation, and writing of the original draft.

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Figures

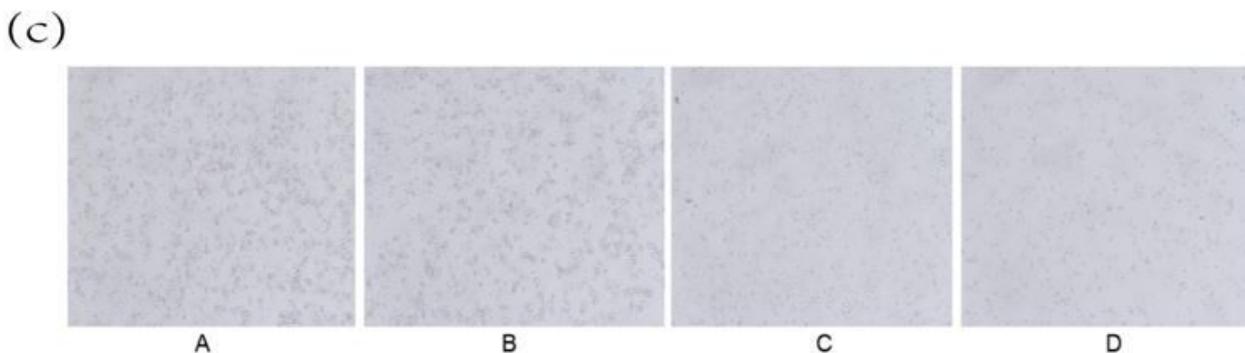
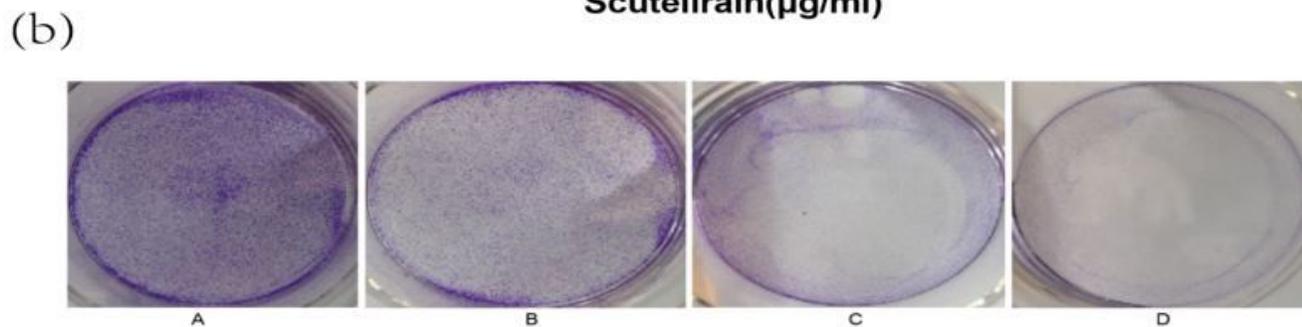
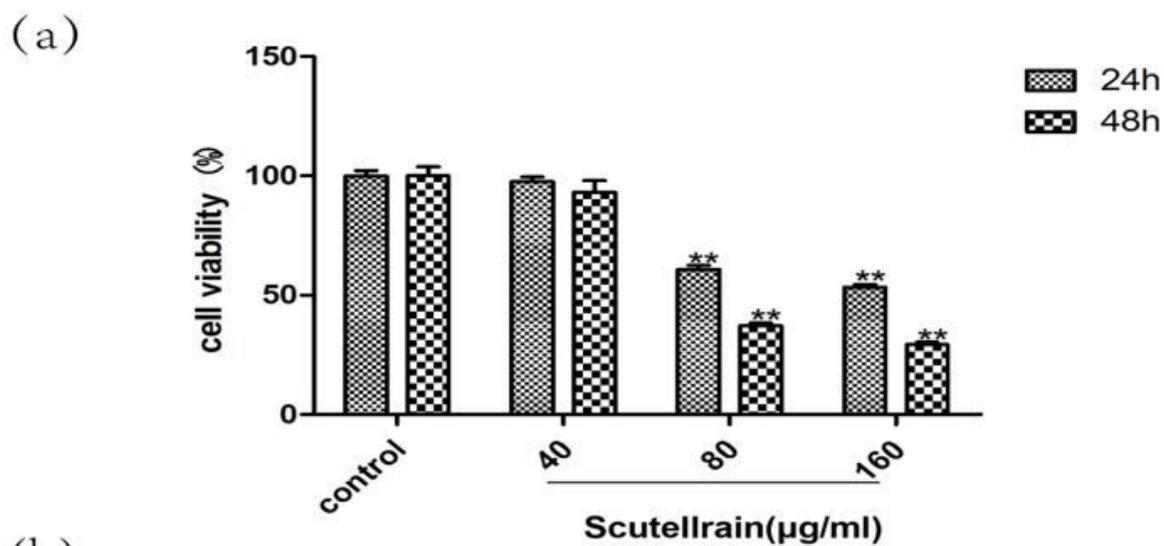


Figure 1

Scutellarin suppressed the proliferation of SW480. (a) Scutellarin with concentrations of 40, 80 and 160ug/ml for 24h and 48h via MTT assay. (b) Scutellarin with concentrations of 40, 80 and 160ug/ml for 7 days, the experiment of soft agar colony formation assay is detailed in the materials and methods section. (c) Scutellarin at concentrations of 40, 80 and 160ug/ml transformed the morphology of SW480 cells.

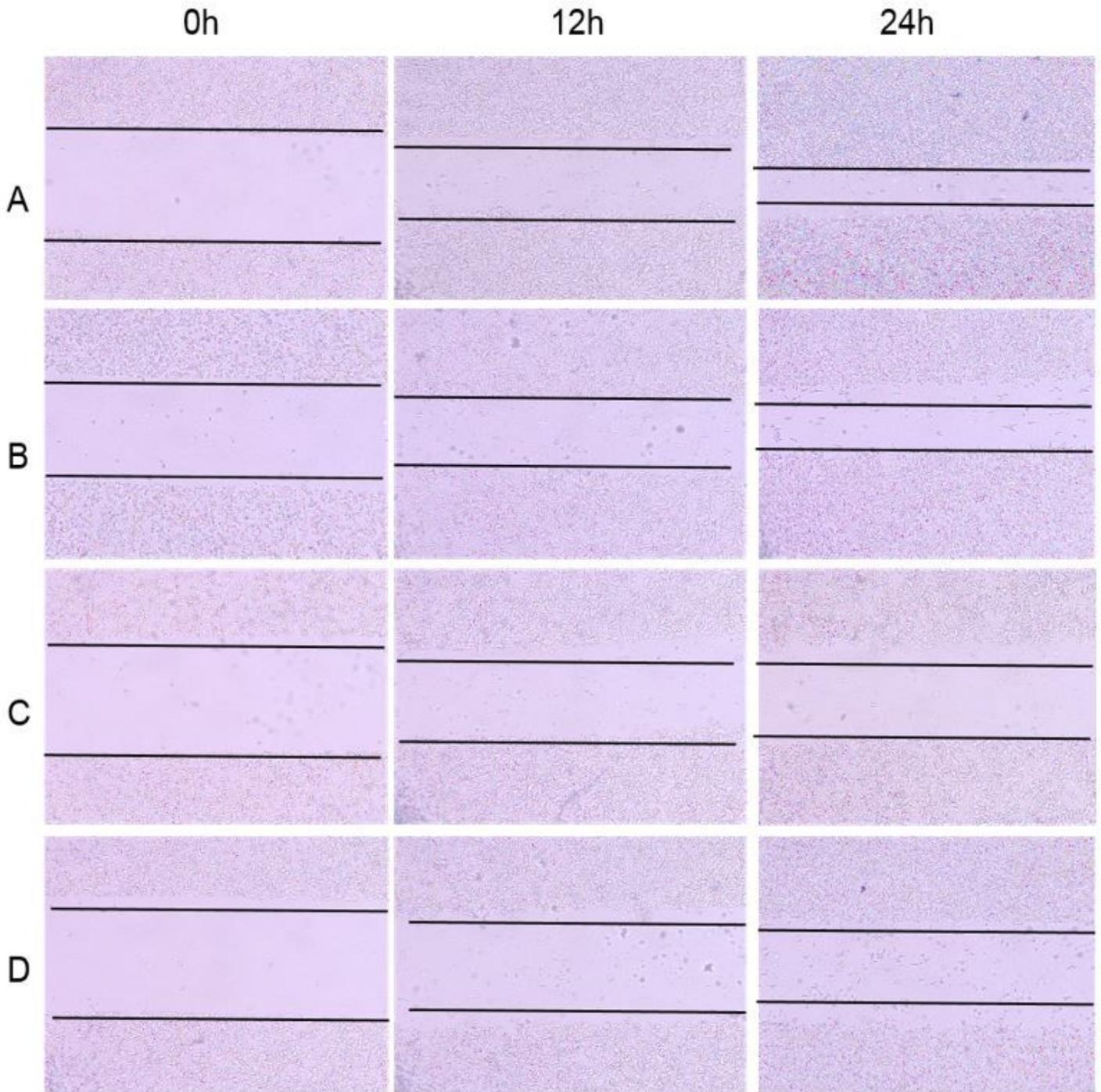
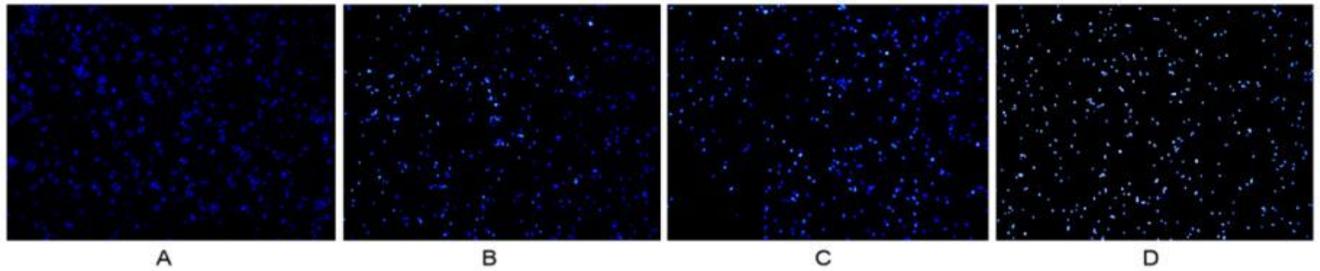


Figure 2

Scutellarin prevented the migration of SW480 cells. SW480 cells with Scutellarin at concentrations of 0(A), 40(B), 80(C) and 160(D)ug/ml for 0h, 24h and 48h, cell migration experiment is detailed in the materials and methods section.

(a)



(b)

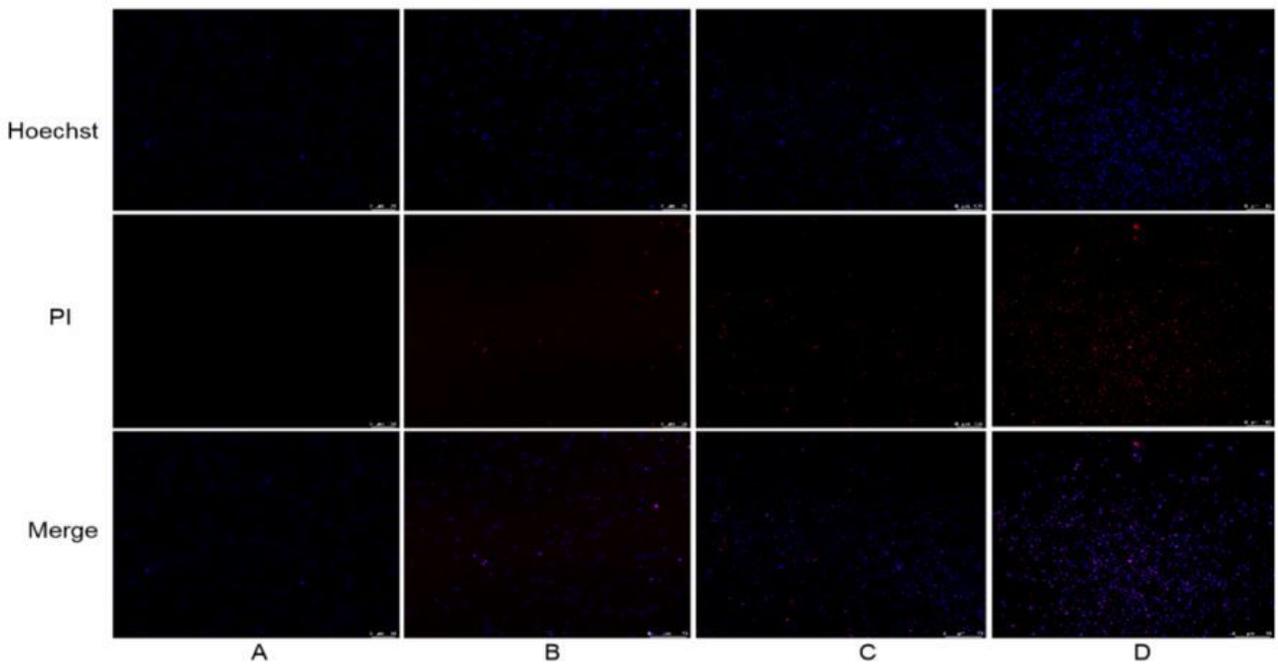


Figure 3

Effect of Scutellarin on apoptosis of SW480 cells. SW480 cells with Scutellarin at concentrations of 40, 80 and 160ug/ml via (a) Hoechst 33342 dye staining (b) apoptosis and necrosis assay.

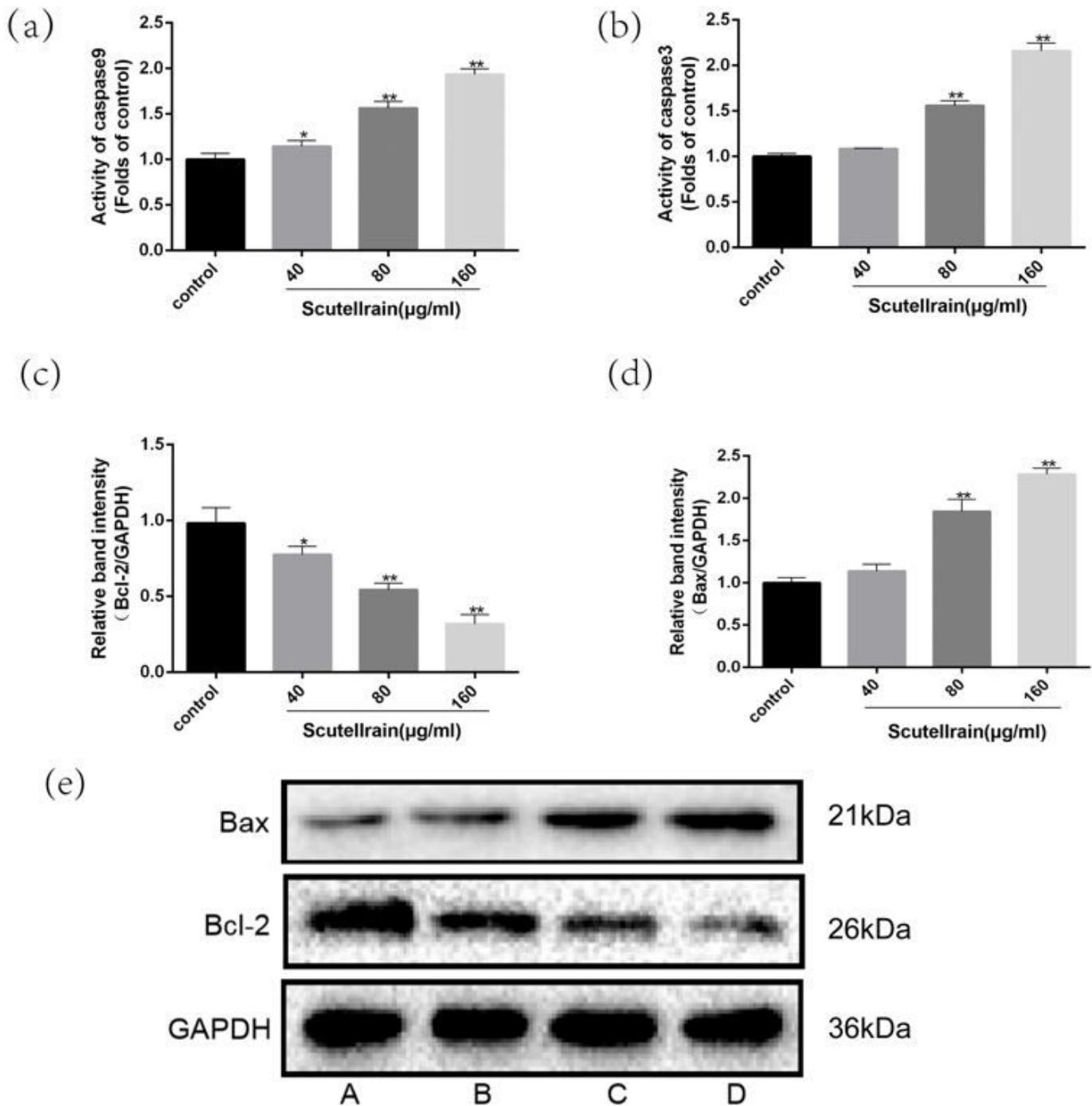


Figure 4

Scutellarin effected the protein levels in SW480 cells. SW480 cells with Scutellarin at concentrations of 40, 80 and 160ug/ml for 48 h can effectively increase (a) caspase-9 (b) caspase-3 protein levels via caspase-3 and caspase-9 kit. (c) Bcl-2 protein was decreased while (d)Bax protein expression was increased via western blotting assay. (e) original blots showed the expression of the Bax, Bcl-2 and GAPDH protein.

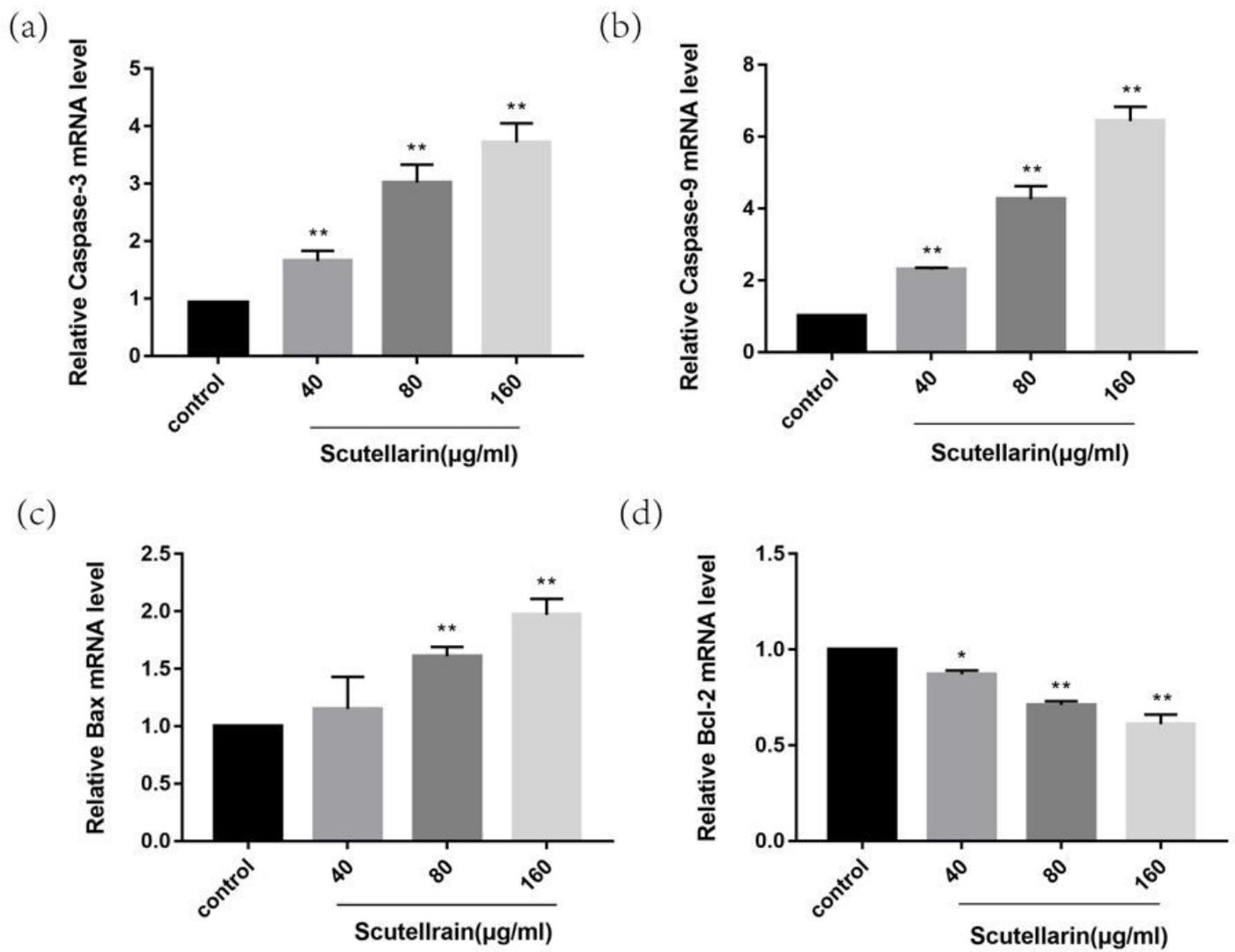


Figure 5

Scutellarin effect the mRNA levels in SW480 cells. After treatment with Scutellarin at concentration of 40, 80 and 160µg/ml for 48h, the expression of (a) Caspase-3 (b) Caspase-9 (c) Bax and (d) Bcl-2 mRNA levels were analyzed.

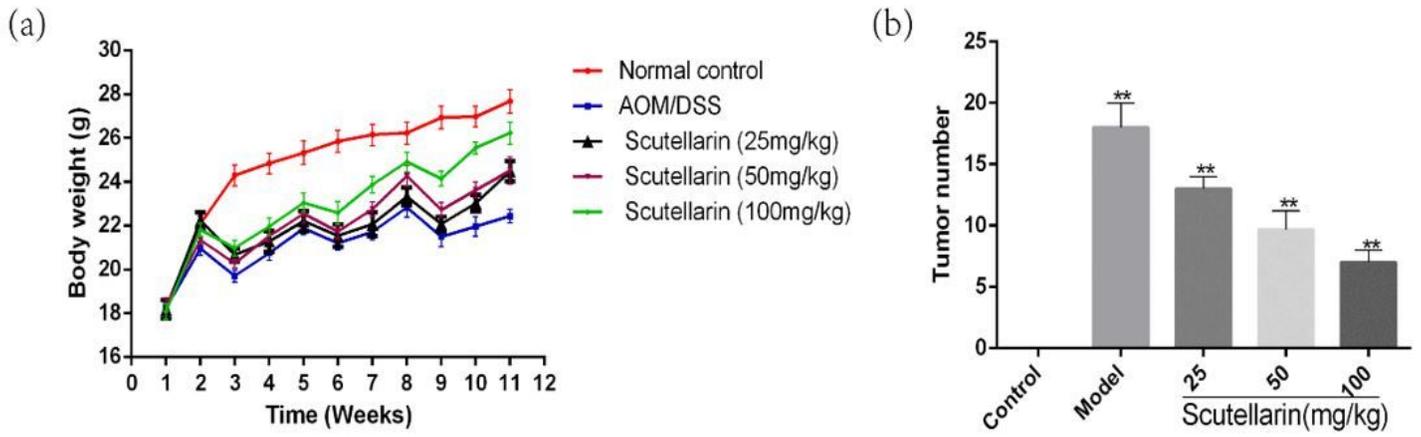


Figure 6

Effects of Scutellarin on animal models of AOM/DSS. (a) Weight changes and (b) tumor number of C57BL/6 male mice after treating with Scutellarin.

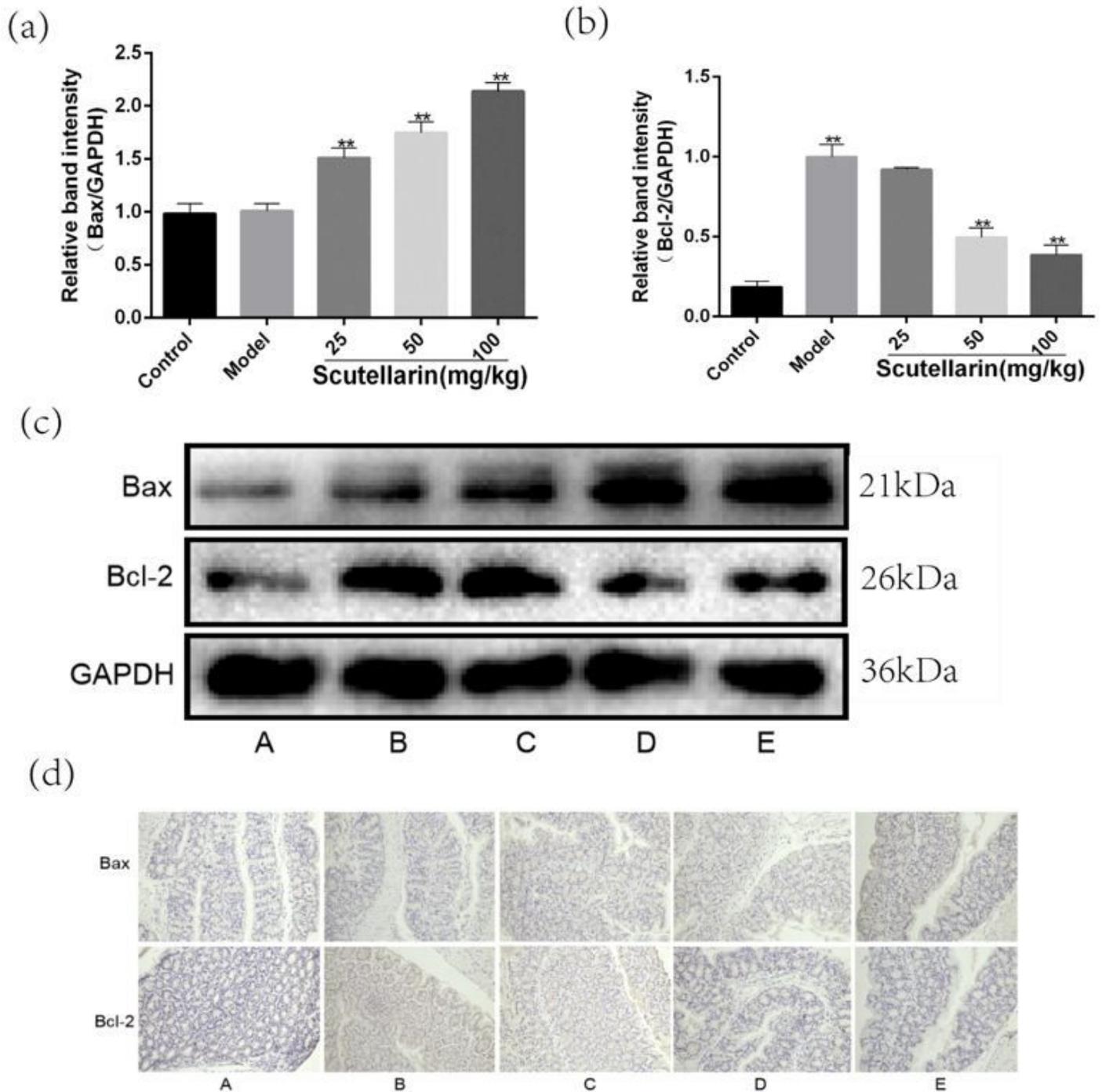


Figure 7

Scutellarin effected the protein expression in AOM/DSS animals. After treatment with Scutellarin 25 mg/kg, 50 mg/kg, and 100 mg/kg, the expression of (a) Bax protein was obviously increased while (b) Bcl-2 protein was decreased which compared with model group via western blotting assay. (c) Original blots showed the expression of the Bax, Bcl-2 and GAPDH protein. (d) The expression of Bax and Bcl-2 protein after treatment with Scutellarin 25 mg/kg, 50 mg/kg, and 100 mg/kg.

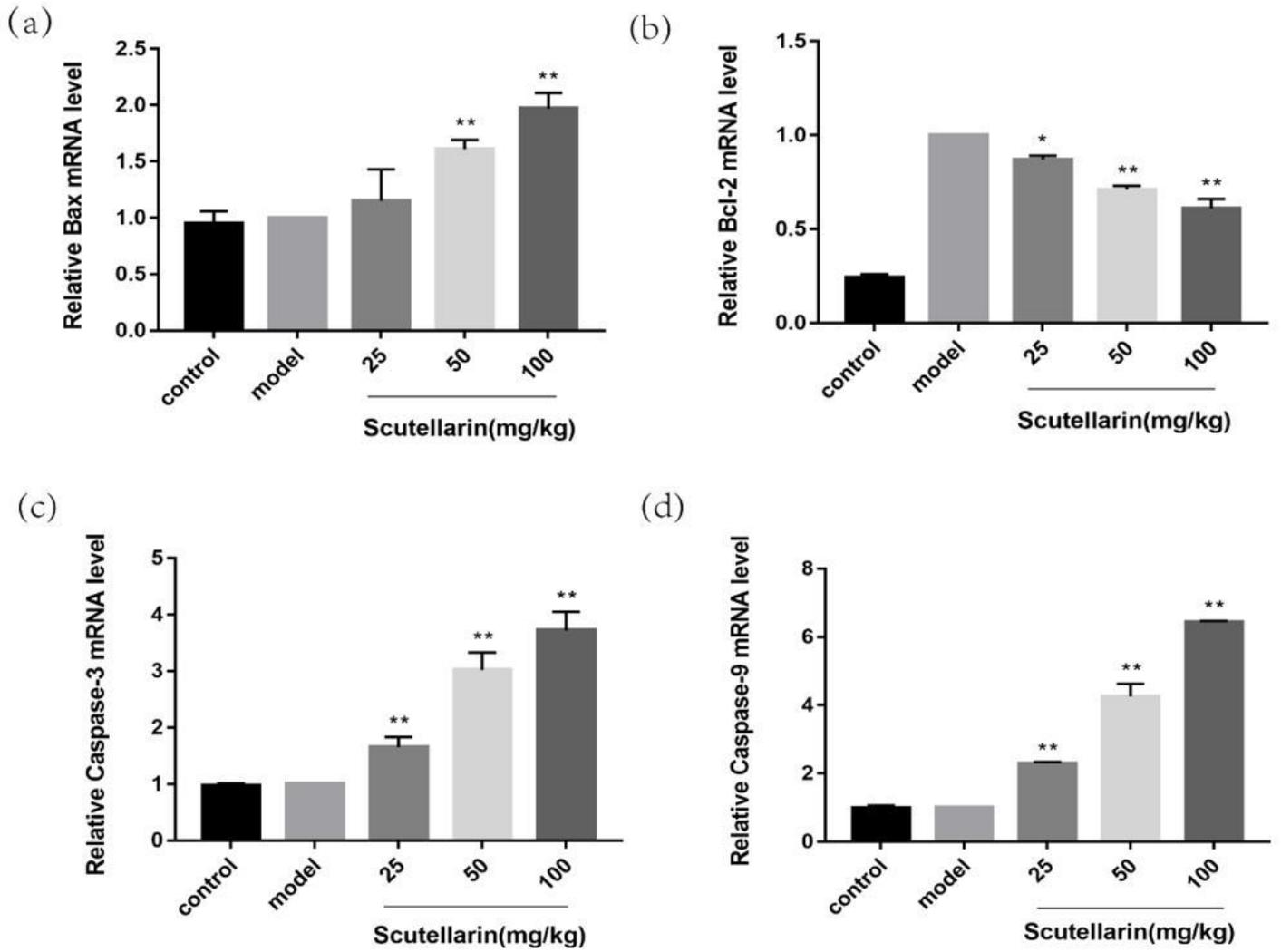


Figure 8

Scutellarin effect the mRNA levels in animals. After treatment with Scutellarin at concentration of 25, 50 and 100mg/kg, the expression of (a) Caspase-3 (b) Caspase-9 (c) Bax and (d) Bcl-2 mRNA levels were analyzed.

Supplementary Files

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