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ORIGINAL ARTICLE

Comparative Study on the Antitumour Activity of 20(R)-ginsenoside Rh2 and 20(S)-ginsenoside Rh2 from *Panax ginseng* against Non-small Cell lung Cancer *in vitro*

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ABSTRACT

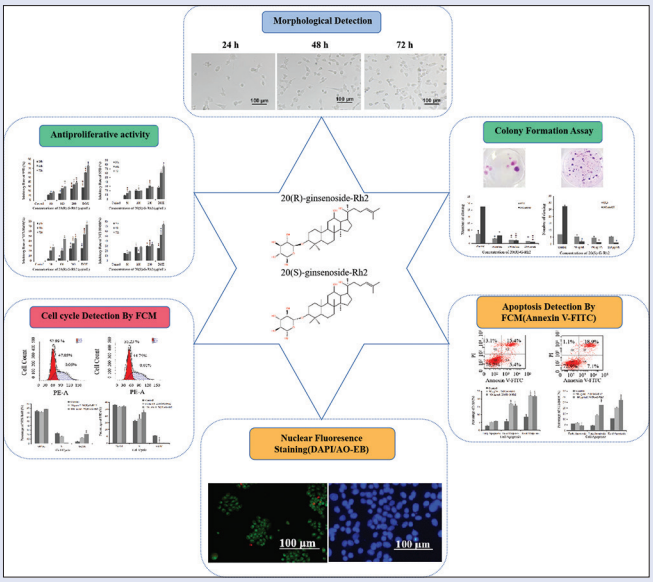
Background: Non-small cell lung cancer (NSCLC) is often harmful to human health. It is common in people who smoke or smoke passively, and the affected people are older and find it difficult to recover. The main treatment methods are surgery, radiotherapy and chemotherapy. This paper aims to study the effects of different configurations of ginsenoside Rh2 on NSCLC 95D and NCI-H460 cells. To provide clinical value for the treatment of NSCLC. **Materials and Methods:** CCK-8 was used to determine the proliferation of 95D and NCI-H460 cells with different concentrations of ginsenoside Rh2 (0, 0.05, 0.1 and 0.2 mg/mL), and IC_{50} was calculated. Cell cycle and apoptosis were detected by immunofluorescence staining and flow cytometry. **Results:** 20(R)-ginsenoside-Rh2 and 20(S)-ginsenoside-Rh2 inhibited the proliferation and colony formation of NSCLC 95D and NCI-H460 cells induced cell cycle arrest in a time and dose-dependent manner in G1/S and promote apoptosis. **Conclusion:** Ginsenoside Rh2 induces antitumour activity by inhibiting proliferation and colony formation of 95D and NCI-H460 cells and inducing cell cycle arrest and apoptosis. Comparing the two configurations of ginsenoside Rh2, the effect of 20(R)-ginsenoside-Rh2 is stronger.

Key words: Apoptosis, cell cycle arrest, 20(R)-ginsenoside-Rh2, 20(S)-ginsenoside-Rh2, 95D, NCI-H460

SUMMARY

- 20(R)-ginsenoside-Rh2 and 20(S)-ginsenoside-Rh2 inhibits the proliferation of NSCLC cell lines 95D and NCI-H460
- 20(R)-ginsenoside-Rh2 and 20(S)-ginsenoside-Rh2 induces cell cycle arrest in NSCLC cell lines 95D and NCI-H460
- 20(R)-ginsenoside-Rh2 and 20(S)-ginsenoside-Rh2 promotes apoptosis of NSCLC cell lines 95D and NCI-H460
- The inhibitory effect of 20(R)-ginsenoside Rh2 on NSCLC cell lines 95D and NCI-H460 was more significant than that of 20(S)-ginsenoside Rh2.

Abbreviations used: NSCLC: Non-small cell lung cancer; 20(R)-G-Rh2: 20(R)-ginsenoside Rh2; 20(S)-G-Rh2: 20(S)-ginsenoside Rh2.



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INTRODUCTION

Lung cancer is a malignant tumour. At present, the pathogenesis of lung cancer is not very clear, and its external causes are mainly air pollution and smoking. The clinical effective treatment methods are surgical, physical, and drug treatment. In recent years, with the increasing number of smokers, the incidence and mortality of lung cancer have become the first of all kinds of tumours, especially in developed areas such as

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Europe and America.[1,2] Lung cancer occurs in bronchial mucosal epithelium and alveoli. It is asymptomatic in the early stage. When it is found, 70% of patients have advanced cancer. It is common in smokers or passive smokers. Most patients are >65 years old. Non-small cell lung cancer (NSCLC) patients accounted for 85% of the total number of lung cancer patients.[3,4]

Ginseng is a top-grade and precious traditional Chinese medicine. Its main effects are: "greatly tonifying vitality, tonifying spleen and lungs, generating saliva and relieving thirst, calming nerves and increasing intelligence".[5-7] Ginsenosides have been proved to have antitumour,[8] antioxidant,[9] antibacterial,[10] and immunomodulatory effects.[11] Ginsenoside Rh2 is a main active component of ginseng after hydrolysis. Ginsenoside Rh2 has been proved to have antitumour activity,[12] but its effect on NSCLC has not been widely explored and the activity of the two configurations of ginsenoside Rh2 has not been compared.

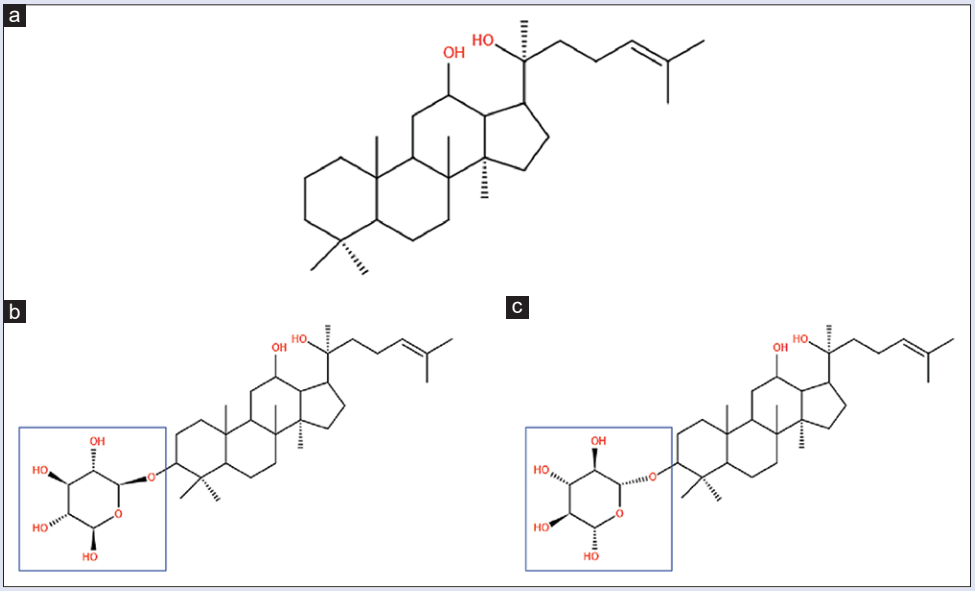

Figure 1: (a) Ginsenoside Rh2 sapogenin. (b) Structural formula of 20(R)-G-Rh2. (c): Structural formula of 20(S)-G-Rh2

Table 1: Inhibitory rates of 20(R)-G-Rh2 and 20(S)-G-Rh2 on NSCLC cell lines 95D and NCI-H460. Effect of ginsenoside-Rh2 on inhibition of cell proliferation of NSCLC cell lines(%)

Type	Cell line	Concentration (μg/mL)	24 h	*P*	48 h	*P*	72 h	*P*
20(R)-G-Rh2	95D	Control	0.00	–	0.00	–	0.00	–
		50	10.49±1.76	0.541	14.72±0.89	0.102	18.68±1.32	0.138
		100	13.62±2.34	0.556	25.56±0.07	0.000***	29.77±3.28	0.296
		200	24.99±0.64	0.019*	33.91±0.61	0.010*	38.55±2.44	0.112
		5 μM DOX	27.25±1.90	0.134	61.09±1.04	0.009**	75.76±0.61	0.002**
	NCI-H460	Control	0.00	–	0.00	–	0.00	–
		50	3.38±0.80	0093	20.38±2.84	0.012*	27.97±3.72	0.395
		100	4.19±1.25	0.044*	20.20±2.28	0.012*	44.17±0.39	0.002**
		200	24.96±0.68	0.000***	35.04±3.48	0.000***	45.18±0.38	0.002**
		5 μM DOX	25.01±0.30	0.000***	53.08±3.34	0.000***	73.66±1.06	0.006**
20(S)-G-Rh2	95D	Control	0.00	–	0.00	–	0.00	–
		50	9.02±0.45	0.070	14.45±0.75	0.000***	17.80±0.99	0.088
		100	20.11±1.95	0.240	19.39±1.42	0.000***	20.15±2.48	0.354
		200	25.24±2.84	0.307	31.76±0.62	0.000***	28.88±1.29	0.058
		5 μM DOX	27.25±1.90	0.134	61.09±1.04	0.009**	75.76±0.61	0.002**
	NCI-H460	Control	0.00	–	0.00	–	0.00	–
		50	15.08±2.34	0.490	14.01±1.91	0.019*	18.88±5.35	0.860
		100	27.42±0.19	0.001**	15.71±1.93	0.010*	27.83±2.04	0.147
		200	30.48±1.25	0.049*	28.92±1.50	0.000***	32.78±0.74	0.015*
		5 μM DOX	25.01±0.30	0.004**	53.08±3.34	0.000***	73.66±1.06	0.006**

Data are presented as mean±SEM, $n \geq 3$; *$P<0.05$; **$P<0.01$; ***$P<0.001$ vs. control

In this study, we investigated the antitumour activity of 20(R)-G-Rh2 and 20(S)-G-RH2 in NSCLC cell lines 95D and NCI-H460 by observing cell inhibition rates, cell colony formation, cell morphology and fluorescence staining of the nucleus, cell cycle and apoptosis of NSCLC.

MATERIALS AND METHODS

Materials

20(R)-G-Rh2 and 20(S)-G-Rh2 (B21729, B21062, Shanghai yuanye Bio-Technology Co), a cell counting kit-8 (CCK-8) (MedChemExpress, China), Newborn Calf Serum (NBS) (Zhejiang Tianhang Biotechnology Co., Ltd.), AO/EB fluorescent staining solution (Beijing solarbio science and technology co., ltd.), DAPI fluorescent staining solution and a cell cycle and apoptosis analysis kit (KeyGENE BioTECH, China).

Cell culture

NSCLC cell lines 95D (CL-0011) and NCI-H460 (CL-0299) were obtained from Procell Life Science and Technology Co., Ltd. Culture 95D and NCI-H460 cells with Roswell Park Memorial Institute-1640 (RPMI-1640, E600028, sangon Biotech) medium added with 10% FBS (164210, Procell Life Science and Technology Co., Ltd) 100 µg/mL Penicillin–Streptomycin Solution in a humidified incubator at 37°C with 5% CO_2.

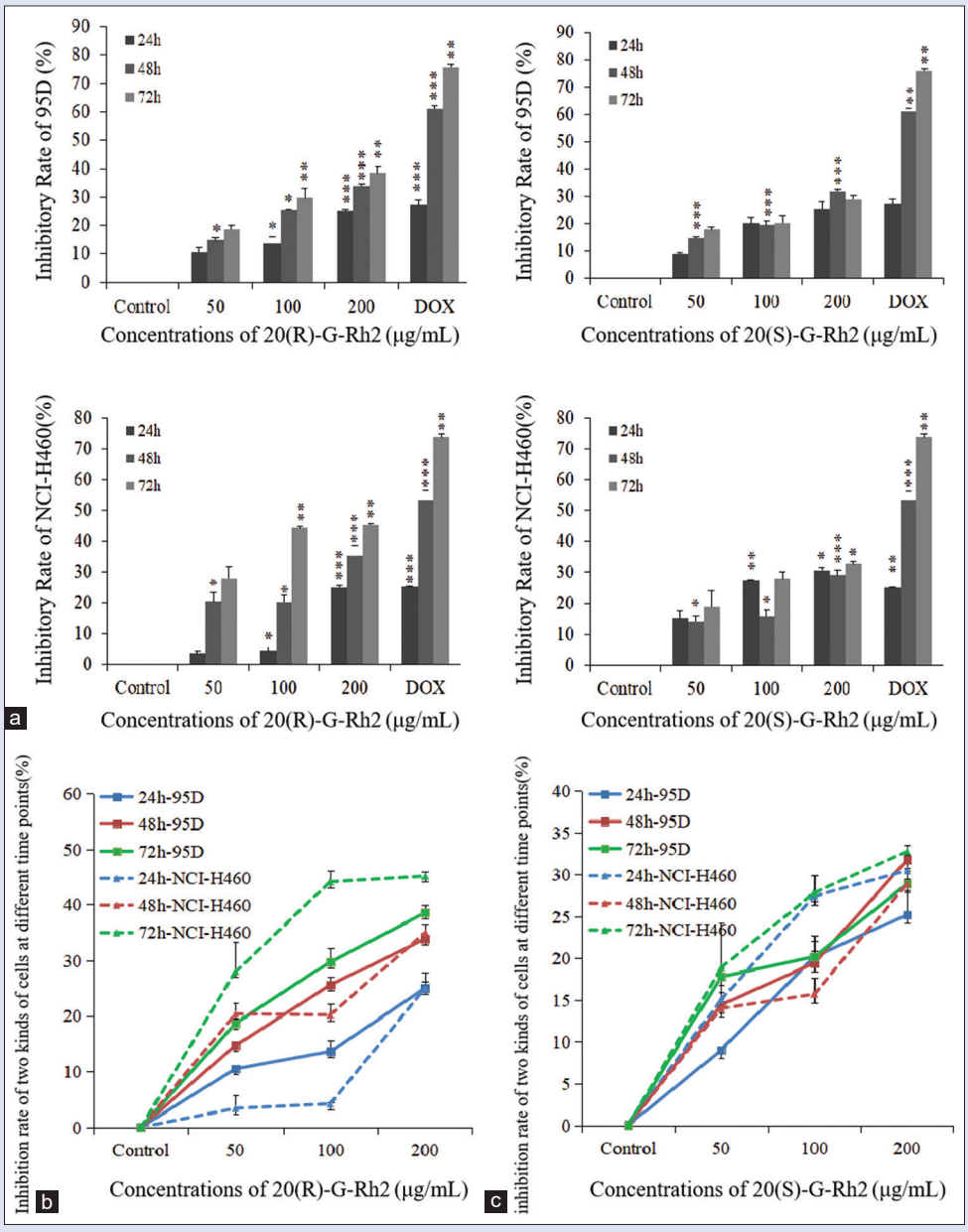

Figure 2: 20(R)-G-Rh2 and 20(S)-G-Rh2 treatment inhibited NSCLC cell lines 95D and NCI-H460 cells viability. (a): The inhibition ability was detected by in 95D and NCI-H460 cells after treatment with different concentrations 20(R)-G-Rh2 and 20(S)-G-Rh2 for 24 h, 48 h and 72 h. (b): The inhibition ability was detected by in 95D and NCI-H460 cells after treatment with different concentrations 20(R)-G-Rh2 for 24 h, 48 h and 72 h. (c): The inhibition ability was detected by in 95D and NCI-H460 cells after treatment with different concentrations 20(S)-G-Rh2 for 24 h, 48 h and 72 h. (Mean ± SEM, n ≥ 3; **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs. control.)

Cell proliferation assay

NSCLC cell lines 95D and NCI-H460 were plated in 96-well plates (2×10^3/well), and culture in a humidified incubator for 24 h. Subsequently, treat two types of cells with 5 μM DOX and different concentrations (0, 50, 100, 200 μg/mL) of 20(R)-G-Rh2 and 20(S)-G-Rh2 for 24, 48 and 72 h. At least three parallel tests should be carried out for each dose concentration. CCK-8 was dissolved in the culture medium at a ratio of 1:10, the cells were placed in a cell culture incubator for 30 min, and then the absorbance at 450 nm was measured with a microplate reader. The inhibition rate was calculated using the following formula:

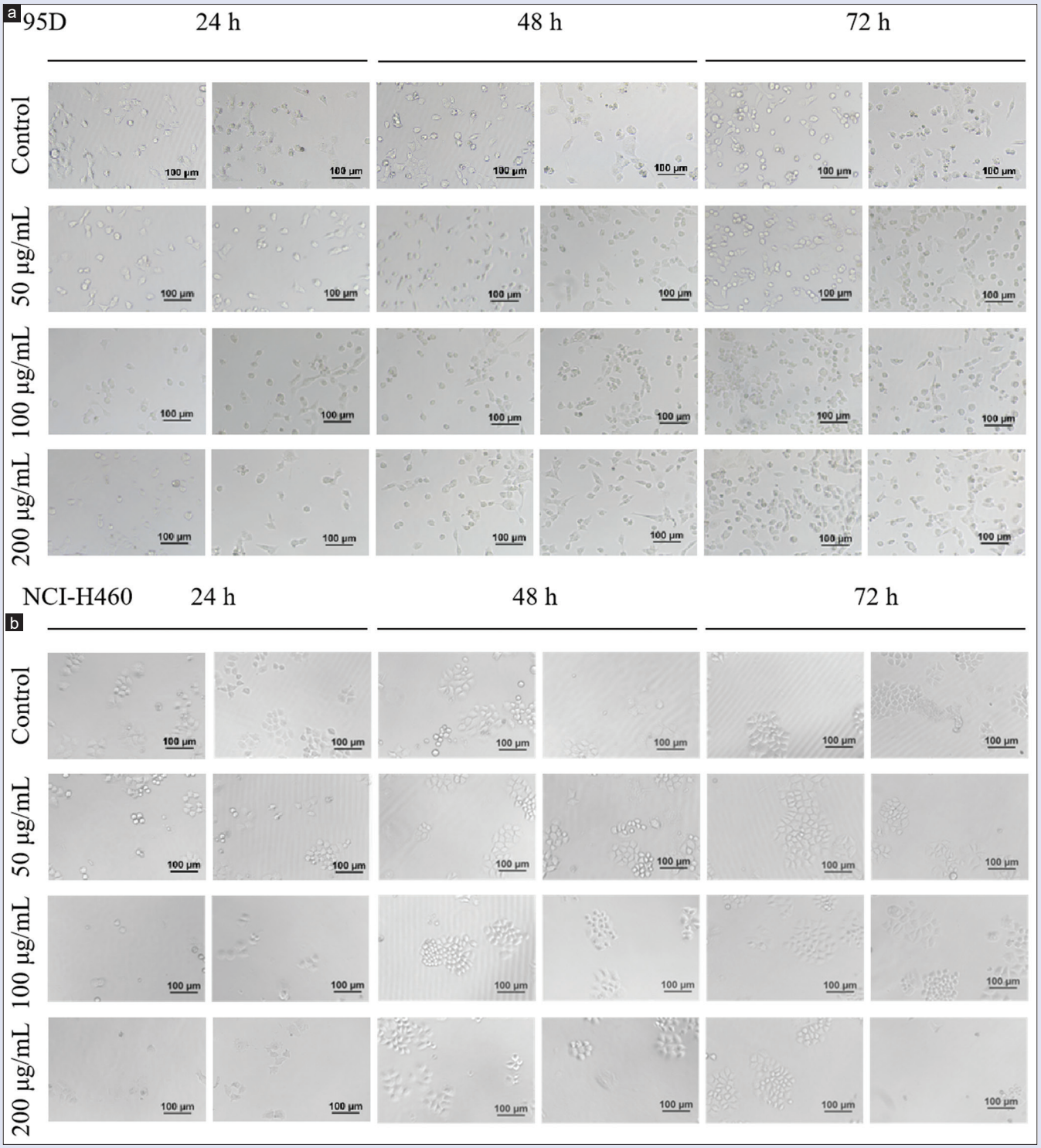

Figure 3: (a) Cell morphology of 95D cells treated with 20(R)-G-Rh2 and 20(S)-G-Rh2 at 24, 48 and 72 h. (b) Cell morphology of NCI-H460 cells treated with 20(R)-G-Rh2 and 20(S)-G-Rh2 at 24, 48 and 72 h. The cells were magnified 100x and 200x by Nikon microscope, and three pictures were retained for each concentration hole. Ginsenoside-Rh2 inhibited colony formation of NSCLC *in vitro*

Inhibition rate (%)

$=([A_{without\ drugs} - A_{with\ drugs}]/[A_{without\ drugs} - A_{blank\ control}]) \times 100\%$

Colony formation assay

The NSCLC cell lines 95D and NCI-H460 (3×10^2/well) were cultured in a six-well plate and RPMI-1640 medium for 24 h. The medium was replaced with 20(R)-G-Rh2 (0, 50, 100, 200 μg/mL) and 20(S)-G-Rh2 (0, 50, 100, 200 μg/mL). For drug culture media, at least three parallel tests should be performed for each concentration. We changed the fresh medium every 4 days and continued cultivating for 28 days. After staining with crystal violet, different areas were selected and photographed under the microscope. In this experiment, the cells were magnified 100x and 200x by a Nikon microscope, and three pictures were retained for each concentration hole.

4′, 6-diamidino-2-phenylindole fluorescence staining

NSCLC cell lines 95D and NCI-H460 (1×10^4/well) were cultured in a six-well plate and RPMI-1640 medium for 24 h, then replaced with a medicated medium. PBS washed the cells. After methanol was fixed and washed, 80 μL DAPI was added to each well, protected from light for 5 min, washed once with PBS, and photographed with a Nikon microscope (100×, 200×).

Acridine orange/ethidium bromide fluorescent staining

NSCLC cell lines 95D and NCI-H460 (1×10^4/well) were cultured in a six-well plate and RPMI-1640 medium for 24 h, then replaced with the medicated medium. A mixture of acridine orange (AO)/ethidium bromide (EB) (1:1) was prepared, and 10 μL of AO/EB mixture was added to each well and mixed and protected from light for 5 min. Nikon microscope was used to take pictures under the 10× and 20× eyepieces and the 10× objective lens, and three photos were kept for each concentration.

Cell cycle analysis

NSCLC cell lines 95D and NCI-H460 (2×10^5/well) were cultured in a 12-well plate and RPMI-1640 medium for 48 h and followed the instructions supplied with the cell cycle and apoptosis analysis kit. Preparation of RNase A: Propidium Iodide (1:9) staining solution. Cells were collected and washed twice with PBS. The collected cells were centrifuged at 2000 rpm for 5 min. About 1 mL of single-cell suspension was taken, centrifuged to remove the supernatant, fixed with 500 μL of 70% ice ethanol, stored at 4°C, and washed with PBS before staining. After fixation, 500 μL of staining solution was added to each assays and tested on the machine. As with other experiments, at least three parallel experiments were carried out.

Cell apoptosis assay

NSCLC cell lines 95D and NCI-H460 (1×10^5/well) were cultured in a 12-well plate and RPMI-1640 medium for 48 h, and then the medium was replaced with a drug medium containing 20(R)-G-Rh2 (0, 50, 100, 200 μg/mL) and 20(S)-G-Rh2 (0, 50, 100, 200 μg/mL). Cells were collected, washed twice with PBS, added the reagents required in the instructions, mixed well, protected from light at room temperature for 5–15 min and analyzed by flow cytometry. Three experiments were performed to ensure the accuracy of the results.

Statistical analyses

The data obtained in this study are expressed as mean ± standard error of the mean (SEM), the Statistical Package for the Social Sciences (SPSS) and Microsoft Excel software are used to analyze the data of each experiment, and the data are expressed as mean ± SEM, which is statistically significant ($P < 0.05$).

RESULTS

Ginsenoside-Rh2 inhibited the proliferation of NSCLC *in vitro*

The structural formula of 20(R)-G-Rh2 and 20(S)-G-Rh2 is shown in Figure 1. We tested whether 20(R)-G-Rh2 and 20(S)-G-Rh2 affected the proliferation of NSCLC cell lines 95D and NCI-H460. As shown in Figure 2, the results of CCK-8 analysis showed that the administration of 20(R)-G-Rh2 and 20(S)-G-Rh2 at serial concentrations (0, 50, 100, 200 μg/mL) inhibited cell viability in a dose and time-dependent manner. As shown in Table 1, 20(R)-G-Rh2 showed the highest inhibitory effect on NSCLC NCI-H460 cells at 200 μg/mL (45.18% ± 0.38%) after 72 h. 20(S)-G-Rh2 showed the highest inhibitory rate at 200 μg/mL (32.78% ± 0.74%) in NCI-H460 lung cancer cells and then calculated the IC_{50} value [Table 2], the inhibition rate of 20(R)-G-Rh2 in NCI-H460 cells is the highest, and the IC_{50} value (368.32 ± 91.28 μg/mL) is the lowest at 72 h. The cell morphology of different drugs at different time points was photographed [Figure 3].

The colony formation experiment is to verify the inhibitory effect of Ginsenoside Rh2 on the proliferation of NSCLC cell lines 95D and NCI-H460. Different concentrations of 20(R)-G-Rh2 and 20(S)-G-Rh2 were applied to lung cancer cells. Compared with the control group, 20(R)-G-Rh2 and 20(S)-G-Rh2 inhibit colony formation and have a greater inhibitory effect on NCI-H460 cells [Figure 4]. As shown in Table 3, the number of 95D and NCI-H460 cell colonies in the control group was 7.00 ± 2.65 and 27.67 ± 1.02. The number of 95D and NCI-H460 cell colonies decreased significantly to 1.67 ± 2.08 and 1.33 ± 0.19 when the concentration of 20(R)-G-Rh2 was 200 μg/mL.

Ginsenoside-Rh2 induced NSCLC cell lines 95D and NCI-H460 cell cycle arrest

To investigate the factors affecting cell proliferation, flow cytometry analysis of cell cycle changes, the results are shown in Figure 5. When the concentration of 20(R)-G-Rh2 is 100 μg/mL, the accumulation rate of 95D cells in S phase increased from 34.70% to 43.05% compared to the control group, and that of NCI-H460 cells in S phase is 3.64% to 22.94%. When the concentration of 20(S)-G-Rh2 is 100 μg/mL, the accumulation rate of 95D cells in S phase increased

Table 2: IC_{50} concentration of 20(R)-G-Rh2 and 20(S)-G-Rh2 in tumour cell lines. IC_{50} of ginsenoside-Rh2 on inhibition of cell proliferation of NSCLC cell lines (μg/mL)

Type	Cell lines	24 h	48 h	72 h
20(R)-G-Rh2	95D	1006.15±219.00	491.46±53.13	596.81±117.37
	NCI-H460	386.77±45.41	783.49±176.97	368.32±91.28
20(S)-G-Rh2	95D	981.81±200.88	588.66±36.20	1514.909±421.71
	NCI-H460	693.57±55.57	802.59±202.11	735.30±196.35

Data are presented as mean±SEM, $n \geq 3$

from 34.70% to 44.75% compared to the control group, and that of NCI-H460 cells in G2 phase is 3.64% to 22.44%. Additionally, after 24h of treatment with 20(R)-G-Rh2 and 20(S)-G-Rh2, the results [Table 4] indicated that ginsenoside Rh2 mediated cell cycle arrest in G1/S phase of 95D cells and cell cycle arrest in G2 phase of NCI-H460 cells.

Intervention effects of ginsenoside-Rh2 on DAPI fluorescence staining

To confirm the inhibitory effect of ginsenoside-Rh2 on NSCLC. DAPI fluorescent staining of 95D and NCI-H460 cells were treated with 0, 50, 100, 200 μg/mL 20(R)-G-Rh2 and 20(S)-G-Rh2. In DAPI staining, compared to the untreated group, the number of apoptotic bodies

Table 3: The CFU of NSCLC cell lines 95D and NCI-H460 under different concentrations of 20(R)-G-Rh2 and 20(S)-G-Rh2. Effect of ginsenoside-Rh2 on CFU of NSCLC cell lines

Type	Cell lines	Control	50 μg/mL	*P*	100 μg/mL	P value	200 μg/mL	*P*
20(R)-G-Rh2	95D	7.00±2.65	3.67±0.58	0.076	2.67±2.08	0.029*	1.67±2.08	0.011*
	NCI-H460	27.67±1.02	6.00±0.88	0.000***	2.33±0.19	0.000***	1.33±0.19	0.000***
20(S)-G-Rh2	95D	7.00±2.65	5.33±4.04	0.532	4.67±3.06	0.387	5.33±2.05	0.532
	NCI-H460	27.67±1.02	2±0.33	0.014*	1.33±0.19	0.021*	1.00±0.00	0.026*

Data are presented as mean±SEM, $n \geq 3$; *$P<0.05$; **$P<0.01$; ***$P<0.001$ vs. control

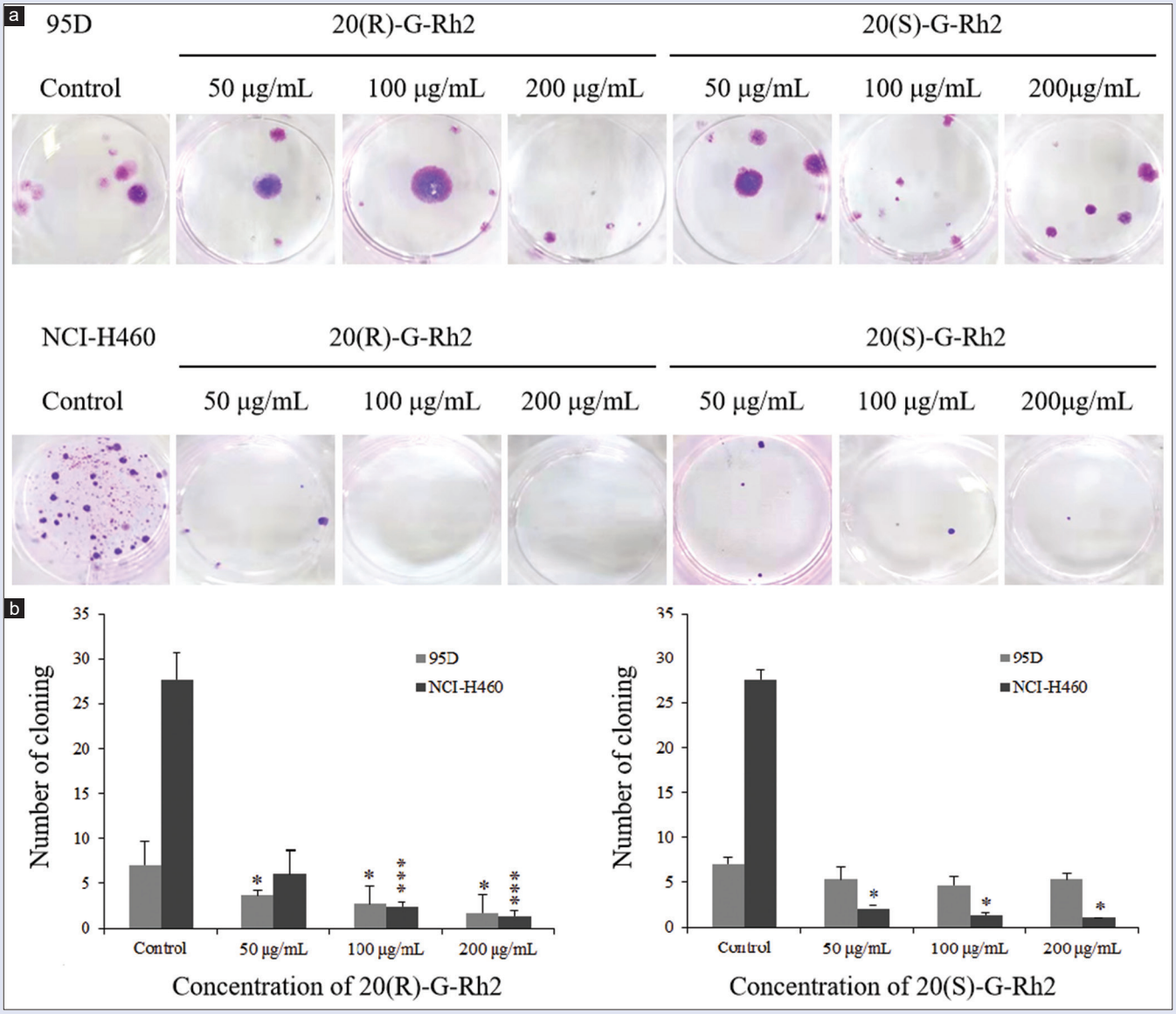

Figure 4: (a) Colony formation of NSCLC cell lines 95D and NCI-H460 under the action of 20(R)-G-Rh2 and 20(S)-G-Rh2 at different concentrations (0, 50, 100, 200 μg/mL). (b) The change of colony forming units (CFU) of NSCLC cell lines 95D and NCI-H460 under the action of 20(R)-G-Rh2 and 20(S)-G-Rh2 at different concentrations (0, 50, 100, 200 μg/mL). (Mean ± SEM, $n \geq 3$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. control.)

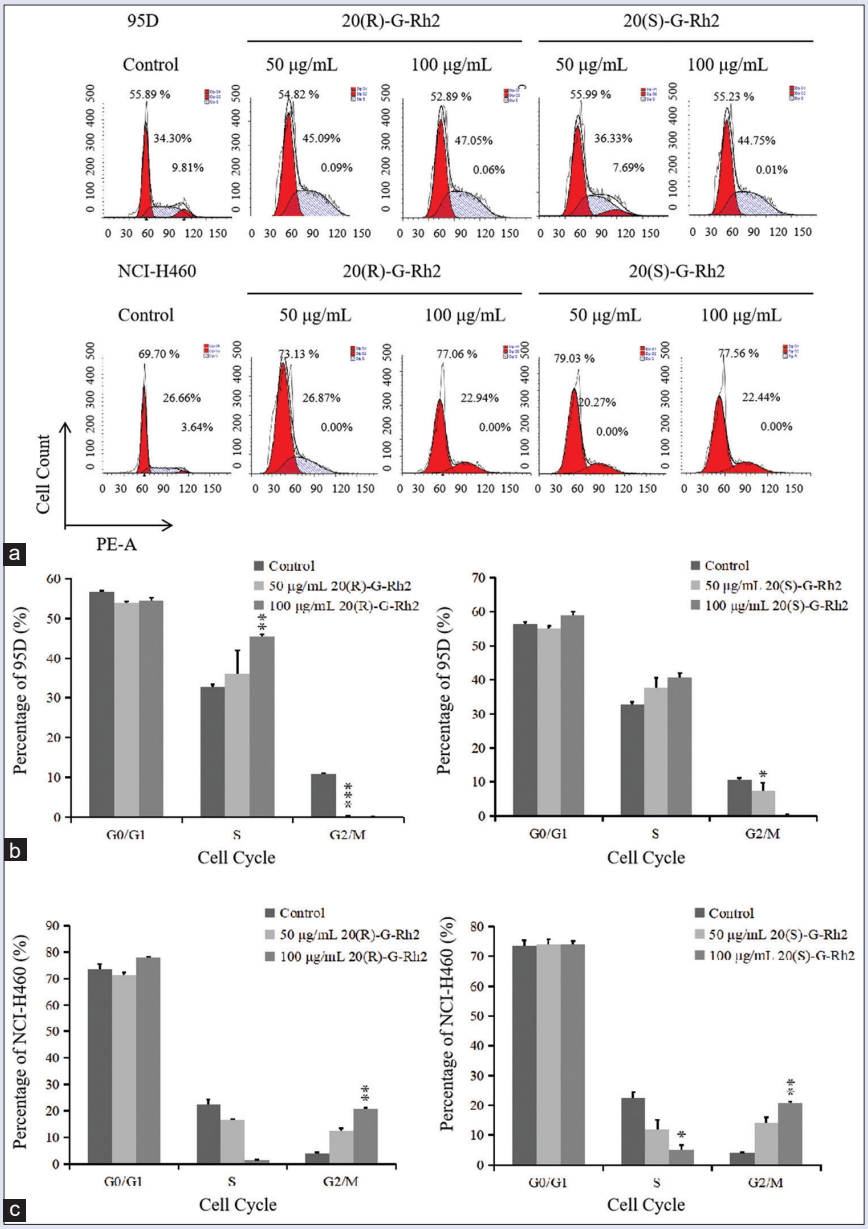

Figure 5: (a) Cell cycle analysis using cell cycle and apoptosis analysis kit and flow cytometry. (b) The percentage of different phases of 95D cell cycle under the action of 0–100 μg/mL 20(R)-G-Rh2 and 20(S)-G-Rh2. (c) The percentage of different phases of NCI-H460 cell cycle under the action of 0–100 μg/mL 20(R)-G-Rh2 and 20(S)-G-Rh2. (Mean ± SEM, $n \geq 3$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. control.)

Table 4: Data of 20(R)-G-Rh2 and 20(S)-G-Rh2 effects on different phases of the 95D and NCI-H460 cell cycle at 24 h. Effect of ginsenoside-Rh2 on cell cycle of NSCLC cell lines(%)

Type	Cell line	Cell cycle	Control	50 μg/mL	*P*	100 μg/mL	*P*
20(R)-G-Rh2	95D	G0/G1	56.51±0.46	53.86±0.31	0.059	54.49±0.59	0.127
		S	32.73±0.72	36.11±5.75	0.987	45.49±0.58	0.005**
		G2/M	10.76±0.31	0.03±0.02	0.000***	0.02±0.01	0.000***
	NCI-H460	G0/G1	73.49±1.90	71.15±1.16	0.927	77.95±0.29	0.666
		S	22.48±1.90	16.51±0.20	0.507	1.33±0.19	0.066
		G2/M	4.03±0.14	12.33±1.07	0.127	20.71±0.46	0.003**
20(S)-G-Rh2	95D	G0/G1	56.51±0.46	55.03±0.73	0.485	58.90±1.12	0.278
		S	32.73±0.72	37.56±3.04	0.346	40.73±1.18	0.142
		G2/M	10.76±0.31	7.42±2.35	0.359	0.37±0.17	0.021*
	NCI-H460	G0/G1	73.49±1.90	74.11±1.63	0.878	74.07±1.13	0.887
		S	22.48±1.90	11.78±3.41	0.122	5.12±1.56	0.027*
		G2/M	4.03±0.14	14.11±1.78	0.225	20.82±0.47	0.003**

Data are presented as mean±SEM, $n \geq 3$; *$P<0.05$; **$P<0.01$; ***$P<0.001$ vs. control

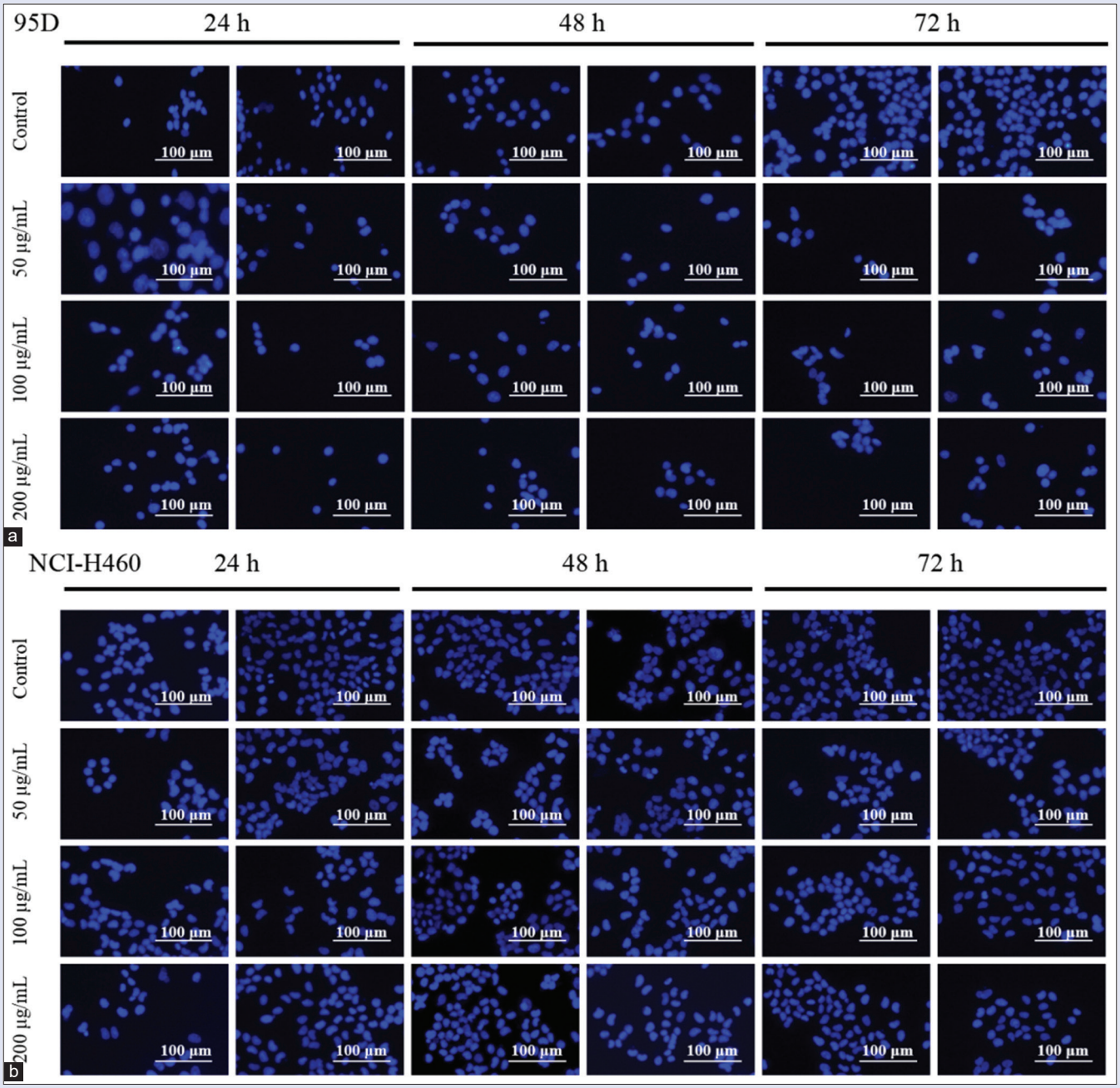

Figure 6: (a and b) DAPI staining of NSCLC cell lines 95D and NCI-H460 treated with 0–200 μg/mL 20(R)-G-Rh2 and 20(S)-G-Rh2 at 24, 48 and 72 h

increases, and as the concentration of the drug increases, the number of apoptotic bodies will increase [Figure 6].

Intervention effects of ginsenoside-Rh2 on AO/EB fluorescence staining

AO/EB fluorescence staining can reflect the apoptosis in different apoptotic stages. Four cell morphology can be observed under fluorescence microscope. Living cells: nuclear chromatin is green and normal. Early apoptotic cells: nuclear chromatin is green pyknosis or bead. Non-apoptotic dead cells: nuclear chromatin is orange and normal. Late apoptotic cells: nuclear chromatin is orange pyknosis or bead. In AO/EB staining, it was observed that compared with the number of apoptotic cells in the control group, the number of apoptotic cells in the administration group increased and the number of living cells and apoptotic cells were drug concentration dependent [Figures 7 and 8].

Ginsenoside-Rh2 induced apoptosis of NSCLC cell lines 95D and NCI-H460

Apoptosis is another factor affecting cell proliferation. The results of apoptotic analysis are shown in Figure 9 and Table 5. Most of the NSCLC cell lines 95D and NCI-H460 survived in the control group. After ginsenoside-Rh2 treatment, the apoptosis rate changed significantly ($P < 0.05$ and $P < 0.001$), At 20(R)-G-Rh2 concentration of 100 μg/mL, 95D cells had the maximum total apoptosis rate of 21.67 ± 0.97% and NCI-H460 cells total apoptosis rate was

Table 5: Data of ginsenoside-Rh2 effects on different type of apoptosis cells after treated by 20(R)-G-Rh2 and 20(S)-G-Rh2. Effect of ginsenoside-Rh2 on cell apoptosis of NSCLC cell lines(%)

Type	Cell line	Apoptosis	Control	50 μg/mL	*P*	100 μg/mL	*P*
20(R)-G-Rh2	95D	Early apoptosis	2.83±0.33	5.27±0.07	0.006**	5.97±0.22	0.002**
		Late apoptosis	5.73±1.23	16.90±0.48	0.004**	15.70±1.13	0.007**
		Total apoptosis	8.57±1.52	22.17±0.42	0.002**	21.67±0.97	0.002**
	NCI-H460	Early apoptosis	6.13±0.10	6.70±0.15	0.180	4.43±0.20	0.004**
		Late apoptosis	4.53±0.08	13.50±0.93	0.079	22.87±2.85	0.005**
		Total apoptosis	10.67±0.17	20.20±1.05	0.057	27.30±2.67	0.006**
20(S)-G-Rh2	95D	Early apoptosis	2.83±0.33	4.97±0.08	0.166	5.27±0.05	0.14
		Late apoptosis	5.73±1.22	12.13±0.07	0.064	15.07±1.58	0.016*
		Total apoptosis	8.57±1.52	17.10±0.03	0.034*	20.33±1.59	0.009**
	NCI-H460	Early apoptosis	6.13±0.10	6.33±0.24	0.606	7.80±0.03	0.004**
		Late apoptosis	4.53±0.08	29.40±3.13	0.127	13.73±0.10	0.000***
		Total apoptosis	10.67±0.17	35.73±2.90	0.108	21.53±0.13	0.000***

Data are presented as mean±SEM, $n \geq 3$; *$P<0.05$; **$P<0.01$; ***$P<0.001$ vs. control

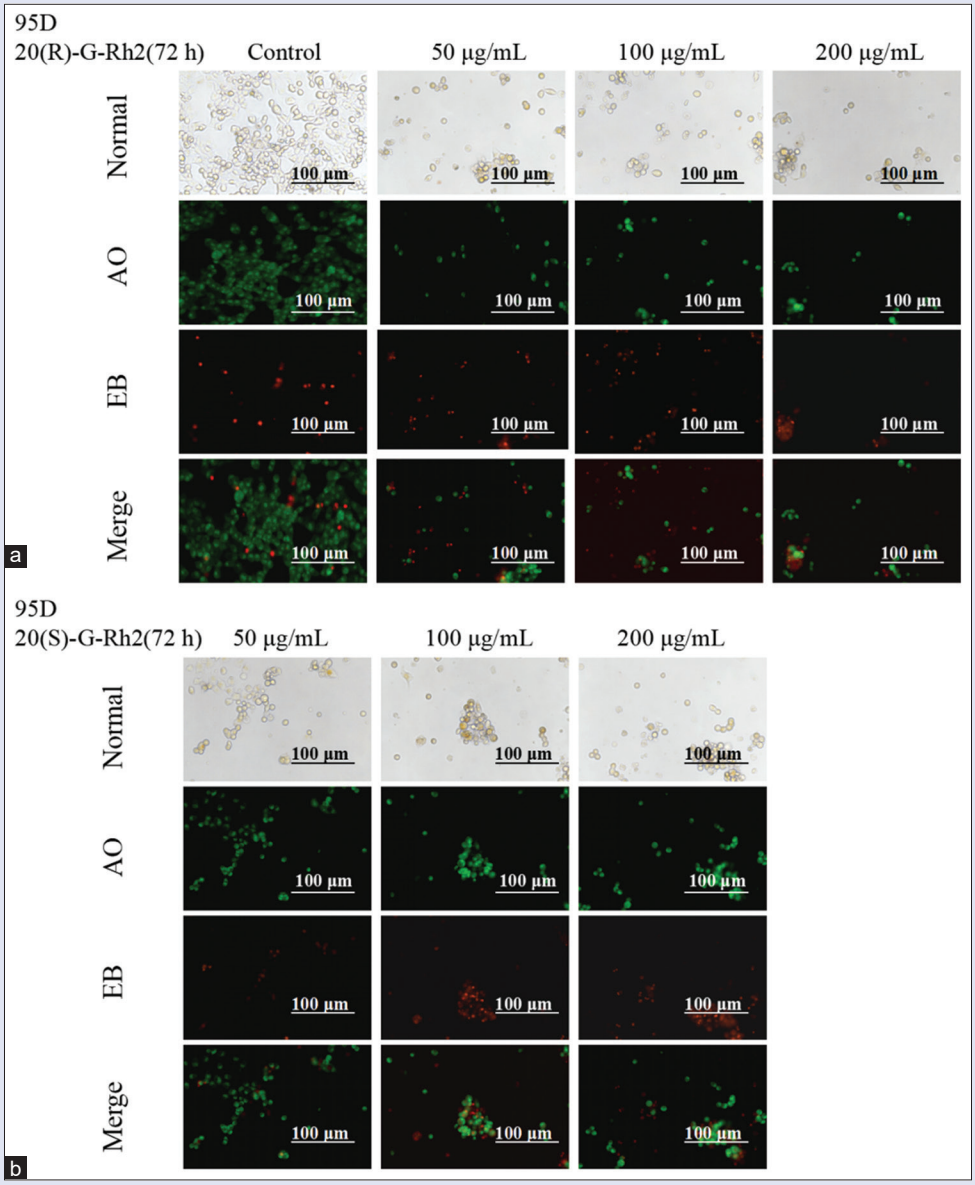

Figure 7: (a and b) AO/EB staining of 95D cells treated with 0–200 μg/mL 20(R)-G-Rh2 and 20(S)-G-Rh2 at 72 h

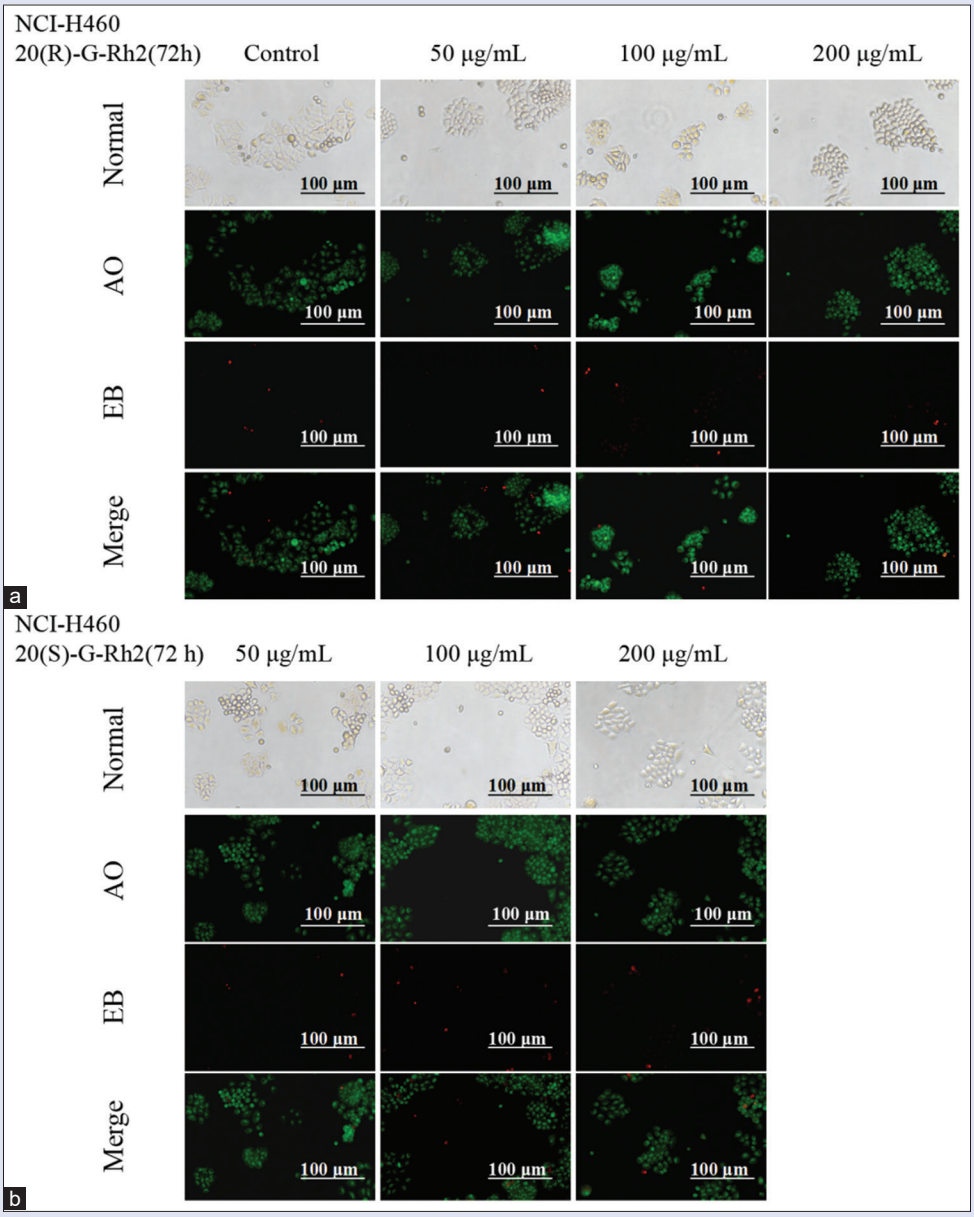

Figure 8: (a and b) AO/EB staining of NCI-H460 cells treated with 0–200 μg/mL 20(R)-G-Rh2 and 20(S)-G-Rh2 at 72 h

27.30% ± 2.67%. At 20(S)-G-Rh2 concentration of 50 μg/mL, 95D cells total apoptosis rate was 17.10% ± 0.03% and NCI-H460 cells had the maximum total apoptosis rate of 35.73% ± 2.90%. These results suggest that 20(R)-G-Rh2 and 20(S)-G-Rh2 has a significant pro-apoptotic effect on NSCLC, and 20(S)-G-Rh2 has a stronger effect on promoting cell apoptosis than 20(R)-G-Rh2.

DISCUSSION

Lung cancer is a respiratory disease. As a malignant tumour, it occurs slowly. In the early stage, patients have no obvious symptoms. However, with the gradual deterioration of the disease, there will be chronic cough, usually irritating dry cough, and sometimes hemoptysis or expectoration. Some sick families cannot afford the high cost of treatment, so the research of traditional Chinese medicine did not only increase the safety in the treatment of lung cancer, but also reduce the treatment cost. Ginsenoside has been proved to have antitumour activity. In this research, we reported that ginsenoside Rh2 can inhibit the proliferation, colony formation, cell cycle and promote apoptosis of NSCLC cell lines 95D and NCI-H460.

Ginsenoside Rh2— the hydrolyzed component of the active substance of ginsenoside.[13] Ginsenoside Rh2 has a variety of biological activities, such as inhibiting the growth of tumour cells,[14] blocking the synthesis and metabolism of important components of tumour cells, inducing tumour cell apoptosis,[15,16] reversing tumour drug resistance and anti-tumour metastasis and reversing the abnormal differentiation of tumour cells. Nowadays, many studies have proved that traditional Chinese medicine has a therapeutic effect on lung cancer. Sun-Bai-Pi and ginseng polysaccharides can induce lung cancer cell apoptosis and inhibit proliferation.[17,18] Traditional Chinese medicine is widely used in the treatment of cancer, not only because of its antitumour activity but also through the combination with Western treatment methods such as chemotherapy to achieve a better therapeutic effects.[19]

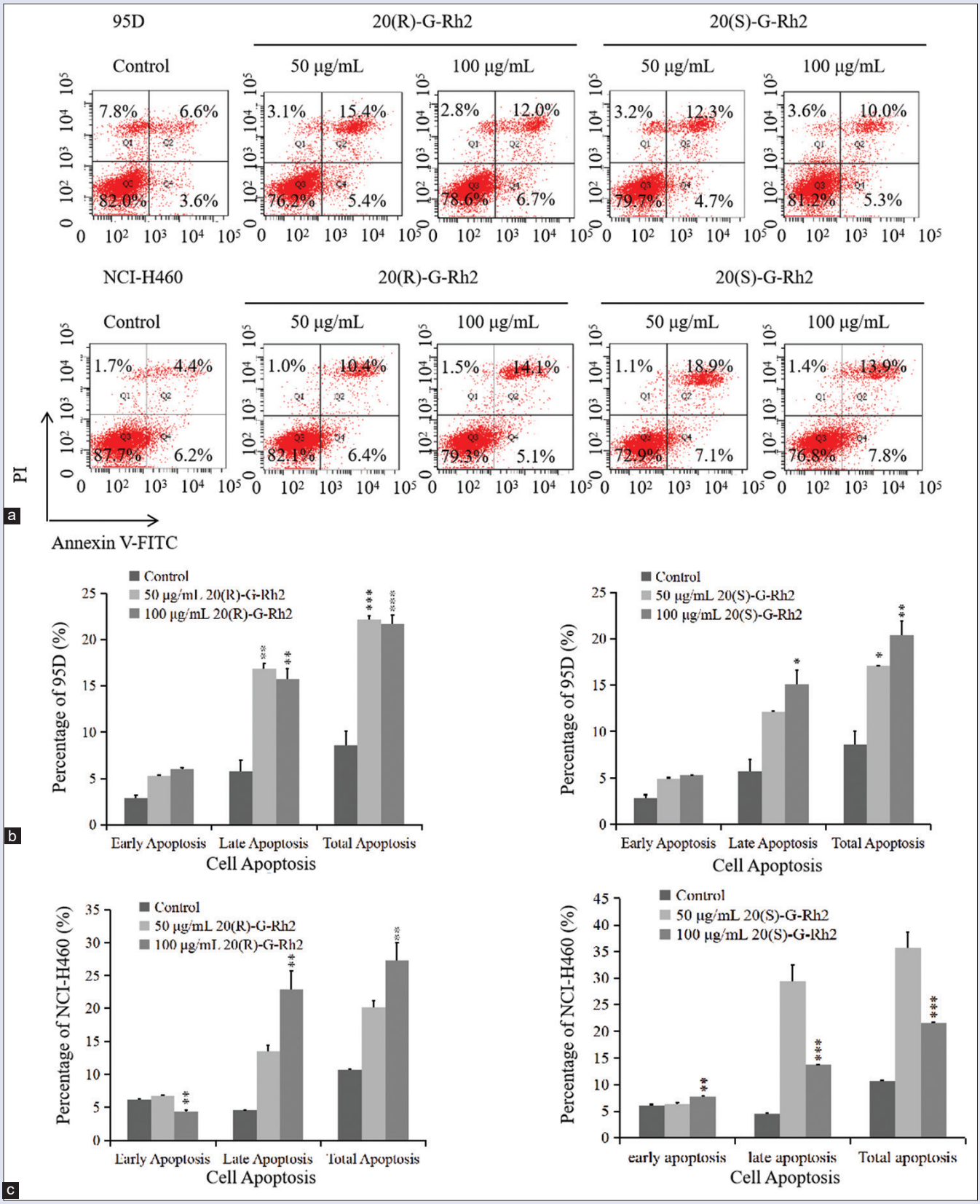

Figure 9: (a) Apoptosis analysis using an Annexin V-FITC/PI apoptosis analysis kit and flow cytometry after 20(R)-G-Rh2 and 20(S)-G-Rh2 treatment NSCLC cell lines 95D and NCI-H460 at 24 h. (b and c) The proportion of NSCLC cell lines 95D and NCI-H460 in different apoptotic stages after 0–100 μg/mL 20(R)-G-Rh2 and 20(S)-G-Rh2 treatment at 24 h. (Mean ± SEM, $n \geq 3$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. control.)

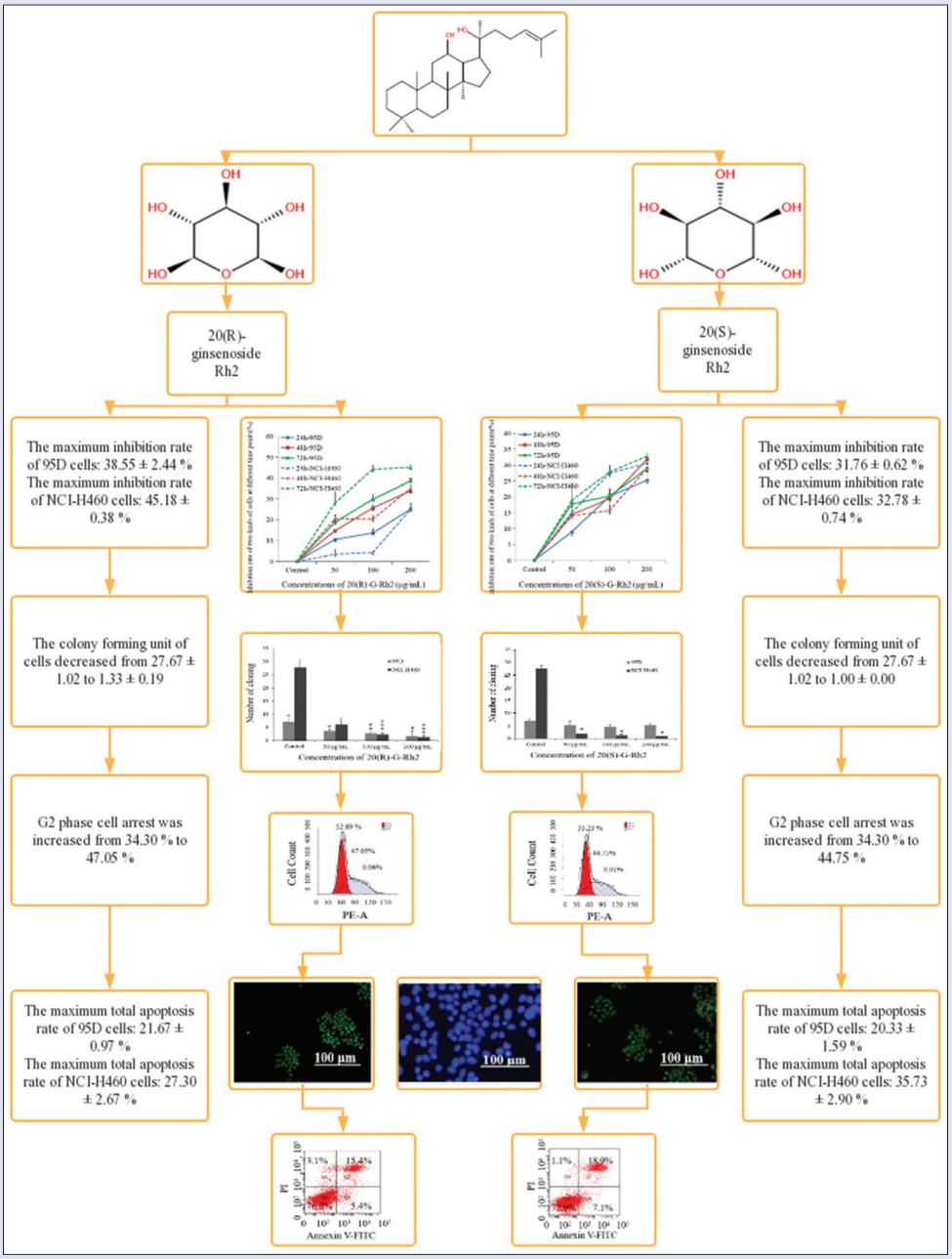

Figure 10: Activity comparison of 20(R)-G-Rh2 and 20(S)-G-Rh2

Previous studies have found that Ginsenoside Rh2 displayed an evident anticancer activity through suppressing cell proliferation.[20] Inducing cell cycle arrest.[21-23] and apoptosis,[24-26] in tumour cells, and it affects the expression of D1 and CDK4, the key regulators of cyclin-dependent kinases.[27] In this study, the results showed that ginsenoside Rh2 can inhibit the proliferation and colony formation of NSCLC 95D and NCI-H460 cells. The maximum inhibition rate of 95D cells was 38.55 ± 2.44 %, and that of NCI-H460 cells was 45.18 ± 0.38 %, 20(R)-ginsenoside Rh2 is more effective.

Ginsenoside Rh2 can differentiate B16 melanoma or F9 teratoma into melanocyte like cells or wall endoderm like cells with normal phenotype, and arrest the HL-60 cell cycle in G1/S phase. These conclusions show that ginsenoside Rh2 has the ability to differentiate tumour cells into morphological and functional granulocytes and induce cell cycle arrest.[28] Ginsenoside Rh2 induce NCI-H460 cell cycle arrest in G2 phase and induce 95D cell cycle arrest in S phase. Studies have shown that ginsenosides such as 20(S)-ginsenoside Rh2 and 20(S)-protopanaxadiol show an obvious structure–activity relationship. 20(S)-ginsenoside Rh2 has a broad-spectrum antitumour effect that is not organ-specific, and its antitumour effect is related to cell cycle arrest. This study shows that 20(S)-ginsenoside Rh2 has a stronger ability to induce an arrest cycle than 20(R)-ginsenoside Rh2.

Studies have proved that ginsenoside Rh2 can induce cell apoptosis, which is induced by ROS-mediated endoplasmic reticulum stress-dependent pathways.[29] Apoptosis and autophagy, as catabolic pathways in various physiological and pathological processes, determine the fate of cancer cells. In the present study, Ginsenoside Rh2 induced cell apoptosis in NSCLC cell lines 95D and NCI-H460, the maximum total apoptosis

rate of 95D cells was 21.67 ± 0.97 %, and that of NCI-H460 cells was 35.73 ± 2.90 %. These results strongly prove that two configurations of ginsenoside Rh2 can significantly promote the apoptosis of NSCLC cells, and the effect of 20(S)-ginsenoside Rh2 is more obvious. However, more detailed information on the genomic and proteomic responses underlying the ginsenoside Rh2 induced anticancer effects remains to be elucidated.

Studies have shown that ginsenoside Rh2 enhances the infiltration of T lymphocytes in tumours and triggers the cytotoxicity of splenic lymphocytes.[30] 20(S)-ginsenoside Rh2 has antitumour activity and can help enhance the anti-cancer effect of other conventional chemotherapy drugs at normal doses.[31] At present, the inhibitory effect of ginsenoside Rh2 on tumour cells has been proved, but the comparison of the activities of two configurations of ginsenoside Rh2 needs to be further studied.

This study compared the inhibitory effects of two configurations of ginsenoside Rh2 on lung cancer [Figure 10]. The maximum inhibitory rates of 20(R)-G-Rh2 and 20(S)-G-Rh2 on NSCLC were 45.18 ± 0.38 % and 32.78 ± 0.74 %, respectively. Ginsenoside Rh2 could inhibit the colony formation of 95D cells and NCI-H460 cells, and the inhibitory effect of 20(R)-G-Rh2 was more obvious. 20(R)-G-Rh2 and 20(S)-G-Rh2 induced cell cycle arrest in G1/S phase of 95D cells and cell cycle arrest in G2 phase of NCI-H460 cells. In the apoptosis experiment, the maximum total apoptosis rate of 20(R)-G-Rh2 and 20(S)-G-Rh2 was 27.3 ± 2.67 % and 35.73 ± 2.90 %. In order to have a deeper understanding of the anti-lung cancer effect of ginsenoside Rh2, the author suggests that we should further study the action mechanism of effective components of traditional Chinese medicine on lung cancer and try to explore the relationship between cyclin and apoptotic protein so that the expression of the sapoptotic protein can be enhanced by regulating cyclin in the future, and vice versa, Then inhibit the growth rate of cancer cells to achieve the maximum therapeutic effect on lung cancer.

CONCLUSION

In this study, our data indicate that ginsenoside Rh2 could inhibit the proliferation and colony formation of NSCLC 95D and 460. In the cell cycle experiment, 20(R)-G-Rh2 and 20(S)-G-Rh2 induced cell cycle arrest in varying degrees. Flow cytometry showed that 20(R)-G-Rh2 and 20(S)-G-Rh2 could promote cancer cell apoptosis. By comparing the inhibitory effects of 20(R)-G-Rh2 and 20(S)-G-Rh2, the effect of 20(R)-G-Rh2 is stronger. Based on these results, 20(R)-G-Rh2 may be a better option for the treatment of lung cancer in the future.

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Conflicts of interest

There are no conflicts of interest.

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