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Cytotoxicity and apoptosis induction of weisiensin B isolated from *Rabdosia weisiensis* C.Y. Wu in human hepatoma cells

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Weisiensin B, a new *ent*-kaurane diterpenoid, was isolated from traditional Chinese herb *Rabdosia weisiensis* C.Y. Wu. In this study, cytotoxicity of weisiensin B was tested on four different tumor cell lines and the effect of growth inhibition and apoptosis in BEL-7402 cell line were investigated *in vitro*. The results indicated that weisiensin B had significant antiproliferation activity on the four cell lines. Further study on BEL-7402 cells involving Hoechst 33258 stain and DNA fragmentation assay revealed the characteristic apoptotic features of nuclear and DNA ladder formation. Flow cytometric (FCM) analysis with propidium iodide (PI) staining demonstrated that BEL-7402 cells treated with weisiensin B were arrested in G_2/M phase. The results demonstrated that a significant fraction of weisiensin B-treated cells died by an apoptotic pathway in BEL-7402 cells.

Keywords: *ent*-kaurane diterpenoids; weisiensin B; cycle arrest; apoptosis

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1. Introduction

Malignant neoplasms still remain as the leading cause of death in many countries. At present, there is an increasing effort for the isolation of bioactive microchemicals from medicinal plants for their possible usefulness in the control of various ailments [1]. Herbal drugs have been widely used for thousands of years in traditional Chinese medicine for the treatment of human diseases. Genus *Isodon* (Labiatae) contains a large amount of *ent*-kaurane diterpenoids, and more than 400 *ent*-kaurane diterpenoids have been isolated from the plants. Many of these diterpenoids display various biological activities, such as antibacterial, anti-inflammatory, and, especially, antitumor actions [2]. Oridonin, one of the extensive-reported antitumor *ent*-kaurane diterpenoids [3], was isolated from *Rabdosia rubescens* Hemsl that was used as a traditional herb in the treatment of gastric tumor in China. Its various antitumor mechanisms were reported in recent years, which include changing Bcl-2 and Bax levels, NF-κB, caspase-3, and the Ras/Raf1-dependent pathway activation, and so on [4–6]; but there are few reports about the antitumor mechanism of other new *ent*-kaurane diterpenoids.

Weisiensin B (Figure 1), a new *ent*-kaurane diterpenoid reported first in our laboratory [7], was isolated from *R. weisiensis* C.Y. Wu., which is one of the *Isodon* family, and sulforhodamine B (SRB) assay showed that it has strong cytotoxicity against human tumor cells BEL-7402 and HO-8910. The aim of this study was to investigate the effects of weisiensin B on human tumor cells, including the inhibitory roles on growth, proliferation, apoptosis induction, and the changes in cell cycle.

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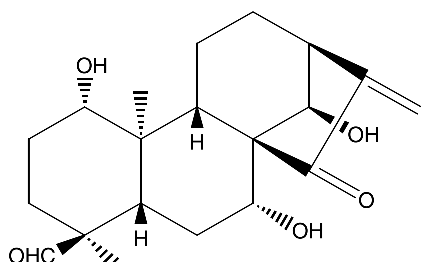

Figure 1. Chemical structure of weisiensin B.

2. Results and discussion

2.1 IC$_{50}$ values of weisiensin B in different tumor cell lines

Cytotoxicity determining, a common method to evaluate the biological activities of nature products, is helpful to confirm whether plant extracts have potential antineoplastic properties [8]. To gain an insight into the effect of weisiensin B, we compared the antiproliferation effects of weisiensin B on four tumor cell lines *in vitro*. The antiproliferative SRB assay was performed to assess growth inhibition by a colorimetric assay that estimates cell number indirectly by staining total cellular protein with the dye SRB. The SRB assay showed that this diterpene has different cytotoxic activities to these human cancer cell lines *in vitro*. Four different cell lines (BEL-7402, HO-8910, SGC-7901, and HepG$_2$) were used to determine the cytotoxicity of weisiensin B and 50% inhibitory concentration (IC$_{50}$) is shown in Table 1.

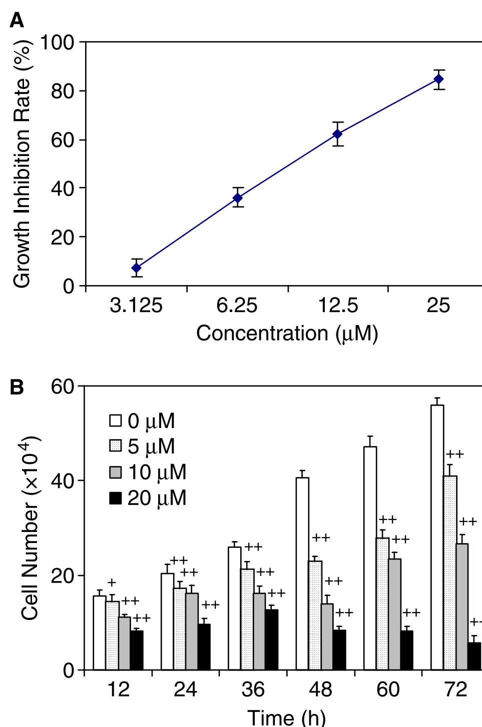

Figure 2. Growth inhibitions of weisiensin B in BEL-7402 cell line. (A) BEL-7402 cells were treated with weisiensin B at the indicated concentrations for 48 h. The growth inhibition rate was determined by SRB assay. Each point is the mean of three replicates; bars represent the SD. (B) Number of viable cells measured by trypan blue exclusion method. Each column represents the mean of the data from three replicates and bars represent the SD. $^{+}P < 0.05$, $^{++}P < 0.01$ versus control.

2.2 Growth inhibition of weisiensin B in BEL-7402 cells

BEL-7402 cells were treated with different concentrations of weisiensin B for different times. The SRB assay shows a significant antiproliferative effect (Figure 2A). The IC$_{50}$ value of weisiensin B in BEL-7402 cells was (10 ± 0.20) μmol/l (Table 1). The cell viability was also evaluated at indicated time respectively based on the ability of the cells to exclude trypan blue. Weisiensin B led to a time- and dose-dependent diminution of cells, indicating its ability to weaken proliferation potential of this tumor cells (Figure 2B).

Table 1. IC$_{50}$ values of weisiensin B in several cultured cell lines.

Cell lines	IC$_{50}$ (μmol/l)
BEL-7402	10.0 ± 0.20
HO-8910	32.0 ± 0.70
SGC-7901	4.34 ± 0.23
HepG$_2$	3.24 ± 0.18

2.3 Apoptosis induced by weisiensin B in BEL-7402 cells

Figure 3 shows the representative morphology of BEL-7402 cells when exposed to

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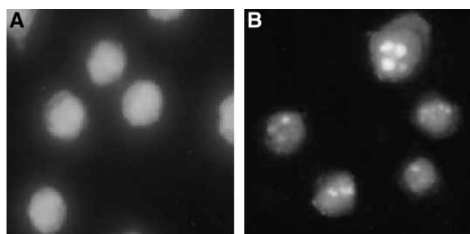

Figure 3. Nuclear morphology of BEL-7402 cells. (A) Control. (B) Apoptotic cells observed under a fluorescence microscope after 48-h treatment with 20 μmol/l of weisiensin B.

weisiensin B (20 μmol/l) for 48 h. The control BEL-7402 cells appeared normal, with the nuclei round and homogeneous (Figure 3A). After treatment, the cells exhibited the characteristic features of apoptosis, such as condensation of nuclear chromatin, nuclear fragmentation, and formation of some blocks of condensed chromatin in the leaflet of nuclear membrane (Figure 3B).

The regulated DNA fragmentation or internucleosomal DNA cleavage is a prominent feature of apoptosis. So, apoptosis was evaluated by the induction of DNA fragmentation. Figure 4 showed the typical DNA fragmentation when BEL-7402 cells were treated with the same concentration of weisiensin B for a specific time. It showed the more treated time, the more noticeable the DNA ladder became. Thus, the results demonstrated that weisiensin B induced endonucleolytic DNA cleavage in a time-dependent manner.

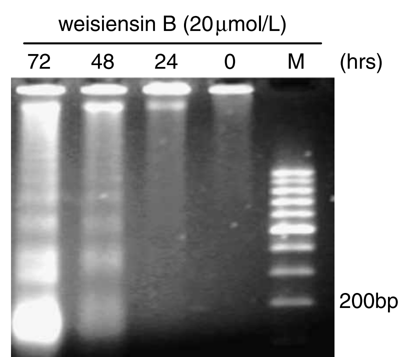

Figure 4. DNA fragmentation of apoptotic BEL-7402 cells treated with 20 μmol/l weisiensin B for the indicated time. M represents DNA marker.

2.4 *Effects of weisiensin B on cell cycle distribution*

The distribution of cell cycle was examined at the indicated times. At a concentration of 20 μmol/l, weisiensin B induced a time-dependent increase of G_2/M cell population in BEL-7402 cells from 0 to 24 h, while a decrease of G_2/M cell population after 24 h was observed. The number of G_2/M cells were 5.8 ± 1.25, 14.9 ± 1.83, 40 ± 4.36, and 17 ± 1.4% at 0, 12, 24, and 48 h, respectively. Figure 5 also shows a hypodiploid (sub-G_1) population increase from 1.6 ± 1.1% at 48 h to 11.2 ± 3.1% at 72 h, but no G_2/M cells were detected at 72 h. The difference between the control and the treatment was statistically significant ($P < 0.05$).

As shown in Figure 6, after treatment with weisiensin B for 24 h, the percentage of cells with hypodiploid DNA was 7.3 ± 1.18%. For another 24 h, the percentage became 28.1 ± 3.4%. But when cells were treated for 60 h, the number of apoptotic cells deceased to 15.4 ± 2.65% ($P < 0.05$ versus control). It indicated when treated with Weisiensin B at the concentration of 40 μmol/l within 48h the apoptosis in BEL-7402 cells was increased in a time-dependent manner.

2.5 *Discussion*

Apoptosis is the process of programmed cell death with a tightly gene-controlled program. Defective apoptosis represents a major causative factor in the development and progression of cancer [9]. Based on the understanding of cancer intervention, induction apoptosis have emerged in the fields of cancer chemoprevention and chemotherapy, and it is considered to be crucial targets of current drug development [9] and a promising approach of cancer therapy [10]. Uncontrolled cellular growth and proliferation are key properties of carcinogenesis [11], and the effectiveness of cytotoxic drugs on malignant cells is often associated

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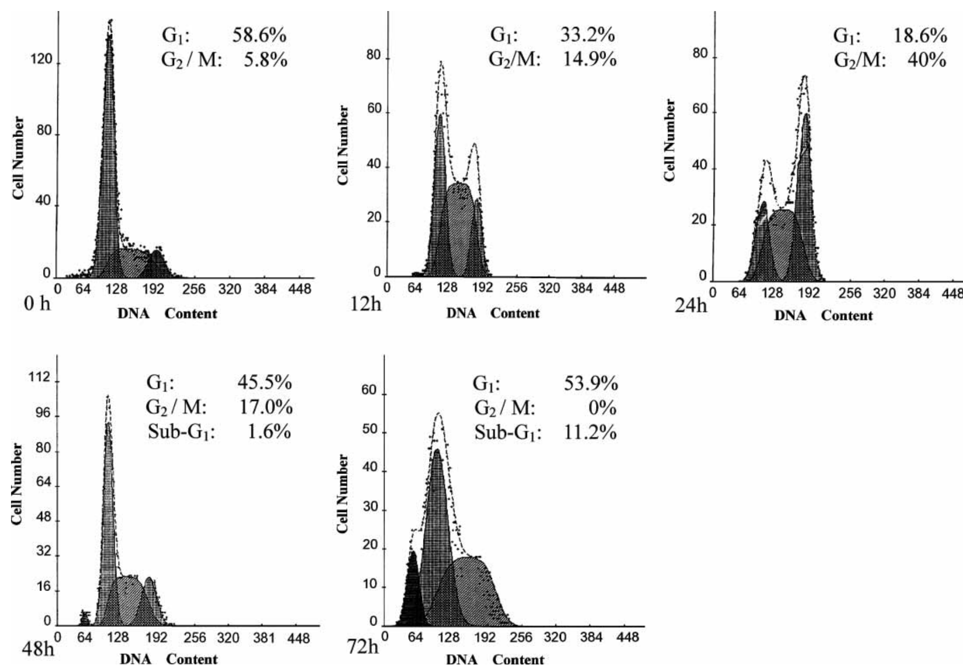

Figure 5. Effects of weisiensin B (20 μmol/l) on cell cycle distribution in BEL-7402 cells for indicated times. Representative of three similar experiments with triplicates.

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with their capacity to inhibit cellular proliferation [12]. The IC_{50} values showed that $HepG_2$ (3.24 ± 0.18 μmol/l) and SGC-7901 (4.34 ± 0.23 μmol/l) are more sensitive to weisiensin B than the HO-8910 (32 ± 0.70 μmol/l) and BEL-7402 (10 ± 0.20 μmol/l) cancer cell lines. It was reported that oridonin, an *ent*-kaurane diterpenoid isolated from the genus *Rabdosia*, was about 27 μmol/l when its inhibitory rate reached 50% on BEL-7402 at 48 h [4]. The results of antiproliferative assay suggested that weisiensin B has extensive and strong cytotoxicity to human tumor cells.

According to the statistics of the World Health Organization, about 6,350,000 cancer cases were reported in the world each year, 4% of which are hepatocellular carcinoma and 42% occur in China [13]; so, further study of weisiensin B in BEL-7402 cell line was specialized in the following works. A growth inhibition in BEL-7402 cells was observed after 48 h exposure to weisiensin B at some dose, and growth inhibition rate is dose dependent as shown in Figure 2A. At the same time, the cells' viability was also determined as shown in Figure 2B. With increasing exposure time, there was a significant decrease in cell viability in the presence of weisiensin B at 5, 10, and 20 μmol/l. Especially, when cells were treated with 20 μmol/l weisiensin B, a marked lethal effect was observed after indicated times. So, the reduction in cell viability is in a time- and dose-dependent manner.

An important biochemical hallmark of apoptosis is the fragmentation of genomic DNA into integer multiples of 180–200 bp units, resulting in a characteristic ladder on agarose gel electrophoresis. The DNA ladder is often considered the definitive biochemical marker for apoptosis [14]. The agar gel electrophoresis was therefore conducted in the experiment and it was found that weisiensin B can induce BEL-7402 cells apoptosis with concentration of 20 μmol/L at 48 and 72 h (Figure 4). At the same time, FCM assay also indicated that cells entered into apoptosis when treated with 20 μmol/l

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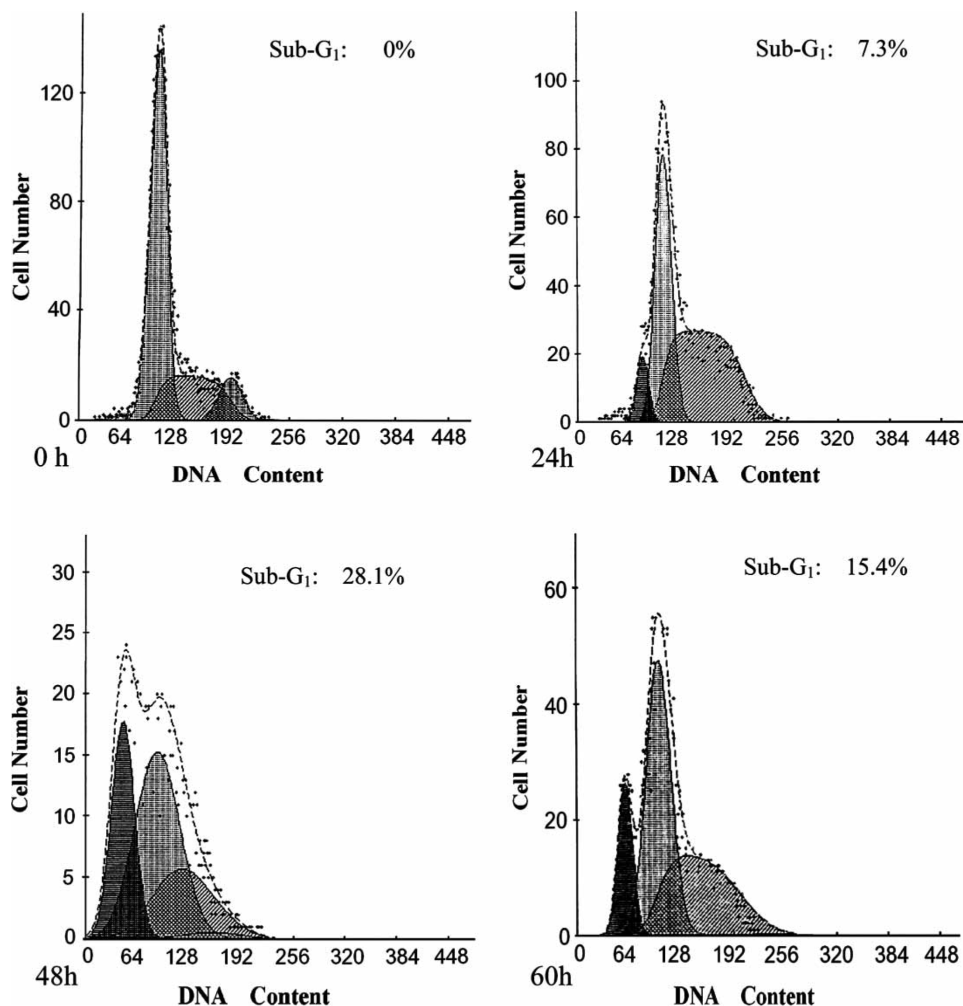

Figure 6. Apoptosis induced by weisiensin B (40 μmol/l) in BEL-7402 cells at the indicated time. Representative of three similar experiments with triplicates.

weisiensin B, and sub-G_1 cells reached 1.6 and 11.2% at 48 and 72 h, respectively (Figure 5). In Figure 6, sub-G_1 cells reached 7.3% at 24 h when they were treated with the concentration of 40 μmol/l weisiensin B, and this is higher than 48-h treatment of 20 μmol/l weisiensin B. At 48 h, apoptotic rate went up to 28.1% with treatment of 40 μmol/l weisiensin B, but it is just 11.2% with the treatment of 20 μmol/l weisiensin B at 72 h. These results suggested that apoptosis induced by weisiensin B is time and dose dependent just within 48 h. When cells were treated with 40 μmol/l for 60 h, a decreased apoptosis rate was observed (Figure 6; 60 h). Maybe it is a result of failing to detect the apoptotic cells that have disintegrated with treatment of higher concentration drugs *in vitro*. Additionally, nuclear morphology is the most accurate indicator of the involvement of apoptosis in cell death [15], and the typical feature of programmed cell death [16] was demonstrated by Hoechst 33258 stain in drug-treated cells (Figure 3B).

In recent years, considerable advances have been made in cell cycle progression, and

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inhibition of deregulated cell cycle progression in cancer cells was considered an effective strategy to halt tumor growth [17]. Actually, many traditional pharmacological agents induce cell death by regulating cell cycle progression [18]. It was reported that oridonin, which was isolated from the traditional Chinese herb *Isodon rubescens*, exhibits antitumor activity through inducing cycle arrest in G_1 phase [19] and G_2/M phase [20], and progression from S to G_2/M phase [21] in different cell lines. In this report, the flow cytometric (FCM) assay indicated that cells treated with 20 μmol/l weisiensin B were arrested in G_2/M phase firstly, then the G_2/M cells declined and apoptotic cells increased (Figure 5). But, when the drug concentration was increased to 40 μmol/l, cells treated with weisiensin B underwent apoptosis in a time-dependent manner (Figure 6). Interestingly, the apoptosis rates are close when the cells were treated with 40 μmol/l for 24 h and 20 μmol/l for 72 h. Cell cycle kinetic studies revealed that the blockade of G_2/M progression is a requisite step for the subsequent apoptosis [22,23], and apoptosis is an end point of stalled cell cycle progression in most cases [24]. Here, the FCM assay indicated that there is a marked G_2/M arrest, firstly when the cells were treated with weisiensin B, then apoptosis follows, and apoptosis can be boosted when the cells were treated with high drug concentration. Our speculation is that weisiensin B induces apoptosis through G_2/M arrest in BEL-7402 cells, and the drug-treatment concentration is a hinge between G_2/M arrest and apoptosis.

2.6 Conclusion

Together, it was demonstrated that weisiensin B inhibited the proliferation of BEL-7402 cells *in vitro* in dose- and time-dependent manners. Based on the morphological changes, DNA fragmentation, and cell cycle distribution assay, it was indicated that a significant fraction of weisiensin B-treated cells died by an apoptotic pathway. But it is not fully understood how weisiensin B causes BEL-7402 cells to undergo G_2/M arrest and apoptosis. Elucidating the molecular basis about the apoptotic sensitivity of tumor cell will be important for understanding the process of tumor progression and also for applying the basic knowledge of tumor to clinical and therapeutic welds [25]. Therefore, further study on apoptosis induced by weisiensin B is needed.

3. Experimental materials and methods

3.1 Materials

RPMI-1640 medium was obtained from Gibco Invitrogen Corporation (Carlsbad, CA, USA), and fetal bovine serum was obtained from Sijiqing Biotech Co. (Hangzhou, China). Hoechst 33258, SRB, propidium iodide (PI), and RNase A were obtained from Sigma Chemical Co. (St Clara, CA, USA). Proteinase K and agarose gel were purchased from BBI (Markham, Canada), and DNA marker from Promega Corporation (Madison, WI, USA). Weisiensin B was isolated from the leaves and twigs of *R. weisiensis* C.Y. Wu. and its structure was established on the basis of modern spectroscopic methods.

3.2 Cell cultures

BEL-7402, HO-8910, SGC-7901, and $HepG_2$ cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (v/v), penicillin (100 IU/ml), and streptomycin (100 μg/ml). Cultures were maintained in a humidified incubator at 37°C with 5% CO_2/95% air.

3.3 SRB assay

A SRB assay was performed as described previously [26]. In brief, cells (2×10^4 cells/ml) were inoculated into 96-well plates. After 24 h, a plate of cells was fixed with 50 μl of 10% trichloroacetic acid (TCA) solution per well at 4°C for 1 h, and it represented a measurement at the time of drug addition. After incubation with the drug for another 48 h, cells

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were directly fixed and the plates were washed five times with distilled water and air-dried. One hundred microliters of SRB solution (0.4% in 1% acetic acid) was added to each well of the 96-well microplates. Staining was done at room temperature for 10 min. Residual dye was washed out with 1% acetic acid and air-dried. To each well, 100 μl of Tris solution (10 mmol/l, pH 10.5) was added, and the absorbance was read at a wavelength of 520 nm. Using the following absorbance measurements, such as time zero ($T0$), control growth (C), and cell growth in the presence of compounds (Tx), the percentage growth was calculated at each of the compound concentration levels. Percentage growth inhibition was calculated as $((Tx - T0)/(C - T0)) \times 100\%$ ($Tx \geq T0$). Each drug concentration was tested in triplicate.

3.4 Cell viability

The cells were dispersed at a final concentration of 1×10^5 cells/ml and inoculated in six-well plates. After 24 h, metabolite was replaced with fresh medium and various concentrations of weisiensin B were added to each well in triplicate. After incubation for the indicated times, the viable cells were counted by the trypan blue exclusion method.

3.5 Detection of apoptosis

Cells (1×10^5 cell/ml) were exposed to weisiensin B for 24 h, collected by centrifugation at 800 r/min for 5 min, and washed with cold phosphate buffer solution (PBS) three times. The pellets were suspended in fixing solution (4% paraformaldehyde) at 4°C for 30 min, and washed again with cold PBS three times. The sediment was stained with Hoechst 33258 (5 μg/ml) for 10 min. Nuclear morphology of the cells was observed under fluorescence microscope (Olympus, Japan).

3.6 DNA fragmentation assay

The untreated and treated cells, approximately 5×10^6, were harvested and washed with PBS twice. Then, the sediment was lysed in 100 ml buffer (10 mmol/l Tris–HCl (pH 7.4), 10 mmol/l EDTA (pH 8.0), and 0.5% Triton X-100) at 50°C for 2 h. The supernatant was acquired through centrifugation at 8000 r/min for 10 min, and then incubated with 5 μl RNase A (20 mg/ml) at 37°C for 60 min. Proteins were removed by incubation with 5 μl proteinase K (20 mg/ml) at 37°C for 60 min. The supernatant with DNA fragment was centrifuged again at 8000 r/min for 10 min, and added with 20 μl 0.5 mol/l NaCl and 250 μl isopropanol deposited at −20°C overnight. After centrifugation at 12,000 r/min for 15 min, the sediment was dissolved in TE (10 mmol/l Tris–HCl (pH 7.4), 1 mmol/l EDTA (pH 8.0)), electrophoresed in a 1.5% agarose gel with 100 V for 1.5 h, stained with ethidium bromide (EB), and photographed under ultraviolet illumination.

3.7 FCM analysis of cell cycle and apoptosis

Cells (1×10^5 cells/ml) were inoculated and cultured for 24 h, and then exposed to weisiensin B. After indicated time, the untreated and treated cells were harvested and washed in cold PBS twice. Then, the cells were fixed by 75% ethanol at 4°C overnight. When they were measured, ethanol was washed off with PBS. After the cell densities were adjusted, PI (50 μg/ml) solution was added to them. At room temperature, they were stained in the absence of light for 30 min. Having been washed with PBS, they were measured by means of a flow cytometer with Cellquest software (version 2) to obtain the information about apoptosis and cell cycle.

3.8 Statistical analysis

The results were expressed as mean ± SD. The differences between the control and the treatment were evaluated using Student's t-test. A P-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

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References

[1] D. Yim, R.P. Singh, C. Agarwal, S. Lee, H. Chi, and R. Agarwal, *Cancer Res.* **65**, 1035 (2005).
[2] H.D. Sun, Y.L. Xu, and B. Jiang, *Diterpenoids from* Isodon *Species* (Science Press, Beijing, 2001), pp. 4–13.
[3] J.J. Liu, R.W. Huang, X.L. Pan, and J. Peng, *Chin. Tradit. Patent Med.* **26**, 1027 (2004).
[4] J.F. Zhang, J.J. Liu, P.Q. Liu, D.G. Lin, X.D. Li, and G.H. Chen, *Hepatol. Res.* **35**, 104 (2006).
[5] J.W. Liu, F. Yang, Y. Zhang, and J.Y. Li, *Int. Immunopharmacol.* **7**, 945 (2007).
[6] Q. Cui, S. Tashiro, S. Onidera, M. Minami, and T. Ikejima, *Biol. Pharm. Bull.* **30**, 859 (2007).
[7] L. Ding, Z.J. Zhang, G.A. Liu, D.J. Yang, G.C. Guo, and H. Wang, *Helv. Chim. Acta.* **88**, 2502 (2005).
[8] A. Itharat, P.J. Houghton, E.E. Amooquaye, P.J. Burke, J.H. Sampson, and A. Raman, *J. Ethnopharmacol.* **90**, 33 (2004).
[9] K. Shailaja and T. Ben, *Mol. Cancer Ther.* **2**, 573 (2003).
[10] R. Johnstone, A. Ruefli, and S. Lowe, *Cell* **108**, 153 (2002).
[11] J. Bartek, C. Lukas, and J. Lukas, *Nat. Rev. Mol. Cell Boil.* **5**, 792 (2004).
[12] T.V. Rakitina, I.A. Vasilevskaya, and P.J. O'Dwyer, *Biochem. Pharmacol.* **73**, 1715 (2007).
[13] W. Yin, X.K. Deng, F.Z. Yin, X.C. Zhang, and B.C. Cai, *Food Chem. Toxicol.* **45**, 1700 (2007).
[14] D. Chu, J.X. Qiu, M. Grafe, R. Fabian, T.A. Kent, D. Rassin, O. Nesic, K. Werrbach-Perez, and R. Perez-Polo, *Neurochem. Res.* **27**, 97 (2002).
[15] X.L. Li, J. Wang, and L.M. James, *Genes Dev.* **19**, 2705 (2005).
[16] Q. Chen, W.L. Peng, and A.L. Xu, *Biochem. Pharmacol.* **63**, 1389 (2002).
[17] R.P. Singh, S. Dhanalakshmi, and R. Agarwal, *Cell Cycle* **1**, 156 (2002).
[18] J. Kluza, A. Lansiaux, N. Wattez, C. Mahieu, N. Osheroff, and C. Bailly, *Cancer Res.* **60**, 4077 (2000).
[19] S. Chen, J. Gao, H.D. Halicka, X. Huang, F. Traganos, and Z. Darzynkiewicz, *Int. J. Oncol.* **26**, 579 (2005).
[20] K.K. Ren, H.Z. Wang, L.P. Xie, D. Chen, X. Liu, J. Sun, Y.C. Nie, and R.Q. Zhan, *J. Ethnopharmacol.* **103**, 176 (2006).
[21] T. Hsieh, E.K. Wijeratne, J.Y. Liang, A.L. Gunatilaka, and J.M. Wu, *Biochem. Biophys. Res. Commun.* **337**, 224 (2005).
[22] A. Skladanowski, *Apoptosis* **7**, 347 (2002).
[23] M.G. Liu, P.Y. Yue, Z. Wang, and R.N. Wong, *Cancer Lett.* **224**, 229 (2005).
[24] A.E. Santiago-Walker, A.J. Fikaris, G.D. Kao, E.J. Brown, M.G. Kazanietz, and J.L. Meinkoth, *J. Biol. Chem.* **280**, 32107 (2005).
[25] S.K. Bae, J.H. Back, Y.M. Lee, and K.W. Kim, *Cancer Lett.* **126**, 97 (1998).
[26] D.T.P. Trafalis, *Cancer Lett.* **243**, 202 (2006).

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