Int J Clin Exp Med 2016;9(6):11084-11089
www.ijcem.com /ISSN:1940-5901/IJCEM0020592

Original Article

Treatment of human cervical cancer cells with butein leads to apoptosis and DNA damage

Xiu-Qin Tong, Wei Lv, Wei-Qi Luo, Wei-Xi Lu, Na Li

Department of Gynecology, Baotou Central Hospital, Baotou 014040, Inner Mongolia, P. R. China

Received September 7, 2015; Accepted March 25, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: The present study demonstrates the effect of butein on the cell viability, induction of apoptosis and DNA damage in the cervical carcinoma cell lines. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for the analysis of cell viability, DNA ladder assays and flow cytometry for apoptosis induction and alkaline comet assay for determination of DNA damage. The results revealed a marked decrease in the viability of MCF-7 and HeLacell growth at a concentration of 20 μM of butein after 36 h. The viability of MCF-7, HeLa and ME-180 cells was reduced to 18, 23 and 19%, respectively after 36 h. It increased the percentage of apoptotic cells in MCF-7 and HeLa cells to 74 and 89%, respectively at the concentration of 20 μM after 36 h compared to 2 and 3% in untreated cells. Butein treatment caused damage to the DNA to a markedly higher level compared to the untreated cells. Therefore, butein exhibits a significant inhibitory effect in the MCF-7 and HeLa cervical cancer cells.

Keywords: DNA damage, apoptosis, inhibition, viability, comet

Introduction

Cervical cancer is the third most commonly detected cancer in females globally and has been shown to originate from pre-malignant precursor lesions [1]. Every year more than 510,000 new cases and 288,000 deaths are reported due to cervical cancer [2]. In cervical cancer the rate of human papilloma viruses (HPV) prevalence is estimated to be 99.7% [3]. Around 100 types of HPV have been identified in humans and among them 40% enter the anogenital tract [3-5]. During its early stage, cervical cancer shows no clear symptoms due to which regular pap tests are performed for its detection [6, 7]. Detection of cervical cancer at the early stage makes treatment possible compared to the late stage detection [8]. The treatment strategies used for the cervical cancer include radiation therapy and chemotherapy [9, 10]. It has been reported that standard therapies in combination with the herbal medicines yield more efficient results [11].

Chalcones, present abundantly in plants are the open chain analogs of flavonoids with an α, β-unsaturated carbonyl three carbon chain joining two aromatic rings [12]. Chalcones possess various biological activities which makes them important target for synthesis [13-15]. The major activities shown by chalcones are antitumor [16, 17], anti-inflammatory [18-20], antioxidant [21], antimicrobial, anti-tubercular [22], anti-HIV [23], antimalarial [24] and anti-allergic activities [25]. Various efforts have been made from time to time to evaluate the biological activities of chalcones [26]. Chalcones are reported to induce apoptosis, inhibit polymerization of tubulin, disrupt cell cycle and inhibit nuclear factor-kappa B (NF-kB) signaling pathway [12, 16, 27, 28]. In the present study role of a chalcone, butein (**Figure 1**) as a potential cytotoxic drug for the cervical carcinoma treatment was studied.

Butein (3,4, 2',4'-tetrahydroxychalone) is a polyphenolic compound isolated from the stem extract of *Rhusverniciflua* and has been shown to inhibit colon adenocarcinoma cell proliferation [29]. It also leads to the development of apoptosis in HL-60 cells through its effect on caspase-3 activity, reduction of Bcl-2 expression and enhancement of Bax expression.

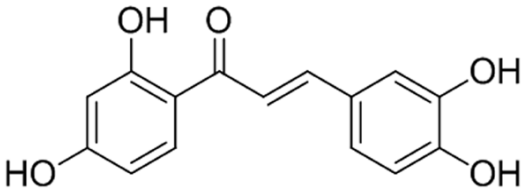

Figure 1. Structure of butein.

Materials and methods

Cell line

MCF-7, HeLa and ME-180 cervical cancer cell lines were purchased from Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All the cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics at 37°C in a 5% CO_2 incubator.

Chemicals and reagents

Butein was provided by Prof. WM Whu. Dimethyl sulfoxide, Trypan blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), N-methyl-N-nitro-N-nitrosoguanidine (MNNG, inducing DNA damage) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Propidium iodide (PI) and DMEM were obtained from Gibco Life Technologies (Carlsbad, CA, USA). The Apoptosis Detection kit was obtained from (BD Biosciences, San Jose, USA). Triton X-100 and ethidium bromide were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cell viability assay

The MCF-7 and HeLacervical cancer cell lines were distributed at a density of 2.5×10^6 cells per well onto 96-well plates (BD Falcon, Franklin, NJ). The cells were left for adherence for a period of 12 h and then the viability of cells was analyzed after 12 and 36 h using an MTT assay. The Automated Microplate Reader (MPR-A4i; Tosoh Corporation, Tokyo, Japan) was used to read the plates at the wavelength of 565 nm.

Extraction of DNA and analysis of DNA fragmentation

The MCF-7 and HeLa cervical cancer cells were treated with various concentrations of butein for 12 or 36 h. The cells treated with DMSO were used as a positive control. Following treatment the cells were harvested and then washed twice with phosphate-buffered saline (PBS). Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA) was employed for the extraction of DNA from the cells. The cells were stained with silver nitrate solution. The electrophoresis on a denaturing urea polyacrylamide gel was used for the analysis of DNA fragmentation.

Apoptosis analysis

For apoptosis, 2.5×10^5 cells were harvested, washed with PBS, incubated with Annexin V-FITC and propidium iodide followed by flow cytometry. For both FL1 (FITC) and FL3 (propidium iodide) channels logarithmic amplification of data was performed. The quantitative analysis of hypodiploid sub-G1 cells was performed using flow cytometry.

Alkaline comet assay

The MCF-7 and HeLacells after treatment with butein for 36 h were collected, washed and suspended in PBS (pH 7.4). 2.5×10^5 cells were then dispersed in molten low-melting-point agarose (1%) at 37°C. The cell suspension was then put onto the microscopic slides covered with a layer of 0.8% regular-melting-point agarose. Olympus BX53 fluorescent microscope (Olympus Corporation, Tokyo, Japan) with a filter of wavelength 515-560 nm was used to capture the images. The image analysis system (CASPLab) was used to measure the level of DNA migration.

Statistical analysis

All the data are expressed as means of ± SD. SPSS software, version 15.0 (SPSS, Inc., Chicago, IL, USA) was used for the analysis of the data. The differences were taken to be significant statistically at $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Inhibition of cervical cancer cell growth by butein

The effect of butein on the growth of cervical cancer cells was examined by analyzing the cell viability after exposing the cells to a range of butein doses. Butein exhibited inhibitory effect

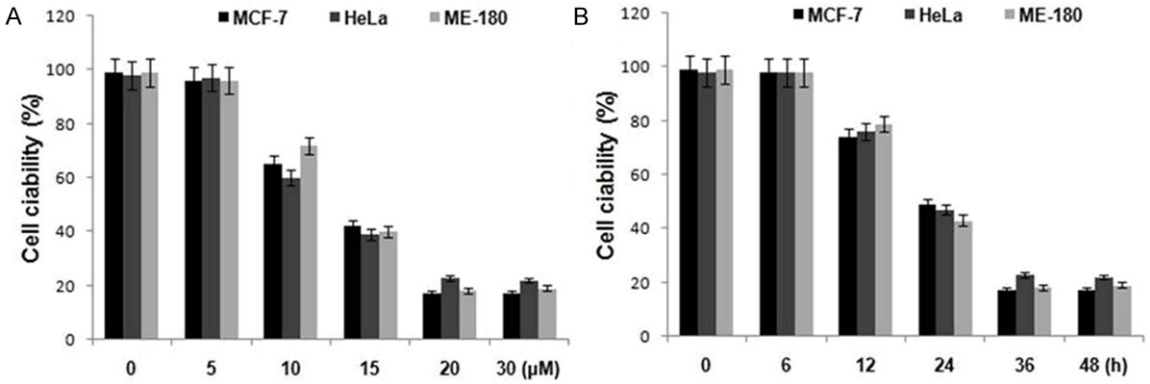

Figure 2. Butein treatment causes inhibition of the growth of MCF-7, HeLa and ME-180 cervical cancer cells.

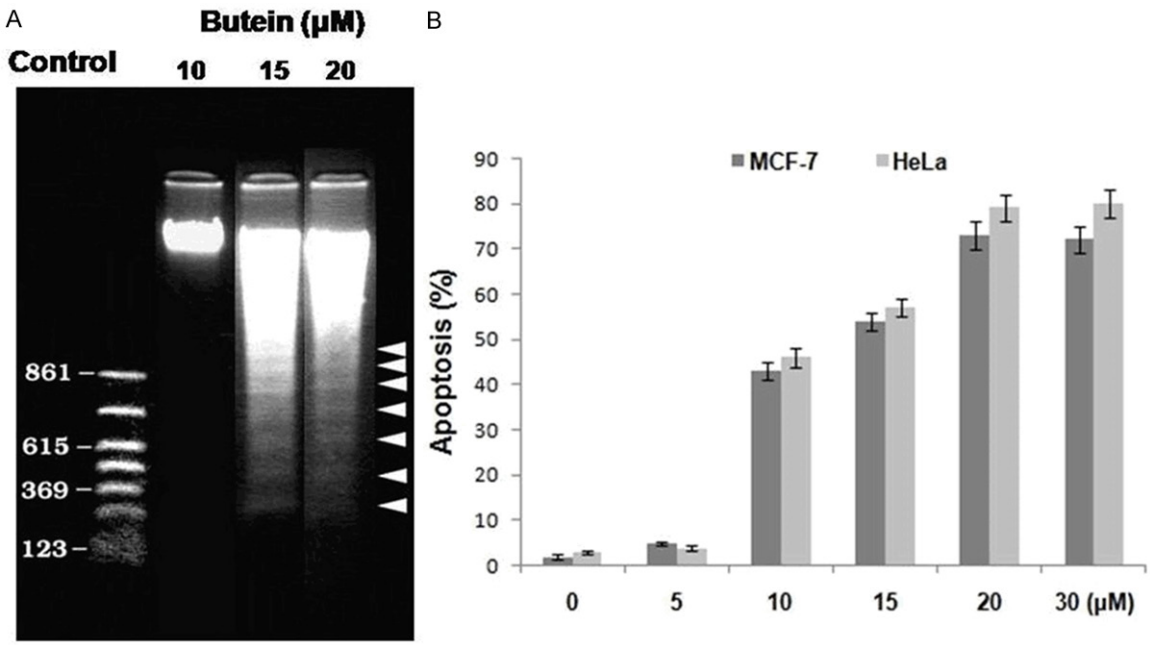

Figure 3. butein induces apoptosis in MCF-7 and HeLa cervical cancer cells. A. Apoptosis-associated DNA fragmentation in MCF-7 cells. B. Apoptotic rates of MCF-7 and HeLa cells treated with butein (20 μM) for 36 h.

on the growth of cervical carcinoma cells in dose and time dependent manner. The viability of MCF-7, HeLa and ME-180 cells was reduced to 18, 23 and 19%, respectively after 36 h of exposure to butein (**Figure 2A**). Treatment of the tested cell lines with butein for 12, 24, 36 and 48 revealed that the inhibition of cell growth was significant after 36 h (**Figure 2B**).

Induction of apoptosis in cervical carcinoma cells by butein

Treatment of MCF-7 and HeLa cervical cancer cells with butein led to the formation of ladder-like pattern of DNA on the urea polyacrylamide gel electrophoresis (PAGE) whereas no such changes were observed in the untreated cells by DNA fragmentation analysis and flow cytometry (**Figure 3A**). Exposure of MCF-7 and HeLa cells to butein at the concentration of 20 μM led to a marked increase in the percentage of apoptotic cells (**Figure 3B**). Thus butein inhibits the growth of cervical cancer cells through the process of apoptosis.

DNA damage in MCF-7 and HeLa cervical cancer cells by butein

The comet assay was used to investigate the effect of butein on the structure of DNA in

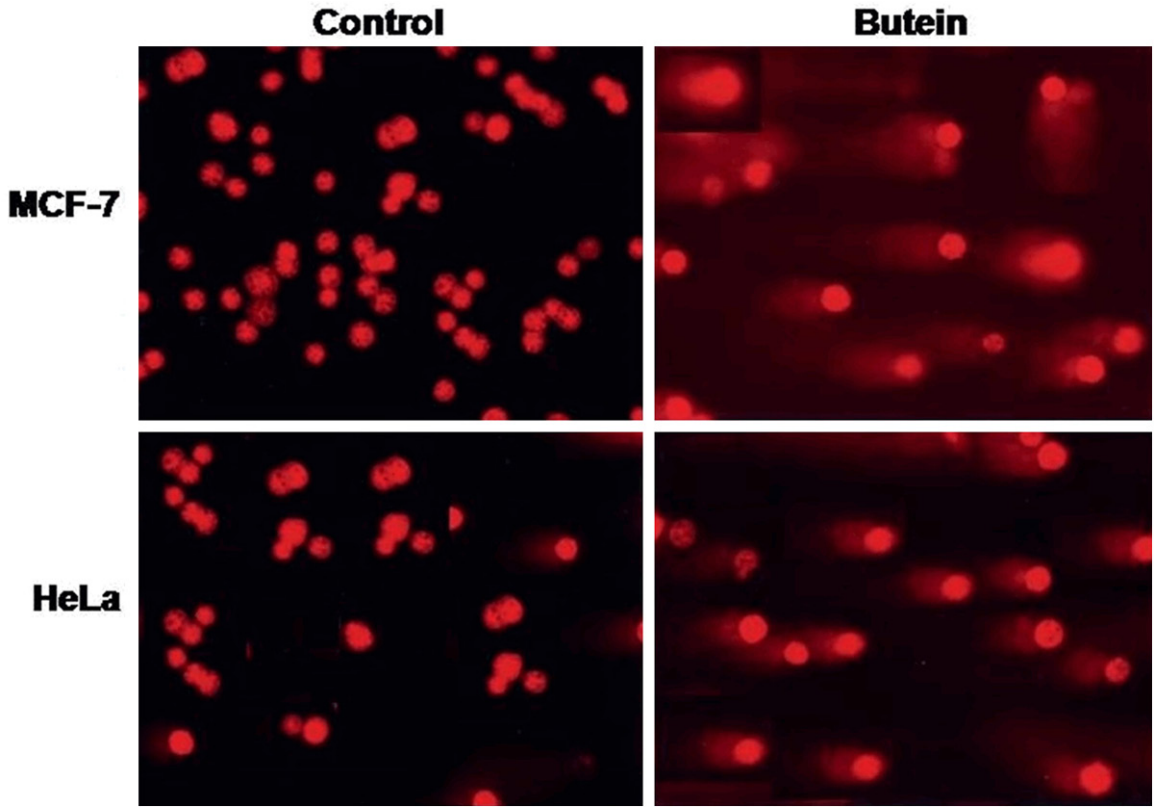

Figure 4. Comet images (magnification, × 200) of alkaline gel electrophoresis demonstrating DNA damage in the MCF-7 and HeLa cells.

MCF-7 and HeLa cervical cancer cells. Exposure of the cells to butein led to transfer of DNA segments and the formation of comet shaped structures. However, the untreated cells showed high-density DNA and normal nuclei. In MCF-7 and HeLa cells a marked increase was observed in comet-positive cells at 20 μM concentration of butein (**Figure 4**).

These results indicated that the cells, which were exposed to different concentrations of butein, exhibited significantly higher DNA damage ($P < 0.01$) compared with the control samples. In these cell lines, butein significantly increased the tail length ($P < 0.01$) and tail moment ($P < 0.01$) when used at concentrations of 1.25 to 5 μM.

Discussion

The discovery of molecules with roles in the treatment of cervical cancer from the natural isolates is an efficient strategy [30]. Chalcones have been reported to possess various biological activities including antitumor [16, 17], anti-inflammatory [18-20], antioxidant [21], antimicrobial, anti-tubercular [22], anti-HIV [23], anti-malarial [24] and anti-allergic activities [25]. The factors responsible for the death of cells include apoptosis, autophagy, paraptosis, mitotic catastrophe and necrosis [31, 32]. These findings suggest that the induction of tumor cell apoptosis by butein may be one of the mechanisms through which these compounds exert their antitumor effects.

Apoptosis is the programmed death of cells and is induced by the process of condensation of chromatin material and fragmentation of DNA [32]. The process of apoptosis has a vital role in the cell development. The treatment strategies like radiation therapy and chemotherapy induce the fragmentation of DNA in various types of cancers. The present study reveals that butein treatment causes breakage of DNA in cervical cancer cells. The results from the alkaline comet assays showed that exposure of cervical cancer cells to butein caused damage to the cell DNA. Our results revealed that butein treatment exhibits inhibitory effect

on the growth of cervical cells and induces apoptotic cell death.

In conclusion, the present study demonstrated that butein was anticarcinogenic, inducing cell DNA damage and apoptosis *in vitro,* and the DNA damage induced by butein may be associated with apoptosis. The results of the present study confirmed the antitumor effects of Butein and the potential of Butein as an agent of chemotherapeutic activity in human cervical cancer cells.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiu-Qin Tong, Department of Gynecology, Baotou Central Hospital, No. 61, Huancheng Road, Donghe District, Baotou 014040, Inner Mongolia, P. R. China. Tel: 0086-472-6955324; Fax: 0086-472-6955324; E-mail: tongxiuqin@hotmail.com

References

[1] Globocan 2008 (IARC), Section of Cancer Information. International Agency for Research on Cancer; 2008.

[2] Saslow D, Castle PE, Cox JT. American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin 2007; 57: 7-28.

[3] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 12-19.

[4] Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518-527.

[5] Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006; 24: S3/1-10.

[6] Steben M, Duarte-Franco E. Human papillomavirus infection: epidemiology and pathophysiology. Gynecol Oncol 2007; 107: S2-S5.

[7] Long HJ 3rd. Management of metastatic cervical cancer: review of the literature. J Clin Oncol 2007; 25: 2966-2974.

[8] Shin YW, Bae EA, Kim DH. Inhibitory effect of ginsenoside Rg5 and its metabolite ginsenoside Rh3 in an oxazolone induced mouse chronic dermatitis model. Arch Pharm Res 2006; 29: 685-90.

[9] Leitao MM Jr, Chi DS. Recurrent cervical cancer. Curr Treat Options Oncol 2002; 3: 105-111.

[10] Yang J, Li J, Sun M, Chen K. Studies of traditional Chinese medicine monomer on HeLa cell of cervical cancer. Pak J Pharm Sci 2014; 27: 1063-1068.

[11] Cohen I, Tagliaferri M, Tripathy D. Traditional Chinese medicine in the treatment of breast cancer. Semin Oncol 2002; 29: 563-574.

[12] Orlikova B, Tasdemir D, Golais F, Dicato M, Diederich M. Dietary chalcones with chemopreventive and chemotherapeutic potential. Genes Nutr 2011; 6: 125-47.

[13] Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. Curr Clin Pharmacol 2010; 5: 1-29.

[14] Sahu NK, Balbhadra SS, Choudhary J, Kohli DV. Exploring pharmacological significance of chalcone scaffold: A review. Curr Med Chem 2012; 19: 209-25.

[15] Iwamura C, Shinoda K, Yoshimura M, Watanabe Y, Obata A, Nakayama T. Naringenin chalcone suppresses allergic asthma by inhibiting the type-2 function of CD4 T cells. Allergol Int 2010; 59: 67-73.

[16] Srinivasan B, Johnson TE, Lad R, Xing C. Structure-activity relationship studies of chalcone leading to 3-hydroxy-4,3,4,5-tetramethoxychalcone and its analogues as potent nuclear factor kB inhibitors and their anticancer activities. J Med Chem 2009; 52: 7228-35.

[17] Modzelewska A, Pettit C, Achanta G, Davidson NE, Huang P, Khan SR. Anticancer activities of novel chalcone and *bis*-chalcone derivatives. Bioorg Med Chem 2006; 14: 3491-5.

[18] Maria K, Dimitra HL, Maria G. Synthesis and anti-inflammatory activity of chalcones and related Mannich bases. Med Chem 2008; 4: 586-96.

[19] Wu J, Li J, Cai Y, Pan Y, Ye F, Zhang Y, Zhao Y, Yang S, Li X, Liang G. Evaluation and discovery of novel synthetic chalcone derivatives as anti-inflammatory agents. J Med Chem 2011; 54: 8110-23.

[20] Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 2007; 42: 125-37.

[21] Miranda CL, Stevens JF, Ivanov V, McCall M, Frei B, Deinzer ML, Buhler DR. Antioxidant and prooxidant actions of prenylated and nonprenylatedchalcones and flavanones*in vitro*. J Agric Food Chem 2000; 48: 3876-84.

[22] Chiaradia LD, Mascarello A, Purificação M, Vernal J, Cordeiro MNS, Zenteno ME, Villarino A, Nunes RJ, Yunes RA, Terenzi H. Synthetic chalcones as efficient inhibitors of Mycobacterium

tuberculosis protein tyrosine phosphatase PtpA. Bioorg Med Chem Lett 2008; 18: 6227-30.
[23] Wu JH, Wang XH, Yi YH, Lee KH. Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus Desmos. Bioorg Med Chem Lett 2003; 13: 1813-5.
[24] Domınguez JN, Charris JE, Lobo G, de Domınguez GN, Moreno MM, Riggione F, Sanchez E, Olson J, Rosenthal PJ. Synthesis of quinolinylchalcones and evaluation of their antimalarial activity. Eur J Med Chem 2001; 36: 555-60.
[25] Dimmock JR, Elias DW, Beazely MA, Kandepu NM. Bioactivities of chalcones. Curr Med Chem 1999; 6: 1125-49.
[26] Go ML, Wu X, Liu XL. Chalcones: An update on cytotoxic and chemoprotective properties. Curr Med Chem 2005; 12: 481-99.
[27] Ducki S. Antimitotic chalcones and related compounds as inhibitors of tubulin assembly. Anticancer Agents Med Chem 2009; 9: 336-47.
[28] Zuo Y, Yu Y, Wang S, Shao W, Zhou B, Lin L, Luo Z, Huang R, Du J, Bu X. Synthesis and cytotoxicity evaluation of biaryl-based chalcones and their potential in TNFk-induced nuclear factor-kB activation inhibition. Eur J Med Chem 2012; 50: 393-404.
[29] Yit CC, Das NP. Cytotoxic effect of butein on human colon adenocarcinoma cell proliferation. Cancer Lett 1994; 82: 65-72.
[30] Kim NY, Pae HO, Oh GS, Kang TH, Kim YC, Rhew HY, Chung HT. Butein, a plant polyphenol, induces apoptosis concomitant with increasedcaspase-3 activity, decreased Bcl-2 expression and increased Bax expression in HL-60 cells. Pharmacol Toxicol 2001; 88: 261-6.
[31] Toh DF, Patel DN, Chan EC, Teo A, Neo SY, Koh HL. Anti-proliferative effects of raw and steamed extracts of *Panaxnotoginseng*and its ginsenoside constituents on human liver cancer cells. Chin Med 2011; 6: 4.
[32] Robertson JD, Orrenius S. Role of mitochondria in toxic cell death. Toxicology 2002; 181-182: 491-496.