# Inhibition of *Corydalis decumbens* Alkaloids on Hydrogen Peroxide-induced Apoptosis of PC12 Cells through Down-regulating Caspase-3 Expression

YAN Ren-jie[1, 2], YANG Yi-fang[1], LUO Yong-ming[2], WU Chun-zhen[1*]

1. Department of Traditional Chinese Medicine, Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Shanghai 200040, China
2. Department of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, China

**Abstract:** **Objective** To extract alkaloids from *Corydalis decumbens* (AsCD) by supercritical $CO_2$ fluid extraction (SFE) and to evaluate protective effects of AsCD against hydrogen peroxide ($H_2O_2$)-induced apoptosis in rat PC12 cells. **Methods** AsCD were extracted by SFE and oxidative damage PC12 cells model was induced by $H_2O_2$. The survival rate of the cells was determined by MTT assay; Lactate dehydrogenase release was determined by ultraviolet spectrophotometry; Flow cytometry was used to detect apoptosis; Caspase-3 mRNA and protein were determined by real-time PCR and Western blotting assay, respectively. **Results** AsCD remarkably reduced the cytotoxicity, prevented membrane damage, and inhibited cell apoptosis. AsCD inhibited Caspase-3 mRNA and protein expression induced by $H_2O_2$ in PC12 cells. **Conclusion** AsCD possess protective effects against $H_2O_2$-induced apoptosis in PC12 cells, and the mechanism of AsCD responsible to the inhibition of apoptosis is possibly attributed to the down-regulating Caspase-3 expression. AsCD might be useful in the treatment of oxidative stress-related neurodegenerative diseases.

**Key words**: alkaloids; apoptosis; Caspase-3; *Corydalis decumbens*; hydrogen peroxide; PC12 cells
**DOI**: 10.3969/j.issn.1674-6384.2011.04.009

## Introduction

Neuronal cell death, which happened in most neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and cerebral ischemia/reperfusion (IR), was approved to be relevant to oxidative stress directly or indirectly (Barnham, Masters, and Bush, 2004; Rao and Balachandran, 2002; Cadet and Brannock, 1998; Kannan and Jain, 2000) because of unregulated products of reactive oxygen species (ROS), such as hydrogen peroxide ($H_2O_2$), nitric oxide (NO), superoxide, and the highly reactive hydroxyl radicals. ROS could damage cells through lipid peroxidation, protein cross-linking, and DNA fragmentation (Wang, Schuschke, and Kang, 1999). $H_2O_2$ is an important member of the ROS family produced by living tissues (Gao, Huang, and Xu, 2001), participates in the pathogenesis of many diseases such as autoimmune disease, cancer, inflammation, and apoptosis, and is usually used as an inducer of the oxidative damage (Halliwell, 1992). Recently studies have shown that $H_2O_2$ induces cytotoxicity to PC12 cells via damaging membrane and nuclear, decreasing mitochondrial membrane potential (MMP) and anti-oxidant enzyme activities, such as superoxide dismutase (SOD), catalase (CAT), and GsPx, and increasing Caspase-3 activation (Hwang and Yen, 2010; Xiao *et al*, 2008).

The rhizomes of *Corydalis decumbens* (Thunb.) Pers. have been used traditionally for the treatment of

* Corresponding author: Wu CZ Address: Department of Traditional Chinese Medicine, Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, 1320 West Beijing Road, Shanghai 200040, China
Tel/Fax: +86-21-6247 3018 E-mail: czw1962@126.com
Received: June 21, 2011; Revised: August 30, 2011; Accepted: September 8, 2011
Fund: Shanghai Natural Science Fund Project (09ZR1431100 ); National "Major Projects for New Drug Development" Science and Technology Project "the 11th-five-year Plan" (2009ZX09301-07); Shanghai Science and Technology Commission "Great Development in Western Regions of China" Project (10495801000); and 2010 Shanghai Municipal Modernization of Traditional Chinese Medicine Project (10DZ1971300)

paralytic stroke. The main components with pharmacological and biological activities are believed to be alkaloids. These alkaloids proved to possess a variety of pharmacological properties, such as preventing the focal cerebral injury in rats (Hu, Sun, and Yu, 2005), enhancing learning and memory ability by increasing brain acetylcholinesterase activity in mice (Sheng, Gu, and Jiang, 2003; Deng, Gu, and Xie, 2003), increasing the content of 5-HT and Dopamine in AD model rats (Zhang, Gu, and Jiang, 2004), decreasing AchE activity, protecting neurons, and anti-apoptosis from injury of cerebral ischemia (Yu *et al*, 2006). However, the protective effects of alkaloids against oxidative stress-induced damage remain unknown, and the mechanism has not been fully investigated yet.

The present study was designed to extract alkaloids from *C. decumbens* (AsCD) by supercritical $CO_2$ fluid extraction (SFE) and to evaluate the protective effects of AsCD against $H_2O_2$-induced apoptosis in rat PC12 cell line by MTT assay, lactate dehydrogenase (LDH) release assay, flow cytometry analysis, and Caspase-3 mRNA and protein expression assay .

## Materials and methods

### Materials

Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco BRL (Gaithersburg, MD, USA). Calf serum was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. (Hangzhou, China). MTT was from Biosharp (USA). DMSO was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Trizol was from Invitrogen Co., Ltd. (Paisley, UK). Revert Aid™ First Strand cDNA Synthesis Kit was from MBI Fermentas (Vilnius, Lithuania). SYBR Green Supermix was from Bio-Rad Laboratories Inc. (Tokyo, Japan). Primer was purchased from Nanjing Genscript Biotechnology Co., Ltd. (Nanjing, China). LDH, Cytotoxicity Assay Kit, malondialdehyde (MDA) Elisa Kit, SOD Elisa Kit, Annexin V-FITC Propidium Iodide (PI) Apoptosis Detection Kit, ECL Western Blotting Detection System Kit, Whole Cell Extraction Kit, High Capacity cDNA Reverse Transcription Kit, and PC12 cell line were obtained from Nanjing KeyGen Biotech. Co., Ltd. (Nanjing, China).

### Extraction of AsCD by SFE

Rhizomes of *Corydalis decumbens* (Thunb.) Pers. were purchased from Jiangxi Tian Shi Kang Chinese Medicine Co., Ltd. Specimens were identified by Prof. GONG Qian-feng in Jiangxi University of Traditional Chinese Medicine (Nanchang, China). AsCD were extracted by SFE. Briefly, the dried tuber powder (200 g) was alkalized with 10% ammonia solution for 1 h at room temperature and then placed in an extraction caldron. The extraction conditions were as follows: extracting for 35 min at 70 ℃ and pressure 35 MPa in ethanol. The yield of AsCD extract was 1.6%. The main alkaloids were determined by HPLC. The contents of bicuculline, egenine, kikemanine, protopine, tetrahydropalmatine, and palmatine in AsCD extraction were 5.450%, 26.946%, 3.283%, 13.340%, 17.912%, and 0.322%, respectively.

### Cell culture and drug treatment

Rat PC12 pheochromocytoma cells were cultured in DEME with 10% heat-inactivated calf serum, 100 U/mL Penicillin, and 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere containing 5% $CO_2$. The medium was changed every 3 d and the cells were passaged weekly. AsCD were freshly prepared as a stock solution in DMSO and diluted with medium before the experiment, and the concentration of DMSO in the final culture medium was 0.05%, which had no protective or toxic effects. Control group was performed in the presence of 0.05% DMSO under the same culture conditions. $H_2O_2$ was freshly prepared in PBS from 3% stock solution before applied to the culture. In all experiments, PC12 cells were transferred to 96- or 6-well tissue culture plates ($10^4$ or $10^5$ cells/well) and incubated for 24 h, then the cultured cells were preincubated with the indicated concentrations of AsCD for 1 h and later the medium containing $H_2O_2$ (final concentration, 25 μmol/L) and AsCD (indicated concentrations) was added. The cells were continued to be incubated for another 24 h.

### MTT assay

Cell viability was assessed by MTT assay. PC12 cells were exposed to indicated concentrations of AsCD and $H_2O_2$ for designated time frames. Then, MTT solution (final concentration, 0.5 mg/mL) was added and further incubated for 4 h at 37 ℃, then the culture medium was removed and the formazan crystal was dissolved in 150 μL DMSO. Finally, absorbance was

measured on an automated microplate reader (Biorad, Hercules, CA, USA) at 490 nm.

**LDH release assay**

The amount of cytoplasmic LDH released into the medium was used as an index to measure cell membrane damage. Briefly, PC12 cells in 6-well plates were cultured and treated according to the procedures described above. The medium was collected and the cells were harvested by centrifugation and spalled in cell lysis buffer (50 mmol/L HEPES, 5 mmol/L EDTA, 100 mmol/L NaCl, 1% Triton X-100, protease inhibitors cocktails; pH 7.4). The amount of LDH release was determined by using an assay kit according to the manufacturer's protocol, and the absorbance of samples was read at 440 nm. The release amount of LDH was calculated according to the following equation: LDH released = LDH activity in the medium / (LDH activity in the medium + LDH activity in the cells) ×100%.

**Detection of apoptotic cells by flow cytometry**

Apoptosis was assayed by PI Apoptosis Detection Kit with fluorescence-activated cell sorting (FACS, Becton-Dickinson, USA). Briefly, the cultured cells were digested by addition of 0.25% pancreatin and harvested by centrifugation. Then, cells ($5 \times 10^5$ cells/mL) were washed twice with PBS (2000 × *g* for 5 min), then were resuspended in binding buffer, and stained with 5 μL annexin V-FITC and 5 μL PI for 15 min at room temperature in dark, then, the cells were detected by flow cytometry. The fraction of cell population in different quadrants was analyzed using quadrant statistics.

**Assay of Caspase-3 mRNA expression**

Total RNA was prepared from the cultured PC12 cells using Trizol reagent. After reverse transcription of RNA, SYBR Green PCR assays were performed in triplicate on samples of cDNA, real-time PCR reactions consisted of 1 μL of cDNA TE buffer solution, 7 μL ultrapure water, 2 μL primer, and 10 μL of iQ SYBR Green Supermix in a total volume of 20 μL, after inactivated at 95 ℃ for 5 min. Amplification was performed under following conditions: 95 ℃ × 15 s, 60 ℃ × 30 s, 72 ℃ × 30 s for 42 cycles, with the last elongation at 72 ℃ for 10 min. Fluorescence intensity of the amplification process was monitored using DA 7600 Single Color Real-time RT-PCR Detection System (Da An Gene Co., Ltd. of Sun Yat-sen University, China). The relative quantification of real time RT-PCR products was performed using the $2^{-\Delta\Delta CT}$ method. PCR primers for Caspase-3 were forward primer 5'-CTTCAGTGGTGGACATGACG-3', and reverse primer 5'-TCAACAATTTGAGGCTGCTG-3'. Primers for β-actin were forward primer 5'-CCCATCTATGAGGGTTACGC-3' and reverse primer 5'-TTTAATGTCACGCACGATTTC-3' (Chen *et al*, 2009).

**Assay of Caspase-3 protein expression**

Approximate $5 \times 10^5$ cells were harvested by centrifugation, and the cell pellets were resuspended in the lysis buffer (50 mmol/L HEPES, 5 mmol/L EDTA, 100 mmol/L NaCl, 1% Triton X-100, protease inhibitors cocktails, pH 7.4) and then the suspension was sonicated. Protein concentration of samples was determined by Bradford Assay (Shimadz Co., Ltd., Japan), which equilibrated to 2 mg/mL with lysis buffer. The protein extract (70 μg) with RIPA lysis buffer was separated on a 10% SDS-polyacylamide gel and transferred to polyvinylidene difluoride membranes (PVDF Pall Corporation, USA). Membranes were blocked with 5% non-fat dry milk for 1 h, subsequently, incubated with rabbit anti-Caspase-3 for 3 h. After being washed with PBST for four times, membranes were incubated with secondary antibody streptavidin-horseradish peroxidase conjugated affinity goat anti-rabbit IgG and exposed to X-rays. Protein bands were detected using an enhanced chemiluminescence Western blotting Detection Kit. The image was measured by image pro plus 6.0 software.

All the assays were carried out at least in three experiments.

**Statistical analysis**

The SPSS 17.0 software package was used for calculations. Data were expressed as $\bar{x} \pm s$. Differences were determined using one-way ANOVA and subsequently the least significant difference (LSD) post hoc test. $P < 0.05$ was considered significant.

## Results

**Protection of AsCD on $H_2O_2$-induced cytotoxicity in PC12 cells**

The cell viability was determined by MTT reduction assay, and the LDH activity was used as an

indicator of relative cell viability as a function of membrane integrity. As shown in Table 1, after the PC12 cells were exposed to 25 μmol/L $H_2O_2$ alone for 24 h, cell viability as MTT reduction was decreased to 46% of the control group and LDH release reflecting cytotoxicity was increased by about 6-fold over the control level, whereas, pretreatment of the cells with AsCD at 0.78, 3.13, and 12.5 μg/mL for 24 h, the cell viability was significantly increased up to 54%—78% of the control group and a markedly reduction in LDH release was observed. These results might suggest that AsCD were able to improve the cell viability and protect plasma membrane from damage.

**Inhibiton of AsCD on $H_2O_2$-induced apoptosis of PC12 cells**

As shown in Fig. 1, after exposure to 25 μmol/L $H_2O_2$ for 24 h, apoptotic rate of PC12 cells increased to 78.8% of the control level. However, co-incubation with AsCD at 0.78, 3.13, and 12.5 μg/mL for 24 h, significantly inhibited the cellular apoptosis by 31.5%—58.0%. These results indicated that AsCD could inhibite $H_2O_2$-induced apoptosis of PC12 cells.

**Table 1 Neuroprotection of AsCD on $H_2O_2$-induced cytotoxicity in PC12 cells ( $\bar{X}$ ± s, *n* = 3)**

| Groups | Dosage / (μg·mL$^{-1}$) | Cell viability / % | LDH release / % |
|---|---|---|---|
| control | | 100 ± 0.178 | 100 ± 0.442 |
| $H_2O_2$ | | 46.30 ± 0.689$^{\#\#}$ | 589.83 ± 4.626$^{\#\#}$ |
| AsCD + $H_2O_2$ | 0.78 | 54.08 ± 2.451$^{*}$ | 511.77 ± 1.647$^{*}$ |
| | 3.13 | 59.66 ± 3.694$^{**}$ | 341.67 ± 2.635$^{**}$ |
| | 12.5 | 77.92 ± 4.126$^{**}$ | 173.02 ± 6.241$^{**}$ |

Cells were pre-incubated with 0.78, 3.13, and 12.5 μg·mL$^{-1}$ AsCD for 1 h, and then were co-incubated with 25 μmol·L$^{-1}$ $H_2O_2$ for 24 h
$^{\#\#}P < 0.01$*vs* control group; $^{*}P < 0.05$ $^{**}P < 0.01$ *vs* $H_2O_2$ group

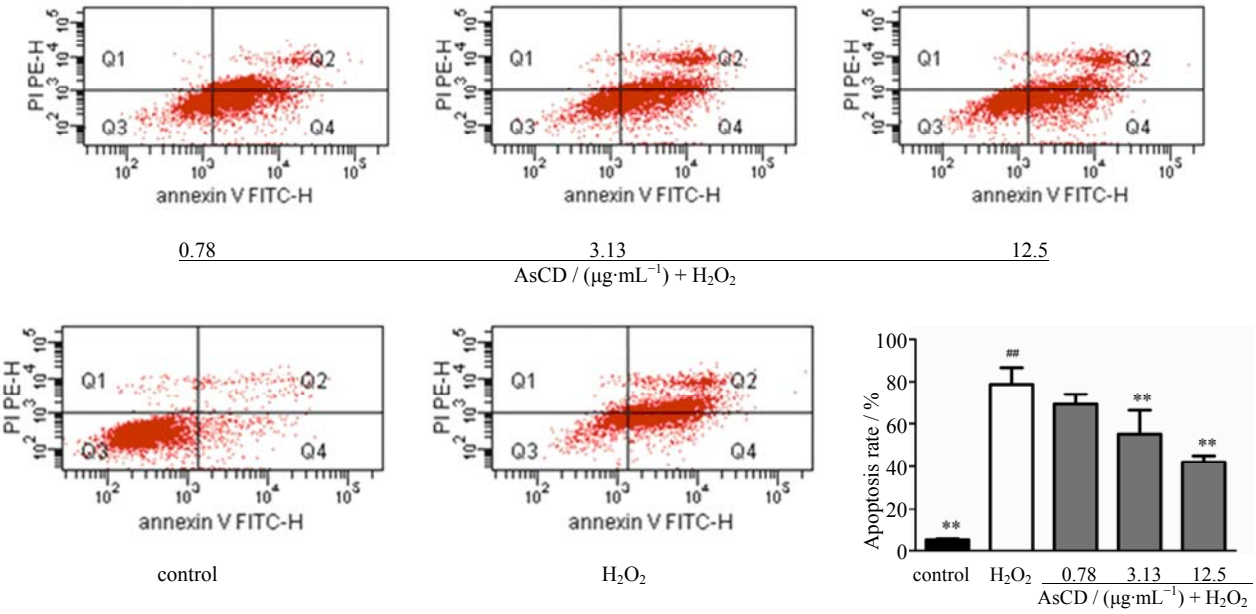


**Fig. 1 Effect of AsCD on $H_2O_2$-induced apoptosis of PC12 cells ( $\bar{X}$ ± s, *n* = 3)**

Cells were pre-incubated with 0.78, 3.13, and 12.5 μg·mL$^{-1}$ AsCD for 1 h, and then were co-incubated with 25 μmol·L$^{-1}$ $H_2O_2$ for 24 h. Cellular apoptosis was assayed by annexin V-FITC and PI staining and analyzed by flow cytometry with fluorescence-activated cell sorting
$^{\#\#}P < 0.01$*vs* control group; $^{**}P < 0.01$ *vs* $H_2O_2$ group

**Effect of AsCD on Caspase-3 mRNA expression in PC12 cells**

The results in Fig. 2 showed that $H_2O_2$ increased Caspase-3 mRNA relative expression in PC12 cells by 40 fold of control levels, whereas, co-incubation with AsCD at 0.78, 3.13, and 12.5 μg/mL could significantly inhibited the Caspase-3 mRNA expression. These results suggested that AsCD might down-regulate Caspase-3 expression induced by $H_2O_2$ in PC12 cells.

**Effect of AsCD on Caspase-3 protein expression in PC12 cells**

As shown in Fig. 3, an increase in Caspase-3 protein expression induced by $H_2O_2$ was observed to be 250% of the control level. However, co-incubation with AsCD at 12.5 μg/mL significantly decreased the Caspase-3 protein expression. It was demonstrated that AsCD might inhibit Caspase-3 protein expression induced by $H_2O_2$ in PC12 cells at high concentration.

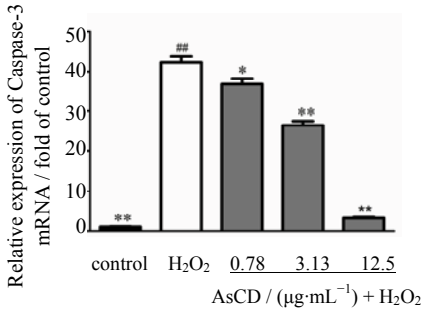


**Fig. 2 Effect of AsCD on Caspase-3 mRNA expression in PC12 cells ( $\bar{X}$ ± s, *n* = 3)**

Cells were pre-incubated with 0.78, 3.13, and 12.5 μg·mL$^{-1}$ AsCD for 1 h, and then were co-incubated with 25 μmol·L$^{-1}$ $H_2O_2$ for 24 h. Caspase-3 mRNA expression was detected by real time PCR assay. All data were normalized using the β-actin signal

$^{\#\#}P < 0.01$ *vs* control group; $^{*}P < 0.05$ $^{**}P < 0.01$ *vs* $H_2O_2$ group

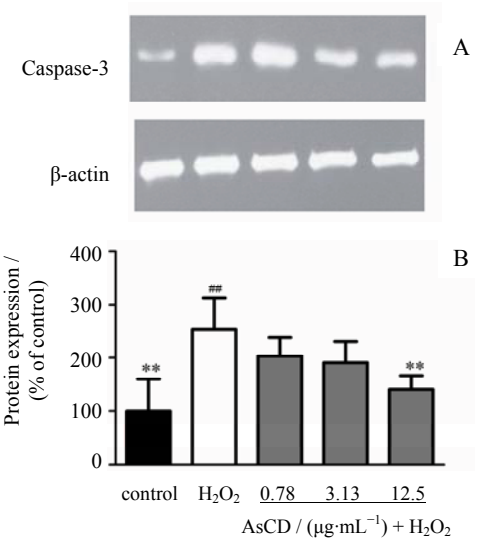


**Fig. 3 Effect of AsCD on Caspase-3 protein expression in PC12 cells ( $\bar{X}$ ± s, *n* = 3)**

Cells were pre-incubated with 0.78, 3.13, and 12.5 μg·mL$^{-1}$ AsCD for 1 h, and then were co-incubated with 25 μmol·L$^{-1}$ $H_2O_2$ for 24 h. Caspase-3 protein expression was detected by chemiluminescence Western blotting assay (A). The results were expressed as percentage of the control value from the normal cells (B). All data were normalized using the β-actin signal

$^{\#\#}P < 0.01$ *vs* control group; $^{**}P < 0.01$ *vs* $H_2O_2$ group

## Discussion

The neuroprotective effect of AsCD on cerebral damage *in vivo* has recently been demonstrated in our laboratory (unpublished data). Our results clearly indicated that pretreatment with AsCD could reduce cerebral infaction volume and improve neurological behavioral deficits in middle cerebral artery occlusion-ischemic/reperfusion (MCAO-I/R) rats. However, the exact mechanism of those actions was not well elucidated. To better understand the oxidative stress-relating mechanisms, we first explored whether AsCD possessed anti-apoptotic effects on $H_2O_2$-inducing damage in PC12 cells and elucidated the potential protective mechanisms.

It is believed that oxidative stress mediated by ROS is the common features in the pathological progress associated with neuronic damage. Although $H_2O_2$ (one of the ROS family) has a limited reactivity, it causes prolonged cell damage by increasing the release of arachidonic acid from the cell membrane (Cantoni *et al*, 1989). In addition, $H_2O_2$ promptly penetrates the cell membrane, and reacts with intracellular metal ions such as $Fe^{2+}$or $Cu^{+}$ to form highly toxic $OH^{-}$, causing DNA alteration (Gille and Joenje, 1992). Thus, $H_2O_2$ causes severe damage to the cultured cells even at lower concentrations (Wink *et al*, 1996). In our experiments, when PC12 cells were incubated with 25 μmol/L of $H_2O_2$ alone for 24 h, the cell viability decreased significantly (Fig .1).

In this study, the cytotoxicity was measured by MTT assay and LDH release assay. LDH is a stable cytoplasmic enzyme in all cells, and it would rapidly release into the cell culture if the plasma membrane was damaged by oxidant. Our data demonstrated that the AsCD could protect the cultured PC12 cells from $H_2O_2$-induced cell death and prevent membrane damage. Furthermore, we tested whether $H_2O_2$ induced cell death via apoptosis and surveyed the anti-apoptotic effect of AsCD.

Apoptosis could be triggered by numerous factors including receptor-mediated signals, withdrawal of growth factors, antitumor drugs, and DNA damage under certain conditions (Kannan and Jain, 2000). The Caspases are central component of this apoptotic program (Salvesen and Dixit, 1999). Excess oxidative stress kills cells either by necrosis or by apoptosis (Zamzami *et al*, 1995; 1996). In many models of apoptosis, alterations in the redox status of the cell to a more oxidizing environment occurs prior to activation of the final phase of Caspase activation (Green and Reed, 1988; Susin, Zamzami, and Kroemer, 1998; Morel and Barouki, 1999; Fernandez *et al*, 1995; Richter, 1993). This argument is supported by the ability of various anti-oxidants such as *N*-acetylcysteine (NAC) to block apoptosis in a similar way that Caspase inhibitors do (MeGowan *et al*, 1996). Caspase-3 is the

key apoptotic executive protein and has been proved to be an important regulator in neuronal apoptosis, which could be activated by both death-receptor pathway and mitochondrial pathway during the final step of apoptosis (Susanna and Clifford, 2004; David *et al*, 1998; Ronit *et al*, 1997). In this experiment, we found that $H_2O_2$ induced Caspase-3 mRNA and protein expression and PC12 cell apoptosis, whereas AsCD depressed the Caspase-3 mRNA and protein expression and subsequent apoptosis induced by $H_2O_2$, which confirmed that AsCD could block the later step of apoptosis. Thus, mechanism of AsCD responsible to inhibition of apoptosis is possibly attributed to down-regulation of Caspase-3 expression. On the other hand, our results might imply that AsCD could not be used in combination with antitumor drugs that are via a mechanism of increasing Caspase-3 expression.

In conclusion, AsCD possess protective effects against $H_2O_2$-induced apoptosis in PC12 cells through down-regulating Caspase-3 expression, demonstrating that AsCD might be useful in the treatment of oxidative stress-related neurodegenerative diseases. Moreover, the mechanisms of AsCD on activation of multiple apoptosis-related proteins in apoptosis need more studies in future.

**References**


Barnham KJ, Masters CL, Bush AI, 2004. Neurodegenerative diseases and oxidative stress. *Nat Rev Durg Discov* 3(3): 205-214.

Cadet JL, Brannock C, 1998. Free radicals and the pathobiology of brain dopamine systems. *Neurochem Res* 32: 117-131.

Cantoni O, Cattabeni F, Stocchi V, Meyn RE, Cerutti P, Murray D, 1989. Hydrogen peroxide insult in cultured mammalian cells: Relationships between DNA single-strand breakage, poly (ADP-ribose) metabolism and cell killing. *BBA Rev Cancer* 1014: 1-7.

Chen HC, Hong JH, Niu RY, Sun ZL, Wang JD, 2009. Fluoride contamination in ground water of Malaprabha sub basin. *Fluoride* 42(2): 121-126.

David SP, Erick JM, Leonidas S, Carol MT, Michael LS, Herbert MG, Lloyd AG, 1998. Multiple pathways of neuronal death induced by DNA-damaging agents, NGF deprivation, and oxidative stress. *J Neurosci* 18(3): 830-840.

Deng XP, Gu ZL, Xie ML, 2003. Effect of total alkaloids from *Rhizoma Corydalis Decumbentis* on scopolamine and *D*-galactose induced learning and memory impairment in rats. *Chin Tradit Herb Drugs* 34(4): 350-352.

Fernandez A, Kiefer J, Fosdick L, McConkey DJ, 1995. Oxygen radical production and thiol depletion are required for $Ca^{2+}$-mediated endogenous endonuclease activation in apoptotic thymocytes. *J Immunol* 155: 5133-5139.

Gao ZH, Huang KX, Xu HB, 2001. Protective effects of flavonoids in the roots of *Scutellaria baicalensis* Georgi against hydrogen peroxide-induced oxidative stress in HS-SY5Y cells. *Pharmacol Res* 43: 173-178.

Gille JJ, Joenje H, 1992. Cell culture models for oxidative stress: Superoxide and hydrogen peroxide versus normobaric hyperoxia. *Res Rev Mutat* 275: 405-414.

Green DR, Reed JC, 1988. Mitochondria and apoptosis. *Science* 281: 1309-1311.

Halliwell B, 1992. Reactive oxygen species and the central nervous system. *J Neurochem* 59(63): 1609-1623.

Hu XY, Sun AS, Yu LM, 2005. Effects of *Corydalis ambailis migo* total alkaloids on experimental cerebral ischemia. *J Chin Integr Med* 3(1): 46-49.

Hwang SL, Yen GC, 2010. Protective effects of the citrus flavanones to PC12 cells against cytotoxicity induced by hydrogen peroxide. *Neurosci Lett* 484(1): 6-11.

Kannan K, Jain SK, 2000. Oxidative stress and apoptosis. *Pathophysiology* 7: 153-163.

MeGowan AJ, Fernandes RS, Samali A, Cotter TG, 1996. Anti-oxidants and apoptosis. *Biochem Soc Trans* 24: 229-267.

Morel Y, Barouki R, 1999. Repression of gene expression by oxidative stress. *Biochem J* 342: 481-496.

Rao AV, Balachandran B, 2002. Role of oxidative stress and anti-oxidants in neurodegenerative diseases. *Nutr Neurosci* 5: 291-309.

Richter C, 1993. Pro-oxidants and mitochondrial $Ca^{2+}$ and their relationship to apoptosis and oncogenesis. *FEBS Lett* 325: 104-107.

Ronit H, Liola L, Hongein Li, Yuan JY, Reuven S, 1997. Need for caspases in apoptosis of trophic factor-deprived PC12 cells. *J Neuronsci Res* 50: 69-80.

Salvesen GS, Dixit VM, 1999. Caspase activation: The induced proximity model. *Proc Natl Acad Sci USA* 96: 10964-10967.

Sheng R, Gu ZL, Jiang H, 2003. Effects of *Rhizoma Corydalis Decumbentis* on learning and memory ability and brain acetylcholinesterase activity in mice. *Chin Tradit Herb Drug* 34(6): 543-545.

Susanna CB, Clifford W, 2004. Adult neuron survival strategies slamming on the brakes. *Neurosci Nature Rev* 5: 686-700.

Susin SA, Zamzami N, Kroemer G, 1998. Mitochondria as regulators of apoptosis: Doubt no more. *Biochim Biophys Acta* 1366: 151-165.

Wang GW, Schuschke DA, Kang YJ, 1999. Metallothionein-verexpressing neonatal mouse cardiomyocytes are resistant to $H_2O_2$ toxicity. *Am J Physiol Heart C* 276: H167-H175.

Wink DA, Cook JA, Pacelli R, DeGraff W, Gamson J, Liebmann J, Krishna MC, Mitchell JB, 1996. The effect of various nitric oxide-donor agents on hydrogen peroxide-mediated toxicity: A direct correlation between nitric oxide formation and protection. *Arch Biochem Biophys* 331: 241-248.

Xiao XH, Liu JT, Hu JW, Zhu XP, Yang H, 2008. Protective effects of protopine on hydrogen peroxide-induced oxidative injury of PC12 cells via $Ca^{2+}$ antagonism and antioxidant mechanisms. *Eur J Pharm* 591: 21-27.

Yu LM, Wen GR, Deng J, Jin F, Sun AS, 2006. Protective effects of injection of *Corydalis decumbens* Pers on cerebral ischemia and

neuron. *Shanghai J Tradit Chin Med* 40(9): 70-72.

Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, Susin SA, Petit PX, Mignotte B, Kroemer G, 1995. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. *J Exp Med* 182: 367-377.

Zamzami N, Susin SA, Marchetti P, Hirsch T, Gomez-Monterrey I, Castedo M, Kroemer G, 1996. Mitochondrial control of nuclear apoptosis. *J Exp Med* 183: 1533-1544.

Zhang Y, Gu ZL, Jiang XG, 2004. Effects of total alkaloids from *Rhizoma Corydalis Decumbentis* (TARCD) on content of brian 5-HT and DA of Alzheimer' disease rats. *J Suzhou Univ Med Sci* 24(2): 134-137.