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Aqueous extract of the Chinese medicine, Danggui-Shaoyao-San, inhibits apoptosis in hydrogen peroxide-induced PC12 cells by preventing cytochrome *c* release and inactivating of caspase cascade

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Abstract

Danggui-Shaoyao-San (DSS), a traditional Chinese medicine used for centuries for the enhancement of women's health, has been shown to display therapeutic efficacy on senile dementia. In the present study, using a rat pheochromocytoma (PC12) cell line, the effect of DSS on hydrogen peroxide (H_2O_2) induced apoptosis was studied. The apoptosis in H_2O_2 -induced PC12 cells was accompanied by downregulation of Bcl-2, upregulation of Bax, the release of mitochondrial cytochrome *c* into cytosol, and sequential activation of caspase-9 and -3. DSS was able to suppress all these changes and eventually protected against H_2O_2 -induced apoptosis. Taken together, these results suggest that treatment of PC12 cells with DSS can block H_2O_2 -induced apoptosis by the regulation of Bcl-2 family members, as well as suppression of cytochrome *c* release and caspase cascade activation.

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Keywords: Danggui-Shaoyao-San; H_2O_2 ; Apoptosis; PC12; Caspase; Cytochrome *c*

1. Introduction

Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder characterized by the progressive deterioration of cognition and memory in association with widespread neuronal loss and deposit of senile plaques (SP). To date, the cause and the mechanism by which neurons die in AD still remain unclear, yet several lines of evidence support the involvement of oxidative stress (Markesbery, 1997; Behl, 1999). Oxidative damage, mediated by reactive oxygen species (ROS) which can be generated following cell lysis, oxidative burst, or the presence of an excess of free transition metals, has been hypothesized to play a pivotal role during the neurodegeneration of AD victims. On the other hand, studies on postmortem tissues provide direct morphological

and biochemical evidence that some neurons in the brain of AD patients degenerate via an apoptotic mechanism including the presence of DNA damage, nuclear apoptotic bodies, and other markers of apoptosis (Levine, 1997). These results suggest a connection between oxidative stress and apoptosis, and therapeutic strategies aimed at preventing and delaying ROS-induced apoptosis might be a reasonable choice for the treatment of the disease.

Danggui-Shaoyao-san (DSS), a famous Chinese complex prescription, first recorded in "JinKuiYaoLue", consists of six Chinese herbs. Its formula is shown in Table 1. In recent years, DSS has been proved to be effective in treating climacteric period syndrome, chronic appendicitis, Parkinson's disease and Meniere's syndrome (Shang and Qiao, 2006), especially in senile dementia (Zhao et al., 2000). Previous studies showed that DSS shortened the latency of reserpine-treated mice in the water maze test (Kou et al., 2002), and inhibited the deposition of the amyloid granules in senescence-accelerated mouse brain (Li et al., 2006). In vitro, DSS also modulates cellular immune functions and attenuates

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Table 1
Recipe of Danggui-Shaoyao-San (DSS) formulation

	Recipe of DSS formulation	Family	Part used	Components ratio	Harvesting places
1	<i>Angelica sinensis</i> (Oliv.) Diels.	Apiaceae	Root	3	Gansu province
2	<i>Paenonia lactiflora</i> Pall.	Paeoniaceae	Root	16	Zhejiang province
3	<i>Poria cocos</i> (Schw.) Wolf.	Polyporaceae	Fungus nucleus	4	Anhui province
4	<i>Atractylodes macrocephala</i> Koidz.	Atractylodes	Root and rhizome	4	Hubei province
5	<i>Ligusticum chuanxiong</i> Hort.	Apiaceae	Rhizome	8	Sichuang province
6	<i>Alisma orientale</i> (Sam.) Juzep.	Alismataceae	Rhizome	8	Jiangxi province

the damage caused by ischemia/reperfusion, glutamate and hydrogen peroxide in hippocampus slices of guinea pigs (Kou et al., 2003) and protects PC12 cells from damage by amyloid- β_{1-42} (Lin et al., 2005). These results provide a pharmacological basis for an AD preventative function of DSS. The aim of the present study was to examine the protective effect of DSS on H₂O₂-induced PC12 cell damage and primarily to investigate its mechanism of anti-apoptosis.

2. Materials and methods

2.1. Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium (MTT), the fluorescent DNA-binding dye Hoechst 33,258, and propidium iodide (PI) were purchased from Sigma–Aldrich (St. Louis, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were obtained from Gibco Life Technologies (NY, USA). The antibody to cytochrome *c* was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). DNA extraction kit and caspase-9, -8 and -3 Activity Kits were acquired from Beyotime Institute of Biotechnology (Jiangsu Province, China). All other chemicals and reagents were of analytical grade.

2.2. Plant materials

Plant materials were purchased from Nanjing Medicinal Materials Company (Jiangsu Province, China) and authenticated by Dr. Minjian Qin (College of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing, China). The vouchers are conserved at the Herbarium of College of Traditional Chinese Medicine, China Pharmaceutical University.

2.3. Extract preparation

Aqueous extract of DSS was prepared by the procedure of Tang et al. (2000). In brief, six medicinal materials were mixed in proportion and macerated for 1 h with 8× (v/w) distilled water, and decocted for 1 h, after which the filtrate was collected and the residue was decocted again for 1 h with 6×(v/w) of distilled water. The filtrates were mixed, condensed and dried by vacuum-drier at 60 °C. The yield of dried powder was 27.9% according to the original herbs. The sample was stored at 4 °C.

2.4. Determination of total phenolic compounds and sugars content in extract

Total phenolic compound contents were determined by the Folin–Ciocalteu method (Ordonez et al., 2006). 0.5 ml DSS (5 mg/ml) was mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent for 5 min and 2 ml 75 g/L Na₂CO₃ were added. The absorbance of reaction was measured at 760 nm after 2 h incubation at room temperature. Results were expressed as gallic acid equivalents. Total sugars were determined by the phenol–sulfuric acid assay using glucose standard (Dubois et al., 1956).

2.5. Cell culture and treatment

PC12 cell line was obtained from Shanghai Institute of Cell Biology and maintained in DMEM supplemented with heat-inactivated 10% fetal bovine serum (Gibco), 100 U/ml penicillin, 100 µg/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C. When the cells reached sub-confluence, they were treated with 0.25% trypsin in 0.02% EDTA solution, after which 1 × 10⁵ cells/ml were seeded onto 96-well culture plate. Experiments were carried out 48 h after cells were seeded. H₂O₂ was freshly prepared from 30% stock solution prior to each experiment and added to the cells for the indicated times. To study the protective effect of DSS, different concentration of DSS was added simultaneously to the medium.

2.6. Cell viability assay

Cell survival was evaluated by MTT reduction. For our purpose, when the cells reached 80% confluence, the media were changed to those containing varying concentrations of DSS (150, 15, 1.5 µg/ml) and 0.5 mM H₂O₂. After incubation for up to 12 h, MTT solution in phosphate-buffered saline (PBS) was added with a final concentration of 0.5 mg/ml. The plates were incubated at 37 °C for an additional 4 h. Finally, the medium with MTT was removed and 200 µl dimethyl sulfoxide (DMSO) was added to each well. The amount of MTT formazan was qualified by determining the absorbance with Multiskan Spectrum (Thermo) at 570 nm, with 630 nm as a reference.

2.7. Morphological assay

PC12 cells were fixed for 10 min with 4% paraformaldehyde in PBS, and stained for 10 min with 10 µg/ml of Hoechst

33,258 to reveal nuclear condensation as described previously (Kruman et al., 1997). Hoechst-stained cells were visualized and photographed under a Leica DMIL microscope.

2.8. DNA fragmentation analysis

Fragmented DNA was isolated by DNA extraction kit (Be-yotime, C0008) according to the manufacturer's instructions. The eluants containing DNA pellets were electrophoresed on a 1.8% agarose gel at 80 V for 1.5 h. The gel was examined and photographed by ultraviolet gel documentation system.

2.9. Flow cytometric analysis

Cells were collected and washed with ice-cold PBS and fixed with 70% ethanol. The fixed cells were harvested by centrifugation at $1000 \times g$ for 5 min and dissolved in 1 ml PBS containing 50 µg/ml RNase A, 50 µg/ml PI, 0.1% Triton X-100 and 0.1 mM EDTA (pH 7.4), and incubated at 37 °C for 30 min. The fluorescence of cells was measured by flow cytometry (American Becton Dickinson, FACSCalibur).

2.10. Analysis of caspase-3, -8 and -9 activities

Caspases activities were measured using Caspase Activity Kit (Be-yotime, C1115, C1151, C1157) according to the manufacturer's instructions. Briefly, cells were washed with cold PBS, resuspended in lysis buffer and left on ice for 15 min. The lysate was centrifuged at $16,000 \times g$ at 4 °C for 15 min. Activities of caspase-3, -8 and -9 were measured using substrate peptides Ac-DEVD-pNA, Ac-IETD-pNA and Ac-LEHD-pNA, respectively. The release of *p*-nitroanilide (pNA) was qualified by determining the absorbance with Multiskan Spectrum (Thermo) at 405 nm.

2.11. Western blotting analysis

Cell lysates were prepared as described previously (Jia et al., 2005). To ensure equal loading of the protein samples, protein concentrations of the cell lysates were determined by Bradford assay. An equal amount (30 µg) of protein was separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% skim milk in $1 \times$ Tris-buffered saline containing 0.05% Tween-20 (TBST) for 1 h. After blocking, the membrane was incubated with 1% skim milk in TBST, containing either the primary mouse monoclonal antibody against cytochrome *c* (1:500, Santa Cruz, sc-13156) overnight. The membrane was washed with $1 \times$ TBST three times, followed by an additional incubation with 1% skim milk in TBST, containing a peroxidase-conjugated Affinipure goat anti-mouse IgG as the secondary antibody (1:5000, ZSGB-BIO). The detection of protein bands utilized the 3,3'-diaminobenzidine tetrahydrochloride substrate kit (ZSGB-BIO).

2.12. RT–PCR analysis

Total RNA of PC12 cells was extracted, and the potential residual genomic DNA was eliminated with RNase-free-Dnase I (BBI) for 30 min at 37 °C. First-strand cDNA was synthesized as follows: 1 h at 42 °C with 100 U MMLV reverse transcriptase (Promega), 15 U Rnasin (Promega), 500 µM each deoxynucleotide triphosphate (dNTP), 0.5 µg oligo(dT) 18 and 2 µg total RNA in a final volume 25 µl, then 5 min at 95 °C. For PCR amplification, the specific primers were as follows:

GAPDH (213 bp): 5'-ATTCAACGGCACAGTCAAGG-3' (forward)
3'-AGTAGAGGCGGGGAAGACG-5' (reverse)
Bcl-2 (303 bp): 5'-GATGACTTCTCTCGTCGCTA-3' (forward)
3'-TACGGAAACACCTTGATATA-5' (reverse)
Bax (331 bp): 5'-GAACTGGACAATAATATGGA-3' (forward)
3'-TCACTGGTAGAAACACCGAC-5' (reverse)

The PCR mixture contained 0.8 pM forward and reverse primers of the bax or bcl-2 gene, 0.4 pM forward and reverse primers of the specific gene, 2.0 mM MgCl₂, 200 µM each dNTP and 1.5 U Taq DNA polymerase. The PCR procedure was performed at 94 °C for 5 min, followed by 28 cycles at 94 °C for 1 min, at 51 °C for 30 s, at 72 °C for 45 s and extension at 72 °C for 10 min. A 10 µl volume of PCR products was mixed with 2 µl gel loading solution, and electrophoresed on agarose-ethidium bromide gel at 100 V for 1 h. The gels were examined and analyzed by an ultraviolet gel documentation system.

2.13. Statistical analysis

All experiments were performed with each assay in triplicate. Data are presented as mean \pm S.D. The Duncan test and a one-way analysis of variance (ANOVA) were used for multiple comparisons (SPSS program, version 12.0).

3. Results

3.1. Content of total phenolic compounds and sugars content in extract

Using gallic acid and glucose as standard substance, the content assay showed that the quality percentage of total phenolic compounds and total sugar in DSS was 1.68% and 20.75%, respectively (Fig. 1).

3.2. Effect of DSS on viability loss and apoptosis in H₂O₂-induced PC12 cells

The results in Fig. 2A show that application of H₂O₂ induced a time-dependent viability loss in PC12 cells. The viability of cells incubated with H₂O₂ at concentration of 0.5 mM for 12 h was 55.3% of the control value. While the cells were treated with DSS (150, 15, 1.5 µg/ml) in the presence of

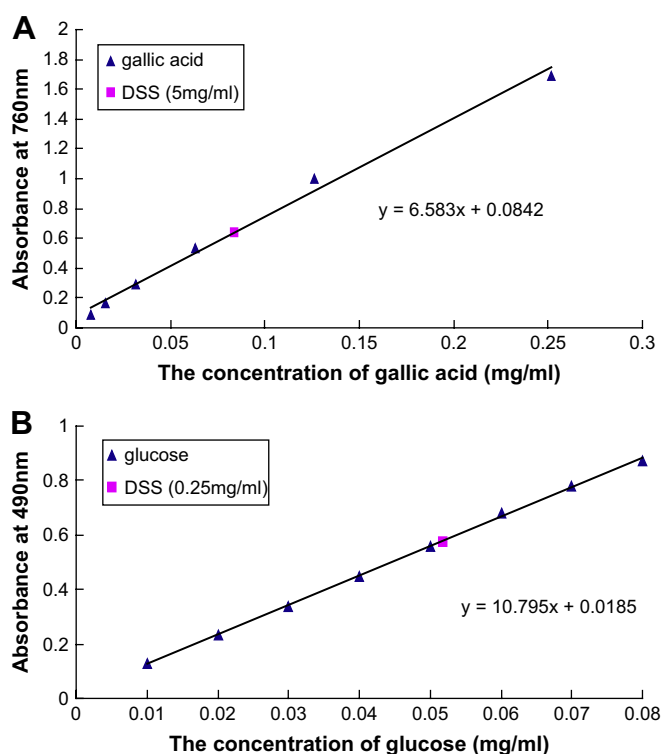


Fig. 1. The content assay of total phenolic compounds and sugars in aqueous extract of DSS. (A) The standard curve of gallic acid. The content of total phenolic compounds in DSS (5 mg/ml) was 0.084 mg/ml and the quality percentage of it was 1.68%. (B) The standard curve of glucose. The content of total sugar in DSS (0.25 mg/ml) was 0.0519 mg/ml and the quality percentage of it was 20.75%.

0.5 mM H_2O_2 the viabilities were significantly increased to 78.2%, 65.7%, and 58.3%, respectively (Fig. 2B).

Hoechst 33,258 staining assay revealed the appearance of apoptotic nuclei upon H_2O_2 treatment at a concentration of 0.5 mM H_2O_2 for 12 h; however, apoptotic nuclei were significantly reduced when cells were treated with 150 μ g/ml DSS in the presence of H_2O_2 . A genomic DNA ladder formation was clearly observed when treatment of PC12 cells with 0.5 mM H_2O_2 for 12 h, and was suppressed by DSS dose-dependently (Fig. 3). A quantitative evaluation of apoptosis was sought using flow cytometry to detect DNA with PI staining. Compared to the control group, the apoptotic rate of PC12 cells which were treated with 0.5 mM H_2O_2 for 12 h significantly increased to 32.23%. When PC12 cells were incubated with DSS (150, 15, 1.5 μ g/ml), the percentage of apoptotic cells decreased from 32.23% to 9.78%, 15.49% and 18.12%, respectively (Fig. 4).

3.3. Effect of DSS on caspase-3, -8, -9 like activities in H_2O_2 -induced PC12 cells

The apoptotic process included the activation of cysteine proteases, which represent both initiators and executors of cell death. Fig. 5 shows that H_2O_2 treatment caused a time-dependent increase in caspase-3 and caspase-9 proteolytic activities. Caspase-3 activity was first detected at 6 h, reached peak at 12 h, and caspase-9 activity was first detected at 3 h then reached peak after 9 h and 12 h of treatment. When cells

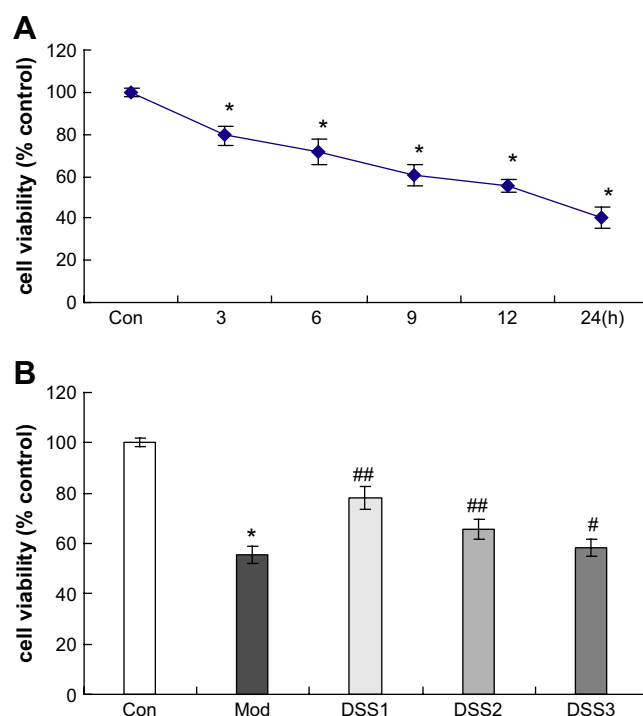


Fig. 2. DSS inhibited H_2O_2 -induced injury in PC12 cells. Cells were treated with 0.5 mM H_2O_2 for indicated times in the presence or absence of DSS. After treatment, cell viability was estimated by MTT method. (A) Cells were treated with 0.5 mM H_2O_2 for indicated times. (B) Exposure to 0.5 mM H_2O_2 and different dose of DSS (150, 15, 1.5 μ g/ml) for 12 h, the cell viability was increased greatly compared with DSS untreated control. * $P < 0.01$ vs. control; # $P < 0.05$; ## $P < 0.01$ vs. H_2O_2 alone.

were incubated with H_2O_2 in the presence of DSS, caspase-3 and caspase-9 activities decreased dose-dependently. We also measured the caspase-8 activity in the PC12 cells apoptosis process and found that treatment of the cells with 0.5 mM H_2O_2 can also affect the activity of caspase-8. When cells were incubated with H_2O_2 in the presence of DSS, caspase-8 activity, as well as caspase-3 and -9 activities, a decrease in a dose-dependent manner was detected.

3.4. Effect of DSS on release of cytochrome c in H_2O_2 -induced PC12 cells

Western blot analysis showed that treatment of PC12 cells with H_2O_2 increased cytochrome c level in the cytosol (Fig. 6A). It first increased at 3 h after 0.5 mM H_2O_2 treatment, and peaked at 9 h. When the PC12 cells were treated with 0.5 mM H_2O_2 in the presence of 150 μ g/ml DSS for 9 h, the band was attenuated remarkably (Fig. 6B), which suggested that treatment with DSS can suppress mitochondrial pore transition and reduce the cytochrome c release into the cytosol.

3.5. Effect of DSS on Bcl-2 family proteins in H_2O_2 -induced PC12 cells

To determine whether Bcl-2 family proteins were involved in H_2O_2 -induced PC 12 cell apoptosis, we used the RT-PCR approach to estimate target cDNA obtained from mRNA

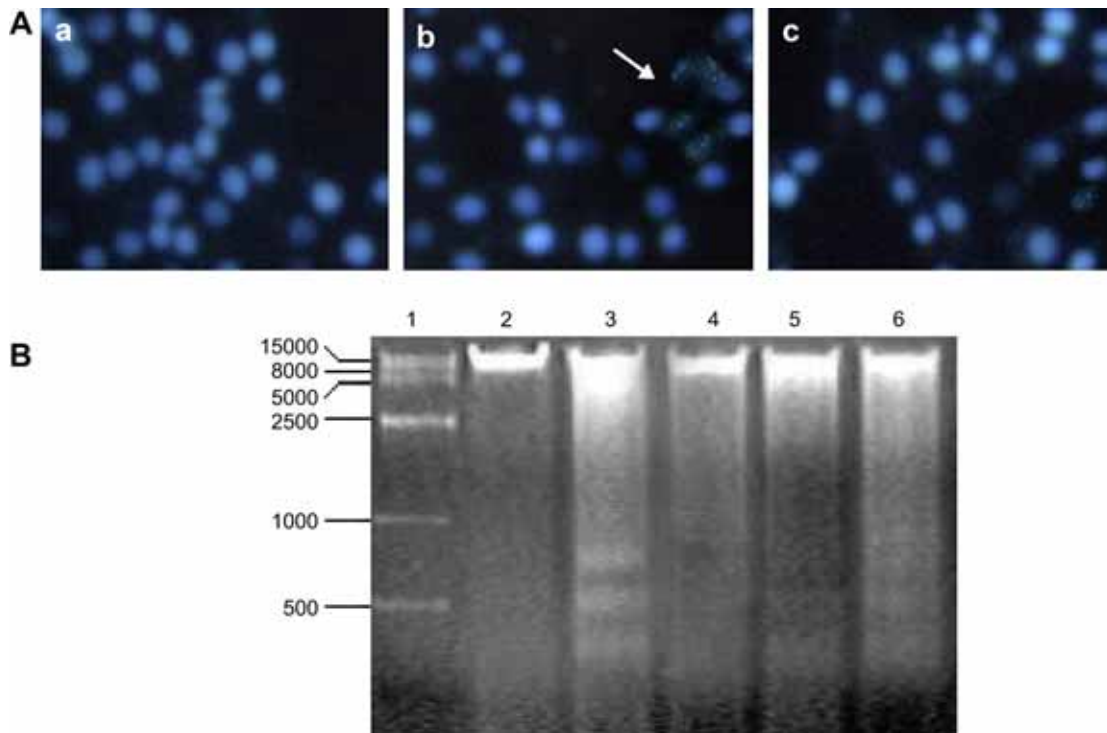


Fig. 3. Morphological analysis of DSS treated PC 12 cells. (A) Morphological analysis of nuclear chromatin in by Hoechst 33,258. (a) Control conditions. (b) After exposure to 0.5 mM H_2O_2 for 12 h, cells displayed apoptotic nuclei (as shown by the arrowhead). (c) After exposure to 0.5 mM H_2O_2 and 150 μ g/ml DSS for 12 h. (B) Agarose gel electrophoresis of DNA fragmentation. Lane 1, DNA ladder mark; lane 2, control; lane 3, 0.5 mM H_2O_2 treated for 12 h; lanes 4–6, DSS (150, 15, 1.5 μ g/ml) and 0.5 mM H_2O_2 treatment for 12 h.

samples. Treatment with H_2O_2 caused upregulation of bax mRNA level with maximal increase at 6 h, a little higher than 9 h, as well as downregulation of bcl-2 mRNA level with maximal decrease at 9 h post-treatment (Fig. 7). To obtain the most evident differences between the model and DSS groups, we chose 6 h to test mRNA expression of the bax gene, and 9 h to test the mRNA expression of bcl-2. However, when the cells were treated with DSS simultaneously, H_2O_2 -induced overexpression of bax level and downregulation bcl-2 level were suppressed.

4. Discussion

DSS is a traditional Chinese medicine which was first recorded early in the third century AD. As a complex prescription, it has success in the treatment of AD, which has a complex etiology (Kou et al., 2005). Nowadays apoptosis in AD has gained much attention due to its role in some pathological neuronal loss (Janicki and Monteiro, 1997; Cotman, 1998).

Apoptosis is a gene-regulated mechanism of cell death. It is driven from the activation of a family of cysteine protease called caspases, which cleave a critical set of cellular proteins to initiate apoptotic cell death. Caspase-9 and caspase-8 are the initiator caspases, which participate in mitochondria- and death receptor-mediated pathways respectively; while caspase-3 is an executioner caspase, which activates caspase-activated DNase, causing apoptotic DNA fragmentation. The

present study showed that caspase-3 activity was upregulated in H_2O_2 -treated cells. To gain insight into the molecular effector pathway of H_2O_2 -induced apoptosis, we detected the level of cytochrome *c* in cytosol and caspase-9 activity in H_2O_2 -treated cells, and found they began to rise after 3 h. Several hours later, some cells began to necrose and the cellular membranes were damaged, which induced the leaking of cell content. As a result, the cytochrome *c* in cytosol peaked at 9 h while the caspase-9 activity crested at 12 h. Furthermore, we observed the downregulation of Bcl-2, and upregulation of Bax prior to the release of cytochrome *c* in H_2O_2 -induced PC12 cell apoptosis. Some studies have shown that Bcl-2 can act as a channel protein in the mitochondrial membrane (Gross et al., 1999). Other studies have demonstrated that an increase of Bcl-2 prevents the mitochondrial release of cytochrome *c*, thereby inhibiting the activation of caspase cascade and apoptosis (Budihardjo et al., 1999; Solange and Martinou, 2000). Our results suggest that the downregulation of Bcl-2 or upregulation of Bax may alter mitochondrial membrane permeability, trigger mitochondrial cytochrome *c* release into cytosol and activate caspase, and probably induce PC12 cell apoptosis via a mitochondria-mediated pathway.

Caspase-8 is a key initiating caspase involved in neuronal apoptosis, which modulates the death receptor-dependent pathway. We detected enhanced caspase-8 activity in H_2O_2 -treated cells, which suggested that a death receptor-mediated pathway may be involved in H_2O_2 -induced apoptosis. However, recent studies have suggested that caspase-8 is not

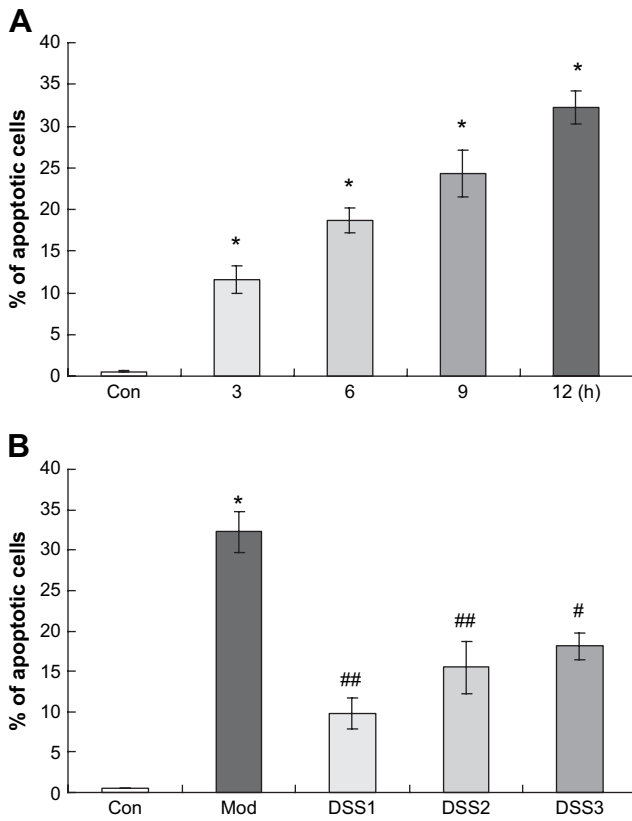


Fig. 4. Effects of DSS on PC12 cells apoptosis by flow cytometric DNA analysis. (A) Cells were treated with 0.5 mM H₂O₂ for indicated times, the apoptotic rate increased time-dependently. (B) Exposure to 0.5 mM H₂O₂ and different dose of DSS (150, 15, 1.5 μg/ml) for 12 h, the cells apoptosis was suppressed dose-dependently. **P* < 0.01 vs. control; #*P* < 0.05; ##*P* < 0.01 vs. H₂O₂ alone.

only activated early in the context of Fas signaling but also in the downstream of caspase-9 activation. In some cells, caspase-9 initiates the processing of caspase-3 in the mitochondria-mediated pathway, which in turn activates caspases-2 and -6. Caspase-6 was found to be required for the activation of downstream caspase-8 (Slee et al., 1999). In short, the present studies suggest that H₂O₂-induced apoptosis in PC12 cells was mediated by at least one pathway through mitochondria with a regulatory role of Bcl-2 family and caspases-3 and -9. Prospectively, further studies to determine whether a death receptor-mediated pathway is involved in H₂O₂-induced apoptosis will be needed.

The present study demonstrated that the aqueous extract of DSS had a great effect on H₂O₂-induced PC12 cell apoptosis. Li et al. (2003) found that a polysaccharide isolated from *Cordyceps sinensis* could significantly attenuate the decreased GSH-Px and SOD activities in H₂O₂-treated PC12 cells. GSH-Px and SOD served as detoxifying systems to prevent cell damage caused by ROS and the combined action of GSH-Px, and SOD provided a repair mechanism for oxidized membrane components. Other studies also revealed that the crude polysaccharide has a strong scavenging activity for superoxide radicals and hydroxyl radicals (Wang and Luo, 2007). It was suggested that perhaps the saccharides, which

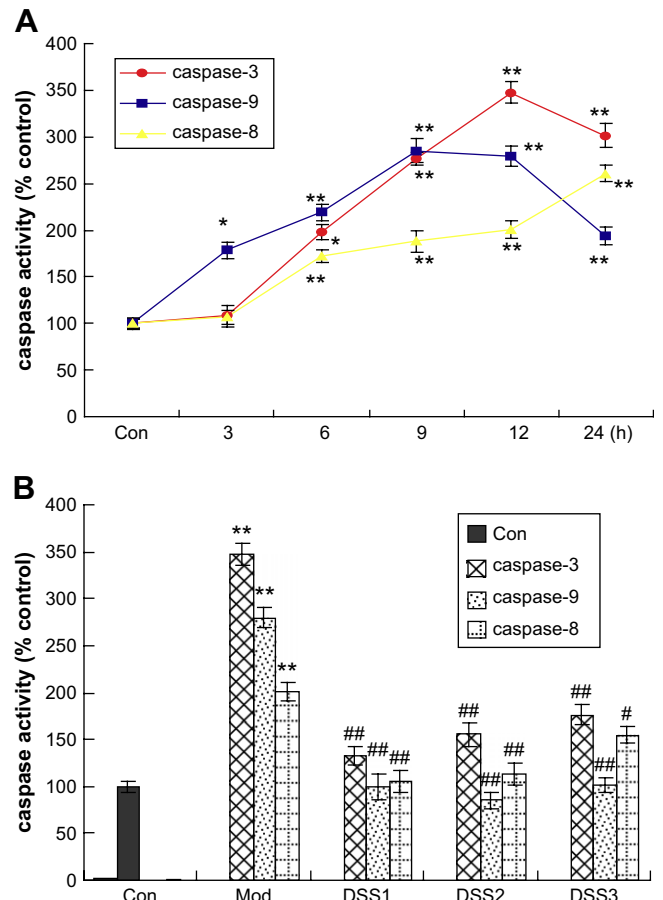


Fig. 5. Activation of caspases-3, -8, -9. (A) Alteration of caspase-3, -8 and -9 activity in 0.5 mM H₂O₂-induced PC12 cells for indicated times. (B) Effect of DSS (150 μg/ml, 15 μg/ml, 1.5 μg/ml) on caspase-3, -8, -9 activity in 0.5 mM H₂O₂-induced PC12 cells for 12 h. **P* < 0.05; ***P* < 0.01 vs. control; #*P* < 0.05; ##*P* < 0.01 vs. H₂O₂ alone at the same time.

occupied 20.75% of the aqueous extract of DSS, had an effect on anti-oxidation by improving the GSH-Px and SOD activities or scavenging superoxide radicals and hydroxyl radicals in H₂O₂-treated PC12 cells, and eventually protected the cells from apoptosis. The content assay also revealed that the aqueous extract contains 1.68% phenolic compounds, which means that one possible mechanism underlying the effectiveness of DSS against oxidative stress involved its polyphenolic composition, since it is known that plant-derived

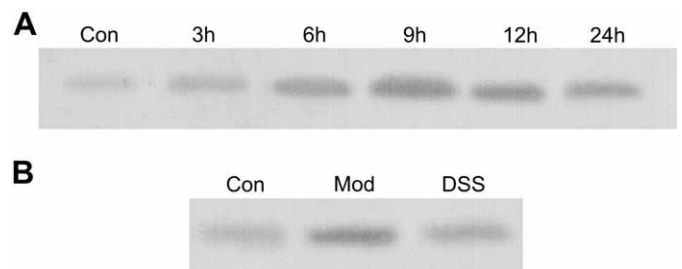


Fig. 6. Western blot analysis of cytosolic cytochrome *c* in H₂O₂ and DSS treated or untreated cells. (A) Cytochrome *c* in cytosol of 0.5 mM H₂O₂-induced PC12 cells for indicated times. (B) Effect of 150 μg/ml DSS on cytochrome *c* release in 0.5 mM H₂O₂-induced PC12 cells for 9 h.

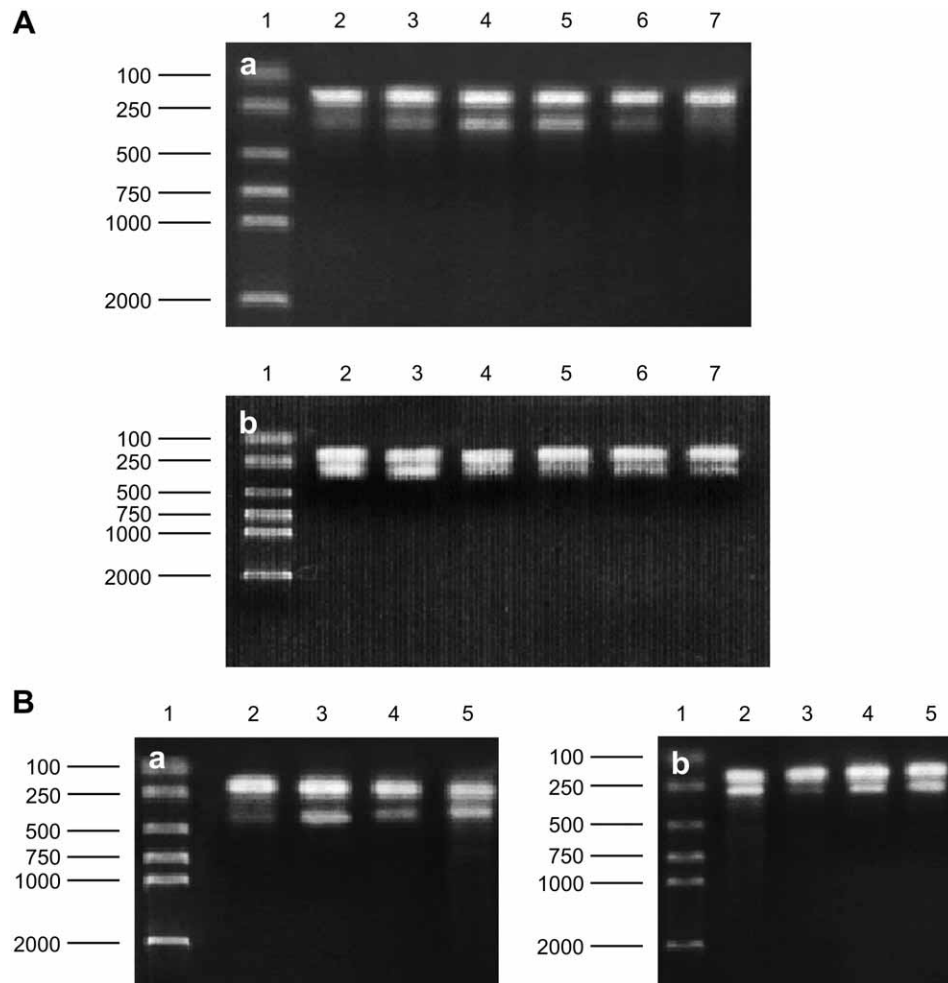


Fig. 7. RT-PCR analysis of Bax and Bcl-2 mRNA in DSS and H₂O₂ treated or untreated cells. (A) Alteration of Bax (a) and Bcl-2 (b) in 0.5 mM H₂O₂-induced PC12 cells for indicated times. Lane 1, DNA mark; lane 2, control; lanes 3–7, 0.5 mM H₂O₂ treated for 3, 6, 9, 12, 24 h. (B) The expression of Bax (a) and Bcl-2 (b) in DSS treated PC 12 cells. Cells were co-incubated with 0.5 mM H₂O₂ and 150 μg/ml, 1.5 μg/ml DSS for 6 h or 9 h. Lane 1, DNA mark; lane 2, control; lane 3, 0.5 mM H₂O₂ treated for 6 h (a) or 9 h (b); lanes 4 and 5, DSS (150, 1.5 μg/ml) and 0.5 mM H₂O₂ treatment for 6 h (a) or 9 h (b).

polyphenolic compounds are potent antioxidants and free radical scavengers (Ishige et al., 2001). Besides saccharides and phenolic compounds, the aqueous extract of DSS also includes various other chemicals, which indicated that perhaps the interactions of these assigned DSS as superior in anti-apoptosis.

In summary, the traditional medicine DSS could upregulate Bcl-2 level and downregulate Bax level first, and these might in turn adjust the mitochondrial membrane permeability, attenuate cytochrome *c* and its release into cytosol, following the suppression of caspase activation. These data provide a further pharmacological basis for the therapeutic efficacy of DSS for Alzheimer's disease.

Acknowledgments

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