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Original article

Neuroprotection of Stilbenes from Leaves of *Cajanus cajan* against Oxidative Damage Induced by Corticosterone and Glutamate in Differentiated PC12 Cells

Ya-min Liu, Sheng-nan Shen, Fang-bo Xia, Qi Chang, Xin-min Liu, Rui-le Pan*

Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Peking Union Medical College, Beijing 100193, China

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ABSTRACT

Objective To investigate the neuroprotective effects of four stilbenes isolated from the leaves of *Cajanus cajan*. **Methods** Neuroprotective effects of the four stilbenes were evaluated using rat pheochromocytoma cell line (PC12 cells) damage models induced by corticosterone or glutamate. In order to elaborate whether the neuroprotective effects of stilbenes are related to anti-oxidant properties, both oxidant and anti-oxidant parameters were measured. **Results** Four stillbenes, namely cajaninstilbene acid (CSA), longistyline A, longistyline C, and cajanolactone A were isolated from the leaves of *C. cajan*. The results of MTT assay and LDH release assay demonstrated that the four stilbenes possessed neuroprotective effects. Moreover, the treatment on PC12 cells with corticosterone or glutamate could significantly increase the levels of ROS and MDA with accompanying decrease in the activities of SOD and CAT. However, the four tested stilbenes could significantly alleviate such situation by dropping out the levels of ROS and MDA, as well as enhancing the activities of SOD and CAT. **Conclusion** These results provide a scientific basis for further studies to explore the potential neuroprotective effects on neurodegenerative diseases.

Key words

anti-oxidants; *Cajanus cajan*; corticosterone; glutamate; stilbenes

1. Introduction

For centuries, neurodegenerative disorders, such as Parkinson's disease (PD), Alzheimer's disease (AD), and major depression, have kept plaguing people's lives. Although neurodegenerative disorders have multiple pathogenesis, most recently available articulations of physiological and pathological process still focus on the damage of hippocampal neurons including the reduced volume and morphological changes (Sapolsky, 2000). Thus, the investigation on the protection of hippocampal neuron injury might be a main way to find the treatments for neurodegenerative diseases.

Hippocampus is a main brain region with the key importance for learning and memory, mediating stress response, and is also involved in the regulation of hypothalamic-pituitary-adrenal (HPA) axis (Lucassen et al, 2001), Usually,

* **Corresponding author: Pan RL** Tel: +86-10-5783 3275 Fax: +86-10-5783 3299 E-mail: rlpan@implad.ac.cn

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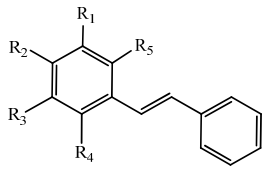
hippocampus participates in the inhibition of the activity of HPA axis. When the hippocampus was impaired, the susceptibility of HPA axis increased, ultimately resulting in the excessive release of glucocorticoid, which could be the most likely reason for the hippocampal volume reduce (Sapolsky et al, 1988; Herman and Cullinan, 1997; Sapolsky, 2001; Syed, 2005; Murray et al, 2008). As one of principals, glucocorticoids synthesized in rodent adrenal cortex, and persistently high levels of corticosterone could induce nerve cells apoptosis (McIntosh and Sapolsky, 1996; Zhu et al, 2006). Thus, the nerve cytoprotection against corticosterone-induced cell injury has become one of the most widely used *in vitro* models to screen and investigate the neuro- protective agents (Li et al, 2003; 2004; Zhou et al, 2009).

Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system stimulating glutamate receptors and playing an important role in both physiological and pathological processes (Meldrum, 1993; Mehta et al, 2013). Glutamate-evoked excitotoxicity has been implicated in the etiology of many neurodegenerative diseases. Moreover, the abnormal metabolism and excess release of glucocorticoid could induce glutamate excitatory neural toxicity, resulting in the death of neurons (Treccani et al, 2014).

Cajanus cajan (L.) Millsp. (Leguminosae) is widely cultivated in the tropical and subtropical areas in the world, and is one of the most important agricultural crops. The plant of *C. cajan* provides a beneficial natural food supplements as well as affords a wide field of medicinal applications. Flowers are prepared for dysentery and menstrual disorders. Seeds are used as diuretic and sickle cell anaemia preparations. Leaves are used to treat anaemia, hepatitis, diabetes, urinary infections, and yellow fever (Akojie and Fung, 1992; Grover et al, 2002).

Previous studies on the chemical constituents in *C. cajan* leaves (CCL) revealed that it contains stilbenes, flavones, coumarins, and phytosterols, some of which are considered to possess anti-oxidant (Ghosh et al, 2009; Sarkar et al, 2009; Wu et al, 2009; Lai et al, 2012), antitumor (Luo et al, 2010; Ji et al, 2011), antimalarial (Duker-Eshun et al, 2004), and anti-bacterial activities (Fu et al, 2009; Nwodo et al, 2011). Recently, the pharmacological effects of stilbenes derived from CCL on nervous system have been reported. In our prophase research, we indicated that stilbenes-containing fraction from CCL could significantly ameliorated the cognitive deficits and neuron apoptosis caused by the injection of $A\beta_{25\text{-}35}$ in mice (Ruan et al, 2009). And the stilbenes-containing fraction and longistyline A had the neuroprotective activity against corticosterone-induced pheochromocytoma (PC12) cells (Jiang, 2012a; 2012b). However, little information has been focused on the separation and the neuroprotective activities of the stilbenes from CCL.

Rat PC12 cell is a kind of rat adrenal chromaffin clone cells with the character of highly serial subcultivation, which is widely used in neural cell experiments of physiology, pharmacology, and pathophysiology. The present study was designed to investigate the protective effects of the four isolated stilbenes (Figure 1) from CCL on PC12 cells induced by corticosterone or glutamate. And whether the neuro-protective effects of stilbenes were related to anti-oxidant properties was also investigated.



1 R_1=OH, R_2=CH_2CH=$C(CH_3)_2$, R_3=OCH_3, R_4=H, R_5=COOH

2 R_1=OCH_3, R_2=CH_2CH=$C(CH_3)_2$, R_3=OH, R_4=R_5=H

3 R_1=OH, R_2=H, R_3=OCH_3, R_4=CH_2CH=$C(CH_3)_2$, R_5=H

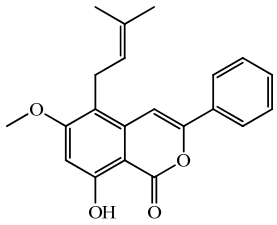


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Figure 1 Structures of four stilbenes

2. Materials and methods

2.1 Materials

Four stilbenes were separated from the leaves of *Cajanus cajan* (L.) Millsp. by our laboratory. The separated method was described in the literature (Liu et al, 2014). The purity is over 95% detected by HPLC.

PC12 cells were purchased from Cell Bank of Peking Union Medical College (Beijing, China). Dulbecco's modified Eagle's medium (DMEM) and 10% heat-inactivated fetal calf serum were from Gibco Co. (USA). Penicillin (100 IU/mL), streptomycin (10 μg/mL), methyl thiazolyl tetrazolium (MTT), dimethylsulfoxide (DMSO), corticosterone (CORT), and glutamate (Glu) were purchased from Sigma-Aldrich Inc. (USA). Lactate dehydrogenase (LDH) kit, intracellular reactive oxygen species (ROS) kit, methane dicarboxylic aldehyde (MDA) kit, methane dicarboxylic aldehyde (SOD) kit, and catalase (CAT) kit were purchased from Nanjing Jiancheng Bioengineering Institute (China). Deionized water was used in all experiments. All the reagents and other chemicals were of analytical grade.

2.2 Cell culture and treatment

The cells were differentiated by adding 50 ng/mL NGF to DMEM [containing 1% fetal bovine serum (FBS)]. PC12 cells were cultured in DMEM medium supplemented with 5% FBS, 10% horse serum, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 °C in humidified atmosphere of 95% air and 5% CO_2. Cultures were allowed to mature for two weeks before used for the experiments.

In order to investigate the cytotoxicity of four individual stilbenes on differentiated PC12 cells, cells were planted into a 96-well culture plate at 6×10^4 cells/well, then treated with

four stilbenes at the concentration of 1, 2, 4, 8, 16, and 32 μmol/L for 4, 8, 12, 24, and 48 h, respectively. After that, the cell viability was determined by MTT assay.

For the assessment of cytotoxicity induced by corticosterone or glutamate, cells were planted into a 96-well culture plate at 6×10^4 cells/well, and exposed to corticosterone (25, 50, 100, 200, and 400 μmol/L) or glutamate (1.875, 3.75, 7.5, 15, and 30 mmol/L), then the cell viability was determined by MTT assay.

For the study on the protective effect of the isolated stilbenes against toxicity induced by corticosterone or glutamate, PC12 cells were pretreated with stilbenes (1, 2, 4, 8, and 16 μmol/L) for 24 h, and then exposed to 200 μmol/L corticosterone for 48 h or 15 mmol/L glutame for 24 h. Then the cell viability was determined by MTT assay.

2.3 Measurement of cell viability

The cell viability was determined using MTT assay. Briefly, at the end of the indicated treatment, the PC12 cells were treated with MTT solution (final concentration of 0.5 mg/mL) for 4 h at 37 °C. After 4 h incubation, the formazan crystals formed in intact cells were solubilized with DMSO, and absorbance at 500 nm was measured with a microplate reader (Model 680, BIO-RAD Laboratories, Hercules, USA). The cell viability is expressed as a percentage of the non-treated group.

2.4 Measurement of LDH release

The release of LDH was another indicator of cellular damage. The determination of LDH release was using the commercial kit according to the manufacturer's instructions. Briefly, at the end of the treatment, the cells in 6-well plate were centrifuged at 1000 *g* for 4 min, 1 mL culture supernatants were collected from each well, 3.4 mL reaction buffer supplied in the kit was then added. After mixing at room temperature for 30 min, the absorbance was assessed using a microplate reader at a test wavelength of 340 nm and the LDH leakage was expressed as a percentage of total LDH activity (LDH in the medium + LDH in the cell), according to the equation as follows: LDH released rate = (LDH activity in medium / total LDH activity) × 100. Each experiment was performed for four times.

2.5 Measurement of intracellular ROS

The intracellular ROS level was measured using DCFH-DA. DCFH-DA is a nonfluorescent compound that is enzymatically converted to the strongly fluorescent compound DCF in the presence of ROS. Briefly, PC12 cells were seeded into a 6-well culture plate at a density of 6×10^5 cells/well. At the end of the treatment, the cells were washed with PBS and incubated with DCFH-DA at a final concentration of 10 μmol/L for 30 min at 37 °C in darkness. The cells were then washed for three times with PBS to remove the extracellular DCFH-DA, and the fluorescence intensity of the DCF was measured with a fluorescent microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 538 nm, and the intracellular ROS levels were expressed as percentage of control.

2.6 MDA, SOD, and CAT assays

After the treatment, the PC12 cells were washed with ice-cold PBS twice, harvested by centrifugation at 1000 *g* for 4 min, pooled in 0.5 mL PBS, and homogenized. The homogenate was centrifuged at 4000 *g* for 15 min, and the supernatant was collected for MDA, SOD, and CAT assays by using commercial kits. All the procedures complied with the manufacturer's instructions.

The MDA content was determined using the thiobarbituric acid method, which formed a red compound with the maximum absorbance at 532 nm. The calculation was performed as follows: MDA level (nmol/mg prot) = (absorbance of test tube − absorbance of standard blank tube)/ (absorbance of standard tube − absorbance of blank tube) × 10 nmol/mL × dilution multiple / protein level (mg prot/mL). The assay of total SOD was based on its ability to inhibit the oxidation of oxymine by the xanthine-xanthine oxidase system at 550 nm. The calculation was performed as follows: SOD activity (U/mg prot) = (absorbance of control tube − absorbance of test tube) / absorbance of control tube / 50% × dilution multiple / protein level (mg prot/mL). CAT activity was determined by monitoring the amount of complex compound at 405 nm due to H_2O_2 decomposition. The calculation was performed as follows: CAT activity (U/mL) = (absorbance of control tube − absorbance of test tube) × 271/ (60 × sample volume) × dilution multiple.

2.7 Statistical analysis

The data were expressed as $\bar{x} \pm s$. Multiple group comparisons were performed using One-way analysis of variance (ANOVA) followed by Dunnett's test to detect any inter-group differences. Differences were considered to be statistically significant at $P < 0.05$.

3. Results

3.1 Effect of individual stilbenes on PC12 cells

The effect of individual stilbenes on PC12 cells was examined at 4, 8, 12, 24, and 48 h. No significant changes were observed on viability of cells treated with individual stilbenes under the concentration of 1−16 μmol/L during the period of 24 h (data were not presented), which was chosen for subsequent experiments.

3.2 Cell toxicity induced by corticosterone or glutamate

The viability of PC12 cells measured by MTT assay was gradually reduced when cells were treated with corticosterone or glutamate with the concentration and time increased.

As shown in Figure 2, the survival rates of PC12 cells were about 82%, 68%, 55%, and 46% at 8, 12, 24, and 48 h, respectively when cells were exposed to 200 μmol/L corticosterone,and the survival rates were 67%, 62%, 42%, and 32% when exposed to 400 μmol/L corticosterone.

As depicted in Figure 3, when exposed PC12 cells to 7.5 mmol/L glutamate, the cell viabilities were about 87%, 82%, 79%, and 65 %; when exposed to 15 mmol/L glutamate, the cell viabilities were about 73%, 66%, 58%, and 52 % at 4, 8, 12, and 24 h, respectively.

Based on these results, the optimal concentration and time of corticosterone or glutamate were 200 μmol/L for 48 h and 15 mmol/L for 24 h, respectively.

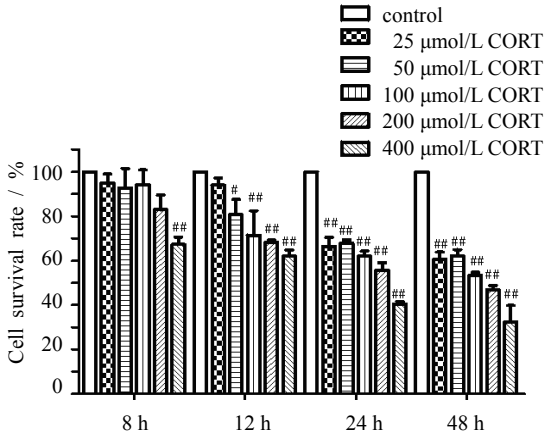

Figure 2 Effect of corticosterone exposure on cell viability by MTT assay ($\bar{x} \pm s$, $n = 4$)

$^{\#}P < 0.05$ $^{\#\#}P < 0.01$ *vs* control group, same as below

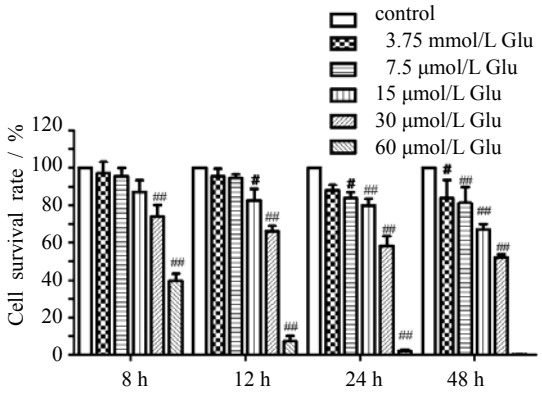

Figure 3 Effect of glutamate exposure on cell viability by MTT assay ($\bar{x} \pm s$, $n = 4$)

3.3 Protection of stillbenes against corticosterone or glutamate-induced cytotoxicity by MTT assay

As illustrated in Figure 4, the cell viability significantly decreased compared with the control group upon the treatment with 200 μmol/L corticosterone ($P < 0.01$), and the value was (54.3 ± 1.9)%. However, when the cells were treated with CSA, longistyline A, longistyline C, and cajanolactone A for 24 h before the corticosterone exposure, the cell viability showed a marked increase with the concentration of 2−8 μmol/L. Among the four tested compounds, CSA possessed a relative strong neuroprotective activity, and the protection displayed in a concentration-dependent manner. The cells treated with CSA (8 μmol/L) showed the best neuroprotection with the cell viability of (86.5 ± 4.8)%.

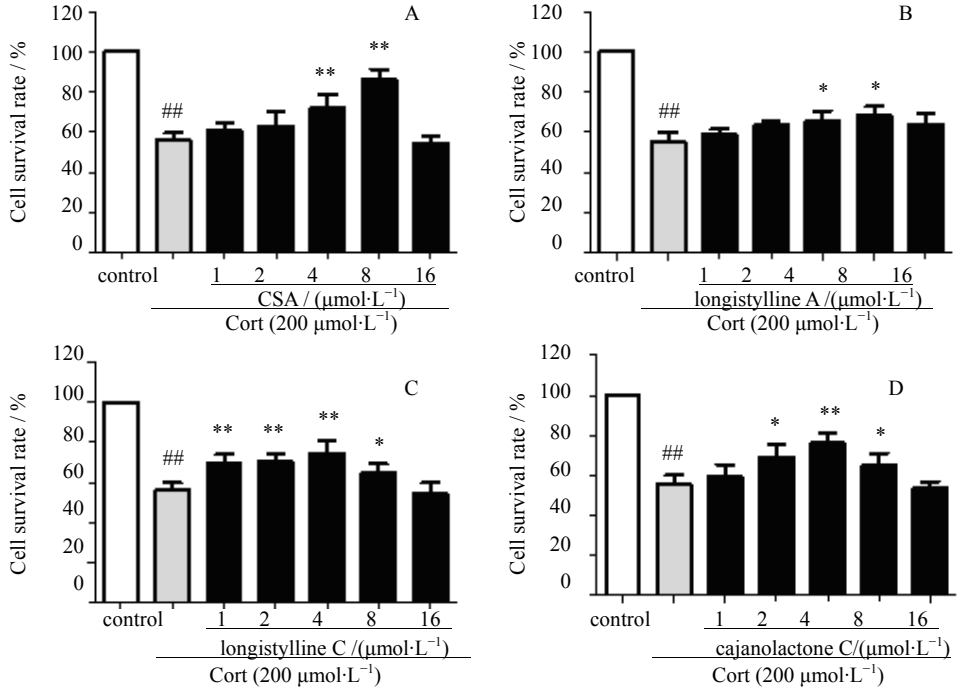

Figure 4 Effects of stilbenes on corticosterone-induced PC12 cells by MTT assay ($\bar{x} \pm s$, $n = 4$)

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* corticosterone group; $^{\#\#}P < 0.01$ *vs* control group; Cort: corticosterone; CSA: cajaninstilbene acid

As shown in Figure 5, cell viability significantly decreased when the cells were treated with glutamate (15 mmol/L) compared with the control group ($P < 0.01$). While cells were incubated with CSA, longistyline A, longistyline C, and cajanolactone A for 24 h before the glutamate exposure, the cell survival of cajanolactone A treated groups showed the significant increase with the concentration of 2−16 μmol/L, but did not show significant increase in a concentration-dependent manner. The 8 μmol/L CSA, longistyline A, longistyline C, and cajanolactone A displayed marked increase in cell survival rate ($P < 0.05$ or 0.01), and the values were (79.7 ± 3.7)%, (68.4 ± 3.8)%, (85.4 ± 4.5)%, and (84.0 ± 4.7)%, respectively.

As described above, we may obviously draw the conclusion that the best cytoprotection of stilbenes against corticosterone and glutamate-induced injuries was 8 μmol/L at which concentration all showed a significant difference compared with the model groups. Thus, we chose 8 μmol/L for the subsequent corticosterone and glutamate-induced experiments.

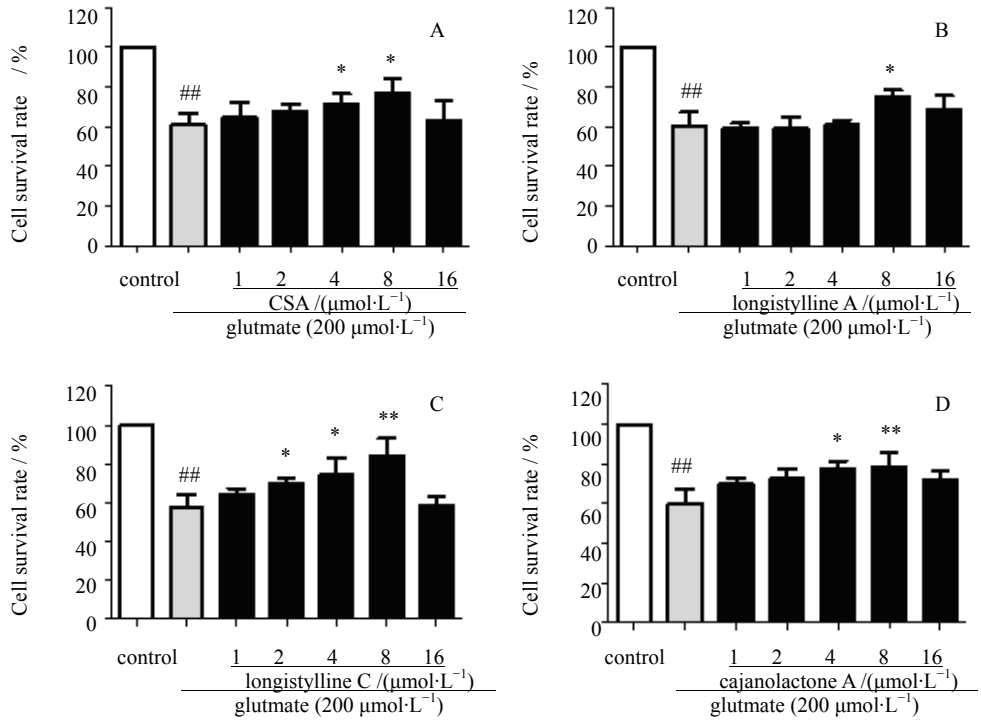

Figure 5 Protection of stilbenes on glutamate-induced PC12 cells by MTT assay ($\bar{x} \pm s$, $n = 4$)

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

3.4 Protection of stillbenes against corticosterone and glutamate-induced cytotoxicity by LDH assay

As shown in Figure 6, the level of LDH release was increased to (82.3 ± 8.6)% after exposure to 200 μmol/L corticosterone. However, pretreatment with stilbenes resulted in a significant decrease in LDH release compared with the corticosterone group, and the LDH releases were (41.4 ± 6.7)% (CSA), (68.3 ± 12.6)% (longistyline A), (56.2 ± 8.9)% (longistyline C), and (60.0 ± 9.8)% (cajanolactone A) at the concentration of 8 μmol/L.

The release of LDH in stilbene treated groups was reduced to (23.2 ± 3.9)% (CSA), (24.8 ± 2.7)% (longistyline A), (22.8 ± 2.5)% (longistyline C), and (23.1 ± 2.7)% (cajanolactone A) at the concentration of 8 μmol/L, while that of glutamate group was (40.5 ± 3.2)% (Figure 7). These results also suggest that pretreatment with the four tested stilbenes has the significant protective effects against glutamate- induced toxicity in PC12 cells.

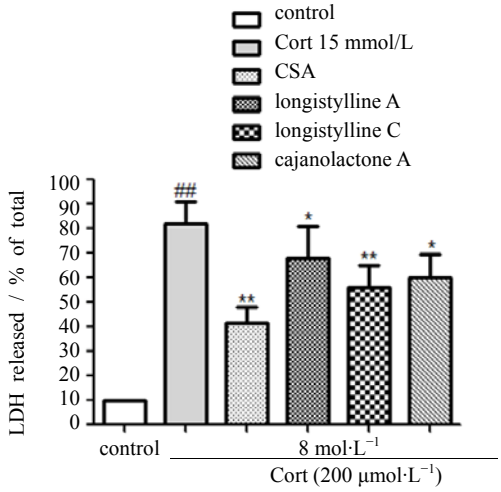

Figure 6 Effect of stilbenes on LDH leakage in corticosterone-treated PC12 cells ($\bar{x} \pm s$, $n = 4$)

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

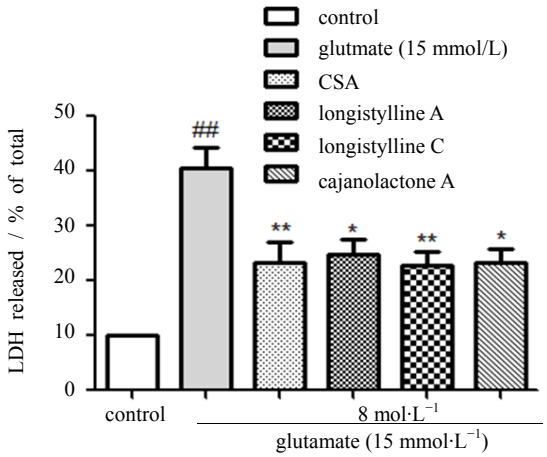

Figure 7 Effect of stilbenes on LDH leakage in glutamate-treated PC12 cells ($\bar{x} \pm s$, $n = 4$)

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

3.5 Effect of stilbenes on corticosterone-induced oxidative stress in PC12 cells

3.5.1 Effect on intracellular ROS and MDA contents

As shown in Figure 8A, the treatment with corticosterone could cause a significant increase in the intracellular ROS levels as compared with the control group ($P < 0.01$). However, the levels were significantly reduced when pretreated with 8 μmol/L stilbenes for 24 h as compared with the corticosterone groups. When pretreated with 8 μmol/L stilbenes before corticosterone treatment, the contents were (122.2 ± 11.3)% (CSA), (115.9 ± 13.6)% (longistyline A), (110.5 ± 5.9)% (longistyline C), and (106.8 ± 6.3)% (cajanolactone A), respectively.

As shown in Figure 8B, the formation of MDA significantly increased as compared with the control group upon the treatment with corticosterone ($P < 0.01$), and the contents was (165.7 ± 8.6)%. However, when the cells were incubated with 8 μmol/L stilbenes before corticosterone treatment, the levels of MDA had a decrease from (165.7 ± 7.5)% (corticosterone-induced group) to (117.2 ± 14.9)% (CSA), (129.3 ± 7.5)% (longistyline A), (117.3% ± 9.4)% (longistyline C) and (118.4 ±14.4)% (cajanolactone A).

3.5.2 Effect of stilbenes on activities of anti-oxidant enzyme in corticosterone-induced PC12 cells

Treated PC12 cells with corticosterone could significantly reduce the activities of SOD and CAT compared with the control group ($P < 0.01$), the SOD activity was (59.1 ± 4.4)% of the control, and CAT activity was (50.4 ± 4.0)%. However, the pretreatment of cells with 8 μmol/L stilbenes markedly attenuated the decrease of SOD and CAT activities induced by corticosterone ($P < 0.01$). When PC12 cells were pretreated with stilbenes in the presence of corticosterone, SOD activities of CSA, longistyline A, longistyline C, and cajanolactone A treated cells increased from (59.1 ± 4.4)% (corticosterone-treated group) to (90.4 ± 5.7)%, (79.3 ± 2.8)%, (82.8 ± 3.1)%, and (81.0 ± 3.2)%, while CAT activities from (50.4 ± 4.0)% (corticosterone-treated group) to (79.5 ± 2.9)%, (74.0 ± 5.4)%, (77.6 ± 7.9)%, and (69.1 ± 8.8)% (Figures 8C and 8D).

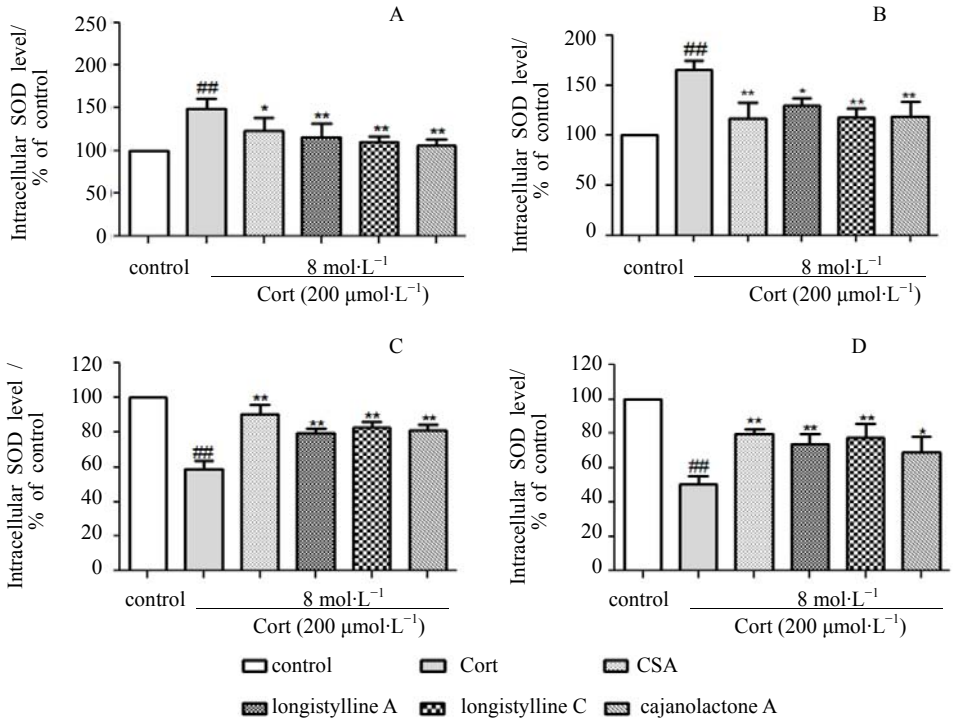

Figure 8 Effects of stilbenes on levels of ROS (A), MDA (B), SOD(C), and CAT (D) in corticosterone-induced PC12 cells ($\bar{x} \pm s$, $n = 4$)

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

3.6 Effect of stilbenes on glutamate-induced oxidative stress in PC12 cells

3.6.1 Effect on intracellular ROS andMDA contents

As shown in Figure 9A, treatment with glutamate could cause a significant increase in the intracellular ROS levels as compared with the control group ($P < 0.01$), and the ROS contents were (154.4 ± 8.49)% of the control group, respectively. However, pretreated with 8 μmol/L stilbenes prior to glutamate treatment, the ROS levels were significantly reduced, with values of (129.5 ± 5.9)% (CSA), (125.3 ± 9.0)% (longistyline A), (112.6 ± 7.5)% (longistyline C), and (124.7 ± 14.7)% (cajanolactone A), respectively. And in the glutamate-induced cells (Figure 9B), the levels of MDA in stilbene treated cells were decreased to (118.6 ± 12.6)% (CSA), (108.1 ± 6.5)% (longistyline A), (100.8 ± 13.1)% (longistyline C), and (137.6 ± 10.7)% (cajanolactone A) compared with glutamate-induced group (173.6 ± 7.6)%.

3.6.2 Effect of stilbenes on activities of anti-oxidant enzyme in glutamate-treated PC12 cells

Exposure of PC12 cells to glutamate obviously reduced SOD and CAT activities compared with the control group ($P < 0.01$)%, the values of which were (53.7 ± 5.5)% and (60.0 ± 7.6)% of the control group. When pretreated PC12 cells with stilbenes in the presence of glutamate, SOD activities of CSA, longistyline A, longistyline C, and cajanolactone A treated cells increased from (53.7 ± 5.5)% (glutamate-treated group) to (86.7 ± 5.5)%, (86.9 ± 2.8)%, (91.8 ± 3.1)% and (88.6 ± 5.1)%, while CAT activities from (60.0 ± 7.6)% (glutamate-treated group) to (69.8 ± 6.2)%, (61.9 ± 4.5)%, (83.1 ± 5.2)% and (81.7 ± 9.0)%, respectively (Figures 9C and 9D).

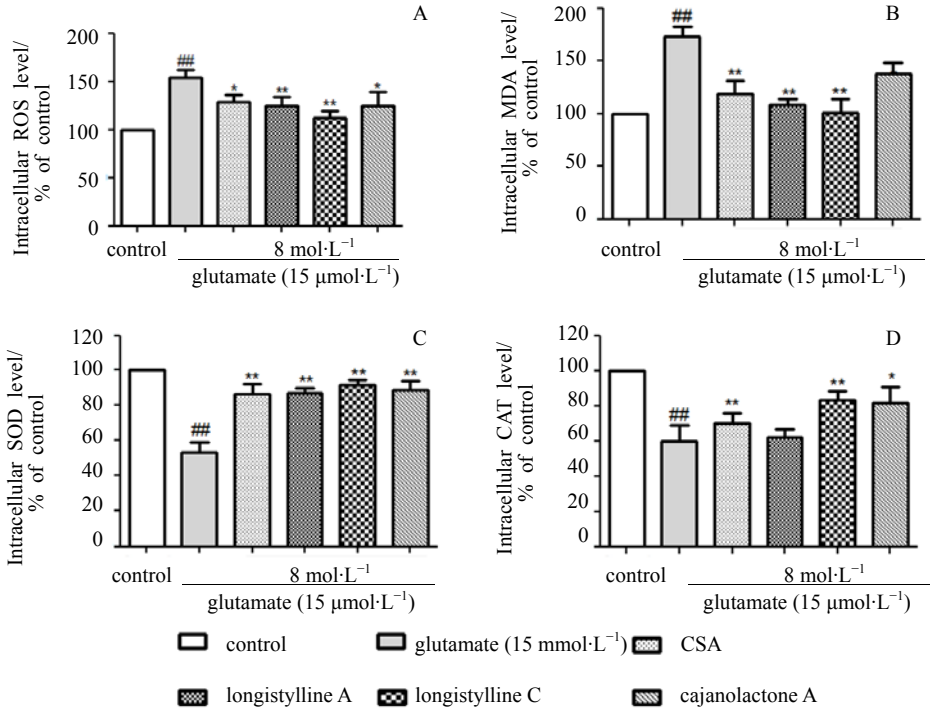

Figure 9 Effects of stilbenes on levels of ROS (A), MDA (B), SOD(C), and CAT (D) in glutamate-treated PC12 cells.

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* glutamate-treated group; $^{\#\#}P < 0.01$ *vs* control group

4. Discussion

Neurodegenerative diseases such as epilepsy, Alzheimer's disease, Amyltropic lateral sclerosis, Parkinson's disease, and major depression, are usually characterized by neuronal loss in the hippocampus and cerebral cortex. Thus, nerve cell protection has become an important strategy for the treatment of neurodegenerative diseases. Previous studies demonstrated that high levels of corticosterone and glutamate-evoked excitotoxicity and oxidative stress burden contributed key roles to the damage of hippocampal neurons (Magarinos and McEwen, 1995; Barnham et al, 2004; Murray et al, 2008; Park et al, 2009; Nam et al, 2012). Meanwhile, a lot of *in vitro* research showed that nerve cells treated with high concentration of corticosterone and glutamate could induce intracellular Ca^{2+} overloading, lipid peroxidation, nuclear DNA strand breaking, mitochondrial disfunction, and result the cell apoptosis (Ankarcrona et al, 1996; Termini, 2000; Li et al, 2004; Penugonda et al, 2006; Fukui et al, 2009; Xia et al, 2009; Zhou et al, 2009; Lu et al, 2010; Yu et al, 2011). In present study, we use corticosterone or glutamate-induced damage in PC12 cells to investigate the neuroprotection of the stilbenes which were isolated from CCL, namely, cajaninstilbene acid, longistyline A, longistyline C, and cajanolactone A, and the results demonstrated that all the four stilbenes possessed obvious protection against the damage induced by corticosterone or glutamate in PC12 cells, which were confirmed by MTT assay and LDH assay (Figures 4−7).

As the neuronal cells possess the low levels of endogenous anti-oxidant enzymes and a feeble anti-oxidant defense system (Jia et al, 2013), the nerve cells are particularly susceptible to oxidative stress-induced injury. Recent findings support that high glucocorticoids treated nerve cells can alter anti-oxidant enzyme capacity and cause the level of ROS to significant increase (McIntosh and Sapolsky, 1996; 1998). And compelling evidences also have led to the fact that neuronal damage induced by glutamate is caused through not only the excitotoxic pathway mediated by ionotropic glutamate receptors, but also through the oxidative glutamate pathway (Rogers and Hunter, 1997; Bleich et al, 2003; Fukui et al, 2010), which have indicated that glutamate-mediated neuronal cell death was closely associated with intracellular ROS accumulation. Our results revealed that incubated PC12 cells with corticosterone or glutamate could increase the production of ROS and induce oxidative stress; While being pretreated with stilbenes could largely relieve the oxidative stress through decreasing the production of ROS which was in accordance with the reported literature.

To relieve the cumulate burden of oxidative stress, cells usually utilize numerous endogenous anti-oxidant defense systems which include both enzymatic and non-enzymatic anti-oxidant mechanisms that can either scavenge ROS or prevent their formation. The enzymatic anti-oxidant defense mechanisms are mediated through SOD, GPX, CAT, and thioredoxin reductase; And non-enzymatic anti-oxidant defense is including anti-oxidants, such as vitamins, phenols, and carotenoids (Dhalla et al, 2000; Maulik and Das, 2002; Seven et al, 2008). SOD, one of acidic protease, plays a part in preventing diseases by catalyzing superoxide ion free radicals to disproportionate, and CAT catalyzes the breakdown of hydrogen peroxide into oxygen and water, to prevent the formation of hydroxyl radicals. In addition, MDA is the production of LPO inside of the cells; Its content reflects the degrees of LPO and oxidant stress (Bonnes-Taourel D, 1992). In the present study, our results have demonstrated the anti-oxidant activities of stilbenes from the view of its endogenous anti-oxidant defense system by increasing SOD and CAT activities in company with decreasing MDA content. Considering that the direct anti-oxidant property of CSA, which was proved through the assays of DPPH radical-scavenging, ABTS, LPO, and XOD inhibition in a cell-free system (Kong et al, 2009; Nan et al, 2009; Wu et al, 2011). It was speculated that the other three stilbenes could also have a direct anti-oxidant property, since the four stilbenes all belong to 1,2-diphenylethylene congeners with hydroxyl groups on their benzene ring. Due to the hydroxyl groups which make it easy to inactivate radicals (Sabuncuoglu et al, 2008). Furthermore, the previous study also proved that the anti-oxidant properties might be interrelated with their structures, the conjugation between rings A and B via a planar C2 unsaturated structure allows electron delocalization across molecules for stabilization of radicals, leading to a strong anti-oxidant activity (Waffo Teguo et al, 1998). In combination of all the findings, it was implied that the four stilbenes suppressed the oxidative stress induced by corticosterone or glutamate could be via both direct and indirect antioxidant actions.

5. Conclusion

Concluded from the results, it has been demonstrated that the four stilbenes, cajaninstilbene acid, longistyline A, longistyline C, and cajanolactone A possess neuroprotection against the damage induced by corticosterone and glutamate in PC12 cells, and the cytoprotection of the four stilbenes may be via inhibiting oxidative stress. These results provide a scientific basis for the further studies to explore the potential neuroprotective effects on neurodegenerative diseases. However, the molecule mechanisms of the protection against oxidative stress need further research.

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; There is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in this manuscript.

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