Research article

Cognitive enhancing and antioxidant effects of tetrahydroxystilbene glucoside in A$\beta_{1\text{-}42}$-induced neurodegeneration in mice

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Abstract

Polyhydroxy stilbenes have been reported to possess various biological activities and have potential for treatment of Alzheimer's disease. Tetrahydroxystilbene glucoside is one of the major polyhydroxy stilbenes, which provides the underlying therapeutic activities for neuroprotective actions in various experimental conditions. This study investigates the impact of a tetrahydroxystilbene glucoside remedy for cognitive disorder and oxidative stress in A$\beta_{1\text{-}42}$-induced Alzheimer's disease mice and clarifies the mechanisms of action through the Kelch-like erythroid cell-derived protein with conserved non-coding homology-associated protein 1/nuclear factor erythroid 2-related factor pathway. It was found that the swimming time of A$\beta_{1\text{-}42}$-induced mice treated with tetrahydroxystilbene glucoside (30, 60, and 120 mg/kg) was significantly increased in the target quadrant of a Morris water maze experiment and the number of avoidances was increased during a passive avoidance experiment, Moreover, tetrahydroxystilbene glucoside attenuated A$\beta_{1\text{-}42}$-induced memory impairment. However, the locomotor and exploratory activity of mice were not affected. Tetrahydroxystilbene glucoside clearly decreased the levels of malondialdehyde and oxidized glutathione in both hippocampus and cortex compared with the A$\beta_{1\text{-}42}$-treated group, and also clearly increased the level of glutathione and activities of catalase and superoxide dismutase in those tissues. The results of this study demonstrated that tetrahydroxystilbene glucoside increased nuclear factor erythroid 2-related factor and heme oxygenase-1 protein expression and decreased Kelch-like erythroid cell-derived protein with conserved non-coding homology-associated protein 1 protein expression in a concentration-dependent manner in A$\beta_{1\text{-}42}$-treated mice, which involved the Kelch-like erythroid cell-derived protein with conserved non-coding homology-associated protein 1/nuclear factor erythroid 2-related factor antioxidant pathway in hippocampus and cerebral cortex tissue. These results demonstrate that tetrahydroxystilbene glucoside as a natural drug that may provide a potential treatment for Alzheimer's disease.

Keywords

Tetrahydroxystilbene glucoside; Alzheimer's disease; β-amyloid; Keap1/Nrf2 pathway; oxidative stress

Submitted: September 8, 2017; **Accepted:** October 23, 2017

Abbreviations

AD	Alzheimer's disease
Aβ	β-amylo
ICV	intracerebroventricular
MDA	malondialdehyde
GSH	glutathione
GSSG	oxidized glutathione
CAT	catalase
SOD	superoxide dismutase
Keap1	Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1
Nrf2	nuclear factor erythroid 2-related factor 2
ARE	antioxidant response elements
HO-1	heme oxygenase-1
Nrf2	NF-E2-related factor 2

1. Introduction

Among various dementia-linked diseases, Alzheimer's disease (AD) is the leading cause of disability and decreased quality of life [1]. It has the characteristics of progressive neuronal loss, intracellular neurofibrillary tangles, and extracellular β-amyloid (Aβ) deposits [2]. Aβ_{1-40} and A$\beta_{1\text{-}42}$ form the majority of the Aβ peptide found in human brain. Furthermore, as the primary core component of senile plaques, A$\beta_{1\text{-}42}$ is more toxic *in vitro* and *in vivo* [3].

Oxidative stress is extensive in AD brain. Many antioxidative defense proteins share common transcriptional control by the Kelch-like erythroid cell-derived protein with conserved non-coding homology (Enoyl-CoA hydratase)-associated protein 1 (Keap1)/nuclear factor erythroid 2-related factor (Nrf2) pathway [4]. Moreover, a recently elucidated pathway that induces antioxidant enzymes involves transcriptional activation through the antioxidant-responsive element (ARE), which is an indispensable cis-acting element for transcriptional activation of phase-II genes by electrophiles. Several lines of evidence suggest the involvement of the N rf2/ARE pathway

in the pathogenesis of AD [5]. Among phase-II enzymes, heme oxygenase-1 (HO-1) has attracted special attention for its therapeutic effects against neurodegenerative diseases. HO-1 oxidatively cleaves heme to biliverdin, forms CO, and releases chelated Fe^{2+} [6].

The Keap1/Nrf2 pathway can be activated by various exogenous and endogenous small molecules (inducers), such as some antioxidants [7]. Polyhydroxy stilbenes are neuroprotective through Nrf2-coordinated induction of endogenous cytoprotective proteins [8]. Tetrahydroxystilbene glucoside is one of the major polyhydroxy stilbenes. It has anti-inflammatory, antioxidant, and antiapoptotic properties which are useful for combating various diseases including, tumor, hepatic fibrosis, hypertension, and liver diseases [9]. Furthermore, tetrahydroxystilbene glucoside provides underlying therapeutic neuroprotective effects, effective treatment of learning and memory disorders, and prevents memory deficits in various experimental conditions [9, 10]. However, there is no detailed evidence for the neuroprotective effect of tetrahydroxystilbene glucoside through the Keap1/Nrf2 pathway.

The aim of this research is to investigate the impact of a tetrahydroxystilbene glucosideve remedy for cognitive disorder and oxidative stress in $A\beta_{1-42}$-induced mice and to clarify its mechanism of action by means of its effect on antioxidants in the brain (i.e. hippocampus and cerebral cortex) of $A\beta_{1-42}$-treated mice.

2. Materials and methods

2.1. Chemicals

Malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG), catalase (CAT), and superoxide dismutase (SOD) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Tetrahydroxystilbene glucoside, donepezil, and $A\beta_{1-42}$ were purchased from Sigma Aldrich (St. Louis, MO, USA). $A\beta_{1-42}$ was dissolved in 0.9% normal saline to get a stock solution of 1.0 mg/ml, then incubated at 37°C for 5 days to gain the fibrillized form.

2.2. Animals and treatment

Male ICR mice (20 ± 2 g) were acclimated to the experimental facility for one week and housed in stainless steel cages in a room with a 12:12-hour light-dark cycle, ambient temperature of 23 ± 1°C, and relative humidity of 55 ± 5%. Mice were allowed a standard rat chow diet and distilled water *ad libitum*. Mice were randomized into six groups and were treated as follows: Group I no treatment, Group II $A\beta_{1-42}$ lesioned plus intracerebroventricular (ICV), saline treated, Group III $A\beta_{1-42}$ lesioned plus ICV, donepezil 10 mg/kg treated, Group IV to VI $A\beta_{1-42}$ lesioned plus ICV, tetrahydroxystilbene glucoside (30, 60 or 120 mg/kg) treated. Mice in the control, donepezil, and tetrahydroxystilbene glucoside treated groups were anesthetized with pentobarbital sodium and injected with aggregated 3 μl $A\beta_{1-42}$ into the left lateral ventricle (AP, −0.5 mm, ML, −1.1 mm, DV, −3.0 mm) according to the mouse brain atlas. After the lesion operation, all lesioned mice received an intramuscular injection of penicillin- G 200 000 IU/ml (0.2 ml/mouse). They were then allowed to recover for three days before ICV injection of tetrahydroxystilbene glucoside or saline. After five consecutive days of ICV injection, behavioral tests such as the Morris water maze test and passive avoidance test were performed from day 8 to day 14, and brain biochemical and protein assessments were performed on day 15.

The investigation was conducted following the Guidelines for Animal Experimentation and the protocol was approved by the Animal Ethics Committee of the Animal Centre of Nanjing University of Chinese Medicine. All mouse tissues were authorized for scientific purpose.

2.3. Morris water maze test

The water maze is a large circular pool (90 cm diameter, 40 cm height) filled with water (20 ± 1°C) and white milk. The water maze area was divided into four equal quadrants and a white escape platform (10 cm diameter, 26 cm height) was submerged 1 cm below the surface of the water in the center of one quadrant. The place navigation test was performed as two trials daily for four consecutive days, and mice were placed in the water at two different starting points and allowed to swim freely to seek the hidden platform. If a mouse found the platform within 90 s and remained on it for at least two seconds, then the test was terminated. If the mice did not find the platform within 90 s, they were guided to it and allowed to stay there for 20 s. On the 5th day, the platform was removed and the mice were allowed to swim freely in the pool for 90 s. The escape latency time (seconds), total distance (cm) traveled to reach the platform, and the dwell time (seconds) in the target quadrant were assessed [11].

2.4. Passive avoidance test

One day after the Morris water maze test an automatic reflex conditioner (shuttle box) for active avoidance was used. Before the first trial of the learning session, the mice received five minutes to explore the shuttle-box so as to familiarize themselves with the learning environment. When the guillotine door was opened after 40 seconds and the mice moved into the dark compartment, the door closed automatically and an electric foot-shock (0.1 mA/10 g.bw) was delivered for two seconds through the grid floor. Twenty hours after the training trial, mice were placed back into the light compartment and the latency time was measured (up to 300 seconds), i.e. time taken to enter the dark compartment [12].

2.5. Brain tissue preparation

On the day after behavioral tests, all mice were sacrificed by cervical dislocation and the brain immediately removed. The hippocampus and cerebral cortex of the 10 mice in each group were dissected out, and stored at − 80°C until the biochemical studies.

2.6. Biochemical analysis

The hippocampus and cerebral cortex tissues were rapidly homogenized in ice-cold saline and centrifuged at 3500 rpm at 4°C for 15 min. The supernatant was collected and used to measure the activities of antioxidative enzymes including CAT and SOD as well as the levels of MDA, GSH, and GSSG by using the assay kits in accordance with manufacturer's directions [13].

2.7. Assessment of Keap1, Nrf2, and HO-1 expression

The expression of Keap1, Nrf2, and HO-1 in the brain were evaluated using Western blot analysis. Whole protein (both nuclear and cytosolic) was isolated from hippocampus and cerebral cortex tissue using a nuclear extraction kit (R&D Systems, USA), following manufacturer's instructions. Briefly, protein concentrations were determined

Table 1. Effect of tetrahydroxystilbene glucoside on escape latency time (seconds) of $A\beta_{1-42}$-treated mice in a spatial probe trial Morris water maze test.

Groups	1st day	2nd day	3rd day	4th day
Normal control	73.35 ± 4.29	70.72 ± 4.18	38.65 ± 2.04	25.71 ± 1.45
$A\beta_{1-42}$-treated	79.95 ± 3.78	$79.47 \pm 3.83^{\#}$	$64.62 \pm 3.20^{\#\#}$	$59.75 \pm 3.25^{\#\#}$
Donepezil + $A\beta_{1-42}$	80.01 ± 4.45	79.26 ± 4.11	$50.22 \pm 2.71^{*}$	$24.92 \pm 1.41^{**}$
TSG30 + $A\beta_{1-42}$	79.77 ± 3.92	79.35 ± 3.98	$54.38 \pm 2.85^{*}$	$30.71 \pm 1.72^{**}$
TSG60 + $A\beta_{1-42}$	79.38 ± 4.01	78.92 ± 4.34	$52.05 \pm 2.66^{*}$	$27.44 \pm 1.67^{**}$
TSG120 + $A\beta_{1-42}$	79.57 ± 4.73	78.79 ± 4.16	$48.97 \pm 3.01^{**}$	$24.69 \pm 1.38^{**}$

TSG30: stilbene glycoside (30 mg/kg), ICV; TSG60: stilbene glycoside (60 mg/kg), ICV; TSG120: stilbene glycoside (120 mg/kg), ICV; Donepezil, 10 mg/kg, ICV. Results expressed as mean S.E.M., $n = 10$. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, vs. $A\beta_{1-42}$-treated group.
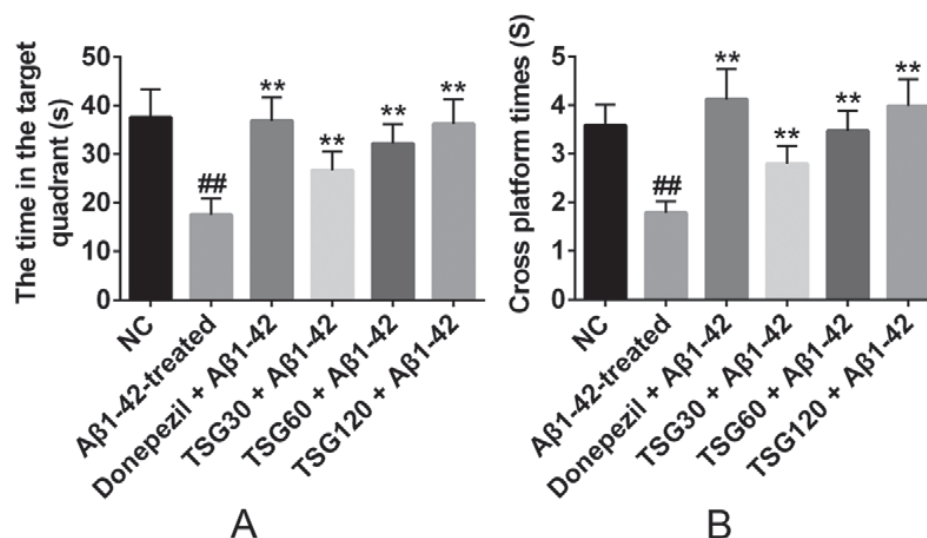

Fig. 1. Effect of tetrahydroxystilbene glucoside on performance of $A\beta_{1-42}$-treated mice in a Morris water maze test. NC: control, without treatment; $A\beta_{1-42}$-treated: received 1.0 mg/ml $A\beta_{1-42}$ 3 μl; Donepezil $A\beta_{1-42}$: received $A\beta_{1-42}$ donepezil (10 mg/kg), ICV; TSG30 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (30 mg/kg), ICV; TSG60 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (60 mg/kg), ICV; TSG120 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (120 mg/kg), ICV. Results are expressed as the mean S.E.M., $n = 10$. $^{\#\#}p < 0.01$, compared with the NC group; $^{**}p < 0.01$, vs. $A\beta_{1-42}$-treated group.

using the bicinchoninic acid (BCA) assay method. Proteins (20 μg per sample) were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane (Millipore, USA). Membranes were immunoblotted with specific primary antibodies (Santa Cruz Biotechnology) for anti-Keap1 (1: 500), anti-Nrf2 (1: 500), anti-HO-1 (1: 500), β-actin (1: 2000), and anti-H2A.Xb (1: 750) overnight at 4°C. After incubation with primary antibody, membranes were incubated with the appropriate secondary antibody for one hour and then incubated with chemiluminescence substrates. Western blot bands were quantified using the ChemiDoc MP system (Bio-Rad, USA).

2.8. Data analysis

All results are presented as mean $\pm$ SEM. Group differences in the escape latency and swimming distance in the Morris water maze test and the number of errors in the passageway water maze test were analyzed by SPSS 10.0 using Windows software to conduct two-way analysis of variance (ANOVA, equal variances assumed by S-N-K) on repeated measurements. Other data were analyzed by SPSS 10.0 using Windows software to conduct one-way ANOVA (equal variances assumed by S-N-K). $p < 0.05$ was considered a significant difference.

3. Results

As shown in Table 1, $A\beta_{1-42}$-treated mice took more time to reach the platform compared to the control group after injection of $A\beta_{1-42}$ ($n = 10, p < 0.01$). However, tetrahydroxystilbene glucoside and donepezil significantly ameliorated the effects of $A\beta_{1-42}$ on escape latency times. As shown in Fig. 1A, mice treated with $A\beta_{1-42}$ spent less time in the target quadrant than other groups. As shown in Fig. 1B, the cross platform times of mice treated with $A\beta_{1-42}$ was less than the control and tetrahydroxystilbene glucoside groups.

The effect of tetrahydroxystilbene glucoside on emotional learning and memory was detected in the passive avoidance test. As shown in Fig. 2, the escape latency time of the $A\beta_{1-42}$-treated group was significantly reduced when compared to that of the control group based on the test trial ($n = 10, p < 0.01$). A clear difference in the latency time was observed in the passive avoidance test. On the contrary, there was no significant difference detected for the latency time between the two groups during the acquisition trial of the passive avoidance test. Moreover, tetrahydroxystilbene glucoside with the 120 mg/kg group mice was observed with a longer latency time

Table 2. Effect of tetrahydroxystilbene glucoside on biochemical variables indicative of hippocampus in $A\beta_{1-42}$-treated mice.

Groups	MDA nmol/mg protein	GSH nmol/mg protein	GSSG nmol/mg protein	CAT U/mg protein	SOD U/mg protein
Normal control	2.5 + 0.12	10.8 + 0.57	0.23 + 0.01	24.5 + 1.23	79.1 + 4.29
$A\beta_{1-42}$-treated	4.6 + 0.23[##]	5.3 + 0.28[##]	0.52 + 0.03[##]	15.8 + 0.82[##]	24.0 + 1.31[##]
Donepezil + $A\beta_{1-42}$	2.7 + 0.13[**]	10.2 + 0.62[**]	0.27 + 0.02[**]	22.7 + 1.31[**]	72.4 + 3.75[**]
TSG30 + $A\beta_{1-42}$	3.6 + 0.17[**]	6.9 + 0.37[*]	0.40 + 0.02[**]	18.3 + 1.05[*]	51.6 + 2.66[**]
TSG60 + $A\beta_{1-42}$	3.1 + 0.21[**]	8.7 + 0.49[**]	0.34 + 0.02[**]	21.6 + 1.14[**]	67.9 + 3.69[**]
TSG120 + $A\beta_{1-42}$	2.5 + 0.14[**]	9.7 + 0.46[**]	0.29 + 0.01[**]	23.4 + 1.07[**]	75.3 + 4.02[**]

TSG30: stilbene glycoside (30 mg/kg), ICV; TSG60: stilbene glycoside (60 mg/kg), ICV; TSG120: stilbene glycoside (120 mg/kg), ICV; Donepezil, 10 mg/kg, ICV. Results expressed as mean S.E.M., $n = 10$. [##] $p < 0.01$, compared with the control group; [*] $p < 0.05$, [**] $p < 0.01$, vs. $A\beta_{1-42}$-treated group.

Table 3. Effect of tetrahydroxystilbene glucoside on biochemical variables indicative of cerebral cortex in $A\beta_{1-42}$-treated mice.

Groups	MDA nmol/mg protein	GSH nmol/mg protein	GSSG nmol/mg protein	CAT U/mg protein	SOD U/mg protein
Normal control	3.7 + 0.20	11.5 + 0.56	0.21 + 0.01	22.6 + 1.17	83.2 + 4.67
$A\beta_{1-42}$-treated	6.1 + 0.32[##]	5.9 + 0.31[##]	0.50 + 0.03[##]	14.7 + 0.85[##]	44.3 + 2.58[##]
Donepezil + $A\beta_{1-42}$	4.4 + 0.23[**]	10.7 + 0.59[**]	0.28 + 0.01[**]	21.3 + 1.06[**]	78.0 + 3.42[**]
TSG30 + $A\beta_{1-42}$	5.3 + 0.27[*]	7.4 + 0.41[*]	0.44 + 0.03[*]	17.9 + 0.92[**]	61.5 + 3.39[**]
TSG60 + $A\beta_{1-42}$	4.7 + 0.24[**]	9.2 + 0.48[**]	0.37 + 0.02[**]	20.8 + 1.09[**]	69.7 + 4.11[**]
TSG120 + $A\beta_{1-42}$	4.2 + 0.25[**]	10.3 + 0.50[**]	0.32 + 0.02[**]	21.5 + 0.93[**]	80.3 + 4.38[**]

TSG30: stilbene glycoside (30 mg/kg), ICV; TSG60: stilbene glycoside (60 mg/kg), ICV; TSG120: stilbene glycoside (120 mg/kg), ICV; Donepezil, 10 mg/kg, ICV. Results expressed as mean S.E.M., $n = 10$. [##] $p < 0.01$, compared with the control group; [*] $p < 0.05$, [**] $p < 0.01$, vs. $A\beta_{1-42}$-treated group.

than the donepezil group. These results indicated that tetrahydroxystilbene glucoside attenuated $A\beta_{1-42}$-induced memory impairment without affecting the locomotor and exploratory activity of the mice.
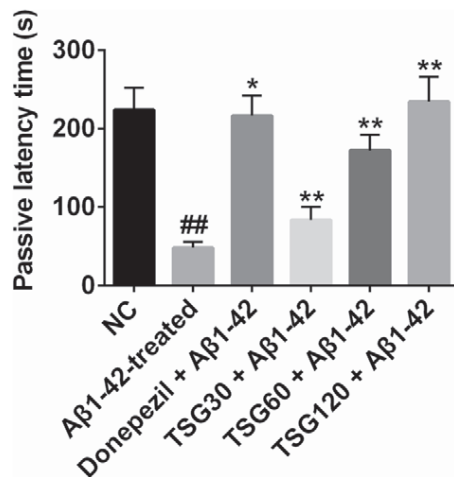

Fig. 2. Effect of tetrahydroxystilbene glucoside on $A\beta_{1-42}$-treated mice in a passive avoidance test. Latency time (seconds) to enter dark compartment was recorded. NC: control, without treatment; $A\beta_{1-42}$-treated: received 1.0 mg/ml $A\beta_{1-42}$ 3 µl; Donepezil $A\beta_{1-42}$: received $A\beta_{1-42}$ donepezil (10 mg/kg), ICV; TSG30 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (30 mg/kg), ICV; TSG60 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (60 mg/kg), ICV; TSG120 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (120 mg/kg), ICV. Results expressed as mean S.E.M., $n = 10$. [##] $p < 0.01$, compared with the NC group; [*] $p < 0.05$, [**] $p < 0.01$, vs. $A\beta_{1-42}$-treated group.

As shown in Table 2 and Table 3, MDA and GSSG levels in hippocampus and cerebral cortexin $A\beta_{1-42}$-treated mice were significantly higher than the control group ($n = 10, p < 0.01$). How-

ever, the GSH level in $A\beta_{1-42}$-treated mice was greatly reduced compared to the control group both for hippocampus and cerebral cortex ($n = 10, p < 0.01$). Also, CAT and SOD activities in hippocampus and cerebral cortexin $A\beta_{1-42}$-treated mice were much lower than the control group ($n = 10, p < 0.01$). After treatment, tetrahydroxystilbene glucoside and donepezil clearly decreased the levels of MDA and GSSG in both hippocampus and cortex compared to the $A\beta_{1-42}$-treated group, and clearly increased the level of GSH and activities of CAT and SOD in those tissues. Moreover, in the three tetrahydroxystilbene glucoside groups, the high dose of tetrahydroxystilbene glucoside was most effective in decreasing MDA and GSSG levels and increasing the GSH level and CAT and SOD activities.

As shown in Fig. 3 and Fig. 4, the expression of Keap1 in hippocampus and cerebral cortex tissue was significantly increased compared with normal mice. The expression of Nrf2 and its downstream gene, HO-1, were significantly decreased compared with normal mice. After treatment with tetrahydroxystilbene glucoside, the expression of Nrf2 and HO-1 was significantly increased and Keap1 expression was decreased in the donepezil and the three tetrahydroxystilbene glucoside groups compared with the $A\beta_{1-42}$-treated group. Immunofluorescence showed Nrf-2 expressed in the cytoplasm and nucleus, so Nrf2 accumulates, translocates to the nucleus, and induces transcription of cytoprotective genes, while simultaneously, the Keap protein was only expressed in the cytoplasm (see Fig. 5).

4. Discussion

In this study, the effects of tetrahydroxystilbene glucoside on $A\beta_{1-42}$-induced memory impairment and brain oxidative stress were evaluated and investigated as a potential treatment agent for AD. Behavioral tests indicated that tetrahydroxystilbene glucoside clearly alleviated cognitive deficits validated by $A\beta_{1-42}$-induced mouse models of AD *in vivo*. Donepezil is one of the few drugs approved by
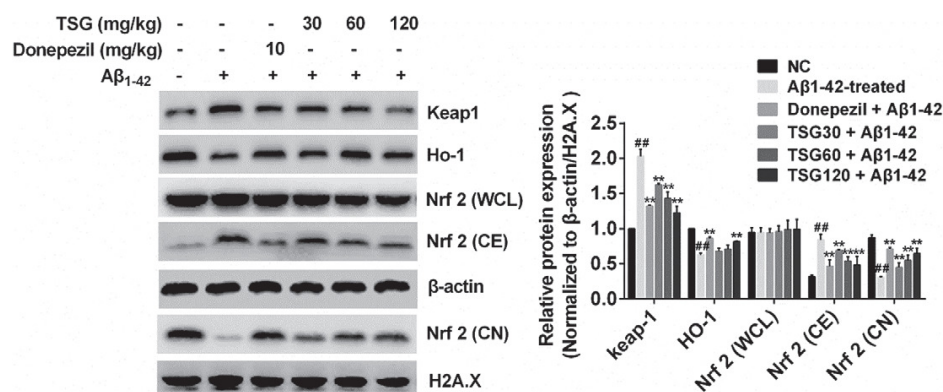
Fig. 3. Tetrahydroxystilbene glucoside activates Keap1/Nrf2 pathway in mouse hippocampus with or without $A\beta_{1-42}$. NC: control, without any treatment; $A\beta_{1-42}$-treated: received 1.0 mg/ml $A\beta_{1-42}$ 3 µl; Donepezil $A\beta_{1-42}$: received $A\beta_{1-42}$ donepezil (10 mg/kg), ICV; TSG30 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (30 mg/kg), ICV; TSG60 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (60 mg/kg), ICV; TSG120 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (120 mg/kg), ICV. Results expressed as mean S.E.M. $^{\#\#}p < 0.01$, compared with the NC group; $^{*}p < 0.05$, $^{**}p < 0.01$, vs. $A\beta_{1-42}$-treated group.
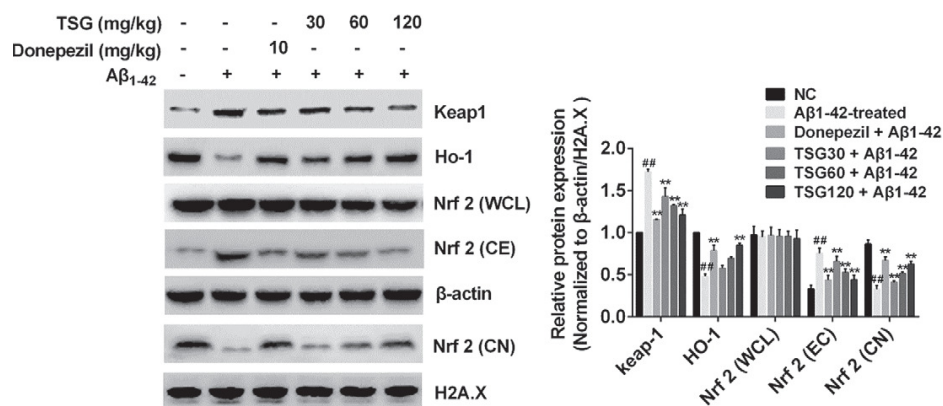

Fig. 4. Tetrahydroxystilbene glucoside activates Keap1/Nrf2 pathway in mouse cerebral cortex with or without $A\beta_{1-42}$. NC: control, without any treatment; $A\beta_{1-42}$-treated: received 1.0 mg/ml $A\beta_{1-42}$ 3 µl; Donepezil $A\beta_{1-42}$: received $A\beta_{1-42}$ donepezil (10 mg/kg), ICV; TSG30 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (30 mg/kg), ICV; TSG60 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (60 mg/kg), ICV; TSG120 $A\beta_{1-42}$: received $A\beta_{1-42}$ tetrahydroxystilbene glucoside (120 mg/kg), ICV. Results are expressed as mean S.E.M. $^{\#\#}p < 0.01$, compared with the NC group; $^{*}p < 0.05$, $^{**}p < 0.01$, vs. $A\beta_{1-42}$-treated group.
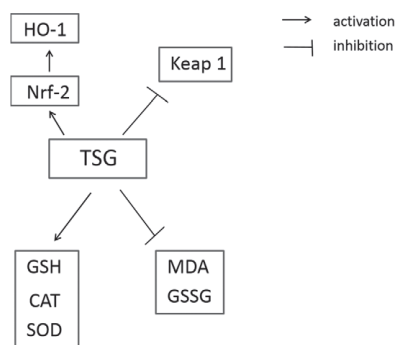

Fig. 5. The schematic pathway.

the Food and Drug Administration for the treatment of AD [14]. Therefore it was used as a positive control based on its clear memory improvement and antioxidant activity.

The Morris water maze test was designed to assess spatial learning and memory and has been linked to long-term potentiation (LTP) [15]. In this test, the escape latency time in the spacial probe trial, the time spent in the platform quadrant and crossing the platform, were used to evaluate spatial learning and memory. Different doses of tetrahydroxystilbene glucoside clearly attenuated increased escape latency time in the target quadrant, when compared with the $A\beta_{1-42}$-treated group ($p < 0.01$). Donepezil, as a positive control drug, exhibited a similar effect compared to that of tetrahydroxystilbene glucoside groups.

The passive avoidance test is a fear-motivated avoidance test used for evaluating long-term memory, or reference memory [11]. Both the tetrahydroxystilbene glucoside and donepezil treated groups significantly extended the time taken by mice to move into the dark compartment, as compared with the time taken by the mice in the $A\beta_{1-42}$-treated group. These results show that tetrahydroxystilbene glucoside treatment ameliorates $A\beta_{1-42}$-induced memory deficit and could improve long-term memory.

Additionally, there were no significant differences among all groups for the mean swimming speed in the Morris water maze test. Also, in the passive avoidance test, the latency time of the acquisition

trial for each group showed no clear differences between groups. However, there was a significant difference for the latency time observed in the test. These results suggested that tetrahydroxystilbene glucoside and donepezil have no effect on the locomotor activity of mice.

Oxidative stress is considered to be a key factor in the pathogenesis of AD and mild cognitive impairment. SOD and CAT form the first line of defense against reactive oxygen species and the decrease in their activity contributes to the oxidative stress in tissue [16]. MDA is one of the main results of lipid peroxidation, which can directly damage cell membranes. The improvement of the activities of CAT, SOD and the level of GSH, and the decrease of the MDA and GSSG indicated that tetrahydroxystilbene glucoside could restore the abnormal biochemical changes. Among the three doses of the tetrahydroxystilbene glucoside treatment groups, the high dose was most effective in increasing the activity of CAT and SOD and decreasing the level of MDA and GSSG in the hippocampus and cerebral cortex tissue.

Keap1 is proposed as a sensor protein of electrophilic compounds and a transducer of the signal from these compounds for transcriptional activation [17]. Nrf2 is a transcription factor that activates expression of antioxidant and cytoprotective genes by binding to AREs within DNA [18]. Many studies show that modification of cysteine residues of the sensor protein Keap1 result in the loss of its Nrf2-binding capacity. As a consequence, Nrf2 accumulates, translocates to the nucleus, and induces transcription of cytoprotective genes [5]. These genes include antioxidative enzymes, anti-inflammatory mediators, the proteasome, and several transcription factors involved in mitochondrial biogenesis [4]. Antioxidant enzymes genes are a set of genes encoding phase-II enzymes, including HO-1, NADPH quinone oxidoreductase 1, and γ-glutamyl cysteine ligase (γ-GCL). These enzymes provide efficient cytoprotection, in part by regulating the intracellular redox state [6].

Currently, various therapeutic approaches are applied to prevent and treat a wide spectrum of neurological disorders. However, their efficacy still remains uncertain [1]. Tetrahydroxystil-bene glucoside, one of the major polyhydroxy stilbenes, displays neuroprotective effects in experimental models of AD *in vitro* and *in vivo* [10]. However, the relationship between the antioxidant activity of tetrahydroxystilbene glucoside and its neuroprotective effect remains unclear. The results of this study confirm that a common oxidative stress mechanism is responsible for the toxicity of $A\beta_{1-42}$, which corroborates previous studies [3, 13, 19].

Moreover, tetrahydroxystilbene glucoside significantly protected brain from $A\beta_{1-42}$-induced damage by activating the Keap1/Nrf2 pathway. It was demonstrated that tetrahydroxystilbene glucoside increases Nrf2 and HO-1 protein expression and decreases Keap1 protein expression in a concentration-dependent manner in $A\beta_{1-42}$-treated mice.

5. Conclusion

The present investigation demonstrated that tetrahydroxystilbene glucoside potentially reversed alterations in cognitive behavioral, biochemical changes, and oxidative damage induced by $A\beta_{1-42}$ in mice. These beneficial effects of tetrahydroxystilbene glucoside were obtained partly by inhibiting the expression of Keap1/Nrf2 pathway in hippocampus and cerebral cortex tissue. These findings indicate

that tetrahydroxystilbene glucoside could be a potent natural drug in the treatment of Alzheimer's disease.

Acknowledgments

Conflict of Interest

All authors declare no conflicts of interest.

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