PHYTOTHERAPY RESEARCH
Phytother. Res. **29**: 1137–1144 (2015)
Published online 21 April 2015 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5358

The Mechanism of Memory Enhancement of Acteoside (Verbascoside) in the Senescent Mouse Model Induced by a Combination of D-gal and $AlCl_3$

Xiao-Ming Peng,[1] Li Gao,[1] Shi-Xia Huo,[1] Xin-Min Liu[2] and Ming Yan[1]*

[1]Prescription Laboratory of Xinjiang Traditional Uyghur Medicine, Xinjiang Institute of Traditional Uyghur Medicine, Urumqi, Xinjiang 830049, China
[2]Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100193, China

Acteoside (verbsacoside), one of the main active phenylethanoid glycosides from *Cistanche deserticola*, is known to have antioxidant and neuroprotective activity, and herbs containing it are used to enhance memory. However, there is relatively little direct experimental evidence to support the use of acteoside in Alzheimer's disease (AD). The purpose of this study was to elucidate the effects of acteoside in improving learning and memory, using a mouse model of senescence induced by a combination of D-galactose and $AlCl_3$, and investigate its potential mechanisms compared with the positive controls vitamin E and piracetam. Acteoside was administered intragastrically at doses of 30, 60 and 120 mg/kg/day for 30 days after AD was induced. Memory function was evaluated using a step-down test. The number of neuron was analysed by haematoxylin and eosin staining and the number of Nissl bodies by Nissl staining. The expression of caspase-3 protein in hippocampus was detected by immunohistochemistry and western blot. Nitric oxide and total nitric oxide synthase level in hippocampus were also assessed. Our results showed that the latency of step down was shortened in AD model mice and the number of errors decreased after treatment with all doses of acteoside. Neurons and Nissl bodies in the hippocampus were increased significantly with higher doses (60 and 120 mg/kg/day) of acteoside. The content of nitric oxide, the activity of nitric oxide synthase and the expression of caspase-3 protein were decreased by 120 mg/kg/day acteoside compared with that of the AD model group. Our results support the results obtained previously using the Morris maze test in the same mouse model of senescence, and the use of traditional medicinal herbs containing acteoside for neuroprotection and memory loss. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: acteoside; D-gal; $AlCl_3$; Alzheimer's disease; mouse; oxidative stress; nitric oxide; nitric oxide synthase; caspase-3; *Cistanche deserticola*.

INTRODUCTION

Alzheimer's disease (AD), the leading cause of dementia, is characterized by a progressive decline in cognitive function, which typically begins with deterioration in memory (Reitz *et al.,* 2011). AD affects 35.6 million people worldwide and is associated with an estimated health-care cost of over US$600 billion in 2010 (Weiner *et al.,* 2012). Currently available therapeutic strategies are mainly targeted at specific symptoms of AD, such as cholinergic activity, neurotrophins, statins, non-steroidal antiinflammatory drugs, hormone replacement therapy, blocking excitotoxicity, Aβ vaccines, immunotherapy and secretase effectors (Castellani *et al.,* 2010). Antioxidant therapy as a therapeutic strategy for AD has also been studied (Feng and Wang, 2012), because oxidative stress is an important pathogenic process associated with AD, and markers of oxidative stress have been shown to precede pathological lesions in AD (Castellani *et al.,* 2010). Antioxidants therefore may blunt the cognitive decline in AD or slow disease progression (Jama *et al.,* 1996; Perrig *et al.,* 1997).

Cistanche deserticola Y. C. Ma (Orobanchaceae) is one of the most popular traditional Chinese herbal medicines and is widely used in China for treating age-related disorders including senile dementia (Jiang and Tu, 2009). Acteoside (verbascoside), one of the main active phenylethanoid glycosides from *Cistanche deserticola* and present in many other important medicinal herbs (He *et al.,* 2011), has been shown to possess antioxidative, hepatoprotective, anti-viral, anti-metastatic, antiinflammatory and neuroprotective activities (Koo *et al.,* 2006; Peng *et al.,* 2013). Previous studies have shown that acteoside can markedly reduce cerebral injury in mice induced by D-galactose (Gao *et al.,* 2013) and significantly alleviate acquired learning disability in mice induced by scopolamine (Lin *et al.,* 2012). In the Morris water maze test, acteoside (30–120 mg/kg/day) reduced the escape latency and increased the number of crossings of the platform as well as significantly increasing the expression of nerve growth factor and tropomycin receptor kinase mRNA in the hippocampus in the same D-galactose and $AlCl_3$-induced mouse model of senescence (Gao *et al.,* 2015). The present study aims to further characterize the effects of acteoside in improving learning and memory in this model.

* Correspondence to: Ming Yan, Prescription Laboratory of Xinjiang Traditional Uyghur Medicine, Xinjiang Institute of Traditional Uyghur Medicine, Urumqi, Xinjiang, 830049, China.
E-mail: yanming21cn@sohu.com

Received 24 October 2014
Revised 27 February 2015
Accepted 24 March 2015

MATERIALS AND METHODS

Chemicals and reagents. Acteoside (Purity ≥ 93.3%) was purchased from the China National Institutes for Food and Drug Control (Beijing, China); D-galactose from Sigma-Aldrich Co. (USA) and $AlCl_3 \cdot 6H_2O$ from Guangfu chemical Co. (Tianjin, China).

Animals and treatment. For 7 days adaptation before the experiment, 140 Kunming strain mice (18 ± 2 g, Experimental Animal Center of Xinjiang) were housed in cages in an air-conditioned room under controlled temperature (23 ± 2 °C) and humidity (40–60%). Briefly, mice were randomly divided into seven groups of 20 animals: the vehicle (negative) control group, the AD model group, two positive control groups, treated with piracetam or vitamin E, and the acteoside-treated AD groups at three doses (30, 60 and 120 mg/kg/day). D-galactose and $AlCl_3$ were dissolved in sterile normal saline. Mice in the model and acteoside-treated groups were intraperitoneally injected with D-gal (60 mg/kg/day) and intragastrically with $AlCl_3$ (5 mg/kg/day) for 90 days (Luo *et al.,* 2009), while mice in the negative control group were treated with the same volume of sterile saline. Starting at day 61, after daily treatment with D-gal and $AlCl_3$, mice in the two positive control groups and the test groups were administered piracetam (300 mg/kg/day), vitamin E (150 mg/kg/day) or acteoside intragastrically at doses of 30, 60 and 120 mg/kg/day in 0.5% sodium carboxymethylcellulose for 30 days. Mice in the negative control and AD model groups were administered with the same volume of sodium carboxymethylcellulose for these 30 days.

Ethics. Animals were treated according to the guidelines of the Regulations for Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China on 14 November 1988. The experiments were carried out under the approval of the Animal Ethics committee of the Xinjiang Institute of Traditional Uyghur Medicine.

Step-down test. On day 89, after two hours of the treatment with D-gal and $AlCl_3$, memory of context in mice was tested by the step-down test. The step-down apparatus (Yiyan, Shandong, China) consisted of a transparent plastic cubicle (25 × 25 × 25 cm) with a stainless-steel grid floor (33 rods 2 mm in diameter) onto which a square wooden platform (7 × 7 × 1.5 cm) was placed, with an electric shock apparatus to deliver an alternating electric current. Ten animals from each group of 20 were trained not to step down from the platform inside the cylinder for 30 s, so as to prevent them from jumping down immediately after being put on the platform. After removal of the cylinder, the time taken for the animal to leave the platform with all four paws was measured as the step-down baseline latency period. Immediately upon step down, mice received a single electric shock (2.0 mA) to the foot, which caused them to return to their home cages. The shock continued to be delivered to the grid and the number of times the animals stepped down from the platform within 5 min was considered the error frequency. Twenty-four hours later, during the recall trial session, the same electric shock as in the training session was given on animal's foot. Latency of step down and error frequency with all four paws was measured until 300 s had elapsed. According to previously validated criteria for the acquisition of the step-down avoidance task, an increase in latency or decrease in error during the recall session is taken as a sign of improvement in memory of context (Wang *et al.,* 2010).

Tissue preparation. After the behavioural tests were completed, the mice were sacrificed by decapitation under anaesthesia with 10% chloral hydrate and the brains rapidly removed and cleaned with saline over the ice. Ten brains from each group of 20 were hemisected, and one hemisphere was immediately stored at −80 °C for western blot analysis. The other hemisphere was submerged in 4% formaldehyde for pathomorphology and immunohistochemistry. The remaining hippocampi were homogenized with ice-cold saline to prepare a 10% cerebral homogenate for the biochemical measurements.

Biochemical measurements. Quantitative detection of nitric oxide (NO) and total nitric oxide synthase (NOS) levels in hippocampus was performed using commercial measurement kits according to the manufactures' protocols (Nanjing Jiancheng Bioengineering Co Ltd., China). Absorbance of each NO sample was measured at 550 nm and NOS at 530 nm using a microplate reader (Multiskan Go 1510, ThermoFisher, USA).

Pathomorphology. To confirm the presence of neuropathological alterations induced by combined treatment with D-gal and $AlCl_3$, haematoxylin and eosin (HE), and Nissl staining, were performed using standard histological techniques. The whole hippocampus was fixed in 4% paraformaldehyde in 0.01 M Phosphate Buffer saline (PBS) (pH 7.4) at 4 °C for 12 h, dehydrated and embedded in paraffin. Sections were cut into 5 μm slices, deparaffinized and then rehydrated using a gradient of high-percentage ethanol to distilled water and were stained with HE for the observation of neuron morphology and with Nissl staining for Nissl bodies.

Haematoxylin/eosin staining. Deparaffinized and rehydrated sections were washed in distilled water three times, stained with alum haematoxylin for 20 min and rinsed with running tap water three times. They were subsequently immersed in 1% acid alcohol for 5 s, stained blue using 1% Scott's tap water substitute for 10 s and then rinsed in tap water for 5 min. The nuclear staining was followed by counterstaining with eosin for 2 min, dehydrated with 95% alcohol, cleared in xylene and mounted with resinous mounting medium for microscopic examination. To evaluate the level of neuron morphology, sections were examined under a light microscope (Leica, Germany). Photos of typical lesions were randomly taken and blindly coded from ten fields in consecutive sections of the hippocampus, using 40-fold magnification and a 1 × 1 mm grid (Fischer *et al.,* 2008).

Nissl staining. Tissue slices (5 μm thick) were mounted on polysine-coated slides and stained for 5 min under agitation

with 0.1% Nissl staining solution (Beyotime, Haimen, China) dissolved in distilled water and filtered. Slices were dehydrated for 2 min using anhydrous ethanol, cleared in xylene for another 2 min and covered with Distyrene Plasticizer Xylene (DPX) mountant (Sigma-Aldridge, USA) and a coverslip. After that, the slices were hydrated Milli-Q water (Millipure Corp, USA) for 30 min and then placed under agitation in Nissl staining solution for 5 min before being rinsed in Milli-Q water. The whole hippocampus slices were progressively dehydrated in 70% alcohol (for 10 min), 95% alcohol (with a few drops of 10% acetic acid; for 3 min) and finally anhydrous ethanol (for 10 min). Slices were cleared in xylene for 5 min before being covered with DPX and a coverslip (Pilati *et al.,* 2008).

Immunohistochemistry and image analysis. Immunohistochemistry was used to detect expression levels of caspase-3 in the samples. The endogenous peroxidase activity of sectioned tissues was blocked with 3% H_2O_2 for 10 min and rinsed with running tap water and PBS (0.01 M, pH 7.2–7.4) three times, respectively. The sections were incubated at 4 °C overnight with rabbit anti-caspase 3 (1:200, ab13847, Abcam, Cambridge, UK) in tris-buffered saline. Subsequently, biotinylated goat anti-rabbit immunoglobulin G (IgG) secondary antibody (ab6721, Abcam, Cambridge, UK) was applied, followed by incubation at 37 °C for 25 min. After rinsing with PBS three times, the hippocampus sections were treated with strept avidin–biotin complex (Boster Co. Wuhan, China) at room temperature for 20 min, and developed in a diaminobenzidine reaction with 3,3N-Diaminobenzidine Tertrahydrochloride (DAB) Horseradish Peroxidase Color Development Kit (Beyotime, Haimen, China), as described for the hippocampus sections. After clearing with xylene, all tissue sections were mounted in general clarity gum for image analysis. Positive cells (coloured yellow) were counted in six fields using the Image-Pro Plus v6.0 analysis system (Media Cybernetics, USA) and were observed at high magnification (×40) (DM2500, Leica, Germany). The numbers of positive cells were calculated as percentages.

Western blot analysis. Protein from the hippocampus was extracted and concentrations determined using a bicinchoninic acid protein assay kit (Nanjing Jiancheng Bioengineering Co Ltd., China). The protein samples (50 μm each) were separated using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and blotted on to polyvinylidene fluoride membranes. The membranes were subsequently probed with primary antibodies against capase-3 (1:1000, ab13847, Abcam, Cambridge, UK). β-actin (1:3000, ab8227, Abcam, Cambridge, UK) was used as loading control. The proteins were detected using horseradish peroxidase-conjugated anti-rabbit secondary antibodies and visualized using WesternBreeze chemiluminescent kit (Thermo Fisher Scientific Inc, USA). The intensity of each band was quantified by densitometry with the 3500R calibrated densitometer (Tannon, China).

Statistical analysis. Results are expressed as mean ± SD. Data were analysed by one-way analysis of variance followed by Tukey post-hoc test for multiple comparisons with $p < 0.05$ considered as being statistically significant.

RESULTS

Effect of acteoside on memory of context deficits in senescent mice

Deficits in memory of context were observed in the AD model group. The times when mice stepped down from the platform within 5 min were considered as acquisition errors. The following day, this procedure was repeated, and the times they stepped down onto the platform within the 5-min interval were recorded as retention errors. Fig. 1B shows that the number of acquisition errors and retention errors in the AD model group was significantly increased ($p < 0.05$), relative to the control group. The acteoside-treated mice showed significantly fewer learning [F (6, 63) = 3.405. $p < 0.05$] and memory errors [F (6, 63) = 4.301. $p < 0.05$] than the AD model (untreated) group, although this effect was not dose-related. The vitamin E positive control showed a similar, statistically significant, effect on reduction of the number of errors as the lowest dose of acteoside, whereas piracetam produced an apparent improvement that was not statistically significant in this experiment (Fig. 1B). Neither of the positive controls, unlike acteoside at all doses, produced a significant reduction in latency (Fig. 1A).

Effect of acteoside on nitric oxide and total nitric oxide synthase activity in hippocampus

Table 1 shows that chronic administration of D-gal and $AlCl_3$ caused a significant increase in NO and total NOS activity in hippocampus as compared with the negative control mice ($p < 0.05$). However, chronic high-dose (120 mg/kg/day) acteoside treatment significantly decreased total NOS activity [F (6, 63) = 5.576. $p < 0.05$] and inhibited the production of NO [F (6, 63) = 3.227. $p < 0.05$], compared with the AD model group.

Pathomorphological changes in the hippocampus

There were typical neuropathological changes in the hippocampus in the senescent model mice induced by a combination of D-gal and $AlCl_3$. Fig. 2A shows the histopathological architecture of the hippocampus after HE staining. In the control group, neurons were arranged tightly and were purplish red in colour, and the nuclear were large, round and lightly stained. Remarkable neuronal damage was seen in the hippocampus of the AD model mice, with the number of neurons significantly decreased ($p < 0.05$) and their arrays sparse and disordered, compared with control mice. After acteoside treatment for 30 days, the number and morphology of neurons improved significantly [F (6, 63) = 2.713. $p < 0.05$] (Fig. 2A), and Nissl bodies in the hippocampus of control mice (stained blue-violet) were more numerous in the mice treated with the higher doses of acteoside (Fig. 2B). Compared with control mice, the number of Nissl bodies was decreased significantly ($p < 0.05$) in the AD model mice (Fig. 2B), but administration of acteoside increased them [F (6, 63) = 2.722. $p < 0.05$]. Administration of either vitamin E or piracetam decreased the number of damaged neurons and

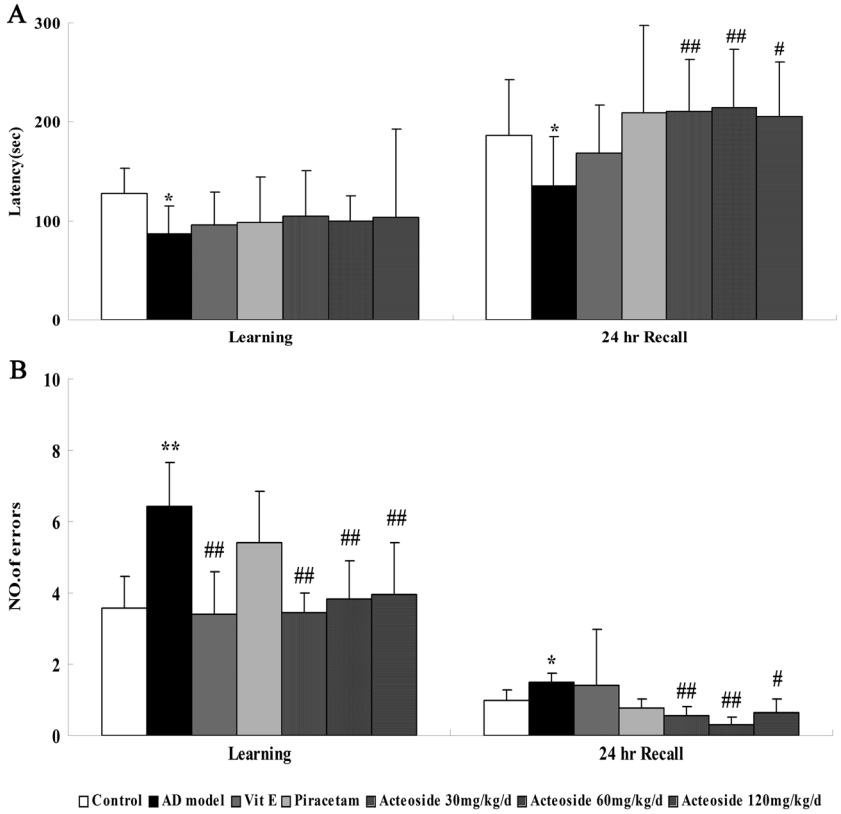

Figure 1. Effect of acteoside on memory of context of Kunming strain mice. The step-down latency (A) and number of errors (B) were automatically recorded in the step-down test. Data are presented as mean ± SD, $n = 10$. $*p < 0.05$ or $**p < 0.01$ versus control group; $\#p < 0.05$ or $\#\#p < 0.01$ versus Alzheimer's disease (AD) model group (one-way analysis of variance).

Table 1. Effect of acteoside on nitric oxide content and total nitric oxide synthase activity in hippocampus (mean ± SD, $n = 10$)

Experimental group	Dosage (mg/kg/day)	NO (μmol/mg protein)	NOS (U/mg protein)
Control	—	2.85 ± 0.78	0.42 ± 0.05
AD model	—	4.93 ± 0.93*	0.81 ± 0.05*
Vitamin E	150	2.51 ± 0.72***	0.46 ± 0.05***
Piracetam	300	1.35 ± 0.36***	0.34 ± 0.03***
Acteoside	30	3.93 ± 0.27	0.47 ± 0.04***
Acteoside	60	3.00 ± 0.58**	0.89 ± 0.05
Acteoside	120	1.55 ± 0.08***	0.51 ± 0.03***

AD, Alzheimer's disease; NO, nitric oxide; NOS, total nitric oxide synthase.
$*p < 0.01$ versus control group.
$**p < 0.05$.
$***p < 0.01$ versus AD model group (one-way analysis of variance).

increased the number of Nissl bodies, but in both cases, the reduction was not statistically significant (Fig. 2A and B).

Effect of acteoside on caspase-3 expression in the hippocampus

Caspase-3 is over-expressed in AD. Levels of caspase-3 were examined in hippocampus by immunohistochemistry. Fig. 3 shows that labelling was visually greater in the model AD group than the controls, and that caspase-3 immunoreactivity in cytoplasm was clearly above that of the background, especially in the deeper layers. After administration of acteoside for 30 days, semiquantitative analysis of positive caspase-3 immunoreactivity in the hippocampus showed a significant decrease [F (6, 63) = 14.384. $p < 0.05$] compared with the model AD group. Piracetam, but not vitamin E, also decreased the levels of caspase-3 in the AD mice (Fig. 3).

Quantitative analysis of caspase-3 in the hippocampus

Quantitative analysis of caspase-3 using western blot was also carried out, and caspase-3 levels in the hippocampus in the AD model mice were significantly higher compared with those in the control mice. These levels were significantly reduced by the administration of 60 and 120 mg/kg/day of acteoside [F (6, 63) = 3.937. $p < 0.05$] (Fig. 4).

DISCUSSION

The aetiology of AD is still incompletely understood. Converging evidence suggests that oxidative stress may be a critical mediator of cognitive impairments such as those found in AD (Harrison *et al.,* 2009). The senescent mouse model induced by D-gal and $AlCl_3$ is widely used for studying the mechanisms of AD and for drug screening (Luo *et al.,* 2009; Sun *et al.,* 2009). These mice show AD-like pathological changes, which are believed to be mediated through various mechanisms including oxidative stress (Yang *et al.,* 2014). D-galactose is a

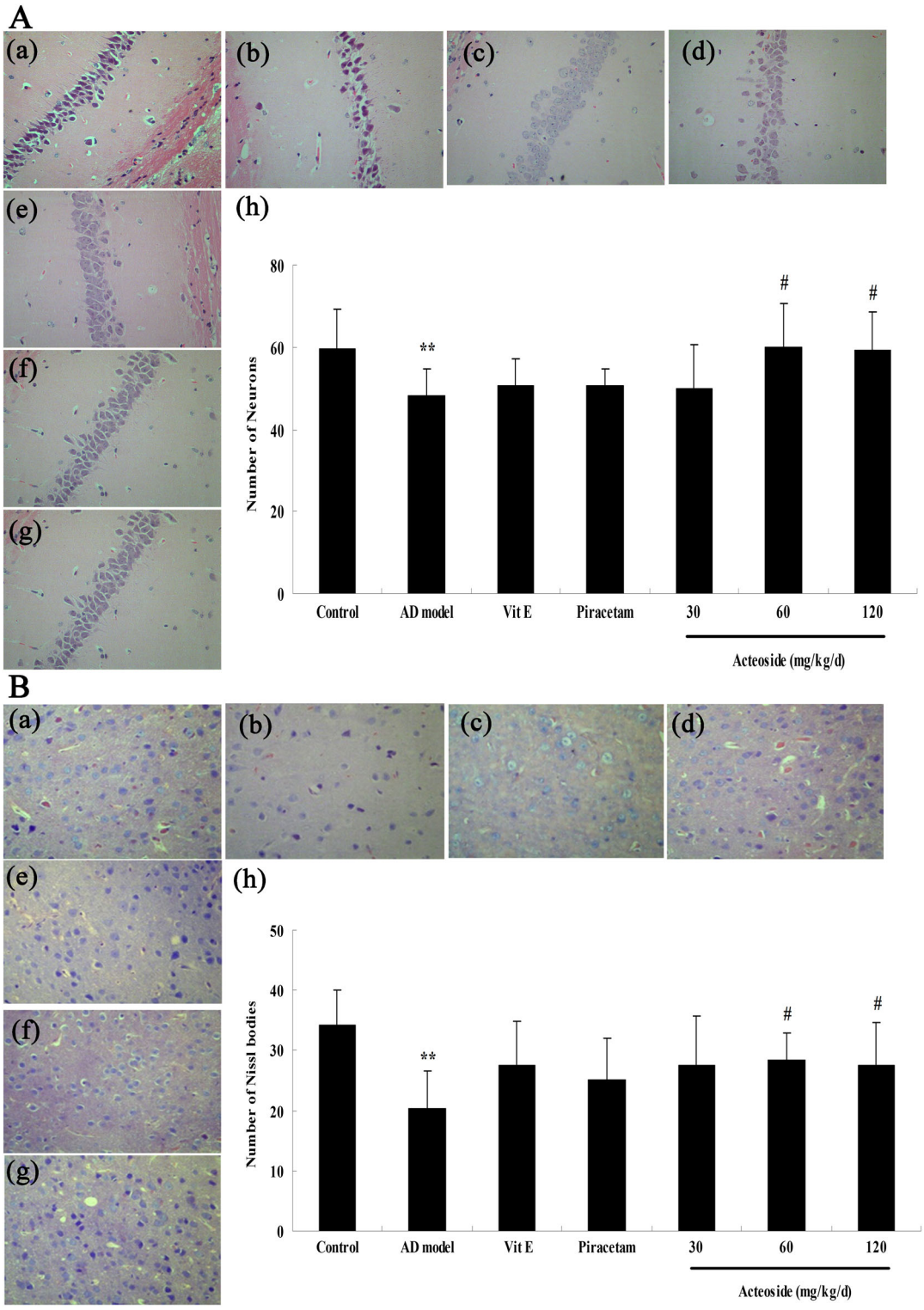

Figure 2. Effect of acteoside on the histopathological architecture of the hippocampus. (A) Haematoxylin and eosin staining of the hippocampus (magnification ×40) revealed the progression of neuronal apoptosis. (B) Nissl staining of the hippocampus (magnification ×40) revealed the progression of neuronal damage. (a) Control group: neurons or Nissl bodies were intact and arrange tightly; (b) Alzheimer's disease (AD) model group: neurons or Nissl bodies arranged dispersedly and the number decreased; (c, d) positive control groups: administration of vitamin E and piracetam increased the number of neurons or Nissl bodies, but not statistically significantly; (e–g) long-term administration of acteoside markedly increased the number of neurons or Nissl bodies; (h) Calculation of the number of neurons or Nissl bodies. Data are presented as mean ± SD, $n = 10$. $^{**}p < 0.01$ versus control group; $\#p < 0.05$ versus AD model group (one-way analysis of variance).

physiological nutrient and a normal reducing sugar in body, which converts into galactitol. Galactose is normally metabolized by D-galactokinase and galactose-1-phosphate uridyltransferase in animals. However, over-supply of D-galactose may contribute to the generation of reactive oxygen species through oxidative metabolism of D-galactose, which can cause oxidative stress and cellular damage (Kumar *et al.*, 2011; Yang *et al.*, 2013). Aluminium is a neurotoxic agent that has been shown to exacerbate oxidative stress (Zaky *et al.*, 2013), so continuous treatment with a combination of D-gal and $AlCl_3$ provides a useful model for studying treatments for AD.

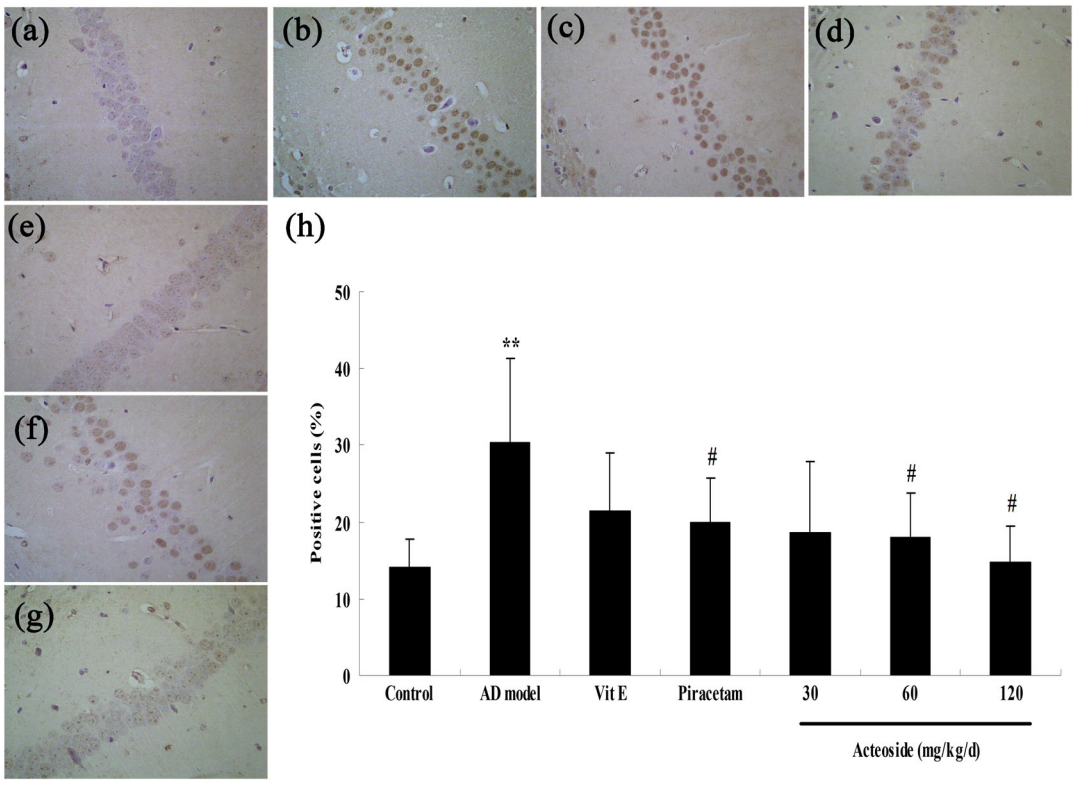

Figure 3. Effect of acteoside on the levels of caspase-3 expression in the hippocampus of senescent model mice (magnification ×40). (a) Control group: neurons arranged tightly and the number of positive cells (coloured yellow) were low; (b) Alzheimer's disease (AD) model group: neurons arranged dispersedly and the number of positive cells were high; (c, d) positive control group: administration of vitamin E and piracetam decreased the number of positive cells, but not statistically significantly in vitamin E group; (e–g) long-term administration of acteoside markedly decreased the number of positive cells; (h) calculation of the number of positive cells. Data are presented as mean ± SD, $n = 10$. $^{**}p < 0.01$ versus control group; $\#p < 0.05$ versus AD model group (one-way analysis of variance). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

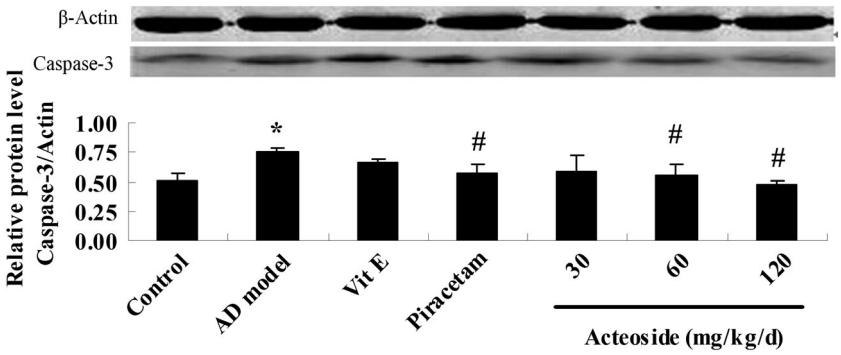

Figure 4. Acteoside decreased the levels of caspase-3 in the hippocampus of senescent model mice. Data are presented as mean ± SD, $n = 10$. $^{*}p < 0.05$ versus control group; $\#p < 0.05$ versus Alzheimer's disease (AD) model group (one-way analysis of variance).

Our results confirmed that the mouse senescence model produced cognitive deficits in the step-down test, indicating a reduction in memory of context. Administration of acteoside attenuated cognitive impairment as evidenced by the extended latency of step-down and decreased number of errors. HE and Nissl staining analysis showed that the number of neurons and Nissl bodies in the hippocampus of the AD model group was reduced compared with those of the untreated control group. This may be because the expression of the caspase-3 gene was increased by the D-gal and $AlCl_3$ treatment. Acteoside increased the numbers of neurons and Nissl bodies compared with those of the AD model group.

Neurodegeneration in AD is considered to be related to activation of various proteases, mainly caspases, calpains and cathepsins (Louneva *et al.,* 2008). Caspases are a class of proteases instrumental in carrying out many cellular functions including cell differentiation, remodelling and cell death (Snigdha *et al.,* 2012). Apoptosis-related caspases have been implicated in progressive neuron death in AD (Louneva *et al.,* 2008). Caspase-3 is a principal effector caspase in apoptotic cascades leading to neuronal apoptosis, and levels are higher in AD brains than in age-matched controls (Snigdha *et al.,* 2012). In our study, immunohistochemical and western blot analysis showed that the expression of caspase-3 protein in the AD model group was significantly higher than in the control group, which is consistent with previous reports (Louneva *et al.,* 2008). After administration of 60 and 120 mg/kg/day of acteoside,

the expression of caspase-3 protein was decreased significantly. Measurement of levels of cleaved caspase-3 and caspase-2 will be carried out in future experiments to confirm this. NO is an important physiological signalling agent, but high concentrations can result in oxidative damage (Aliev *et al.,* 2013). It has been implicated in pathological processes associated with several neurodegenerative disorders including AD (Santos *et al.,* 2012). Most of the neurotoxic effects caused by high concentrations of NO in the development of AD are due to high levels of nitrotyrosine, which have been found in the brain of AD patients (Santos *et al.,* 2012). NO is produced by all brain cells including neurons, endothelial cells and glial cells by NOS. NOS has been suggested to act as a central mediator of amyloid-β activity, contributing to the maintenance, self-perpetuation and progression of the disease in AD (Santos *et al.,* 2012). Our results confirmed that all these parameters were increased in the hippocampus of the AD model group, that the content of NO was decreased and the activity of NOS reduced after administration with acteoside, suggesting that this may be another mechanism involved in the effects observed after treatment with acteoside.

The present study was designed to elucidate the effects of acteoside in improving learning and memory using a mouse model of senescence induced by a combination of D-gal and $AlCl_3$ and investigate its potential mechanisms. Our results showed that the latency of step-down was extended in AD model mice and the number of errors decreased after treatment with all doses of acteoside. Administration of acteoside was thus able to reverse some of the cognitive impairment induced by a combination of D-gal and $AlCl_3$, and the mechanism is likely to be related to the prevention of neuronal apoptosis due to oxidative stress. The numbers of neurons and Nissl bodies in the hippocampus were increased significantly at the higher doses (60 and 120 mg/kg/day) of acteoside. The content of NO, the activity of NOS and the expression of caspase-3 protein in the hippocampus were all decreased by 120 mg/kg/day acteoside compared with the AD model group. Both vitamin E and piracetam reduced the number of damaged neurons and increased the number of Nissl bodies, but the results were not statistically significant. Vitamin E, despite its antioxidant effects and wide promotion as a potential anti-dementia treatment and being used at a high dose, has paradoxical effects in dementia which have been discussed by Brewer (2010). As the aetiology of AD is so complex, there may be no ideal positive control for AD experiments available at present. Together with the memory improvement previously reported for the Morris maze test in the same model (Gao *et al.,* 2015), our results support the use of traditional medicinal herbs containing acteoside for neuroprotection and suggest that acteoside (verbascoside) has potential for further investigation, including measuring levels of cleaved caspase-3 and caspase-2 and other relevant markers, towards development as a treatment for the early stages of AD.

Acknowledgements

This research was supported by Xinjiang Uyghur autonomous Region (grant no. 201110104).

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Aliev G, Obrenovich ME, Tabrez S, *et al*. 2013. Link between cancer and Alzheimer disease via oxidative stress induced by nitric oxide-dependent mitochondrial DNA overproliferation and deletion. *Oxid Med Cell Longev* **2013**: 962984.

Brewer G. 2010. Why vitamin E therapy fails for treatment of Alzheimer's disease. *J Alzheimers Dis* **19**: 27–30.

Castellani RJ, Rolston RK, Smith MA. 2010. Alzheimer disease. *Dis Mon* **56**: 484–546.

Feng Y, Wang XC. 2012. Antioxidant therapies for Alzheimer's disease. *Oxid Med Cell Longev* **2012**: 472932.

Fischer AH, Jacobson KA, Rose J, *et al*. 2008. Hematoxylin and eosin staining of tissue and cell sections. *CSH Protoc*, **2008**: pdb. prot 4986.

Gao L, Lin J, Zhang FC, *et al*. 2013. Antioxidant effects of acteoside on aged mice induced by D-galactose. *Chin Pharmacol Bull* **29**: 1440–1443.

Gao L, Peng XM, Huo SX, *et al*. 2015. Memory enhancement of acteoside (verbascoside) in a senescent mice model induced by a combination of D-gal and $AlCl_3$. *Phytother Res* (*in press*)

Harrison FE, Allard J, Bixler R, *et al*. 2009. Antioxidants and cognitive training interact to affect oxidative stress and memory in APP/PSEN1 mice. *Nutr Neurosci* **12**: 203–218.

He J, Hu XP, Zeng Y, *et al*. 2011. Advanced research on acteoside for chemistry and bioactivities. *J Asian Nat Prod Res* **13**: 449–464.

Jama JW, Launer LJ, Witteman JC, *et al*. 1996. Dietary antioxidants and cognitive function in a population-based sample of older persons. The Rotterdam Study. *Am J Epidemiol* **144**: 275–280.

Jiang Y, Tu PF. 2009. Analysis of chemical constituents in *Cistanche* species. *J Chromatogr A* **1216**: 1970–1979.

Koo KA, Kim SH, Oh TH, *et al*. 2006. Acteoside and its aglycones protect primary cultures of rat cortical cell from glutamate-induced excitotoxicity. *Life Sci* **79**: 709–716.

Kumar A, Prakash A, Dogra S. 2011. *Centella asiatica* attenuates D-galactose induced cognitive impairment, oxidative and mitochondrial dysfunction in mice. *Int J Alzheimers Dis* **2011**: 347569.

Lin J, Gao L, Huo SX, *et al*. 2012. Effect of acteoside on learning and memory impairment induced by scopolamine in mice. *China J Chin Mat Med* **37**: 2956–2959.

Louneva N, Cohen JW, Han LY, *et al*. 2008. Caspase-3 is enriched in postsynaptic densities and increased in Alzheimer's disease. *Am J Pathol* **173**: 1488–1495.

Luo Y, Niu F, Sun Z, *et al*. 2009. Altered expression of Aβ metabolism associated molecules from D-galactose/$AlCl_3$ induced mouse brain. *Mech Ageing Dev* **130**: 248–252.

Peng XM, Gao L, Gan P, *et al*. 2013. Study on protective effects of acteoside on oxidative damage of PC12 induced by H_2O_2. *Pharmacol Clin Chin Mat Med* **29**: 35–38.

Perrig WJ, Perrig P, Stahelin HB. 1997. The relation between antioxidants and memory performance in the old and very old. *J Am Geriatr Soc* **45**: 718–724.

Pilati N, Barker M, Panteleimonitis S, *et al*. 2008. A rapid method combining Golgi and Nissl staining to study neuronal morphology and cytoarchitecture. *J Histochem Cytochem* **56**: 539–550.

Reitz C, Brayne C, Mayeux R. 2011. Epidemiology of Alzheimer disease. *Nat Rev Neurol* **7**: 137–152.

Santos RM, Lourenco CF, Ledo A, *et al*. 2012. Nitric oxide inactivation mechanisms in the brain: role in bioenergetics and neurodegeneration. *Int J Cell Biol* **2012**: 391914.

Snigdha S, Smith ED, Prieto GA, *et al*. 2012. Caspase-3 activation as a bifurcation point between plasticity and cell death. *Neurosci Bull* **28**: 14–24.

Sun ZZ, Chen ZB, Jiang H, *et al*. 2009. Alteration of A beta metabolism-related molecules in predementia induced by $AlCl_3$ and D-galactose. *Age* **31**: 277–284.

Wang Q, Sun LH, Jia W, *et al*. 2010. Comparison of ginsenosides Rg1 and Rb1 for their effects on improving scopolamine-induced learning and memory impairment in mice. *Phytother Res* **24**: 1748–1754.

Weiner MW, Veitch DP, Aisen PS, *et al*. 2012. The Alzheimer's disease neuroimaging initiative: a review of papers published since its inception. *Alzheimers Dement* **8**: S1–S68.

Yang WN, Han H, Hu XD. 2013. The effects of perindopril on cognitive impairment induced by D-galactose and aluminum trichloride via inhibition of acetylcholinesterase activity and oxidative stress. *Pharmacol Biochem Behav* **114-115**: 31–36.

Yang WN, Hu XD, Han H, *et al*. 2014. The effects of valsartan on cognitive deficits induced by aluminum trichloride and D-galactose in mice. *Neurol Res* **36**: 651–658.

Zaky A, Mohammad B, Moftah M, *et al*. 2013. Apurinic/apyrimidinic endonuclease 1 is a key modulator of aluminum-induced neuroinflammation. *BMC Neurosci* **14**: 26.

