

Effects of caffeic acid on learning deficits in a model of Alzheimer's disease

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Abstract. Caffeic acid is a type of phenolic acid and organic acid. It is found in food (such as tomatoes, carrots, strawberries, blueberries and wheat), beverages (such as wine, tea, coffee and apple juice) as well as Chinese herbal medicines. In the present study, we examined the effects of caffeic acid on learning deficits in a rat model of Alzheimer's disease (AD). The rats were randomly divided into three groups: i) control group, ii) AD model group and iii) caffeic acid group. Caffeic acid significantly rescued learning deficits and increased cognitive function in the rats with AD as demonstrated by the Morris water maze task. Furthermore, caffeic acid administration resulted in a significant decrease in acetylcholinesterase activity and nitrite generation in the rats with AD compared with the AD model group. Furthermore, caffeic acid suppressed oxidative stress, inflammation, nuclear factor- κ B-p65 protein expression and caspase-3 activity as well as regulating the protein expression of p53 and phosphorylated (p)-p38 MAPK expression in the rats with AD. These experimental results indicate that the beneficial effects of caffeic acid on learning deficits in a model of AD were due to the suppression of oxidative stress and inflammation through the p38 MAPK signaling pathway.

Introduction

With an aging global population, the morbidity of Alzheimer's disease (AD), an age-related illness, is increasing significantly (1). In 2008, the cost of treatment in Europe reached 177 billion euros, far more than cost of treating tumors and heart disease which are considered as diseases with the highest morbidity and death rate (2). Furthermore, the available treatment options for AD are restricted and therefore

optimal outcomes are not achieved (3). Thus, novel therapies producing reliable effects and offering ease of administration and minimal adverse effects, which can be used in clinical practices, would benefit patients with AD worldwide.

AD is a progressive, neurodegenerative disease characterized by worsening of cognition and memory, progressive interference with daily living activities accompanied by neuropsychiatric symptoms and behavioral disorders (4). The pathogenesis of AD is characterized by abnormalities in amyloid precursor proteins which result in the leakage of proteins from the cytomembrane, thereby causing neurofibrillary tangles and cell death, i.e., selective neuronal loss. Drugs used to treat AD principally improve the neurotransmission of choline, cerebral circulation and the metabolism of brain cells (5). Furthermore, drugs including calcium antagonists, hormone therapy, nonsteroidal anti-inflammatory drugs, free radical scavengers, antioxidants and muscarinic receptor agonists, are also used in the treatment of AD. without achieving optimal therapeutic outcomes (6). Thus, the development of effective treatments for AD is proving to be challenging.

Caffeic acid possesses a number of pharmacologic functions including anti-inflammatory, antibacterial and antiviral effects (7-9). Furthermore, it may also increase the levels of white blood cells and blood platelets (10). Thus, caffeic acid has the potential to be used to minimize oxidative stress and inflammatory responses in cardiovascular diseases and brain damage as well as to prevent and treat viral diseases such as HIV, in addition to treating leukopenia and thrombocytopenia (11). By exerting antioxidant, anti-inflammatory, immunoregulatory and antibacterial effects, caffeic acid may potentially be of value in the treatment of diseases associated with oxidative stress and inflammatory responses (7,11,12). To the best of our knowledge, this is the first study to directly examine the effects of caffeic acid in AD by evaluating cognitive function and to explore the possible mechanisms responsible for these effects.

Materials and methods

Animals. Healthy male Sprague-Dawley (SD) rats (7-8 weeks of age) were purchased from the Experimental Center of Henan University School of Medicine and were housed in a room

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maintained at 23°C under a 12-h light/dark cycle, with *ad libitum* access to food and water. The present study was approved by the Animal Care and Use Committee of Zhengzhou University (Zhengzhou, China), and all experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Firstly, the amyloid β -peptide A β 1-40 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in deionized water stored at -80°C until use. The rats were subjected to an intraperitoneal injection of 350 mg/kg chloral hydrate. The hippocampus was localized at 3.8 mm posterior, 2.9 mm below the top of the skull and 2.4 mm lateral to the Bregma. Each rat received 5 μ l A β 1-40 in the left side over 10 min in order to establish a model of AD.

Grouping. The rats were randomly divided into 3 groups (n=12/group): i) control group, ii) AD model group, and iii) caffeic acid group. The rats in the caffeic acid group were injected with 100 mg/kg caffeic acid (Sigma-Aldrich Chemie GmbH) for 2 weeks; the rats in the control and AD model groups were injected with normal saline for 2 weeks.

Morris water maze task. A circular water pool (150x60 cm; containing water at 24 \pm 2°C) was divided into 4 equally spaced quadrants containing various prominent visual cues. An invisible escape platform (10x10 cm, 1 cm below the water surface) was hidden in the center of quadrant II during the training period and removed at the time of the probe task. Five days after the A β 1-40 injection, memory training was initiated. The training was recorded twice a day for 5 days. Each rat was allowed to swim onto the platform or until a 120 sec time period had elapsed. A video tracking system (SMART; Panlab SL, Barcelona, Spain) was used to record the escape latency, average swim speed, mean path length, time spent in the target quadrant, and the number of times the animal crossed the previous location of the platform.

Hematoxylin and eosin (H&E) staining. We examined synaptophysin expression in our model of AD to analyze the effects of caffeic acid on cerebral damage by H&E staining. After the rats were sacrificed by decollation under anesthesia, the hippocampal tissues were quickly removed, washed with saline and embedded in paraffin. The hippocampal tissues were then serially sectioned into 30- μ m-thick slices. The slices were stained with H&E and observed under a Mirax Scan digital microscope slide scanner (Mirax 3D Histech; Carl Zeiss, Oberkochen, Germany).

Determination of acetylcholinesterase (AChE) activity in the brain. The hippocampal tissues were quickly removed, washed with saline and embedded in paraffin. AChE activity was collected from brain homogenate using the method developed by Ellman *et al* (13). A spectrophotometer (Infinite H200 PRO; Tecan, Männedorf, Switzerland) was used to measure absorbance at 412 nm and the results are expressed as μ M of acetylthiocholine iodide hydrolysed/min/mg of protein.

Measurement of nitrite generation in the brain. A total of 100 μ l homogenate was incubated with an equal volume of Griess reagent at room temperature for 10 min. A spectrophotometer (Infinite H200 PRO; Tecan) was used to measure

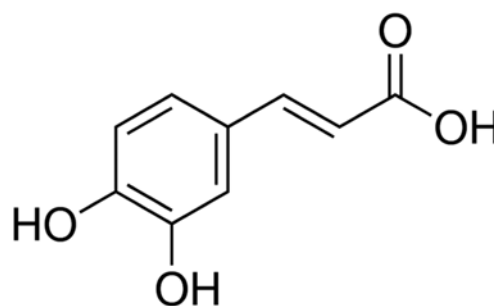


Figure 1. Chemical structure of caffeic acid.

nitrite generation in the brain at 550 nm and the results were calculated from a sodium nitrite standard curve.

Measurement of catalase (CAT), glutathione (GSH), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) activities. CAT, GSH, IL-6 and TNF- α ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to analyse the supernatant (200 μ l, which was removed using a pipette). A spectrophotometer (Infinite H200 PRO; Tecan) was used to measure the activities of CAT, GSH, IL-6 and TNF- α .

Western blot analysis. The hippocampal tissues were quickly removed and pre-cooled radio-immunoprecipitation assay (RIPA) buffer was used to extract the proteins. Miscible liquids were centrifuged at 12,000 x g for 15 min at 4°C and used to determine the protein content using the bicinchoninic acid method (Joincare Pharmaceutical Group Industry Co., Ltd., Zhuhai, China). Equal amounts of protein from each group were subjected to 12% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Berkeley, CA, USA). The PVDF membranes were blocked with 5% fat milk in Tris-buffered saline for 1 h and incubated with anti-nuclear factor (NF)- κ B-p65 (1:2,000), anti-p53 (1:4,000) and anti-phosphorylated (p)-p38 MAPK (1:4,000) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA); and anti- β -actin (1:500; Wuhan Boster Biological Technology Ltd., Wuhan, China) at 4°C overnight. The membranes were washed three times with 0.1% Tween-20 TBS and incubated with horseradish peroxidase-conjugated anti-sheep secondary antibodies (Wuhan Boster Biological Technology Ltd.). The proteins were detected with an enhanced chemiluminescence kit (Millipore Corp., Billerica, MA, USA).

Analysis of caspase-3 activity. The hippocampal tissues were quickly removed and a pre-cooled RIPA buffer was used to extract proteins. Miscible liquids were centrifuged at 12,000 x g for 15 min at 4°C and the protein content was determined using the bicinchoninic acid method (Joincare Pharmaceutical Group Industry Co., Ltd.). Equal amounts of protein from each group were incubated with the colorimetric substrate for caspase-3, Ac-DEVD-pNA, at 37°C for 2 h in the dark, and detected using a spectrophotometer (Infinite H200 PRO, Tecan) at a wavelength of 405 nm.

Statistical analysis. All data are expressed as the means \pm standard error of the mean. Statistical analysis was performed

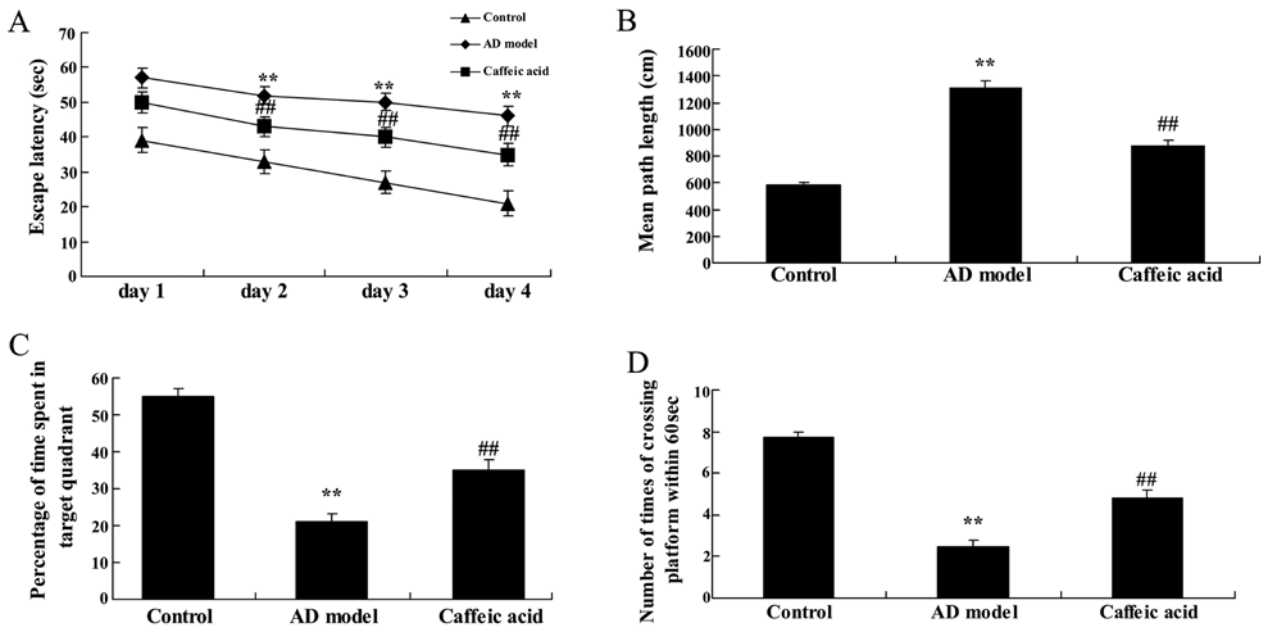


Figure 2. Effect of caffeic acid on cognitive functions in a model of Alzheimer's disease (AD). Effect of caffeic acid on (A) escape latency, (B) mean path length, (C) target quadrant and (D) number of times the animals crossed the former platform location. ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

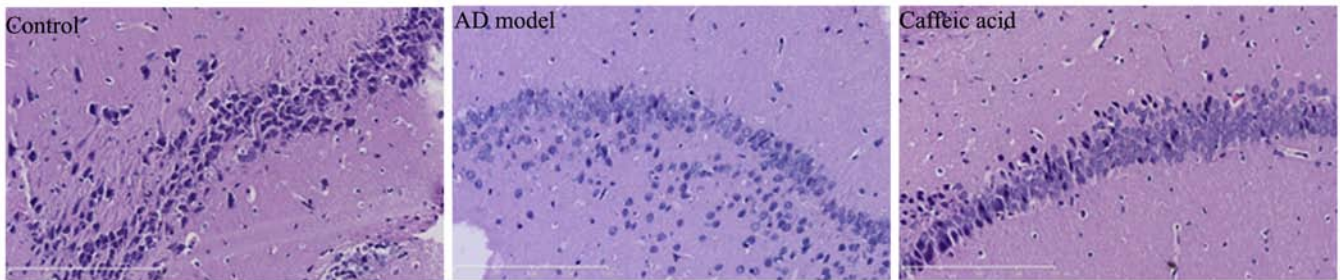


Figure 3. Effect of caffeic acid on synaptophysin in a model of Alzheimer's disease (AD). Scale bar, 200 μm .

using one- or two-way ANOVA followed by Dunnett's post hoc test. A p -value < 0.05 was considered to indicate a statistically significant difference.

Results

Effect of caffeic acid on cognitive function in a model of AD.

The chemical structure of caffeic acid is presented in Fig. 1. The results of the Morris water maze task are shown Fig. 2. Escape latency in the AD model group significantly increased compared with that in the control group at different time points (Fig. 2A). However, there was a significant decrease in escape latency in the caffeic acid-treated group compared with that in the AD model group (Fig. 2A). The mean path length taken by the rats in the AD group was significantly longer than that taken by the rats in the control group (Fig. 2B). As shown in Fig. 2B, treatment with caffeic acid significantly decreased the mean path length taken by the rats. Moreover, the rats in the AD model group spent significantly less time in the target quadrant and also, crossed the former platform location within 60 sec fewer times, compared with the rats in the control group (Fig. 2C and D). Following caffeic acid

treatment, the time spent in the target quadrant and the number of times the animals crossed the former platform location were significantly increased compared with the AD model group (Fig. 2C and D).

Effect of caffeic acid on cerebral damage in model of AD.

To examine the effect of caffeic acid on cerebral damage in a model of AD, the hippocampal tissues from each group were stained with H&E to determine synaptophysin expression. As shown in Fig. 3, synaptophysin expression was observably weakened and severe cerebral damage had occurred in the AD model group compared with the control group. Treatment with caffeic acid markedly increased synaptophysin expression and weakened the AD-induced cerebral damage in the rats compared with the AD model group (Fig. 3).

Effect of caffeic acid on AChE activity in a model of AD.

To examine the effect of caffeic acid on AChE activity in a model of AD, we measured AChE activity in the rat brain tissue. As shown in Fig. 4, enhanced AChE activity was observed in the hippocampal tissues from rats in the AD model group compared with the tissues from the control group. Furthermore,

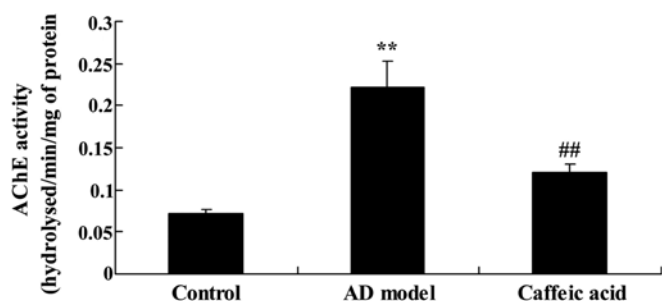


Figure 4. Effect of caffeic acid on acetylcholinesterase (AChE) activity in a model of Alzheimer's disease (AD). ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

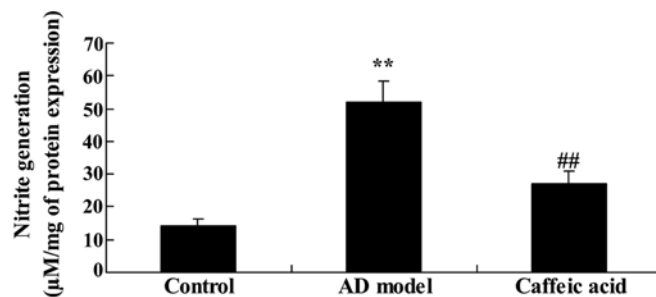


Figure 5. Effect of caffeic acid on nitrite generation in a model of Alzheimer's disease (AD). ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

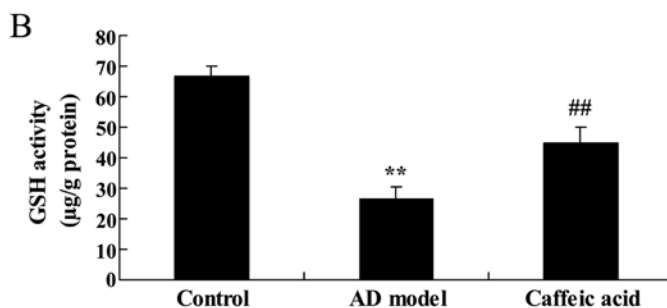
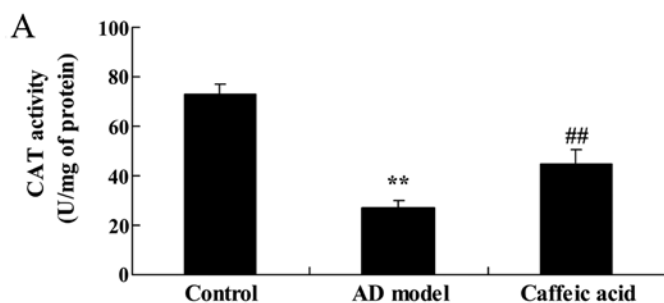


Figure 6. Effect of caffeic acid on oxidative stress in a model of Alzheimer's disease (AD). Effect of caffeic acid on (A) catalase (CAT) and (B) glutathione (GSH) activity in a model of AD. ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

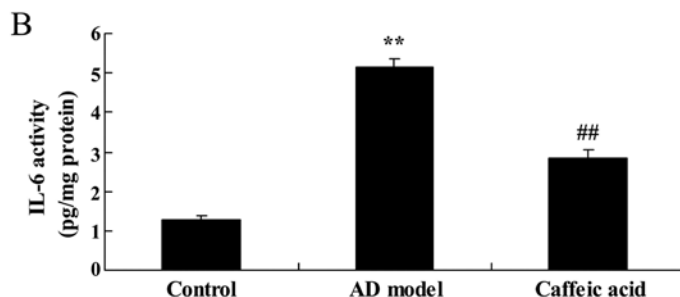
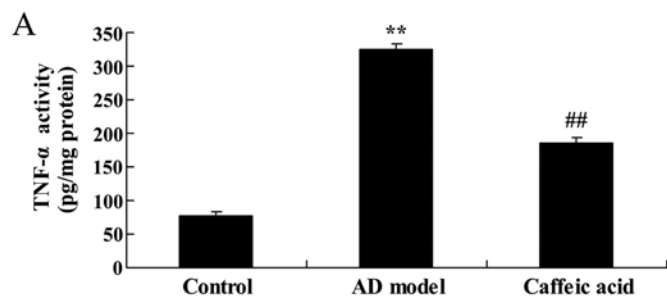


Figure 7. Effect of caffeic acid on inflammation in a model of Alzheimer's disease (AD). Effect of caffeic acid on (A) interleukin (IL)-6 and (B) tumor necrosis factor- α (TNF- α) activity in a model of AD. ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

the enhanced ChE activity was significantly suppressed by treatment with caffeic acid compared with that in the AD model group (Fig. 4).

Effect of caffeic acid on nitrite generation in a model of AD. We next examined the effect of caffeic acid on nitrite generation in a model of AD. There was a significant increase in nitrite generation in the hippocampal tissues of the AD model group compared with that in the control group (Fig. 5). Compared with the AD model group, caffeic acid significantly reduced the elevated generation of nitrite (Fig. 5).

Effect of caffeic acid on oxidative stress in a model of AD. The activities of CAT and GSH were significantly reduced in the hippocampal tissues in the AD model group compared with the control group (Fig. 6). Treatment with caffeic acid significantly

elevated the inhibition of CAT and GSH activities in the hippocampal tissues compared with the AD model group (Fig. 6).

Effect of caffeic acid on inflammation in a model of AD. Brain IL-6 and TNF- α activities in the AD model group were increased compared with the control group (Fig. 7). The elevated IL-6 and TNF- α activities were significantly reduced by caffeic acid treatment (Fig. 7).

Effect of caffeic acid on NF- κ B-p65 expression in a model of AD. The brain tissues from rats in the AD model group were observed to have significantly increased NF- κ B-p65 protein expression as compared with the control animals (Fig. 8). Caffeic acid treatment suppressed NF- κ B-p65 protein expression significantly compared with the AD model group (Fig. 8).

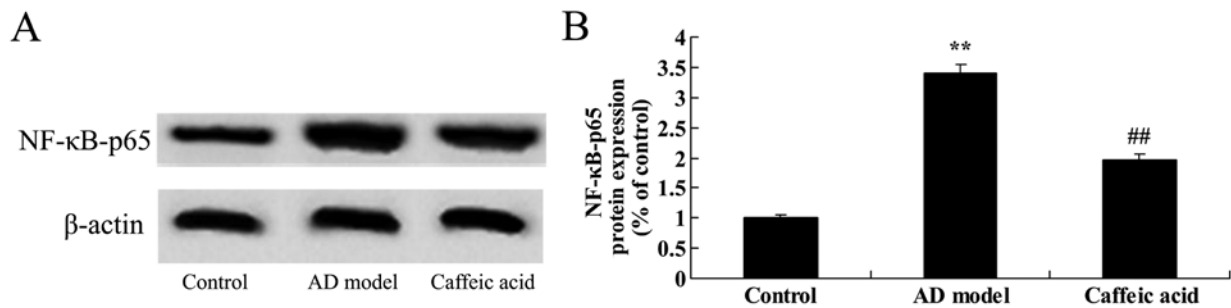


Figure 8. Effect of caffeic acid on nuclear factor (NF)-κB-p65 expression in a model of Alzheimer's disease (AD). Effect of caffeic acid on NF-κB-p65 protein expression was evaluated by (A) western blot analysis and (B) statistical analysis of NF-κB-p65 protein expression in a model of AD. ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

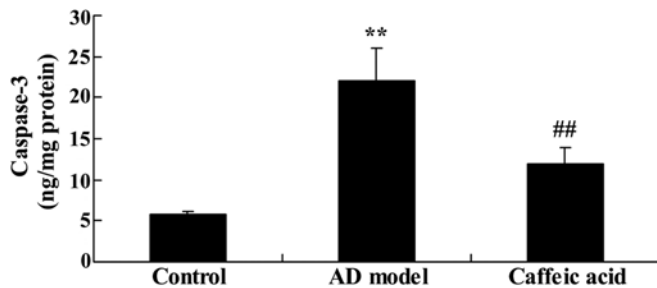


Figure 9. Effect of caffeic acid on caspase-3 activity in a model of Alzheimer's disease (AD). ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

the AD-induced caspase-3 activity compared with that in the AD model group (Fig. 9).

Effect of caffeic acid on p53 expression in a model of AD. As shown in Fig. 10, a significant increase in p53 protein expression was observed in the hippocampus of the rats in the AD model group compared with that in the control group. Moreover, a significant decrease in p53 protein expression was found in the caffeic acid-treated group compared with that in the AD model group (Fig. 10).

Effect of caffeic acid on p-p38 MAPK expression in a model of AD. There was a significant increase in the protein expression of p-p38 MAPK in the AD model group, compared with that in the control animals (Fig. 11). By contrast, caffeic acid significantly suppressed the protein expression of p-p38 MAPK compared with that in the AD model group (Fig. 11).

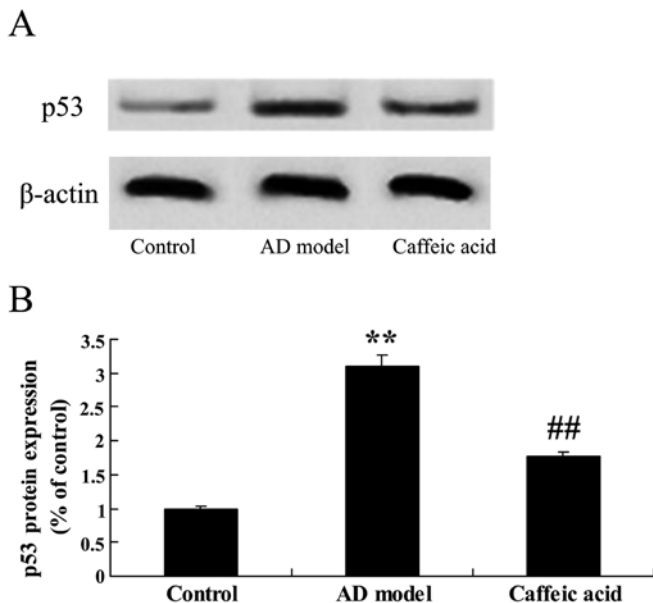


Figure 10. Effect of caffeic acid on p53 expression in a model of Alzheimer's disease (AD). Effect of caffeic acid on p53 protein expression was evaluated by (A) western blot analysis and (B) statistical analysis of p53 protein expression in a model of AD. ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

Discussion

AD is a neurodegenerative disease of the central nervous system characterized by cognitive decline and the impairment of memory (14). With an aging population, the incidence of AD increases which places a heavy burden on society and families (15). Therefore, medical researchers and scholars are presented with a great challenge; to discover a therapeutic drug with high treatment efficacy and low toxicity. The present study revealed that caffeic acid increased cognitive function and attenuated cerebral damage compared with the rats in the AD model group. Khan *et al* suggested that caffeic acid prevented $AlCl_3$ -induced dementia in rats (16). Pinheiro Fernandes *et al* suggested that caffeic acid prevents memory deficits induced by focal cerebral ischemia (17). Consistent with these findings, our results indicate that caffeic acid may be a novel drug for use in the treatment of AD.

In the human brain, there are two types of cholinesterase: AChE and butyrylcholinesterase (BChE). Research has shown that BChE also exists in the brain and it may degrade acetylcholine (ACh). Thus, at times when AChE levels are insufficient or inhibition has occurred, BChE is capable of compensating for a lack of AChE (18). In fact, in the brains of patients with worsening symptoms of AD, the activity of BChE is evidently increased, which may be due to the development of tolerance to AChE inhibitors (19). We found that caffeic acid inhibited the AD-induced AChE activity and nitrite generation in a rat

Effect of caffeic acid on caspase-3 activity in a model of AD. The rats in the AD model group showed a significant increase in caspase-3 activity compared with the control animals (Fig. 9). However, treatment with caffeic acid significantly weakened

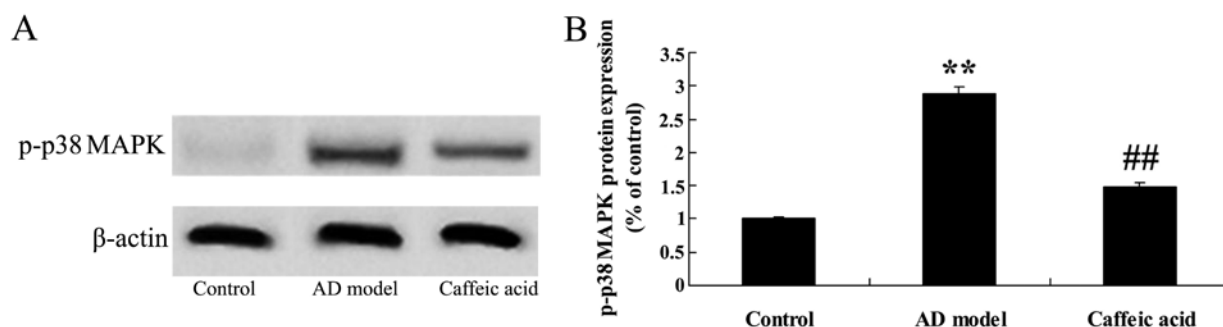


Figure 11. Effect of caffeic acid on phosphorylated (p)-p38 MAPK in a model of Alzheimer's disease (AD). Effect of caffeic acid on the protein expression of p-p38 MAPK was evaluated by (A) western blot analysis and (B) statistical analysis of p-p38 MAPK protein expression in a model of AD. ** $p < 0.01$ compared with the control group, ## $p < 0.01$ compared with the AD model group.

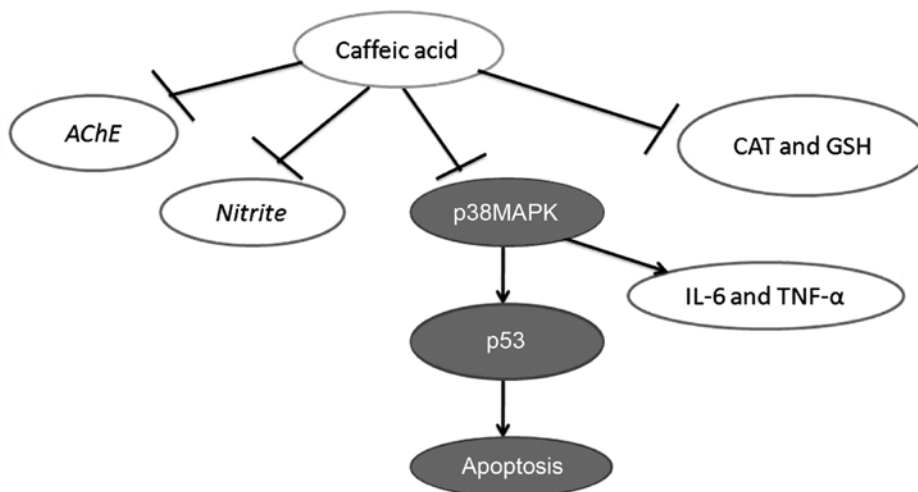


Figure 12. Effect of caffeic acid on learning deficits in a model of Alzheimer's disease through p53 and p38 signal pathways. CAT, catalase; GSH, glutathione; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; AChE, acetylcholinesterase.

model of AD. Khan *et al* suggested that caffeic acid prevents against AlCl_3 -induced dementia by reducing brain AChE activity and nitrite levels in rats (16). Mehrotra *et al* also reported that caffeic acid inhibited myeloperoxidase, malondialdehyde and nitrite generation in mice and rats (20).

In the brains of patients with AD, neurons in the nucleus basalis of Meynert are seriously denatured or lost and the activity of choline acetyltransferase is decreased (21). The generation and release of ACh is lessened and the numbers of presynaptic cholinergic receptors are diminished. In $\text{A}\beta_{42}$ -induced AD, choline acetyltransferase in synaptosomes experiences oxidative modification and enzymatic activities are decreased (22). The pathogenesis of AD involves numerous factors and oxidative stress may be the trigger for a series of complex events. Oxidative stress and inflammation leads to the abnormal peroxidation, glycosylation or nitration of structural and functional materials. Furthermore, the accumulation of abnormal materials damages cellular functions (23). Previous findings have indicated that caffeic acid suppresses the oxidative state and colonic inflammation in the inflamed colon (24). In the present study, we have shown that caffeic acid increased the activities of CAT and GSH which were inhibited in the AD model group and reduced

the promotion of IL-6 and TNF- α activity induced by AD as well as NF- κB -p65 protein expression in the rat brains.

Learning and memory formation are closely associated with the formation and plasticity of synapses in hippocampal neuron. In the hippocampus, the sites and degree of positive expression of synaptophysin correspond with the number and distribution of synapses and neurons (25). The apoptosis of nerve cells induced by drugs and the central nervous system affects the expression of synaptophysin. Research has shown that in patients with AD, neurons in the hippocampal formation are lost and synaptophysin is significantly decreased, which is mediated by the p38 MAPK signaling pathway (26). Moreover, the p38 MAPK signaling pathway as a regulatory center is associated with the degree of cognitive impairment (27). In the present study, we demonstrated that caffeic acid suppressed the protein expression of p53 and p-p38 MAPK in a rat model of AD. Lee *et al* suggested that caffeic acid exerts an antidepressant-like effect through the suppression of p38 MAPK (28). Yang *et al* also reported that caffeic acid inhibited the migratory capability and cancer stem cells-like properties of malignant human keratinocytes by downregulating the p38 NF- κB /snail signaling pathway (12).

Taken together, the findings of the present study demonstrated that caffeic acid attenuated the development of AD, by increasing cognitive function, attenuating cerebral damage, and inhibiting the AD-induced increase in AChE activity and nitrite generation in a model of AD. Furthermore, caffeic acid induced the inhibition of oxidative stress, inflammation and apoptosis through the p53 and p38 MAPK signaling pathways (Fig. 12). These findings suggest that the effects of caffeic acid in the treatment of AD occur through the p53 and p38 MAPK signaling pathways.

References

- Muir SW, Speechley M, Wells J, Borrie M, Gopaul K and Montero-Odasso M: Gait assessment in mild cognitive impairment and Alzheimer's disease: the effect of dual-task challenges across the cognitive spectrum. *Gait Posture* 35: 96-100, 2012.
- Pitkala KH, Raivio MM, Laakkonen ML, Tilvis RS, Kautiainen H and Strandberg TE: Exercise rehabilitation on home-dwelling patients with Alzheimer's disease - a randomized, controlled trial. Study protocol. *Trials* 11: 92, 2010.
- Spires-Jones TL, Mielke ML, Rozkalne A, Meyer-Luehmann M, de Calignon A, Bacskai BJ, Schenk D and Hyman BT: Passive immunotherapy rapidly increases structural plasticity in a mouse model of Alzheimer disease. *Neurobiol Dis* 33: 213-220, 2009.
- Olazarán J, Reisberg B, Clare L, Cruz I, Peña-Casanova J, Del Ser T, Woods B, Beck C, Auer S, Lai C, *et al*: Nonpharmacological therapies in Alzheimer's disease: a systematic review of efficacy. *Dement Geriatr Cogn Disord* 30: 161-178, 2010.
- Wang WY, Tan MS, Yu JT and Tan L: Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Ann Transl Med* 3: 136, 2015.
- Götz J, Ittner LM, Schonrock N and Cappai R: An update on the toxicity of Abeta in Alzheimer's disease. *Neuropsychiatr Dis Treat* 4: 1033-1042, 2008.
- Pittalà V, Salerno L, Romeo G, Siracusa MA, Modica MN, Romano GL, Salomone S, Drago F and Bucolo C: Effects of novel hybrids of caffeic acid phenethyl ester and NSAIDs on experimental ocular inflammation. *Eur J Pharmacol* 752: 78-83, 2015.
- Pinho E, Henriques M and Soares G: Caffeic acid loading wound dressing: Physicochemical and biological characterization. *Ther Deliv* 5: 1063-1075, 2014.
- Tanida I, Shirasago Y, Suzuki R, Abe R, Wakita T, Hanada K and Fukasawa M: Inhibitory effects of caffeic acid, a coffee-related organic acid, on the propagation of hepatitis C virus. *Jpn J Infect Dis* 68: 268-275, 2015.
- Paracatu LC, Faria CM, Quinello C, Rennó C, Palmeira P, Zeraik ML, da Fonseca LM and Ximenes VF: Caffeic Acid phenethyl ester: consequences of its hydrophobicity in the oxidative functions and cytokine release by leukocytes. *Evid Based Complement Alternat Med* 2014: 793629, 2014.
- Bailly F and Cotellet P: Anti-HIV activities of natural antioxidant caffeic acid derivatives: toward an antiviral supplementation diet. *Curr Med Chem* 12: 1811-1818, 2005.
- Yang Y, Li Y, Wang K, Wang Y, Yin W and Li L: P38/NF- κ B/snail pathway is involved in caffeic acid-induced inhibition of cancer stem cells-like properties and migratory capacity in malignant human keratinocyte. *PLoS One* 8: e58915, 2013.
- Ellman GL, Courtney KD, Andres V Jr and Feather-Stone RM: A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 7: 88-95, 1961.
- Paillard T, Rolland Y and de Souto Barreto P: Protective effects of physical exercise in Alzheimer's disease and Parkinson's disease: a narrative review. *J Clin Neurol* 11: 212-219, 2015.
- Xu H, Finkelstein DI and Adlard PA: Interactions of metals and Apolipoprotein E in Alzheimer's disease. *Front Aging Neurosci* 6: 121, 2014.
- Khan KA, Kumar N, Nayak PG, Nampoothiri M, Shenoy RR, Krishnadas N, Rao CM and Mudgal J: Impact of caffeic acid on aluminium chloride-induced dementia in rats. *J Pharm Pharmacol* 65: 1745-1752, 2013.
- Pinheiro Fernandes FD, Fontenele Menezes AP, de Sousa Neves JC, Fonteles AA, da Silva AT, de Araújo Rodrigues P, Santos do Carmo MR, de Souza CM and de Andrade GM: Caffeic acid protects mice from memory deficits induced by focal cerebral ischemia. *Behav Pharmacol* 25: 637-647, 2014.
- Yusuf Ali M, Jung HA and Choi JS: Anti-diabetic and anti-Alzheimer's disease activities of *Angelica decursiva*. *Arch Pharm Res* 38: 2216-2227, 2015.
- Zimmermann M: Neuronal AChE splice variants and their non-hydrolytic functions: redefining a target of AChE inhibitors? *Br J Pharmacol* 170: 953-967, 2013.
- Mehrotra A, Shanbhag R, Chamallamudi MR, Singh VP and Mudgal J: Ameliorative effect of caffeic acid against inflammatory pain in rodents. *Eur J Pharmacol* 666: 80-86, 2011.
- Sassi C, Ridge PG, Nalls MA, Gibbs R, Ding J, Lupton MK, Troakes C, Lunnon K, Al-Sarraj S, Brown KS, *et al*: Influence of coding variability in APP-A β metabolism genes in sporadic Alzheimer's disease. *PLoS One* 11: e0150079, 2016.
- Gonzalez P, da Costa VC, Hyde K, Wu Q, Annunziata O, Rizo J, Akkaraju G and Green KN: Bimodal-hybrid heterocyclic amine targeting oxidative pathways and copper mis-regulation in Alzheimer's disease. *Metallomics* 6: 2072-2082, 2014.
- Hatanaka H, Hanyu H, Hirose D, Fukusawa R, Namioka N and Iwamoto T: Peripheral oxidative stress markers in individuals with Alzheimer's disease with or without cerebrovascular disease. *J Am Geriatr Soc* 63: 1472-1474, 2015.
- Kim H, Kim W, Yum S, Hong S, Oh JE, Lee JW, Kwak MK, Park EJ, Na DH and Jung Y: Caffeic acid phenethyl ester activation of Nrf2 pathway is enhanced under oxidative state: structural analysis and potential as a pathologically targeted therapeutic agent in treatment of colonic inflammation. *Free Radic Biol Med* 65: 552-562, 2013.
- Ashabi G, Alamdary SZ, Ramin M and Khodaghli F: Reduction of hippocampal apoptosis by intracerebroventricular administration of extracellular signal-regulated protein kinase and/or p38 inhibitors in amyloid beta rat model of Alzheimer's disease: involvement of nuclear-related factor-2 and nuclear factor- κ B. *Basic Clin Pharmacol Toxicol* 112: 145-155, 2013.
- Yang WN, Ma KG, Qian YH, Zhang JS, Feng GF, Shi LL, Zhang ZC and Liu ZH: Mitogen-activated protein kinase signaling pathways promote low-density lipoprotein receptor-related protein 1-mediated internalization of beta-amyloid protein in primary cortical neurons. *Int J Biochem Cell Biol* 64: 252-264, 2015.
- Kim TI, Lee YK, Park SG, Choi IS, Ban JO, Park HK, Nam SY, Yun YW, Han SB, Oh KW and Hong JT: L-Theanine, an amino acid in green tea, attenuates beta-amyloid-induced cognitive dysfunction and neurotoxicity: reduction in oxidative damage and inactivation of ERK/p38 kinase and NF- κ B pathways. *Free Radic Biol Med* 47: 1601-1610, 2009.
- Lee MS, Kim YH, Lee BR, Kwon SH, Moon WJ, Hong KS, Song YS, Morita K, Hahm DH, Shim I and Her S: Novel antidepressant-like activity of caffeic acid phenethyl ester is mediated by enhanced glucocorticoid receptor function in the hippocampus. *Evid Based Complement Alternat Med* 2014: 646039, 2014.