Original Article

Neuroprotective effects of tenuigenin on neurobehavior, oxidative stress, and tau hyperphosphorylation induced by intracerebroventricular streptozotocin in rats

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Abstract:

BACKGROUND: Tenuigenin (TEN), a major active component of the Chinese herb Polygala tenuifolia root, has been used to improve memory and cognitive function in Traditional Chinese Medicine for centuries.

PURPOSE: The present study was designed to explore the possible neuroprotective effect of TEN on the streptozotocin (STZ)-induced rat model of sporadic Alzheimer's disease (sAD).

METHODS: STZ was injected twice intracerebroventrically (3 mg/kg, ICV) on alternate days (day 1 and day 3) in Rats. Daily treatment with TEN (2, 4, and 8 mg/kg) starting from the first dose of STZ for 28 days. Memory-related behaviors were evaluated using the Morris water maze test. Hyperphosphorylation of tau proteins in hippocampus were measured by western blot assay. Superoxide dismutase activities, malondialdehyde, glutathione peroxidase and 4-hydroxy-2-nonenal adducts contents were also measured in the hippocampus.

RESULTS: Treatment with TEN significantly improved STZ-induced cognitive damage, markedly reduced changes in malondialdehyde and 4-hydroxy-2-nonenal adducts, and significantly inhibited STZ-induced reduction in superoxide dismutase and glutathione peroxidase activities in the hippocampus. In addition, TEN decreased hyperphosphorylation of tau resulting from intracerebroventricular STZ (ICV-STZ) injection, and Nissl staining results showed that TEN has protective effects on hippocampal neurons.

CONCLUSION: These results provide experimental evidence demonstrating preventive effect of TEN on cognitive dysfunction, oxidative stress, and hyperphosphorylation of tau in ICV-STZ rats. This study indicates that TEN may have beneficial effects in the treatment of neurodegenerative disorders such as AD.

Keywords:

Intracerebroventricular streptozotocin, oxidative stress, tau hyperphosphorylation, tenuigenin

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Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by a progressive decline in cognitive functions.[1] The exact etiology of AD has not been clearly identified, but numerous pharmacological research studies have demonstrated that oxidative stress is involved in AD-type neurodegeneration with cognitive impairment and age-related cognitive deficit.[2] Oxidative stress is an imbalance between an oxidant (reactive oxygen species [ROS]/reactive nitrogen species [RNS]) and antioxidant defense systems. This increase in the ROS/RNS can lead to mitochondrial dysfunction,

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tau hyperphosphorylation, and β-amyloid (Aβ) overexpression.[3-5]

Streptozotocin (STZ), a glucosamine- nitrosourea chemical compound, can generate a cytotoxic product when metabolized, which induces preferential damage in pancreatic β cells. Intracerebroventricular (ICV) injection of STZ in rats can produce long-term and progressive loss of memory. It also impairs cerebral glucose and energy metabolism, resulting in oxidative stress and inflammation.[6,7] Furthermore, ICV-STZ leads to increased Aβ accumulation and hyperphosphorylation of tau proteins in the hippocampus.[8,9] Thus, the method of ICV injection of STZ provides a useful model for studying the mechanism and therapeutic intervention of AD.[10]

Tenuigenin (TEN), the active component of the Chinese herb Polygala tenuifolia root, has been used to improve memory and intelligence in Traditional Chinese Medicine for approximately 2000 years. Figure 1 shows the structural formula of TEN. TEN has been reported to have various biological and pharmacological activities such as antioxidation, anti-inflammation, antidementia, and anti-aging.[11,12] Pharmacological data indicate that TEN could suppress secretion of Aβ in SH-SY5Y APP 695 cells[13] and protect SH-SY5Y cells against the injury induced by 6-OHDA.[14] Our previous study has shown that TEN could improve the proliferation and differentiation of hippocampal neural stem cells *in vitro*.[15] It also protected hippocampal neuronal cells against damage induced by methylglyoxal through its antioxidant and antiapoptotic properties.[16]

In the present study, we use *in vivo* model to evaluate the protective effects of TEN against learning and memory impairments, oxidative stress, and tau protein phosphorylation induced by ICV injection of STZ.

Materials and Methods

Animals

Adult male Sprague-Dawley rats, weighing 250–300 g (obtained from Beijing Vital River Laboratory Animal Technology, Co. Ltd., China), were used in the present experiments. All rats were housed under a 12/12 h light/dark cycle and specific pathogen-free conditions. Food and water were provided *ad libitum*. All animal care and experimental procedures were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of Xuan Wu Hospital of Capital Medical University.

Chemicals and reagents

TEN (purity >98%) was purchased from the National Institute of Pharmaceutical and Biological Products (Beijing, China). STZ, 4-hydroxy-2-nonenal (4-HNE), thiobarbituric acid (TBA), paraformaldehyde, hematoxylin, and eosin were purchased from Sigma (St. Louis, USA). Superoxide dismutases (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) kits were obtained from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). Total tau, ser396-phosphorylated tau, thr181-phosphorylated tau, and β-actin-specific antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA).

Intracerebroventricular administration of streptozotocin

ICV injection of STZ was performed as previously described.[17] Rats were anesthetized with 10% chloral hydrate (3 ml/kg, i. p.), the head was fixed in a stereotactic frame and a midline sagittal incision was made in the scalp. Two holes were drilled in the skull on both sides over the lateral ventricles using the following coordinates: 0.8 mm posterior to the bregma, 1.5 mm lateral to the sagittal suture, and 3.6 mm beneath the surface of the brain. Rats in the STZ and three TEN groups were given ICV injections of STZ (3 mg/kg bilaterally, on day 1 and 3). STZ was dissolved in citrate buffer (pH 4.4) shortly before application. Rats in the control group were given an ICV injection of the same volume of artificial cerebral spinal fluid (ACSF, 147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$, and 2.2 mM dextrose) on day 1 and day 3.

Animal groups and drug administration

After a week of adaption to colony room conditions, all rats were randomly divided into five groups consisting of 12 animals each (n = 12): Group I: Sham operated, vehicle-treated control (Sham); Group II: ICV-STZ-infused and vehicle-treated (ICV-STZ); Group III: ICV-STZ-infused and 2 mg/kg TEN treated (TEN 2 mg/kg); Group IV: ICV-STZ-infused and 4 mg/kg TEN treated (TEN 4 mg/kg); and Group V: ICV-STZ-infused and 8 mg/kg TEN treated (TEN 8 mg/kg). The administration was conducted once daily after the first STZ infusion until the water maze trial. Thus, the total period of administration was 28 days. The TEN dose used in this study was determined from previous studies. The animals in the Sham and STZ groups were administered with the same amount of double-distilled water. All drugs were administered by intragastric administration.

Morris water maze test

The Morris water maze (MWM) procedure is most commonly used to test the spatial memory. The water maze is a circle-shaped black pool (150 cm diameter, 40 cm high, and filled up to 30 cm with water at 28 ± 1°C). The pool is divided into four quadrants (1, 2, 3, and 4),

and a platform is placed in the 4th quadrant submerged 1 cm below the water surface. Animals performed four trials daily for five consecutive days. Each trial was started by placing the animal into the tank, facing the wall of the tank. Before each trial, the releasing position was randomly selected by computer. The latency time to find the hidden platform and the swim velocity were recorded; when 120 s had elapsed, the trial was terminated automatically. The rat was allowed to stay on the platform for 5 s. If they were not able to find the platform after 120 s of swimming, they were put on the platform gently by the examiner and allowed to stay on it for 15 s. After each trial, rats were gently dried and placed in their home cage. Using the ANY-maze video tracking system (Caterpillar instrumentation Pvt.), we analyzed the path of each rat. On the 6th day, a probe trial was done by removing the platform and allowing the rats swim freely in the pool for 60 s. The results were obtained by calculating the time spent in the target quadrant where the platform had previously been located during the training phase. This parameter provided a measure of task comprehension.

Nissl staining

After being anesthetized with 10% chloral hydrate, the rats were subjected to transaortic perfusion with phosphate-buffered saline followed by 4% paraformaldehyde to fix the brain. After fixation, the tissue was dehydrated and embedded in paraffin. Coronal sections at the level of the hippocampus were collected and cut into 4 mm thickness. Nissl staining was done as described in the protocol of the instructions. Sections were observed under light microscope (Olympus, CX-21) for histopathological changes. Six random visual fields of the hippocampal CA1 were photographed in each section. At high magnification (×400), the number of staining cells in each field was calculated, and the data were represented as the number of staining cells.

Assessment of the lipid peroxidation

Preparation of brains samples

After completing the MWM test, rats were sacrificed by decapitation and brain tissues were dissected and rinsed with ice-cold isotonic saline. The hippocampus samples were quickly separated and then homogenized with phosphate buffer solution (pH 7.4). The homogenate was centrifuged at 1500 rpm for 10 min (4°C) and aliquots of supernatant were separated and used for assays of SOD, GSH-Px, and MDA. Protein concentrations were determined in the hippocampus samples using the Bradford assay.

Biochemical analysis

Activities of SOD, GSH-Px, and MDA in the hippocampus were measured according to the manufacturer's instructions.

The activity of SOD was determined by detecting its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unit (U) of SOD activity is defined as the amount of enzyme required to inhibit the rate of NBT reduction by 50%. The maximum absorbance was read at 550 nm and enzymatic activity was expressed as U/mg protein/min.

GSH-Px activity was measured by quantifying the rate of oxidation of the reduced glutathione to the oxidized glutathione, which was catalyzed by GSH-Px. One unit of GPX is defined as the amount of enzyme required to oxidize 1 nmol GSH/min. The GSH-Px activity in the supernatant was expressed as U/mg protein.

MDA is a biomarker to measure the level of lipid peroxidation. MDA can react with TBA, which produces a pink complex with a peak absorbance at 532 nm. The MDA level was expressed as nmol per mg protein.

Western blot analysis

Analyses of immunocontent of 4-HNE protein adduct, tau, and phosphorylated tau (ser396 and thr181) in the hippocampus were performed using Western blotting. Briefly, the hippocampus was homogenized in ice-cold lysis buffer containing protease and phosphatase inhibitors. The protein concentrations of the samples were determined using a BCA protein assay kit (Bio-Rad, Hercules, CA, USA). After electrophoresis, the proteins were transferred to a PVDF membrane. Then, the membranes were blocked in Tris-buffered saline mixed with Tween-20 (TBST) containing 10% skim milk for 60 min.

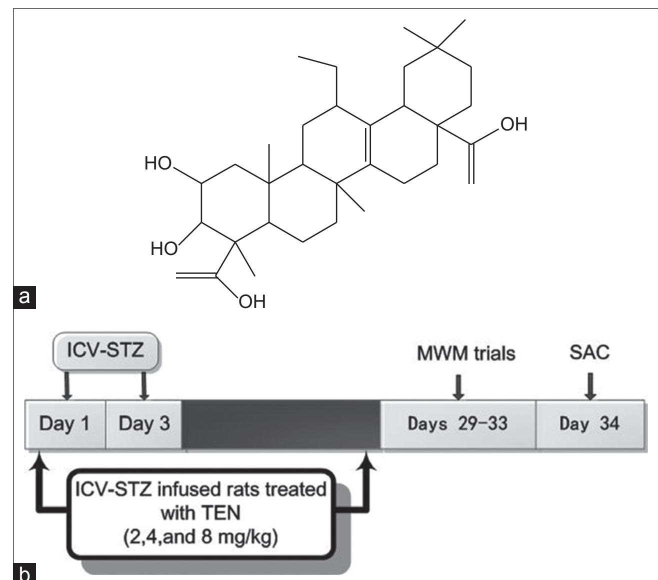

Figure 1: (a) Chemical structure of tenuigenin. (b) The experimental design treatment schedule and intervals for estimation of various parameters. ICV-STZ = intracerebroventricular streptozotocin, MWM = Morris water maze, SAC = sacrificed

In the incubation overnight, we used a blocking solution containing the respective antibody 4-HNE (1:2000), total tau (1:1000), primary rabbit anti-ser396-p-tau (1:1000), rabbit anti-thr181-p-tau (1:1000), and mouse anti-β-actin (1:1000). After washing with TBST, the membranes were incubated with anti-rabbit or anti-mouse secondary antibodies for 2 h at room temperature and washed again in TBST buffer. Finally, the membranes were developed using a chemiluminescence detection system and densitometry was analyzed with the ImageJ software. β-actin was used as a housekeeping protein.

Statistical analysis

Data are expressed as the mean ± standard deviation. Statistical analysis of the results was performed by applying the analysis of variance, followed by Tukey's test for biochemical parameters, and the Mann–Whitney test for behavioral observations. $P < 0.01$ was considered statistically significant.

Results

Effects of tenuigenin on spatial learning and memory deficits induced by intracerebroventricular injection of streptozotocin

Learning and memory functions were assessed using the MWM test, which includes the place navigation task and spatial probe test. The results showed that after 5 days of training, the escape latency to reach the submerged platform decreased significantly in experimental rats of all groups. From day 3 onward, the STZ group rats had a significant increase in the latency time required to reach the platform throughout the training period than did the Sham group rats, indicating poorer learning and memory performance ($P < 0.01$). Treatment with TEN significantly decreased escape latency compared with the STZ group. In addition, 8 mg/kg of TEN was more effective than 2 mg/kg and 4 mg/kg ($P < 0.05$, $P < 0.01$). The effects of 2 mg/kg and 4 mg/kg TEN on escape latency were not significantly different [Figure 2a].

Similar effects were observed in the spatial probe test with the platform removed. STZ treated rats had a significantly decreased amount of time spent in the target quadrant than the Sham group rats ($P < 0.01$), as they could not remember the precise location of the platform. However, the amount of time spent in the target quadrant by the rats in the TEN groups was much longer than that in the STZ group ($P < 0.05$, $P < 0.01$) [Figure 2b], supporting a protective effect on learning and memory.

Effects of tenuigenin on hippocampal neurons damage induced by intracerebroventricular injection of streptozotocin

The results of Nissl staining are shown in Figure 3. Neuronal loss and damaged neurons were observed in the hippocampus of the STZ group. The histopathology of the injured neurons showed shrinkage with condensed nuclei and sparse Nissl bodies. In the TEN (2, 4, and 8 mg/kg) groups, it can be clearly observed that neurons had larger cell bodies, and obvious Nissl bodies were seen in most of the neurons compared with the STZ group [Figure 3a]. Our results showed that the number of Nissl staining cells decreased remarkably in the STZ group, and this effect can be attenuated by TEN in a dose-dependent manner ($P < 0.05$, $P < 0.01$) [Figure 3b].

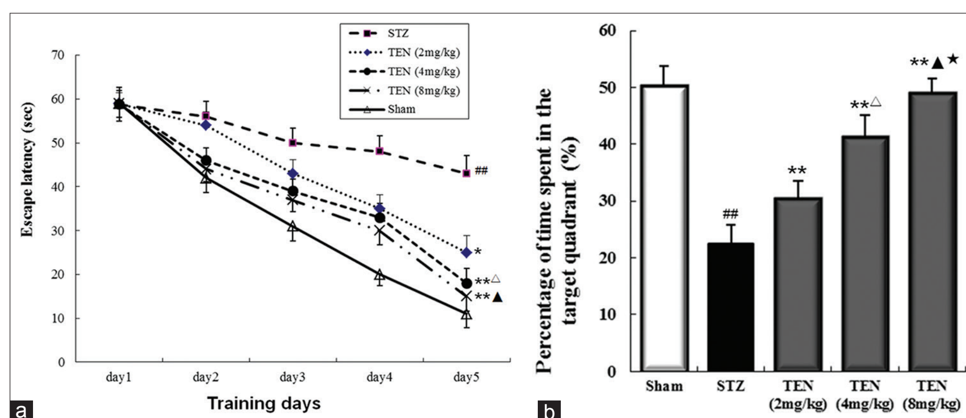

Figure 2: (a) Effects of tenuigenin on memory deficits induced by intracerebroventricular injection of streptozotocin, as evaluated by the Morris water maze test. Streptozotocin-injected rats had longer escape latency than the Sham group, but administration of tenuigenin showed a dose-dependent reduction in escape latency as compared with the streptozotocin group. All values are expressed as mean ± standard deviation. ##$P < 0.01$ vs. Sham group and **$P < 0.01$ versus streptozotocin group. △$P < 0.05$ or ▲ $P < 0.01$ versus tenuigenin 2 mg/kg group. (b) Comparison of the time spent in the target quadrant with other quadrants. The average time in the target quadrant was significantly reduced in the streptozotocin group as compared with the Sham group and which was reversed by tenuigenin treatments. All values are expressed as mean ± standard deviation. ##$P < 0.01$ versus Sham group and **$P < 0.01$ versus streptozotocin group. △$P < 0.05$ or ▲ $P < 0.01$ versus tenuigenin 2 mg/kg group. ★$P < 0.05$ versus tenuigenin 4 mg/kg group

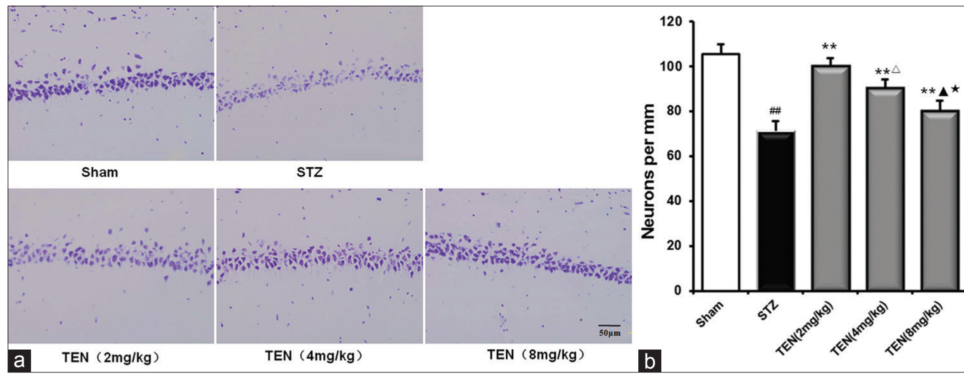

Figure 3: (a) Nissl stain in the CA1 region of hippocampus. Sham group indicates the normal neurons (①). Streptozotocin group revealed neuronal loss (②). Tenuigenin markedly decreases the loss of the cells (③-⑤). Scale bar is 20 μm. (b) The number of Nissl staining cells in the hippocampal CA1 regions. Quantitative cell count revealed an increase in the number of surviving neurons in the tenuigenin groups in a dose-dependent manner. All values are expressed as mean ± standard deviation (n = 5). ##P < 0.01 versus Sham group and **P < 0.01 versus streptozotocin group. △P < 0.05 or ▲ P < 0.01 versus tenuigenin 2 mg/kg group. ★P < 0.05 versus tenuigenin 4 mg/kg group

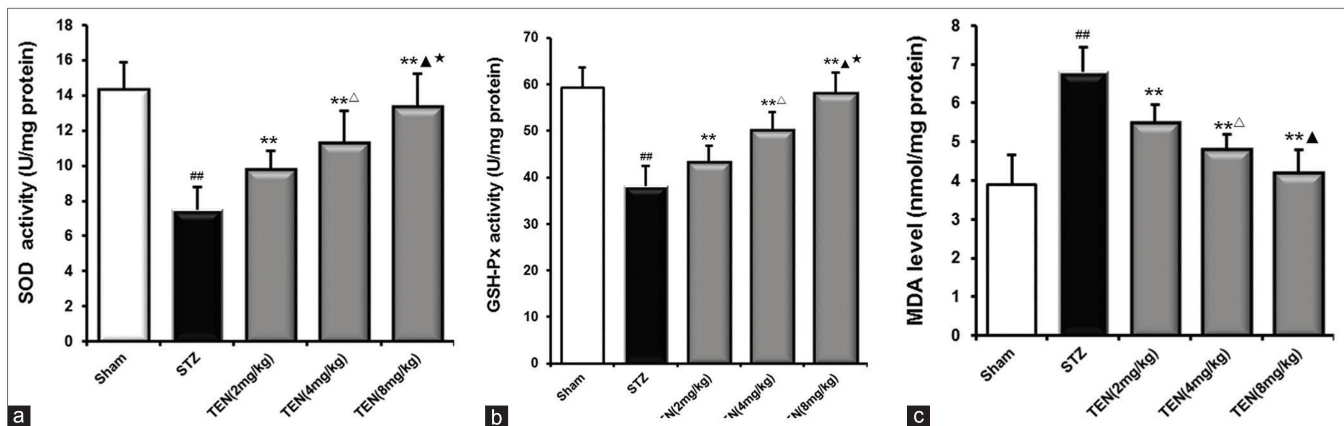

Figure 4: (a) Effects of tenuigenin on superoxide dismutases in the hippocampus induced by intracerebroventricular injection of streptozotocin. Streptozotocin significantly decreased the superoxide dismutases activity in the hippocampus as compared with the Sham group (##P < 0.01 vs. Sham group), whereas tenuigenin had significant effect on increasing the activity of superoxide dismutases in a dose-dependent manner (**P < 0.01 vs. streptozotocin group, △P < 0.05 or ▲ P < 0.01 vs. tenuigenin 2 mg/kg group, ★P < 0.05 vs. tenuigenin 4mg/kg group). (b) Effects of tenuigenin on glutathione peroxidase in the hippocampus induced by intracerebroventricular injection of streptozotocin. The activity of glutathione peroxidase in the hippocampus of the streptozotocin group was significantly lowered compared with the Sham group (##P < 0.01 vs. Sham group), but treatment with tenuigenin notable restored the glutathione peroxidase activity in a dose-dependent manner (**P < 0.01 vs. streptozotocin group, △ P < 0.05 or ▲ P < 0.01 vs. tenuigenin 2 mg/kg group, ★P < 0.05 vs. tenuigenin 4mg/kg group). (c) Effects of tenuigenin on malondialdehyde level in the hippocampus induced by intracerebroventricular injection of streptozotocin. Intracerebroventricular - streptozotocin caused a significant increase in the levels of malondialdehyde in the hippocampus (##P < 0.01 vs. Sham group). Administration of tenuigenin significantly restored the levels of malondialdehyde. (**P < 0.01 vs. streptozotocin group, △P < 0.05 or ▲ P < 0.01 vs. tenuigenin 2 mg/kg group)

Effects of tenuigenin on superoxide dismutases activity, glutathione peroxidase, and malondialdehyde contents in the hippocampus induced by intracerebroventricular injection of streptozotocin

To understand the antioxidant effects of TEN, the SOD activity in the hippocampus was measured and summarized in Figure 4a. The levels of SOD in the hippocampus decreased significantly in the STZ group compared to the Sham group (P < 0.01). TEN (2, 4, and 8 mg/kg) treatment for 28 days significantly inhibited the reduction of SOD activity in a dose-dependent manner in the hippocampus (P < 0.05, P < 0.01) [Figure 4a].

GSH-Px activity was measured to evaluate the enzymatic defense potential of the cells against the oxidative stress. As shown in Figure 4b, a significant reduction in the GSH-Px activity in the hippocampus was observed in the STZ group compared to the Sham group (P < 0.01). However, following the application of TEN (2, 4, and 8 mg/kg), the GSH-Px content was significantly higher in a dose-dependent manner than that of the STZ group (P < 0.05, P < 0.01).

The degree of free radical damage following STZ injection was assessed using lipid peroxidation, which was measured as the MDA level. As shown in Figure 4c, compared to the Sham group, MDA levels in the brain tissue significantly increased in the STZ group (P < 0.01); however, treatment with TEN (2, 4, and 8 mg/kg) significantly ameliorated the increases (P < 0.05, P < 0.01).

Effect of tenuigenin on 4-hydroxy-2-nonenal adducts levels

4-HNE is an α, β unsaturated aldehyde produced during

oxidation in the membranes. 4-HNE can react with proteins to form 4-HNE adducts, and the levels of 4-HNE adducts in the hippocampus were also investigated as oxidative parameters. As shown in Figure 5a and b, the hippocampal tissues of the STZ group had significantly higher 4-HNE levels compared to the Sham group ($P < 0.01$). Furthermore, 4-HNE in the rats treated with 2 mg/kg of TEN was lower than that of 4 and 8 mg/kg ($P < 0.05$, $P < 0.01$). The effects of 4 mg/kg and 8 mg/kg TEN on hippocampal 4-HNE were not significantly different.

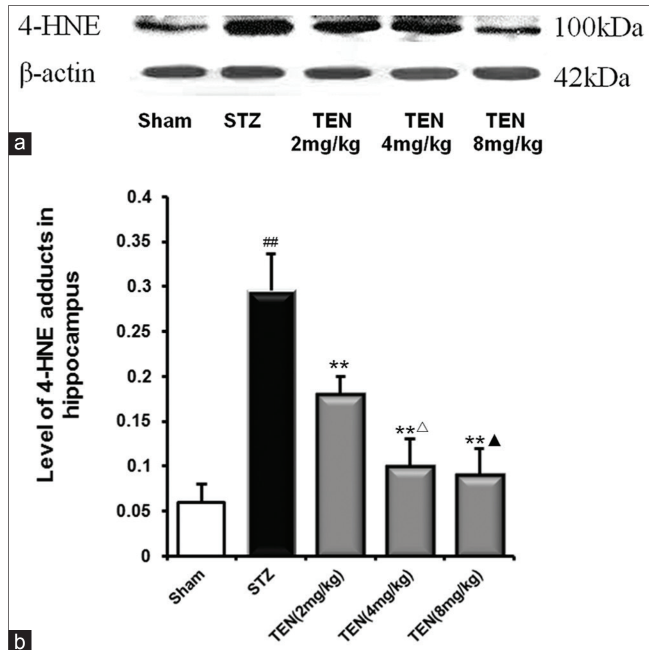

Figure 5: (a) Effect of tenuigenin treatment on 4-hydroxy-2-nonenal adducts. The relative values from each group were presented as mean ± standard deviation. Equal loading of proteins was illustrated by actin bands. (b) The level of 4-hydroxy-2-nonenal adducts was significantly increased in the intracerebroventricular - streptozotocin group as compared to the Sham group (##$P < 0.01$ vs. Sham group). Treatment with tenuigenin significantly restored these enhanced levels of 4-hydroxy-2-nonenal adducts as induced by intracerebroventricular - streptozotocin (**$P < 0.01$ vs. streptozotocin group, △$P < 0.05$ or ▲ $P < 0.01$ vs. tenuigenin 2 mg/kg group)

Effect of tenuigenin on phosphorylated tau proteins

We evaluated levels of tau phosphorylation and observed an increase at ser396 and thr181 sites in the hippocampus in ICV-STZ rats compared with the Sham group ($P < 0.01$). As illustrated in Figure 6a and b, the total tau protein expression was not significantly changed among the groups ($P > 0.05$). Treatment with TEN (2, 4, and 8 mg/kg) obviously reversed the hyperphosphorylation of tau in the hippocampus of ICV-STZ rats [Figure 6c and d]. In addition, treatment with the highest dose of TEN was more effective in decreasing the hyperphosphorylation of tau when compared to the effect of the medium and lowest TEN doses ($P < 0.05$, $P < 0.01$).

Discussion

AD is the most common cause of dementia characterized by the loss of memory and cognitive dysfunctions,[18,19] accounting for approximately 65%–70% of all dementia.[20] AD is increasing rapidly as life expectancy is prolonged, and the disease has become a critical health-care issue and an important social problem worldwide.[21]

ICV-STZ causes desensitization of insulin receptors, resulting in impaired insulin signaling.[22-24] Our

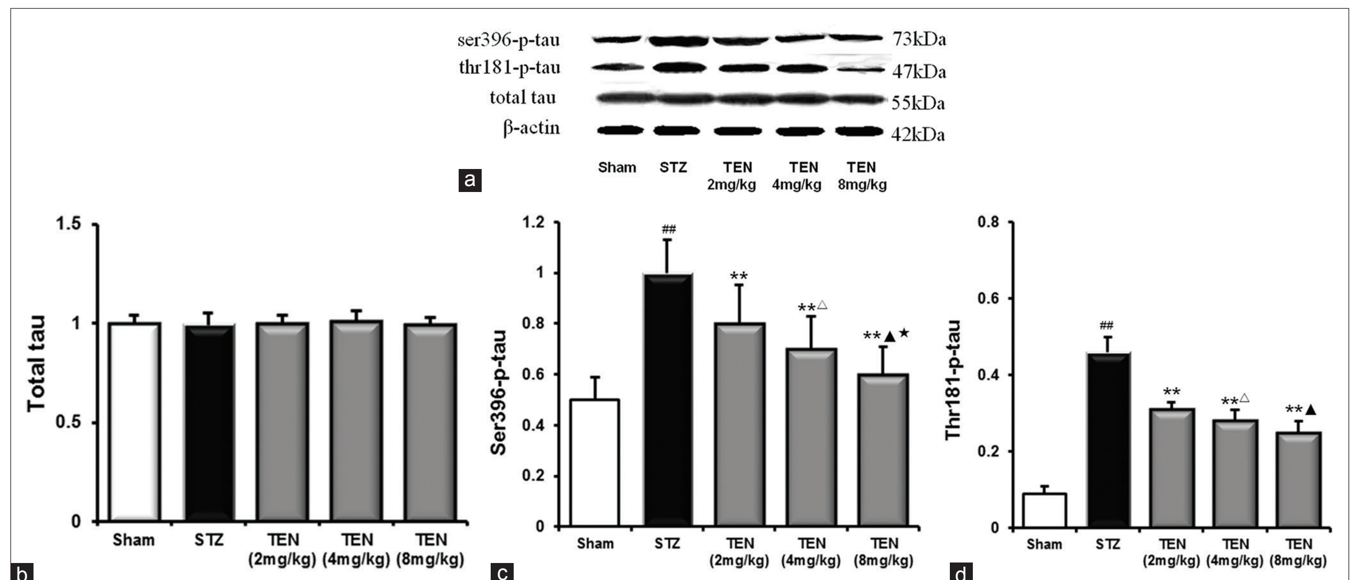

Figure 6: (a) Effects of tenuigenin on intracerebroventricular - streptozotocin -induced phosphorylated tau. The level of ser396-phosphorylated tau and thr181-phosphorylated tau as induced by streptozotocin was significantly decreased after administration of tenuigenin. All values are expressed as mean ± standard deviation ($n = 5$). (##$P < 0.01$ vs. Sham group). **$P < 0.01$ versus streptozotocin group. △$P < 0.05$ or ▲ $P < 0.01$ versus tenuigenin 2 mg/kg group. ★$P < 0.05$ versus tenuigenin 4 mg/kg group. (b) The level of total tau. (c) The level of ser396-phosphorylated tau. (d) The level of thr181-phosphorylated tau

results today are consistent with previous reports[25-27] stating that ICV injection of STZ triggered an obvious impairment in memory and a broad range of neural changes including impaired metabolism and increases in oxidative stress, while administration of TEN prevented this cognitive damage.

TEN, a major active component of the Chinese herb Yuanzhi, has been described as having a wide range of activities on the pathological state of the organism by many studies.[11-15] Recently, our laboratory has demonstrated that TEN has significant protective effects against the neurotoxicity induced by methylglyoxal in cultured hippocampal neural stem cells.[16] Hence, the present study was designed to explore the neuroprotective effect of TEN on STZ-induced cognitive decline in a model of sporadic AD.

The MWM escape task was employed to assess spatial learning and memory abilities of animals by observing the escape latency to reach a hidden platform. In the present study, animals in the STZ group showed a slower rate of decline in the escape latency compared to the Sham group, whereas those treated with TEN showed shorter escape latencies in a dose-dependent manner. Furthermore, treatment with TEN showed a marked increase in the percentage of distance and time spent in the target quadrant. The MWM test suggests that TEN could protect rats from STZ-induced learning and memory impairment.

Previous reports have demonstrated that oxidative stress plays an important role in the advancement of ageing and age-related neurodegenerative disorders such as AD.[28,29] Oxidative stress is the imbalance between the oxidative-antioxidative systems, which is a key mechanism involved in STZ-induced neurotoxicity. Free radical-induced damage to macromolecules such as lipid, protein, and nucleic acids is considered an important factor in the acceleration of AD.[30]

MDA, an end-product of lipid hydroperoxide, is an indicator of ROS. SOD is a catalyzing enzyme that can reduce O_2 – to hydrogen peroxide (H_2O_2). GSH-Px protects cells by catalyzing the reduction of H_2O_2 and other peroxides.[31] A decrease in the level of GSH-Px may inhibit H_2O_2 clearance and increase the formation of $^{\bullet}$OH. $^{\bullet}$OH is the most toxic molecule to the brain, resulting in more oxidant load and consequently oxidative damage. Too much H_2O_2 might accelerate the generation of the peroxidation of polyunsaturated fatty acids and lead to the formation of lipid peroxidation.[32,33] Lipid peroxides and hydroperoxides can generate MDA and HNE, which are more stable and diffusible cytotoxic agents. The brain is a main metabolizer organ of oxygen and seems to be more susceptible to oxidative stress.[34]

In the present study, the increased MDA level in the ICV-STZ animals was attenuated by TEN treatment. TEN could also increase the SOD and GSH-Px activities in the hippocampus of STZ-treated rats. Previous studies have revealed that TEN has antioxidative properties and can protect against oxidative stress *in vitro*.[35] In this work, we guess that ameliorating oxidative stress may be one of the reasons TEN improving learning and memory abilities. Our previous study suggested that TEN was involved in regulating the proliferation and differentiation of hippocampal neural stem cells *in vitro*. However, whether there is similar effect of TEN on hippocampal neurons *in vivo* not yet known and we hope that our today's work can clarify the potential therapeutic value of TEN in the treatment of cognitive dysfunction.

4-HNE is a kind of aldehyde produced during lipid peroxidation of unsaturated lipids in the membrane. It can conjugate to specific amino acid residues of proteins and play key roles in signal transduction pathways, cellular adhesion, and numerous cell cycle events. The previous report suggested that there exist increased levels of specific HNE-histidine Michael adducts in AD hippocampus compared to age-matched controls.[36] The results of the present study are similar to the previous report demonstrating that HNE was significantly increased in ICV-STZ rats. Moreover, after TEN treatment, the HNE level decreased markedly.

Tau protein is a kind of microtubule-associated protein, and the main function of tau is to maintain intracellular microtubule stabilization. Hyperphosphorylation of tau decreases the capacity for binding and stabilizing microtubules.[37] Phosphorylated tau protein aggregates to neurofibrillary tangles (NFTs), which are one of the typical characteristics of the hallmark pathologies of AD.[38] Phosphorylation of tau proteins on many sites is toxic to neurons and inhibits normal tubulin,[39] leading to axonal transport dysfunction,[40] proteasome activity inhibition,[41] consequent impairment of the structure and function of neurons, and eventually neurodegeneration.

In the present study, we also investigated the tau hyperphosphorylation expression induced by ICV-STZ. Previous reports showed that the accumulation of HNE produced hyperphosphorylated tau proteins; furthermore, under conditions of modified HNE, normal tau proteins are vulnerable to be hyperphosphorylated, and epitopes of these modified tau represent the major conformational changes contributing to the formation of NFTs.[42] In this study, we found that increased 4-HNE was accompanied by elevation of phosphorylated tau at ser396 and thr181 in ICV-STZ rats. After TEN administration, 4-HNE decreased and TEN could reduce hyperphosphorylation of tau protein at these two sites,

suggesting the role of TEN in the suppression of p-tau activation and associated pathogenesis caused by STZ.

Conclusion

The present study shows that TEN can ameliorate behavioral impairments of memory deficit, inhibit oxidative stress, and block tau hyperphosphorylation in rat hippocampus induced by the administration of ICV-STZ. Taken together, these data suggest that TEN may be a useful agent for the treatment of AD.

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Conflicts of interest
There are no conflicts of interest.

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