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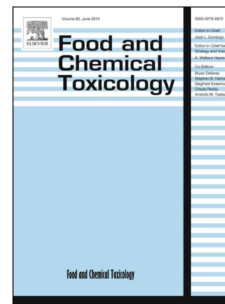

Acteoside protects against 6-OHDA-induced dopaminergic neuron damage via Nrf2-ARE signaling pathway

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Title

Acteoside protects against 6-OHDA-induced dopaminergic neuron damage via Nrf2-ARE signaling pathway

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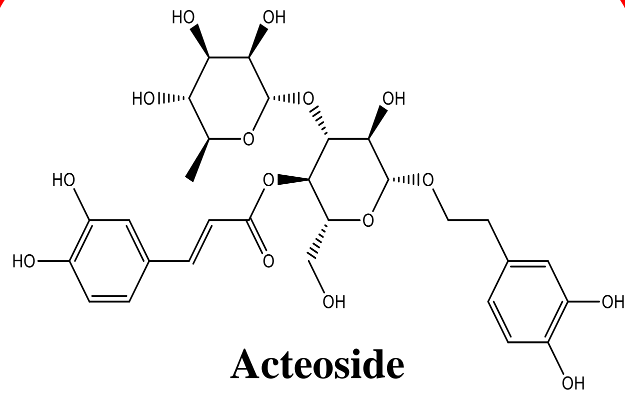
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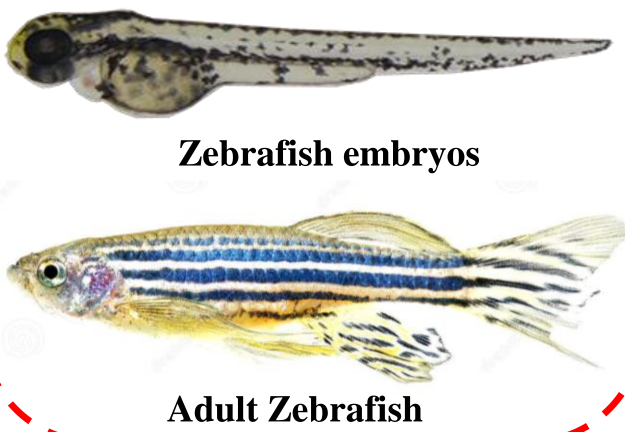
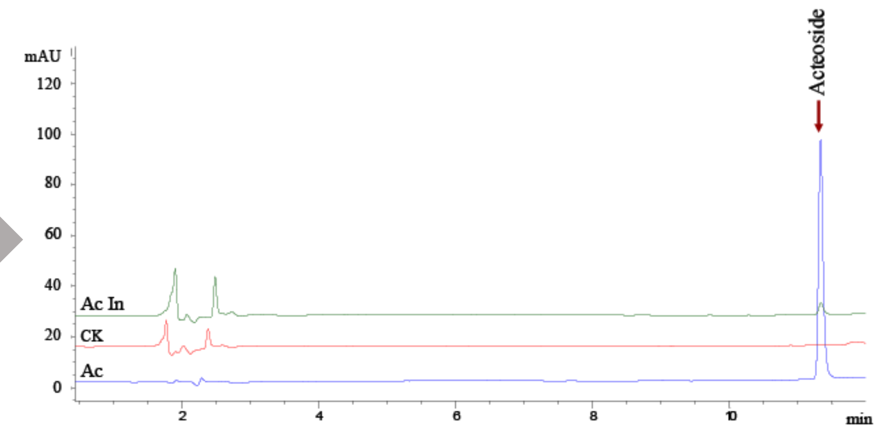

Zebrafish embryos
Adult Zebrafish

BBB permeability

Intraperitoneal Injection


Acteoside
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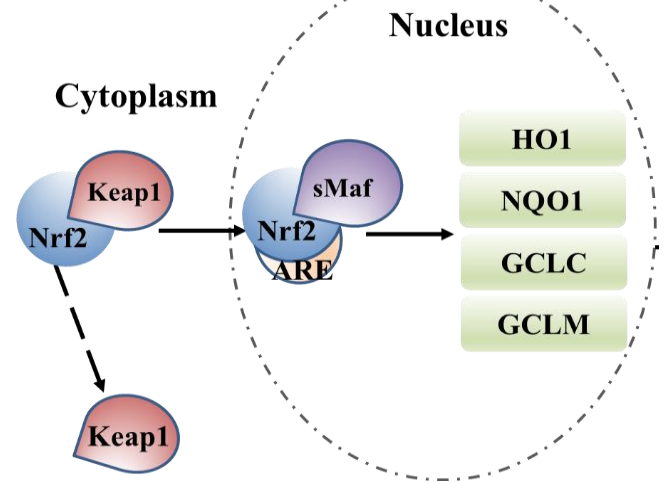
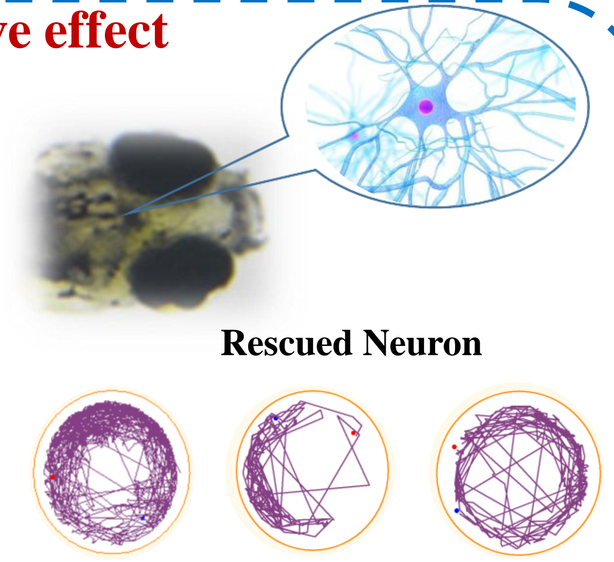
UPLC Analysis

Neuroprotective effect


Nucleus
Cytoplasm
Keap1
Nrf2
sMaf
ARE
HO1
NQO1
GCLC
GCLM
qRT-PCR Analysis


Rescued Neuron
Recovered Behavior

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1. Introduction[1]

Parkinson's disease (PD) is the second most common neurodegenerative disease (Zhang et al., 1900) characterized by a loss of dopaminergic neurons in the substantia nigra (Savitt et al., 2006). Although the exact mechanisms underlying PD are largely unknown, oxidative stress is thought to be one of the critical factors that induce the onset of the disease (Hwang, 2013). Antioxidant defense system is one of the major mechanisms to protect cells from such stress. There are three cellular components: Kelch-like ECH-associated protein 1 (Keap1), nuclear factor erythroid 2-related factor 2 (Nrf2) and antioxidant response elements (ARE) in this system (Itoh et al., 1999). Under physiological conditions, repressor Keap1 holds Nrf2 in the cytoplasm and promotes its ubiquitination (Kumar et al., 2014), followed by 26S proteasomal degradation (Biswal, 2007). Oxidative stress dissociates Nrf2 from Keap1 and increases translocation and transcription of Nrf2 dependent genes including: heme oxygenase-1 (HO-1), glutathione cysteine ligase regulatory subunit (GCLC) and glutathione cysteine ligase modulatory subunit (GLCM). Loss of Nrf2 exacerbates the vulnerability of dopaminergic neurons to oxidative stress (Rojo et al., 2010; Burton et al., 2006; Jakel et al., 2007), whereas Nrf2 activation provides a neuroprotective response (G et al., 2016). Due to these facts, the Nrf2/ARE pathway is considered to be a promising target in neurodegenerative diseases such as PD.

6-hydroxydopamine (6-OHDA) is widely used to generate experimental cell and

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[1] Abbreviations: **6-OHDA**, 6-hydroxydopamine; **ARE**, antioxidant response elements; **BBB**, the blood-brain-barrier; **CAT**, catalase; **DA**, dopaminergic; **dpf**, day post fertilization; **GLCM**, glutathione cysteine ligase modulatory subunit; **GCLC**, glutathione cysteine ligase regulatory subunit; **GSH-Px**, glutathione peroxidase; **HO-1**, heme oxygenase-1; **Keap1**, Kelch-like ECH-associated protein 1; **MDA**, lipid peroxidation; **Nrf2**, nuclear factor erythroid 2-related factor 2; **PD**, Parkinson's disease; **PDA**, photodiode array; **SOD**, superoxide dismutase; **TH**, tyrosine hydroxylase

animal models of PD because it could induce cell damage by producing oxidative stress (Blesa et al., 2012). Zebrafish is a well-established model in studies on drug discovery for neurological disorders. Recent studies showed that 6-OHDA could induce dopaminergic neuronal death and Parkinson's pattern in zebrafish (Feng et al., 2014).

The bioactivity of natural product was a hot topic. Dietary flavonoids and their modifications was reported to show antioxidant capacity and anti-inflammatory capacity (Chen et al., 2018). Studies showed that some of them was good Nrf2 activators, such as agrimonolide and desmethyl agrimonolide (Chen et al., 2017). Acteoside is a widespread disaccharide caffeoyl esters and plants with high concentrations of acteoside have been used in folk medicine (Georgiev et al., 2012). Acteoside was reported to have antioxidant and neuroprotective effect. In previous studies, the acteoside-rich *Osmanthus fragrans* flower extract was reported to be a natural antioxidant using ABTS· assay, DPPH· assay, FRAP assay, cell antioxidant assays, and aging ICR mouse model (Lu et al., 2014a; Lu et al., 2014b; Xiong et al., 2015). Acteoside could alleviate MPP^+-induced apoptosis and oxidative stress in PC12 neuronal cells (Sheng et al., 2002). and protect SH-SY5Y cells from Aβ25-35-induced injury (Wang et al., 2009). However, the potential mechanism have not been clarified. Based on the previous study, we hypothesize that acteoside may have therapeutic effects in 6-OHDA-induced zebrafish model of PD via upregulating Nrf2 activity and the potential to penetrate the BBB.

2. Experimental procedures

2.1 Chemical compounds and reagents

Acteoside (CAS NO. 61276-17-3), 6-OHDA (CAS NO. 636-00-0), nomifensine (CAS NO. 32795-47-4), were purchased from Yuanye Biotechnology Company (Shanghai, China). MDA, SOD, CAT, GSH-Px diagnostic kits were purchased from Beyotime Institute of Biotechnology (Jiangsu, China).

2.2 Fish maintenance

All studies were conducted in accordance with and approved by the Institutional Animal Care and Use Committee of the Laboratory Animal Center, Zhejiang University. We followed the relevant guidelines from the Laboratory Animal Center of Zhejiang University. The AB strain of wild-type zebrafish (*Danio rerio*) was used for this study. Adult zebrafish were maintained at 28℃ under the 14 : 10 h light : dark cycle in an aquatic recirculation system (SPX-150, Shanghai Haisheng Biotechnology Company, Shanghai, China). They were fed twice daily with *Artemia nauplii*. The adult zebrafish were placed in breeding tanks one night in prior to mating. After 2 h, embryos were collected after natural spawning and raised at 28 ℃ in EGG water. The EGG water was change once a day until the 3 day post fertilization (dpf).

2.3 Locomotor behavioral test

The locomotor behavioral test was performed using standard method (Zhang et al., 2016). Briefly, zebrafish larvae at 3 dpf were co-incubated with 250 μM 6-OHDA and nomifensine or 100, 200, 400 μg/mL acteoside for 4 days, the medium was changed every day. Zebrafish at 7 dpf were transferred into 96-well plate (1 fish per well). After they accommodated to the new environment for 30 min, the swimming pattern

and total distance traveled of each fish were recorded for 10 min. Zebrafish behavior was analyzed using an automated video tracking system (Any-maze 4.73, Stoelting, Wood Dale, USA).

2.4 Anti-tyrosine hydroxylase (TH) whole-mount immunostaining

Zebrafish embryos at 1 dpf were treated for 2 days with indicated concentrations of acteoside in the presence of 6-OHDA. The medium was changed every day. Then zebrafish larvae were fixed in 4% paraformaldehyde in PBS for 30 min, rinsed, and stored at −20 °C in 100% MtOH. Wholemount immunostaining was performed using standard method (Zhang et al., 2011). Briefly, fixed samples were blocked by 2% lamb serum and 0.1% BSA in PBST for 1 h at room temperature. A mouse monoclonal anti-tyrosine hydroxylase antibody (MAB318, Millipore, diluted 1:200 in blocking buffer) was used as the primary antibody and incubated with the sample overnight at 4°C. The next day, samples were washed six times with PBST (30 min each wash), followed by incubation with secondary antibody according to the instructions that accompany the Vectastain ABC kit (Vector Laboratories, Burlingame, USA). Then, the zebrafish were flat-mounted with 3.5% methylcellulose and photographed.

2.5 Measurement of intracellular lipid peroxidation (MDA) production, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px)

MDA, SOD, CAT, GSH-Px were measured by assay kits (Beyotime Biotechnology, Nanjing, China). All procedures completely complied with the manufacturers' instructions. The contents of MDA and the activities of SOD, CAT and GSH-Px were

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normalized with the corresponding total protein content.

2.6 Total RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction

Zebrafish embryos at 3dpf were treated with 250 μM 6-OHDA and nomifensine or 100, 200, 400 μg/mL acteoside for 4 days, the medium was changed every day. Zebrafish at 7 dpf were sacrificed in 0.9% tricaine. Total RNA was extracted using the RNAiso Plus (Takara, Shiga, Japan), following the manufacturer's instructions. Total RNA samples were reverse transcribed with PrimeScript™RT reagent Kit with gDNA Eraser (Takara, Shiga, Japan) according to the instruction. Quantitative real-time PCR was performed by using SYBR® Premix Ex Taq™ II (Takara, Shiga, Japan) in Applied Biosystems ViiA™ 7 Real-Time PCR System. Relative expression of target genes was normalized to *β-Actin*, analyzed by 2-ΔΔCt method. The primer sequences used in this study are as follows:

β-Actin	Forward primer	CACTGAGGCTCCCCTGAATC
	Reverse primer	GGGTCACACCATCACCAGAG
NRF2	Forward primer	CTGCTGTCACTCCCAGAGTT
	Reverse primer	GCCGTAGTTTTGGGTTGGTG
HO-1	Forward primer	AAGAGCTGGACAGAAACGCA
	Reverse primer	AGAAGTGCTCCAAGTCCTGC
GCLC	Forward primer	CTCCTCACAGTCACGGCATT
	Reverse primer	TGAATGGAGACGGGGTGTTG
GCLM	Forward primer	AAGCCAGACACTGACACACC

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	Reverse primer	ATCTGGAGGCATCACACAGC
NQO1	Forward primer	AAGCCTCTGTCCTTTGCTCC
	Reverse primer	TGCTGTGGTAATGCCGTAGG

2.7 Assessment of the acteoside permeability to the BBB

Zebrafish embryos at 3 dpf were treated with the solvent (water with 0.1% formic acid), 100, 200, 400 and 2000 μg/mL acteoside for 4 days, the medium was changed every day. Zebrafish at 7 dpf were sacrificed in 0.9% tricaine. The head samples were obtained and rinsed with cold saline. The samples were then placed 1 mL methanol, homogenized with an electric blender (OSE-Y10, TIANGEN, Beijing, China), and stored at -80 □ until further analysis.

Adult zebrafish (1 year old, male : female=1:1), weighing approximately 0.26 g, were randomly assigned into two groups, the solvent and the acteoside (200 mg/kg) was intraperitoneally administered them respectively. The adult fish were slightly anesthetized with 0.3% tricaine, and moved on to a moistened sponge in a vertical position, then the acteoside (20 mg/mL) was injected using a microinjector (10 μL) at the position of ventral fins. The fish were then individually placed into a tank of fresh water and incubated until sample collection. After the fish were sacrificed at the designated times (15 min, 30 min, 45 min, 1 h, 2 h and 3 h). The brain samples were rinsed with cold saline and the wet weights were determined. The samples were then placed in 1 mL methanol, homogenized with an electric blender (OSE-Y10, TIANGEN, China), and stored at -80 C until further analysis.

The samples were filtered through a 0.22 μm nylon syringe filter (ANPEL,

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Shanghai, China), and were analyzed by UPLC (ACQUITY, Waters, Milford, MA, USA) equipped with a photodiode array (PDA) detector (ACQUITY), as was performed in previous study (Zhou et al., 2017). A BEH-C18 Symmetry column (100 mm × 2.1 mm; 1.7 μm) attached with a VanGuard precolumn (2.1 mm × 5 mm, 1.7 μm) (Waters, Milford, MA, USA) was maintained at 40°C. Mobile phase A: water with 0.1% formic acid, mobile phase B: acetonitrile with 0.1% formic acid. The gradient elution programme was as follows: 0-1 min, 94% A; 1-3 min, from 94% to 85% A; 3-8.5 min, from 85% to 82% A; 8.5-9.9 min, from 82% to 28% A; 9.9-10 min, from 28% to 0% A; 10-12 min, 0% A; 12-12.1 min, from 0% to 94% A; and 12.1-15 min, 94% A. The injection volume was 1 μL, and flow rate was 0.2 mL/min. The PDA detector was set from 200 nm to 400 nm.

2.8 Statistical analysis

One way ANOVA with LSD analysis were performed as appropriate to the data using SPSS. All results were confirmed from three independent experiments. Data were expressed as means ± standard error of the mean. Data were considered as statistically significant differences at $p < 0.05$.

3 Results

3.1 Acteoside rescued the 6-OHDA-induced deficit of locomotor activity in zebrafish larvae

In zebrafish larvae, 6-OHDA-treatment resulted in deficits of locomotive behavior. As shown in Fig. 1, treatment with 6-OHDA markedly altered the swimming behavior and reduced the total swimming distance of zebrafish larvae (31.89 mm), whereas

nomifensine significantly reduced this deficit to 46.84 mm ($p < 0.01$). Under the same conditions, 100, 200 and 400 μg/mL of acteoside reversed 6-OHDA-induced reduction in total movement distance in a concentration-dependent manner (39.84, 42.45 and 47.86 mm, respectively), and partly recovered the swimming behavior.

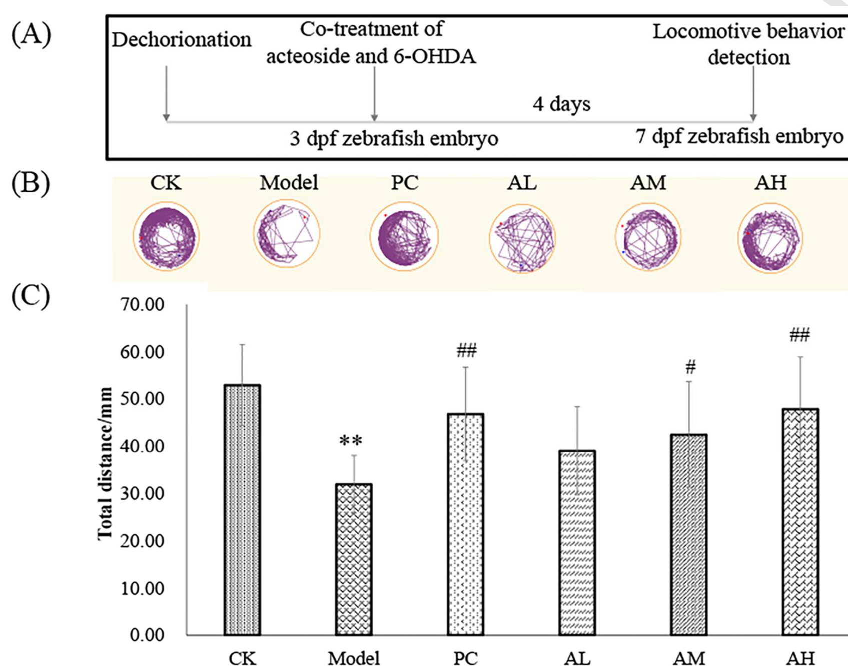

Fig.1 The effect of acteoside on 6-OHDA-induced deficits in the locomotor behavior of zebrafish. Zebrafish larvae at 3 day post fertilization were exposed to 250 μM 6-OHDA with or without acteoside for 4 days. Then larvae were collected to perform locomotion behavior test using the Any-maze 4.73; the total distance traveled and motion trail in 10 min was calculated. (A) A schematic illustration of how zebrafish was treated. (B) Typical swimming patterns of control, 6-OHDA, nomifensine and acteoside treated groups. (B) Statistical analysis of total distance moved of control, 6-OHDA, nomifensine and acteoside treated groups, 10 fish/group were used. Nomifensine used as a positive control. CK: normal group, Model: 6-OHDA treated group, PC: nomifensine treated group; AL: 100 μg/mL acteoside treated group, AM: 200 μg/mL acteoside

treated group, AH: 400 μg/mL acteoside treated group. All data are expressed as means ±SD and represent three independent experiments. ** $p<0.01$ versus untreated group; # $p<0.05$, versus 6-OHDA treated group; ## $p<0.01$, versus 6-OHDA treated group.

3.2 Acteoside prevented 6-OHDA-induced dopaminergic neuronal loss in zebrafish larvae

To confirm the neuroprotective effect of acteoside in vivo, we evaluated the dopaminergic neuronal loss of zebrafish larvae by immunofluorescent staining. As shown in Fig. 2, 6-OHDA treatment decreased the number of dopamine (DA) neurons markedly in the diencephalon of zebrafish. Acteoside significantly alleviated the loss of DA neurons, suggesting that acteoside could protective effect against 6-OHDA-induced dopaminergic neuron death in zebrafish.

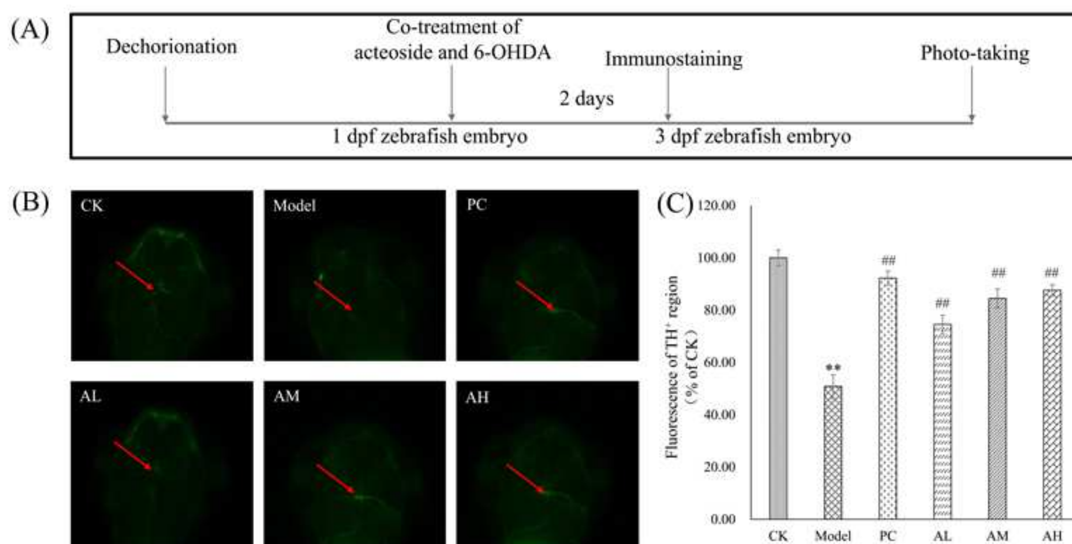

Fig. 2 The effect of acteoside on 6-OHDA-induced dopaminergic (DA) neuron loss in zebrafish. Zebrafish embryos at 1 day post fertilization were exposed to 250 μM 6-OHDA with or without acteoside for 2 days. Then zebrafish larvae were fixed for whole-mount immunostaining. (A) A

schematic illustration of how zebrafish was treated. (B) Representative morphology of DA neurons in the zebrafish brain indicated by immunostaining with antibody against tyrosine hydroxylase (TH). (C) Statistical analysis of TH^+ neuron in each group, 10 fish/group were used. Nomifensine used as a positive control. CK: normal group, Model: 6-OHDA treated group, PC: nomifensine treated group; AL: 100 μg/mL acteoside treated group, AM: 200 μg/mL acteoside treated group, AH: 400 μg/mL acteoside treated group. All data are expressed as means ±SD and represent three independent experiments. ***p*<0.01 versus untreated group; ## *p*<0.01, versus 6-OHDA treated group.

3.3 Acteoside suppressed 6-OHDA-induced accumulation of MDA in zebrafish larvae

As shown in Fig. 3, exposure of zebrafish larvae to 6-OHDA for 4 h significantly increased MDA level, from 44.403 μg/mg Pro to 49.723 μg/mg Pro ($p < 0.01$). Acteoside treatment significantly attenuated MDA (46.40, 43.36 and 35.29 μg/mg Pro, respectively),

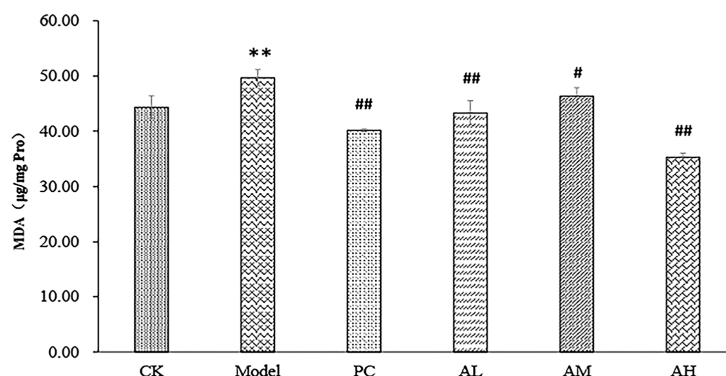

Fig. 3 Acteoside blocked MDA accumulation in 6-OHDA treated zebrafish. Zebrafish larvae at 3 day post fertilization were exposed to 250 μM 6-OHDA with or without acteoside for 4 days.

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Then larvae were collected to detect the MDA level. Nomifensine used as a positive control. CK: normal group, Model: 6-OHDA treated group, PC: nomifensine treated group; AL: 100 μg/mL acteoside treated group, AM: 200 μg/mL acteoside treated group, AH: 400 μg/mL acteoside treated group. All data are expressed as means ±SD and represent three independent experiments. ** $p<0.01$ versus untreated group; # $p<0.05$, versus 6-OHDA treated group; ## $p<0.01$, versus 6-OHDA treated group.

3.4 Acteoside reversed 6-OHDA-induced decrease of SOD, CAT, GSH-Px activity

Exposure of zebrafish larvae to 6-OHDA for 4 h significantly reduced SOD ($p < 0.05$), CAT ($p < 0.01$), GSH-Px ($p < 0.01$) activities (Fig. 4). Acteoside treatment significantly recovered the activities of these antioxidative enzymes above, and the medium-concentration-group showed best effects.

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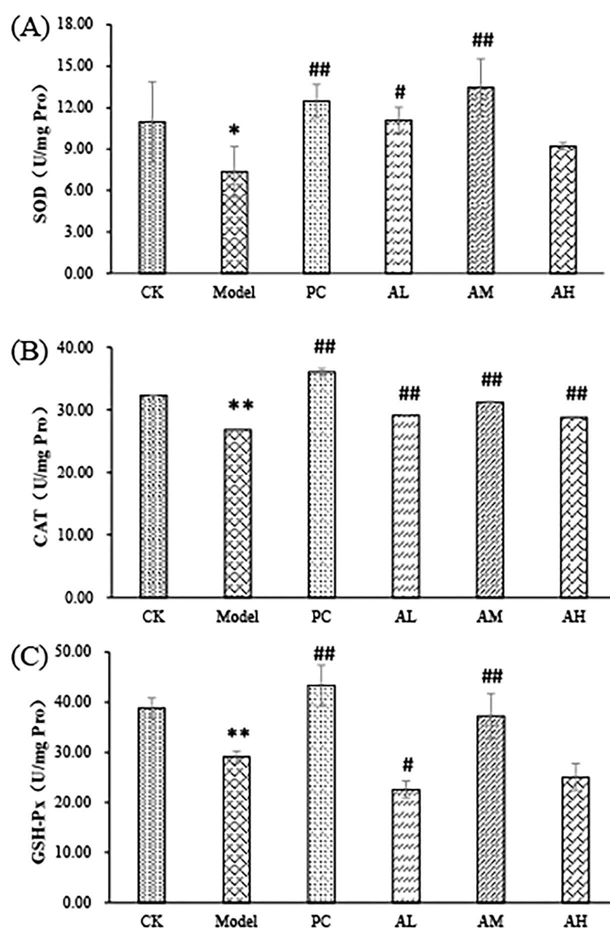

Fig.4 Acteoside reversed the activities of antioxidantive enzymes in 6-OHDA treated zebrafish. Zebrafish larvae at 3 day post fertilization were exposed to 250 μM 6-OHDA with or without acteoside for 4 days. Then larvae were collected to perform detect the antioxidantive enzymes level. (A) SOD level. (B) CAT level. (C) GSH level. Nomifensine used as a positive control. CK: normal group, Model: 6-OHDA treated group, PC: nomifensine treated group; AL: 100 μg/mL acteoside treated group, AM: 200 μg/mL acteoside treated group, AH: 400 μg/mL acteoside treated group. All data are expressed as means ±SD and represent three independent experiments. *p<0.05 versus untreated group; **p<0.01 versus untreated group; # p<0.05, versus 6-OHDA treated group; ## p<0.01, versus 6-OHDA treated group.

3.5 Acteoside activated Nrf2/ARE pathway in 6-OHDA-treated zebrafish larvae

The Nrf2-ARE pathway, as one of the major antioxidant pathways, is a therapeutic target in neurodegenerative diseases. *HO-1*, *NQO1*, *GCLC*, and *GCLM* are Nrf2-regulated downstream genes closely related to antioxidant capacity. Therefore, we investigated whether the Nrf2-ARE pathway is involved in 6-OHDA-induced zebrafish PD model. The mRNA expression of *NRF2*, *HO-1*, *NQO1*, *GCLC*, and *GCLM* was observed after treatment. Obvious decrease was found in the mRNA expression of *NRF2*, *HO-1*, *GCLC*, and *GCLM* after 6-OHDA treatment (Fig. 5A–D). Acteoside upregulated these three genes significantly ($p < 0.01$), and the high-concentration-group showed the best effects.

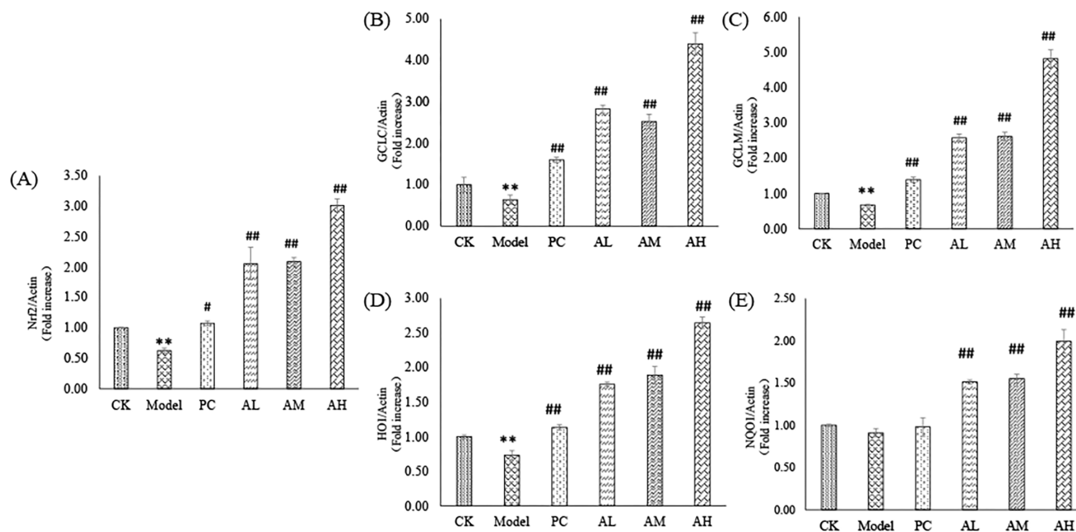

Fig.5 Acteoside increased the expression of *NRF2*, *GCLC*, *GCLM*, *HO-1*, and *NQO1 genes*. Zebrafish larvae at 3 day post fertilization were exposed to 250 μM 6-OHDA with or without acteoside for 4 days. Then larvae were collected to detect the gene expression. (A) Quantitative analysis of *Nrf2* fold increase. (B) Quantitative analysis of *GCLC* fold increase. (C) Quantitative analysis of *GCLM* fold increase. (D) Quantitative analysis of *HO-1* fold increase. (E) Quantitative analysis of *NQO1* fold increase. CK: normal group, Model: 6-OHDA treated group, PC:

nomifensine treated group; AL: 100 μg/mL acteoside treated group, AM: 200 μg/mL acteoside treated group, AH: 400 μg/mL acteoside treated group. Nomifensine used as a positive control. All data are expressed as means ±SD and represent three independent experiments. ***p*<0.01 versus untreated group; # *p*<0.05, versus 6-OHDA treated group; ## *p*<0.01, versus 6-OHDA treated group.

3.6 Acteoside permeated the BBB of zebrafish

No acteoside was detected in the head of zebrafish embryos treated with 100, 200, 400 μg/mL acteoside. But acteoside was detected when the zebrafish embryos were treated with 2000 μg/mL acteoside (Fig. 6).

In the intraperitoneal injection groups, acteoside was detected to show the highest content in zebrafish fish brain tissue in 15 min after injection. The content decreased with administration time. The acteoside was almost undetectable 3 h after injection.

These results showed that acteoside could penetrate the BBB.

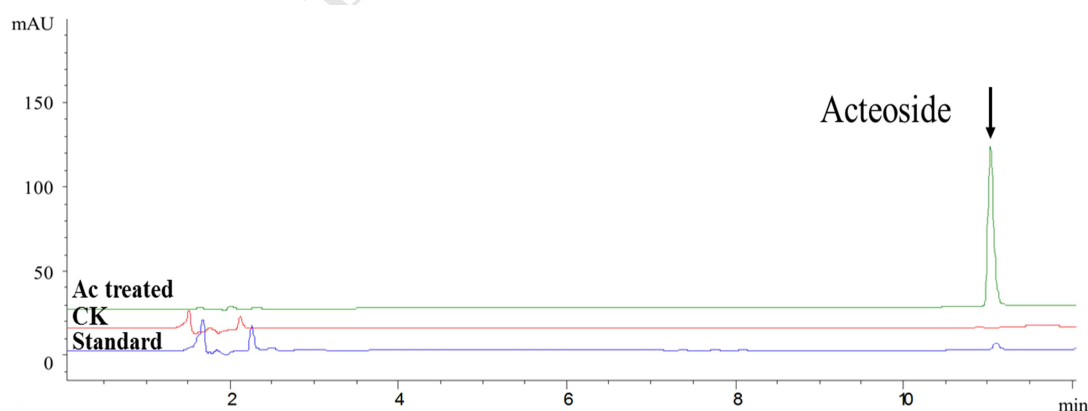

Fig.6 UPLC analysis of acteoside in the head of zebrafish embryo after acteoside exposure. Ac: 2000μg/mL Acteoside treated group; CK: Solvent treated group; Standard: Acteoside standard.

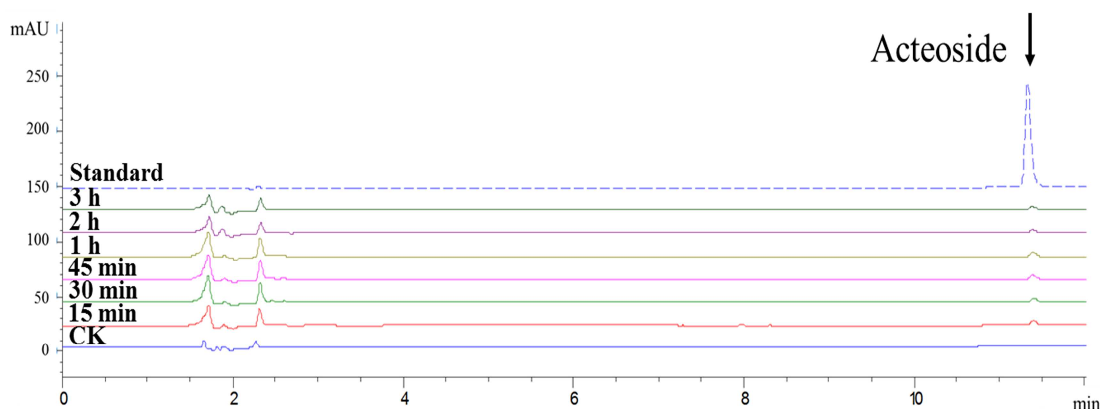

Fig.7 UPLC analysis of acteoside in the brain of adult zebrafish after intraperitoneal injection. Standard: Acteoside standard; CK: Solvent intraperitoneal injection group; 15 min, 30 min, 45 min, 1 h, 2 h, 3 h: brain sample from acteoside treated group 15 min, 30 min, 45 min, 1 h, 2 h, 3 h after intraperitoneal injection.

4 Discussion

The present study showed that acteoside is a potent neuroprotective agent in zebrafish in vivo and this neuroprotective activity might be exerted by permeating the BBB, activating the Nrf2-ARE pathway and upregulating the downstream genes in zebrafish. To the best of our knowledge, this is the first study to demonstrate the neuroprotective of acteoside in zebrafish.

6-OHDA, one of the most common neurotoxins used to experimentally model nigra degeneration in vitro and in vivo, is a hydroxylated analogue of the natural dopamine neurotransmitter. 6-OHDA was suggested to induce nigrostriatal dopaminergic lesions via the generation of hydrogen peroxide and derived hydroxyl radicals (Heikkila and Cohen, 1971). 6-OHDA immersion could induce a significant reduction in zebrafish locomotor activity (Feng et al., 2014). Therefore, 6-OHDA-induced injury model of

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zebrafish can be used an appropriate model for PD. In the present study, TH immunostaining of zebrafish showed that the immunopositive area of DA neuron has been significantly reduced by 6-OHDA-induced oxidative stress, while the loss of DA neuron can be effectively attenuated by acteoside. This provides evidence supporting the hypothesis that acteoside is a therapeutic agent in PD. Other natural products, such as quercetin (Zhang et al., 2011), danshensu (Chong et al., 2013), and berberine (Zhang et al., 2017) were reported to exhibit potent neuroprotective activities in 6-OHDA-induced zebrafish model by mitigating the dopaminergic neuron loss and behavior movement deficiency. Further mechanism study showed that berberine could upregulate PI3K/AKT/Bcl-2 cell survival and Nrf2/HO-1 antioxidative signaling pathways.

The Nrf2-ARE signaling pathway plays an important role in cellular antioxidant defense and survival, and is of great interest in the treatment of neurodegenerative diseases (van Muiswinkel and Kuiperij, 2005). Nrf2$^{(-/-)}$ knock-out mice, and primary astroglial and neuronal culture models derived from these mice are more susceptible to oxidative stress (Calkins et al., 2005; Lee et al., 2003) while Nrf2 overexpression in mixed rat neuronal-glial cortical cultures enhanced antioxidant capacity in both neuronal and astroglial cells and to protect cortical neurons from excitotoxicity (Shih et al., 2003; Sun et al., 2005). Our results show that acteoside increases the activity of antioxidative enzymes including SOD, CAT, GSH-Px, decreases the level of MDA in PD mode and the medium-concentration-group showed best effects. Considering the relatively high expression of *NRF2*, *HO*-1, *GCLC*, *GCLM* genes, we speculated that

in high-concentration-group the high expression of these genes caused a negative feedback mechanism and ultimately suppressed the transcription and translation of antioxidative enzymes. The neuroprotective effects of acteoside may link to the activation of Nrf2, because the increased mRNA level of *NRF2* was observed. In addition, *HO*-1, *GCLC*, *GCLM*, the Nrf2 downstream phase II detoxifying and antioxidative enzymes are also upregulated. These results indicated that acteoside showed neuroprotective activity on PD and was a potential Nrf2 activator. Similarly, in the Aβ-induced Alzheimer's disease model acteoside was also reported to show neuroprotective effect by induction of HO-1 via ERK and PI3 K/Akt signaling (Wang et al., 2012). Even though we used different model, these results together prove that acteoside is an activator of Nrf2 and inducer of HO-1 expression and play important role in the prevention and treatment of neurodegenerative diseases.

The BBB, a tightly regulated barrier in the central nervous system, is essential for maintaining brain homeostasis and protecting the brain from toxic substances (Abbott et al., 2010). However the penetration into the brain remains one of the in the pharmaceutical in main obstacles in the development of drugs used to treat central nervous system (CNS), such as the neurodegenerative diseases. Therefore, the successful development of any kind of CNS-targeting drug depends on the penetration of the BBB (Desai et al., 2007). The endothelial tight junction-based BBB of zebrafish is similar to that of higher vertebrates, and zebrafish may be an excellent model for studying development and maintenance of the BBB (Jeong et al., 2008). Here, we show that acteoside can be detected after intraperitoneal injection,

supporting its permeability to the BBB. In accordance with our study, pharmacokinetics study showed that acteoside extensively and rapidly distributed in most tissues including brain after oral administration (Wen et al., 2015), providing evidence for the BBB permeability of acteoside.

5 Conclusion

In summary, we demonstrate that acteoside exerts a neuroprotective effect in zebrafish. The molecular mechanism may be that acteoside penetrates the BBB and directly acts on brain, meanwhile it activates the Nrf2-AER signaling pathway and enhances the expression of HO-1 against 6-OHDA-induced antioxidant stress. Taken together, it can be concluded that the BBB permeability and the activation of antioxidant mechanisms may be involved in the neuroprotective effects of acteoside.

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Conflict of interest

The authors hereby declare there is no conflict of interests.

Declaration

All animal experiments was complied with the ARRIVE guidelines and was carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines.

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Acteoside showed neuroprotective effect of on 6-OHDA-induced zebrafish PD model.

Acteoside activated the Nrf2/ARE signaling pathway in zebrafish PD model.

Acteoside could penetrate the blood-brain-barrier in zebrafish.

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