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Fisetin improves lead-induced neuroinflammation, apoptosis and synaptic dysfunction in mice associated with the AMPK/SIRT1 and autophagy pathway

Wei Yang[a,b,1], Zhi-Kai Tian[a,1], Hui-Xin Yang[a], Zhao-Jun Feng[a], Jian-Mei Sun[a], Hong Jiang[a], Chao Cheng[a], Qing-Lei Ming[a], Chan-Min Liu[a,*]

[a] School of Life Science, Jiangsu Normal University, No.101, Shanghai Road, Tongshan New Area, 221116, Xuzhou City, Jiangsu Province, PR China
[b] Shanxi Key Laboratory of Ecological Animal Science and Environmental Veterinary Medicine, College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801, China

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ABSTRACT

Fisetin, a natural flavonoid found in plants, fruits and vegetables, exerts anti-cancer, anti-oxidant, anti-inflammatory and anti-mitotic effects. The current study instigates the protective effect of fisetin against lead-induced synaptic dysfunction, neuroinflammation and neurodegeneration in mice, and explores its underlying mechanisms. The results indicated fisetin can significantly ameliorated behavioral impairments in Pb-treated mice. Fisetin inhibited Pb-induced the apoptotic neurodegeneration, as indicated by the decreased levels of Bax and cleaved caspase-3. Fisetin suppressed activations of Toll-like receptor 4 (TLR4), myeloid differentiation factor 88 (MyD88), NF-κB and subsequently inactivate pro-inflammatory factor including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). It can also decrease the accumulation of p-tau and amyloid-beta (Aβ) and increased the expression of the Aβ remover neprilysin (NEP) in brains of mice. Fisetin also reversed Pb-induced synaptic dysfunction by increasing the levels of synaptosomal associated protein-25 (SNAP-25), post-synaptic density-95 (PSD-95), cyclic-AMP-response element-binding protein (CREB) phosphorylation and calcium/calmodulin kinase II (CaMKII) phosphorylation. Fisetin promoted Pb-induced autophagy in the brains of mice. Moreover, fisetin can increase levels of the denosine 5′-monophosphate-activated protein kinase (AMPK) phosphorylation and SIRT1. Fisetin may be developed as a potential nutritional target for the prevention of Pb-induced neurotoxicity.

1. Introduction

Neurological disorders affect mostly to the patients and their families, which often take out the qualities of being human. Alzheimer's disease (AD) is a complex multifactorial disease characterized by synapse loss, memory impairment and other cognitive problems (Weiner et al., 2013; Currais et al., 2014). Parkinson's disease (PD) is a complex disease marked by a movement disorder, with the typical symptoms being resting tremor, rigidity, bradykinesia and postural instability. PD is the second most common neurodegenerative disorder of the central nervous system (CNS) after AD (Koppula et al., 2012; Maher, 2017). Huntington's disease (HD), a rare neurodegenerative disorder of CNS, is characterized by motor, cognitive and psychiatric disorders, and a range of somatic symptoms (Bachoud-Lévi et al., 2015). Therefore, many natural products and medicinal plants could be a good source for treatment of nervous system diseases with fewer or no side effects (Koppula et al., 2012; Maher, 2017).

Fisetin (3,7,3′,4′-tetrahydroxyflavone, $C_{15}H_{10}O_6$) is a natural flavonoid found in various fruits, vegetables, nuts and teas such as persimmon, mango, grape, apple, strawberry, peach, cucumber, tomato, onion and lotus root at concentrations ranging from 0.1 to 539 μg/g (Khan et al., 2013; Sundarraj et al., 2018). Previous studies have shown that oral administration of fisetin (50 mg/kg or 100 mg/kg) had no effect on animals (Wu et al., 2016; Hussain et al., 2019; Ge et al., 2019).

Abbreviations: Aβ, amyloid-beta; AMPK, the denosine 5′-monophosphate-activated protein kinase; CaMKII, calcium/calmodulin kinase II; CREB, the cyclic-AMP-response element-binding protein; IL-6, interleukin-6; MyD88, myeloid differentiation factor 88; NEP, neprilysin; NF-κB, nuclear factor-κB; Pb, lead; PSD-95, postsynaptic density-95; SNAP-25, synaptosomal associated protein-25; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-alpha

* Corresponding author.
E-mail address: lcm9009@126.com (C.-M. Liu).
[1] These authors contributed equally to this work.

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Since its special structure with benzoquinone pharmacophore, fisetin exhibited numerous biological activities, including anti-oxidant, anti-mitotic, anti-inflammation and anti-cancer (Zheng et al., 2008; Currais et al., 2014; Maher, 2015). Previous studies have indicated that fisetin could promote the induction of long-term potentiation (LTP) and enhance object recognition memory in a behavioral experiment with rat (He et al., 2018). Fisetin had the ability to improve memory impairment and other neurological diseases including AD, PD, HD, stroke, depression and the neurological complications of diabetes (Currais et al., 2014; Maher, 2015, 2017). Fisetin treatment suppressed the production of pro-inflammatory cytokines, astrocyte, activation and loss of nerve cell function in the brains of the AD mice (Currais et al., 2014). Further evidence showed that fisetin inhibited lipopolysaccharide (LPS)-induced neurotoxicity by regulating Toll-like receptor 4 (TLR4)-mediated NF-κB pathway (Zheng et al., 2008). Moreover, fisetin can inhibit neuroinflammation by reducing amyloid-beta (Aβ) deposition and activating adenosine monophosphate-activated protein kinase (AMPK) (Ahmad et al., 2017).

Lead (Pb), a widespread heavy metal in the environment, which can result in many health problems, especially nervous system damage (Gąssowska et al., 2016; Feng et al., 2016; Hossain et al., 2016; Zhang et al., 2017). Previous studies showed that Pb exposure increases cognitive deficits, inflammation, oxidative stress and apoptosis via regulating AMPK/SIRT1 signal pathways (Liu et al., 2018; Feng et al., 2016; Zhang et al., 2017). Pb could induce nervous damage by modulating the activations of SIRT1 and cAMP response element-binding protein (CREB) (Feng et al., 2016; Zhang et al., 2017). Prenatal Pb exposure could increase tau hyperphosphorylation-mediated autophagy and apoptosis in brains of rats (Zhang et al., 2012; Meng et al., 2016). Moreover, studies demonstrated that Pb can cause the cognitive deficits, synaptogenesis impairments and neurotoxicity via regulating the protein expression of CREB, calcium/calmodulin kinase II (CaMKII), synaptosomal associated protein-25 (SNAP-25) and postsynaptic density-95 (PSD-95) in brains of rats (Liu et al., 2013; Gąssowska et al., 2016). Pb could cause inflammation, microgliosis and astrogliosis through modulating TLR4-MyD88-NFkappaB pathways (Liu et al., 2015).

In the present study, we instigated the protective effect of fisetin against lead-induced synaptic dysfunction, neuroinflammation and neurodegeneration in mice, and explored its underlying mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

Fisetin (98%), Dimethyl sulfoxide (DMSO) and lead acetate ((Pb $(CH_3COO)_2$)) were obtained from Aladdin Bio-Chem Technology Co. (Shanghai, China). The phosphorylated-tau (p-tau), Aβ, neprilysin (NEP), AMPK, phosphorylated-AMPK (p-AMPK), SIRT1, PSD-95, SNAP-25, CREB, phosphorylated-CREB (p-CREB), phosphorylated-CaMKII (p-CaMKII), TLR4, MyD88, Bcl-2, Bax, cleaved casapase-3, NF-κB p65, IL-6, TNF-α and β-actin antibodies were supplied by Santa Cruz Biotechnology (Santa Cruz, CA) and Abcam (Cambridge, MA, USA).

2.2. Animals and ethics

Forty male ICR (mice (10 ± 1 g) were provided from Beijing HFK Bioscience CO., LTD, (Beijing, China). The mice were kept for 1 week in a room with an circumambient temperature of 23 ± 1 °C, a 12 h dark/light cycle and relative humidity (55 ± 5)%. Then, the mice were randomly divided into four groups (10 mice/group): (1) Control group (saline 0.9% NaCl), (2) Pb group, (3) Pb + fisetin (25 mg/kg b.w) group and (4) Pb + fisetin (50 mg/kg b.w) group. In group (1), mice were provided with distilled water and received saline 0.9% NaCl intragastrically once daily. In group (2), (3) and (4), lead acetate (200 mg/L) was dissolved in the drinking water of mice. In group (3) and (4), mice were also supplied with fisetin 25 or 50 mg/kg b.w (dissolved in 0.1% dimethyl sulfoxide), intragastrically once daily. The doses of fisetin and Pb were based on previously described protocols (Zhou et al., 2015; Ahmad et al., 2017; Liu et al., 2018).

At the end of 4 weeks, mice were sacrificed by decapitation. Blood and brains were collected immediately and stored at −80°Cfor future experiments.

All experiments process was approved by Jiangsu Normal University committees (No. IACUC-1.0.11) and performed according to national institutes of health guidelines for the care and use of animals and Chinese laws on care of laboratory animals.

Table 1
Effects of fisetin(Fis) on behaviors in lead-exposed mice in the open-field test.

Group	Decreased crossing number	Decreased rearing number
Control	16.16 ± 1.85[a]	6.23 ± 1.03[a]
Pb	42.02 ± 1.92[b]	15.32 ± 2.17[b]
Pb + Fisetin(25 mg/kg))	34.85 ± 2.17[c]	11.13 ± 1.28[c]
Pb + Fisetin(50 mg/kg)	30.24 ± 1.26[d]	10.24 ± 2.05[d]

Data are expressed as mean ± S.E.M. (n = 10). One-way ANOVA was used for comparisons of multiple group means followed by post hoc testing. Values not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$.

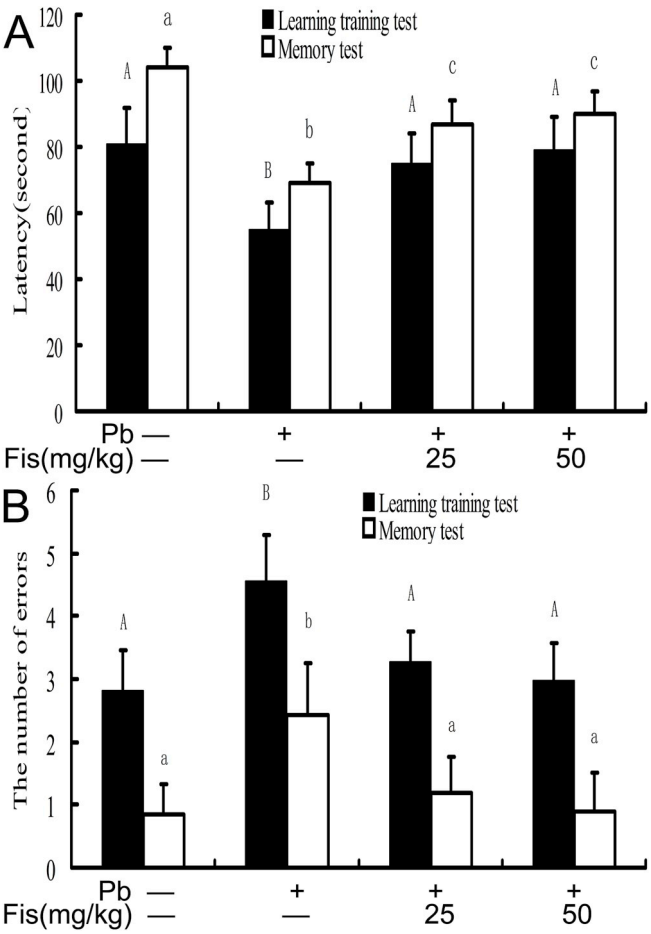

Fig. 1. Effects of fisetin(Fis) on learning and memory abilities in lead-exposed mice in the step-down test. (A) Latency; (B) The number of errors. Data are expressed as mean ± S.E.M. (n = 10). Values not sharing a common superscript letter (a–c or A-C) differ significantly at $P < 0.05$.

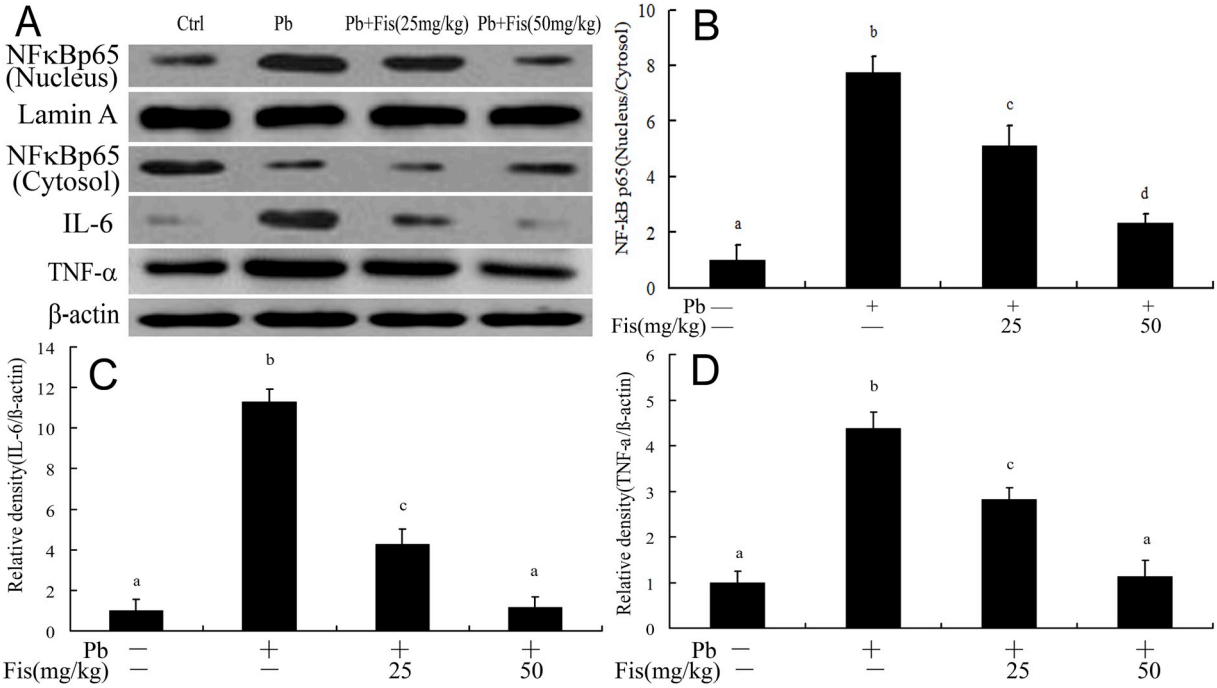

Fig. 2. Fisetin(Fis) inhibited Pb-induced inflammation in the brains of mice. (A) Western blot analysis of the NF-κB p65, TNF-α and IL-6 proteins in the brains; (B) Relative density analysis of the NF-κB p65 protein bands in cytosol and nucleus; (C) Relative density analysis of the IL-6 protein bands; (D) Relative density analysis of the TNF-α protein bands. Lamin A and β-actin were probed as an internal control in relative density analysis. The vehicle control is set as 1.0. Data are expressed as mean ± S.E.M. and representative of at least three independent experiments (individual animals). Values not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$.

2.3. Habituation to the open field task

Work memory was assessed by performance in the training section and non-associative memory in the retention test session (Comim et al., 2012). Habituation to an open field was carried out in a 40 cm × 60 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 9 equal rectangles by black lines. The animals were gently placed on the left rear quadrant and left to explore the area for 5 min (training session). Immediately following this, the animals were taken back to their home cage and submitted again to a similar open-field session 24 h later (test session). Crossing of the black lines and rearing performed in both sessions were counted. The decrease in the number of crossings and rearings between the two sessions was taken as a measure of the retention of habituation.

2.4. Step-down test

The learning and memory ability was measured using Step-down test according to a previous report (Zhang et al., 2014). Briefly, Step-down test training was performed 24 h after the final administration of fisetin. The step-down test is a one-time stimulus avoidance response test. During the experiment, the mice were put on the copper grid and the current was switched on. Mice received an electric shock. The normal reaction of the mice is to avoid the shock and jump on to the platform, but most mice would jump off the platform even after repeatedly receiving an electric shock. The training lasted for 3 min, and the time that each mouse remained on the platform for the first time before jumping off was recorded as the latency, and the number of shocks within 3 min was recorded as the number of errors. After 24 h, the test was repeated as the memory test, and the latency and number of errors were recorded as memory test scores.

2.5. Western blotting analysis

The brain protein expressions of p-tau, Aβ, NEP, AMPK, p-AMPK, SIRT1, PSD-95, CREB, p-CREB, p-CaMKII, TLR4, MyD88, Bcl-2, Bax, Cleaved caspase-3, NF-κB p65, IL-6, TNF-α and β-actin were analyzed by western blot according to the manufacturer's guidelines (Bio/Rad, Hercules, CA, USA)(Liu et al., 2018).

2.6. Statistical analysis

Statistical significant differences between means were evaluated by Student's t-test and one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Fisetin rescues Pb-induced learning and memory deficits

Exposure to lead increased the crossing of the open field squares, as well as the rearing behavior in test session, when compared to the negative control group. As shown in Table 1, compared with the control group, Pb exposure increased the decreased number of crossing and rearing behaviors by 160% and 146%, respectively. Whereas, compared with the Pb group, the decreased number of crossing behaviors in the Pb + fisetin treatment groups (25 and 50 mg/kg) was decreased by 17% and 28%, respectively, and the decreased number of rearing behaviors in the Pb + fisetin treatment group (25 and 50 mg/kg) was decreased by 27% and 33%, respectively ($P < 0.01$). No significant difference in crossing and rearing behaviors was found between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

The learning and memory ability was measured using Step-down test. As shown in Fig. 1, when compared with the control group, Pb exposure reduced latency of mice ($P < 0.01$), in both the learning

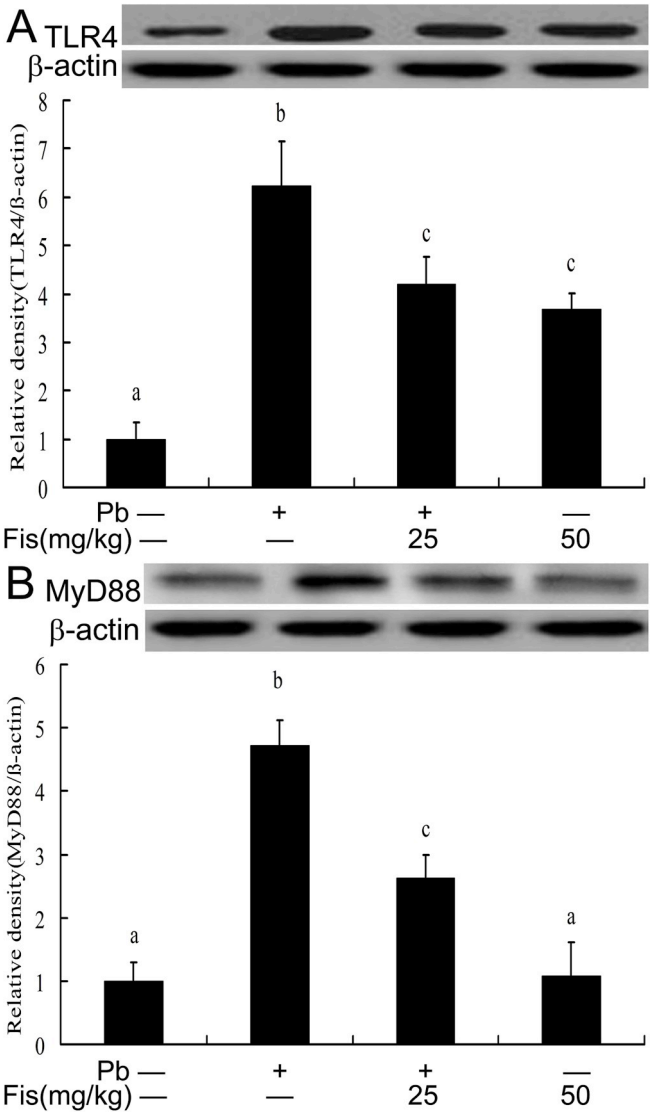

Fig. 3. Fisetin (Fis) inhibited the Pb-induced activation of TLR4 and MyD88 in the brains of mice. (A) Relative density analysis of the TLR4 protein bands; (B) Relative density analysis of the MyD88 protein bands. β-Actin was probed as an internal control in relative density analysis. The vehicle control is set as 1.0. Data are expressed as mean ± S.E.M. and representative of at least three independent experiments (individual animals). Values not sharing a common superscript letter (a–c) differ significantly at $P < 0.05$.

training (by 32%) and memory tests (by 34%), while Pb exposure increased the number of errors in the learning training (by 62%) and memory tests (by 188%) as compared with the control group ($P < 0.05$). This shows that Pb exposure caused learning and memory deficits in the mice. These findings are consistent with a previous report (Zhang et al., 2014). However, fisetin treatment increased latency and decreased the number of errors in mice compared with Pb-exposed group. No significant difference in the learning and memory ability was found between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

3.2. Fisetin suppressed the inflammation in the brains of mice

In order to examine the anti-inflammatory activity of fisetin, we determine the expressions of NF-κB p65, IL-6 and TNF-α in brains of mice. As is showed in Fig. 2, Pb increased the activations of pro-inflammatory factors IL-6, TNF-α and NF-κB p65 nuclear translocation. However, the expressions of these inflammatory factors were inhibited by the fisetin supplement ($P < 0.01$). No significant difference in these inflammatory factors was found between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

3.3. Fisetin inhibited activation of TLR4 and MyD88 in brain of mice

TLR4 and MyD88 were key factors that regulated inflammatory response (Liu et al., 2015). In this study, we further measured the expressions of TLR4 and MyD88 in brains of mice. As shown in Fig. 3, Pb exposed increased the expressions of TLR4 and MyD88 as compared with control mice. However, the presence of fisetin reduced the levels of these proteins in the Pb group ($P < 0.01$). There was no significant difference between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

3.4. Fisetin suppressed Pb-induced apoptosis in the brains

In order to examine the anti-apoptosis property of fisetin, we determined the expression of Bcl-2, Bax and cleaved casapase-3 in brains of mice. As is showed in Fig. 4, the expression of Bax and cleaved casapase-3 was increased in the brains of Pb group, which was reversed by fisetin supplement. In contrast, the expression of Bcl-2, one of anti-apoptotic proteins, was reduced in the brains of Pb group, which was increased by fisetin treatment ($P < 0.01$). No significant difference in these proteins was found between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

3.5. Fisetin reduced p-tau and Aβ accumulation in the brains of mice

Over expression of phosphorylated-tau (p-tau) and Aβ peptide could induce neurotoxicity (Ahmad et al., 2017; Liu et al., 2018). Therefore, we measured these protein expressions in the brains of mice. As showed in Fig. 5, the protein expressions p-tau and Aβ were remarkably higher in the brains of Pb group in comparison to control. Interestingly, treatment with fisetin reduced the deposition of these proteins in mouse brains of Pb group. In addition, the expression of NEP, a remover of Aβ, was reduced in the brains of Pb group, which was remarkably increased by fisetin treatment ($P < 0.05$). There was no significant difference between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

3.6. Fisetin promoted Pb-induced autophagy in the brains of mice

To examine the effect of fisetin on the regulation of autophagy in Pb-induced neurotoxicity, we measured protein expression of the autophagy-related markers. As Fig. 6 show, Pb increased the expressions of LC3-II and Beclin-1. Intriguingly, fisetin supplement further increased these protein expressions and promoted the Pb-induced autophagy in brains of mice ($P < 0.05$). No significant difference in these autophagy-related proteins was found between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

3.7. Fisetin attenuated synaptic dysfunction in brains of mice

SNAP-25, PSD-95, CREB and CaMKII are proteins that involved in synaptic plasticity (Liu et al., 2013; Gąssowska et al., 2016). As a result, Pb exposure markedly suppressed the PSD-95, SNAP-25, p-CREB and p-CaMKII as compared to control mice. In contrast, the fisetin treatment showed a remarkably reversal of the aberrant expression of these proteins in brains of Pb group (Fig. 7). There was no significant difference between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

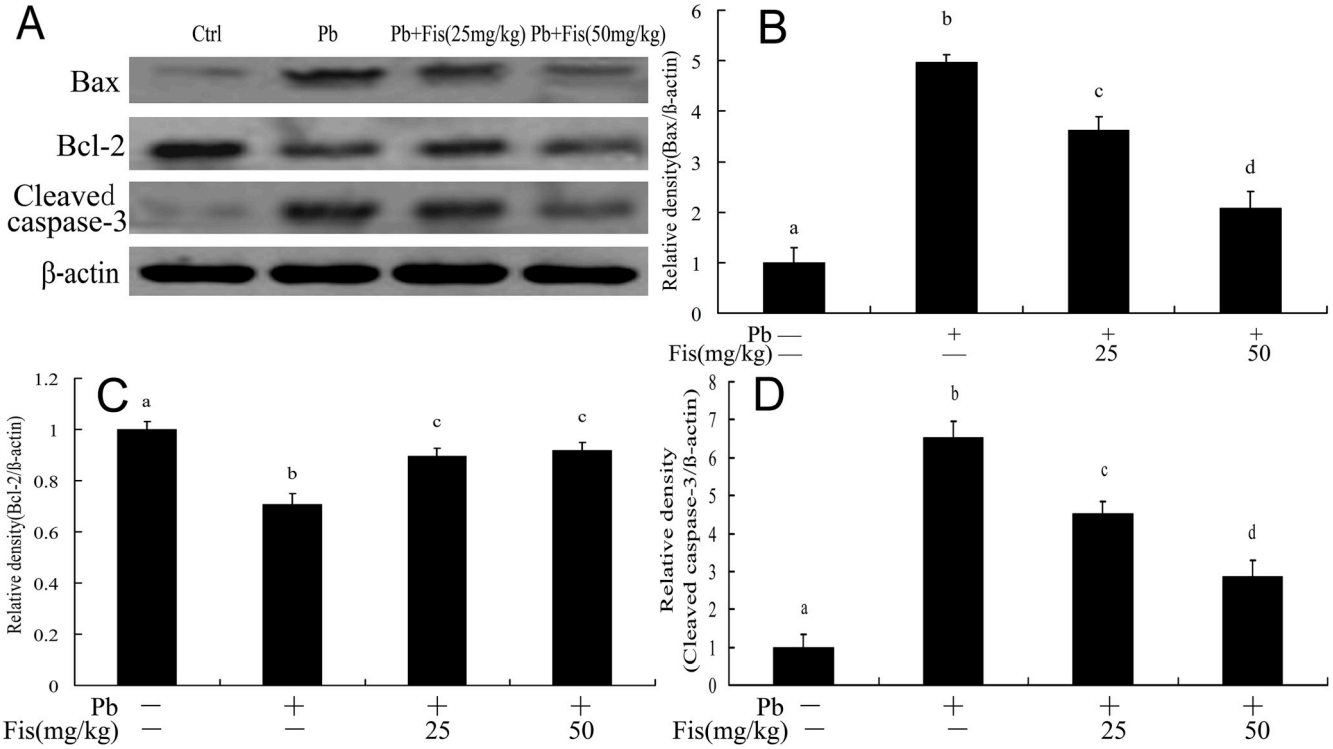

Fig. 4. Fisetin(Fis) inhibited Pb-induced apoptosis in the brains of mice. (A) Western blot analysis of the Bax, Bcl-2 and cleaved caspase-3 proteins in the brains; (B) Relative density analysis of the Bax protein bands; (C) Relative density analysis of the Bcl-2 protein bands; (D) Relative density analysis of the cleaved caspase-3 protein bands. β-actin was probed as an internal control in relative density analysis. The vehicle control is set as 1.0. Data are expressed as mean ± S.E.M. and representative of at least three independent experiments (individual animals). Values not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$.

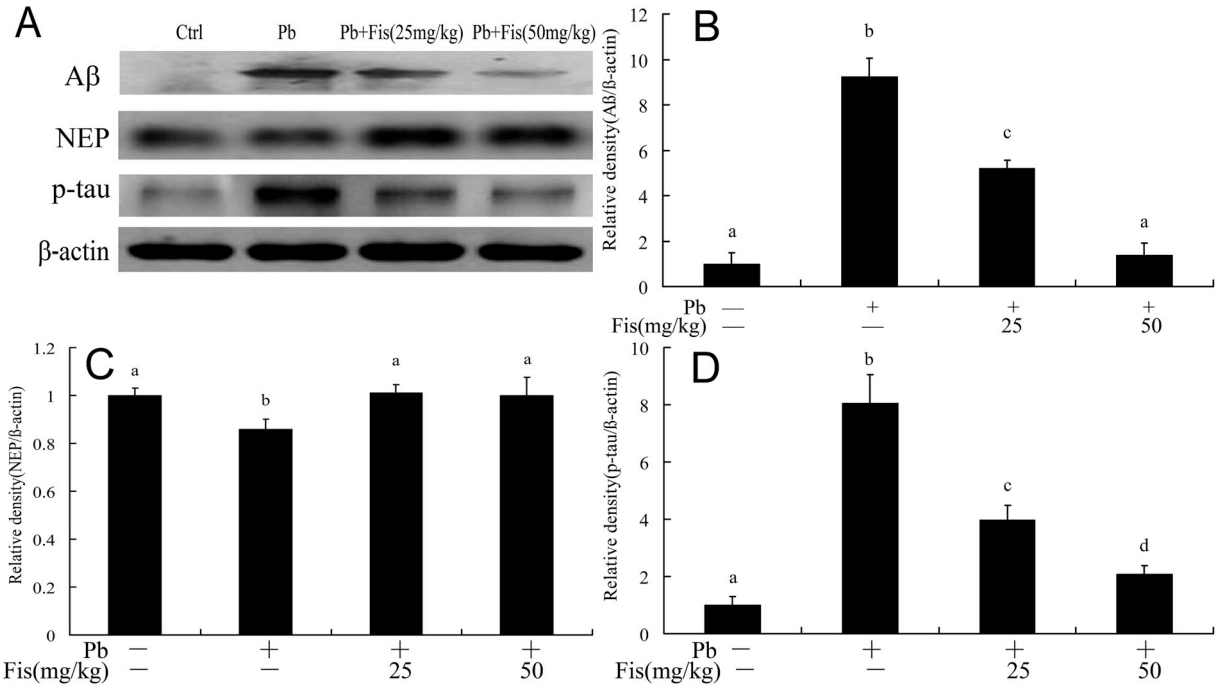

Fig. 5. Fisetin(Fis) reduced p-tau and Aβ accumulation in the brains of Pb-treated mice. (A) Western blot analysis of the p-tau, Aβ and NEP proteins in the brains; (B) Relative density analysis of the p-tau protein bands; (C) Relative density analysis of the NEP protein bands. The vehicle control is set as 1.0. Total tau or β-actin were probed as an internal control in relative density analysis. The vehicle control is set as 1.0. Data are expressed as mean ± S.E.M. and representative of at least three independent experiments (individual animals). Values not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$.

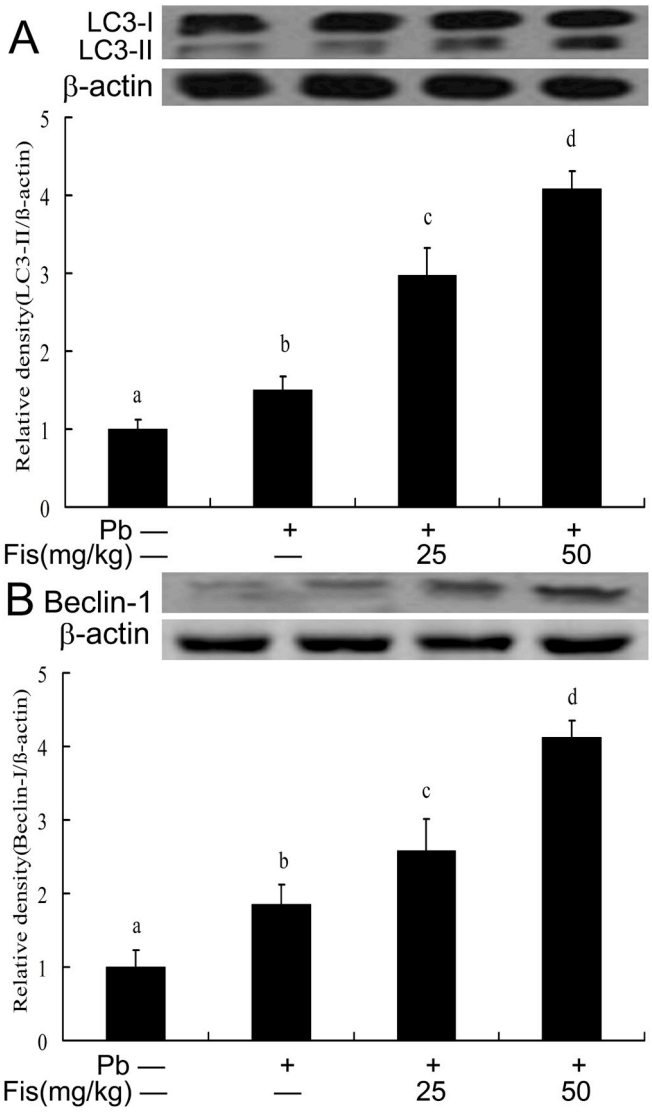

Fig. 6. Fisetin(Fis) promoted Pb-induced autophagy in the brains of mice. (A) The protein expression of the LC3-II; (B) The protein expression of the Beclin-1. Data are expressed as mean ± S.E.M. and representative of at least three independent experiments (individual animals). Values not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$.

3.8. Fisetin activated AMPK and SIRT1 in the brains of mice

AMPK and SIRT1 have been suggested to act as the regulators of neurodegeneration, neuroinflammation and autophagy pathways (Ng and Tang, 2013; Liu et al., 2014). Therefore, we next measure the expressions of p-AMPK and SIRT1 in the brains. There result indicated that Pb exposure markedly decreased the expressions of p-AMPK and SIRT1 as compared to control mice. Fisetin supplement obviously increased the level of p-AMPK and SIRT1 in Pb group (Fig. 8). There was no significant difference between the control group and the group of only 50 mg/kg fisetin treatment (data not shown).

4. Discussion

Fisetin, a kind of flavonoid existed in fruit and vegetables, was reported to exhibit many biological functions, such as antioxidant, anti-apoptosis, anti-inflammatory, anti-diabetic and neuroprotective effects (Khan et al., 2013; Maher, 2015; Sundarraj et al., 2018). In present study, we investigate the protective effects of fisetin on Pb-induced neurodegeneration, neuroinflammation and memory impairment by regulating AMPK, SIRT1 and autophagy signaling pathway.

Pb, a ubiquitous pollutant, can cause widespread neurotoxicity and memory deficits in humans and animals (Gąssowska et al., 2016; Zhang et al., 2017). It is reported that fisetin could increase long-term potentiation and memory function in rats (Maher et al., 2006). Fisetin treatment could inhibit neuroinflammation and maintain cognitive function in AD mice (Currais et al., 2014). Fisetin improved Aβ-induced memory deficits, neuroinflammation and neurodegeneration in adult mice (Ahmad et al., 2017). In our study, fisetin treatment can dramatically ameliorate the Pb-induced learning and memory deficits (Fig. 1) and behavioral abnormalities (Table 1) in mice, this finding suggest that fisetin exerts a protective effect on Pb-induced cognitive impairment.

TLR4, a transmemnerane protein belongs to PRR family, plays an important role in inflammatory response (Liu et al., 2015; Zhou et al., 2015). Previous study has demonstrated that Pb could activate TLR4/MyD88 pathway and increase the release of inflammatory factors through regulating NF-κB signaling pathways in brains (Liu et al., 2015; Chibowska et al., 2016). Study demonstrated fisetin could improve neurological function and suppressing LPS-induced inflammatory response in rat brains by TLR4/NF-κB pathways (Zhou et al., 2015). Fisetin could also attenuate intracerebral hemorrhage-induced brain edema and inflammation in aged mice via inhibiting NF-κB signaling pathways (Chen et al., 2018). Consistently, we found that Pb markedly increased the activation of TLR4/MyD88/NF-κB signaling pathway and further increased the expression of pro-inflammatory mediators IL-6 and TNF-α. However, fisetin supplement obviously decreased the NF-κB activation and the expression of pro-inflammatory factors in the brains (Figs. 2 and 3). The results suggested that fisetin could ameliorate the Pb-induced brain damage via suppressing inflammatory response.

Many reports had demonstrated that Pb could cause apoptosis in brains via the mitochondrial apoptosis pathway (Zhang et al., 2012; Liu et al., 2018). Consistently, our data indicated that the Bcl-2 expression was significantly attenuated and the expression of Bax and cleaved casapase-3 was observably enhanced in brains of the Pb group, suggesting that Pb induced apoptosis in mouse brains. Several evidences have shown that fisetin exerts neuroprotective properties by suppressing apoptosis in the experimental models of nerve injury (Ahmad et al., 2017; Singh et al., 2018; Zhou et al., 2015). Fisetin could also prevent neuronal apoptosis in a mouse model of aluminium chloride-induced neurodegeneration (Prakash and Sudhandiran, 2015). In our experiments, fisetin treatment increased the Bcl-2 expression and reduced the levels of Bax and cleaved casapase-3 in brains of Pb group (Fig. 4). These results confirmed that fisetin treatment exhibits neuroprotective effects by inhibiting Pb-induced apoptosis.

Aβ, a fragment of amyloid precursor protein (APP), its accumulation can result in many neurological diseases such as AD, cognitive dysfunction and memory loss, synaptic impairments (Ahmad et al., 2017; Zhang et al., 2017; Liu et al., 2018). Tau is a protein associated with microtubule in the brain. Over expressions of p-tau and Aβ were two signs associated with cognitive impairs, neuroinflammation and neurodegeneration in the brains (Ahmad et al., 2017; Liu et al., 2014; Singh et al., 2018). It was reported that Pb exposure increased the expression p-tau and Aβ and decreased the protein expression of the Aβ remover NEP (Liu et al., 2014; Zhang et al., 2017; Wang et al., 2019). Researches had demonstrated that fisetin could improve cognitive function impairs, neuroinflammation and neurodegeneration via decreasing the expressions of p-tau and Aβ (Currais et al., 2014; Prakash and Sudhandiran, 2015; Ahmad et al., 2017). In our study, we observed that Pb exposure markedly increased the levels of p-tau and Aβ and decreased the expression of NEP. However, treatment with fisetin, the expression of p-tau and Aβ were noticeably reduced in the brains of Pb group. In contrast, fisetin treatment increased the expression of NEP in the brains of Pb group (Fig. 5). The results suggest that p-tau and Aβ were participated in the neuroprotection of fisetin in brains of mice.

Autophagy is a highly regulated process in which the misfolded

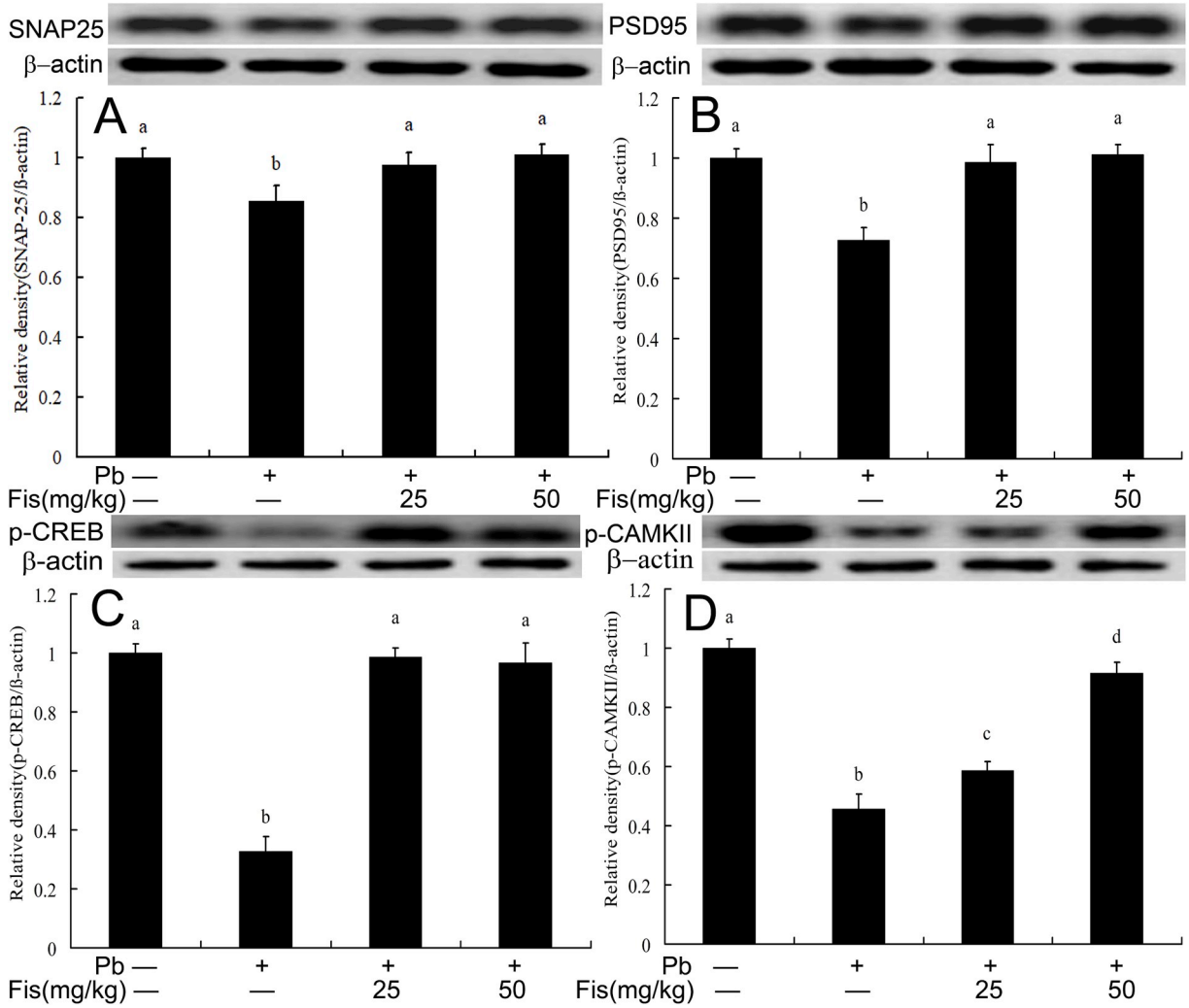

Fig. 7. Fisetin(Fis) alleviated Pb-induced synaptic dysfunction in the brains of mice. (A) Relative density analysis of the SNAP-25 protein bands; (B) Relative density analysis of the PSD-95 protein bands; (C) Relative density analysis of the p-CREB protein bands; (D) Relative density analysis of the p-CaMKII protein bands. β-actin was probed as an internal control in relative density analysis. The vehicle control is set as 1.0. Data are expressed as mean ± S.E.M. and representative of at least three independent experiments (individual animals). Values not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$.

proteins and damaged organelles are delivered to lysosomes for degradation (Meng et al., 2016; Ng and Tang, 2013). Cumulative data had revealed that Pb could cause memory injury, p-tau accumulation, type I program death (apoptosis) and type II program death (autophagy) in brains of rats (Zhang et al., 2012; Meng et al., 2016). Recent study had demonstrated that Pb could induce autophagy to protect neural cells from severe cellular toxicities (Gu et al., 2019). It is found that fisetin could protect the brains in aging models of rats by promoting autophagy and inhibiting apoptosis, neuroinflammation, and neurodegeneration (Singh et al., 2018). Fisetin treatment significantly increased autophagy and p-tau level in cortical cells or primary neurons (Kim et al., 2016). Our results are consistent with previous reports that Pb markedly increased the expression of the autophagy markers Beclin-1 and LC3-II. Intriguingly, fisetin supplement obviously increased these markers of autophay in the brains (Fig. 6). The results suggested that fisetin could ameliorate the Pb-induced nerve damage via promoting autophagy.

Presynaptic protein SNAP-25 and postsynaptic protein PSD-95 are essential for brain development, synaptic connection, neuronal maturation and plasticity (Hossain et al., 2016; Ahmad et al., 2017). Pb exposure caused alterations of synaptic morphology and reduced the expression of SNAP-25 and PSD-95 in brains, which could induce synaptic dysfunction and spatial memory loss (Gąssowska et al., 2016; Hossain et al., 2016). In this study, we found that fisetin increased the expression of SNAP-25 and PSD-95 in the brains of Pb-treated mice, which suggests that fisetin could alleviate Pb-induced learning and memory impairment by enhanced presynaptic and postsynaptic protein expression (Ahmad et al., 2017). The transcription factor CREB and CAMKII participate in improve cognitive/synaptic dysfunction and protect neurological damage in the brains (Liu et al., 2013; Feng et al., 2016). Previous study had indicated that Pb exposure suppressed the SIRT1/CREB pathway and caused cognitive impairs in rats (Feng et al., 2016). Pb also inhibited CREB activation, which resulted in memory impairment, neuroinflammation, apoptosis and other nerve disease (Liu et al., 2013, 2018; Feng et al., 2016). It had been revealed that fisetin could facilitate long-term memory and induced CREB phosphorylation in rat brains (Maher et al., 2006). Fisetin also ameliorate Aβ-induced cognitive dysfunction, neuroinflammation and neurodegeneration by increasing the levels of SNAP-25, PSD-95, p-CREB and p-CAMKII (Ahmad et al., 2017). In our study, as expected, Pb exposure remarkably decreased the expression of SNAP-25, PSD-95, p-CREB and p-CAMKII, which inevitably interfered with neuronal survival, cell differentiation, postnatal synaptophysin and synapse formation (Maher et al., 2006; Hossain et al., 2016; Gąssowska et al., 2016). However, fisetin supplement markedly increased the expression of these cognition-related proteins in Pb group (Fig. 7). This result indicated that

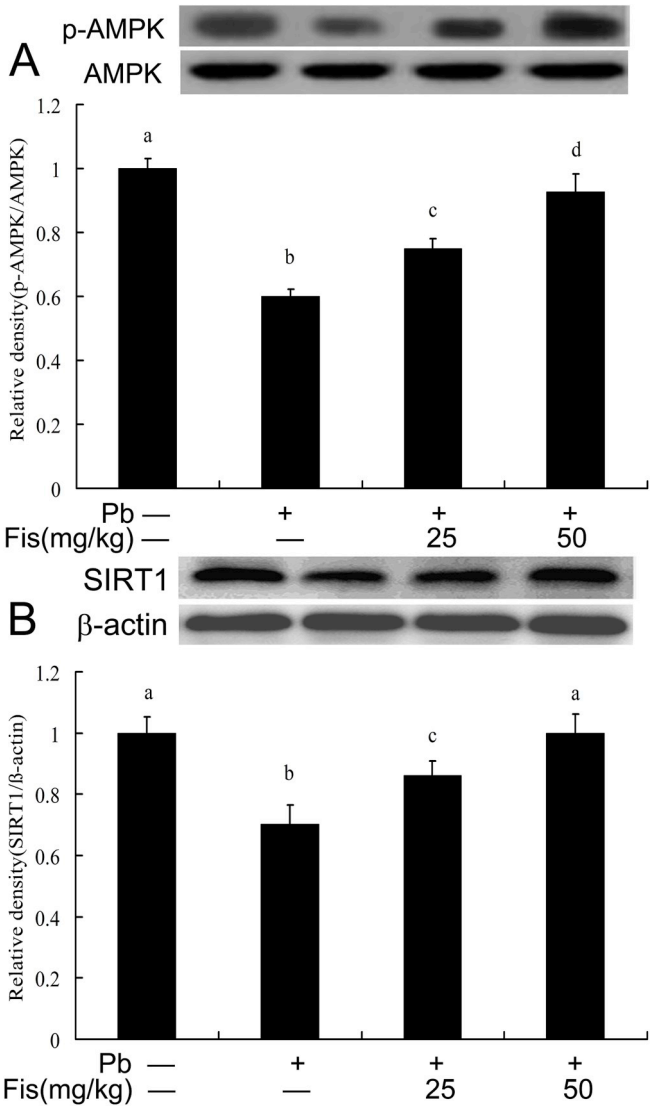

Fig. 8. Fisetin (Fis) increased the phosphorylation levels of AMPK and SIRT1 in the brains of Pb-treated mice. (A) Relative density analysis of the p-AMPK protein bands; (B) Relative density analysis of the SIRT1 protein bands; Total AMPK or β-actin was probed as an internal control in relative density analysis. The vehicle control is set as 1.0. Data are expressed as mean ± S.E.M. and representative of at least three independent experiments (individual animals). Values not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$.

fisetin displayed its neuroprotective effects on Pb-induced synaptic dysfunction by modulating pre- and postsynaptic proteins.

AMPK and SIRT1 are considered as pivotal regulators in many diseases, including oxidative stress, inflammation, apoptosis, autophagy, diabetes, aging, proliferation, cardiovascular disease, hypertension, neurological disease and kidney disease (Ng and Tang, 2013; Liu et al., 2018). Activated SIRT1 could enhance autophagy and inhibit apoptosis (Ng and Tang, 2013; Lee et al., 2015). AMPK/SIRT1 pathway is closely correlated with Aβ accumulation, neuroinflammation and neurodegeneration (Zhang et al., 2017; Liu et al., 2018). Previous studies has demonstrated that Pb could cause inflammation, apoptosis and autophagy through suppressing AMPK/SIRT1 signaling pathways in brains (Feng et al., 2016; Zhang et al., 2017; Liu et al., 2018). Study demonstrated fisetin could inhibit tunicamycin-mediated cell death in PC12 cells by activating SIRT1 pathway (Yen et al., 2017). Here, our results indicated Pb exposure inhibited the expression of p-AMPK and SIRT1. However, administration of fisetin remarkably attenuated the

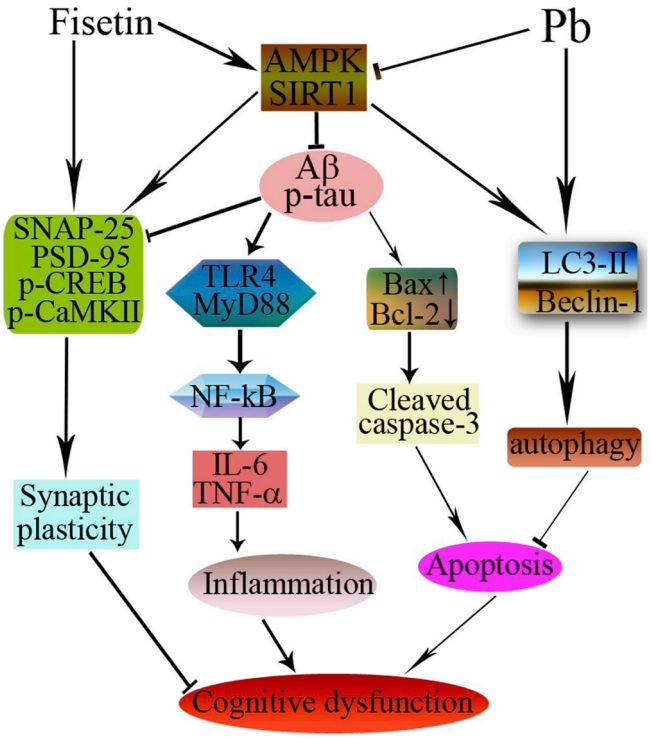

Fig. 9. Schematic diagram showed the possible protective effects of fisetin in Pb-induced cognitive injury. The → indicates activation or induction, and ⊣ indicates inhibition or blockade.

abnormal expression of these proteins in mouse brains (Fig. 8). The results revealed that AMPK and SIRT1 were involved in the neuroprotective effects of fisetin on Pb-induced neuroinflammation and neurodegeneration.

The prior studies showed that Pb exposure can impact on cognitive function and behavior in human, and that this exposure is also associated with an increase in biomarkers involved in AD, PD, HD and neuroinflammation (Chibowska et al., 2016; Gąssowska et al., 2016; Hossain et al., 2016). In this study, we found that fisetin (at the dose of Fis 50 mg/kg/day for 4 weeks) possess protective properties against Pb-induced neurotoxicity in mice. Our results suggest that fisetin can decrease Pb-induced synaptic dysfunction, neuroinflammation and neurodegeneration in brains through regulating AMPK/SIRT1 and autophagy pathway (Fig. 9). The results suggested fisetin (at the dose of Fis 50 mg/kg/day for 4 weeks) may be developed as a potential nutritional target for the prevention of Pb-induced neurotoxicity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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