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ORIGINAL ARTICLE

# Attenuation of Aluminum Chloride-Induced Neuroinflammation and Caspase Activation Through the AKT/GSK-3β Pathway by Hesperidin in Wistar Rats

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## Abstract

Hesperidin, a flavanoglycone abundantly present in citrus fruits, is reported to have antioxidant, anti-inflammatory, and neuroprotective properties. Previous reports from our laboratory indicated the neuroprotective effect of hesperidin against aluminum chloride ($AlCl_3$)-induced memory loss, acetylcholine esterase hyperactivity, oxidative stress, and enhanced expression of amyloid β protein biosynthesis-related markers. However, their role on $AlCl_3$-induced inflammation, caspase activation, Tau pathology, altered Akt/GSK 3β signaling pathway, and Aβ clearance marker has not yet been fully elucidated. Intraperitonial injection of $AlCl_3$ (100 mg/kg body weight) for 60 days significantly elevated the expressions of insulin-degrading enzyme (IDE), cyclin-dependent kinase 5 (CDK 5), and phosphoTau (pTau); inflammatory markers such as glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule 1 (Iba-1), NF-kB, cyclooxygenase-2 (COX-2), interleukin (IL)-1β, IL-4, IL-6, tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS); and apoptotic markers including cytosolic cytochrome c (cyto c), caspase-3, caspase-8, and caspase-9, and lowered expressions of mitochondrial cyto c, phospho-Akt (pAkt) and phospho-glycogen synthase kinase-3β (pGSK-3β) in the hippocampus and cortex. Co-administration of hesperidin to $AlCl_3$ rats for 60 days significantly ameliorated the aluminum-induced pathological changes. The behavioral studies also supported the above findings. Our results imply that treatment with hesperidin might be a potent option for treating the symptoms of cognitive impairment in Alzheimer's disease by targeting its most prominent hallmarks.

**Keywords** Aluminum · Alzheimer's disease · Hesperidin · Inflammation · Tau pathology · Akt/GSK 3β signaling pathway

## Introduction

Aluminum (Al) is abundantly present in nature and reported to have a role in dementia (Khan et al. 2013), Alzheimer's disease (AD) (Kawahara et al. 2011), and Parkinson's disease (PD) (Ahmed and Santosh 2010). Al exposure could produce clinical and pathological features of AD such as memory deficits, cholinergic neuronal loss, senile plaques (SP), and neurofibrillary tangles (NFTs) formation in the hippocampus and cerebral cortex. It also affects numerous cellular signaling pathways of the brain by inducing oxidative stress and inflammation (Prakash et al. 2013a; Ali et al. 2014; Justin Thenmozhi et al. 2017; 2015; Dhivya Bharathi et al. 2015). Therefore, strategies to attenuate Al-induced impairments could provide a potential therapeutic intervention for AD. Flavonoids are the richest class of natural polyphenolic compounds and widely present in fruits, grains, leaves, roots, bark, stems, flowers, tea, and wine (Nijveldt et al. 2001). Numerous clinical experiments have revealed the beneficial effects of flavonoids on the human brain (Kean et al. 2015) which are

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related to their antioxidant activity and the ability to control neuronal function, survival, synaptic plasticity, and long-term potentiation (LTP) (Bhullar and Rupasinghe 2013). We have reported that hesperidin (5, 9-dihydroxy-40-methoxy-7-orutinosyl flavanone) offers benefit against AD and PD (Justin Thenmozhi et al. 2017; 2015; Tamilselvam et al. 2013) and others reported on Huntington's disease (HD) as well (Menze et al. 2012).

Over the last several years, studies and therapies aimed at amyloid beta (Aβ) and Tau clearance have blossomed. Al significantly reduced the activity of insulin-degrading enzyme (IDE) involved in the Aβ clearance and induced hyperphosphorylation of Tau by enhancing protein kinases such as CDK 5 (Amador et al. 2014). Massive neuronal cell death during most neurodegenerative diseases (NDDs) occurs due to uncontrolled inflammation where activated astrocytes and microglia secretes various cytotoxic agents including reactive oxygen species (ROS), nitric oxide (NO), and inflammatory cytokines (Teismann and Schulz 2004; Reale et al. 2009). Caspases 8, 9, and 3 are situated at pivotal junctions in both intrinsic and extrinsic apoptotic pathways that induce neurodegeneration. Abnormal production of Aβ plaque and neurofibrillary tangles resulted in neuronal damage and inflammation that leads to phosphorylation of PI3K. It activates Akt phosphorylation, which inhibits phosphorylation of GSK-3β by $A\beta_{1-42}$ to protect neuronal cells in the brain (Kitagishi et al., 2014). Results from our lab demonstrated the neuroprotective efficacy of hesperidin against Al intoxication (Justin Thenmozhi et al. 2017; 2015). The present study was designed and executed to strengthen the results of our previous findings and to address knowledge gap regarding the mechanism behind the neuroprotective effect of hesperidin against impaired Aβ clearance, Tau pathology, neuroinflammation, and caspase activation in a rat model of $AlCl_3$-induced neurotoxicity.

## Materials and Methods

### Animals

Male Albino Wistar rats (200–225 g; 10–12 weeks age) were procured from Central Animal House, Rajah Muthiah Medical College & Hospital, Annamalai University, and maintained at standard conditions with food and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee (Reg. No. 160/1999/CPCSEA, Proposal No. 1005) according to the National Guidelines on the Proper Care and Use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, 2000).

### Chemicals

$AlCl_3$, hesperidin, and horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG were purchased from Sigma–Aldrich, Bangalore, India. Primary antibodies for cyto c; caspase-3, caspase-8, and caspase-9; tTau; pTAU; tGSK-3β; pGSK-3β (ser 9); tAkt; pAkt (ser 473); GFAP; NF-kB; COX-2; CDK 5; interleukin (IL)-1β, IL-4, IL-6; TNF-α; iNOS; Iba-1; and anti-β-actin were purchased from Cell Signaling Technology, USA. IDE was procured from Santa Cruz Biotechnology, USA. All other chemicals used were of analytical grade.

### Experimental Design

Forty-eight rats were randomized and divided into four groups ($n = 12$) as follows:

Group I: Rats were intraperitoneally injected with saline (0.5 ml) daily once for 60 days.

Group II: Rats were intraperitoneally injected with $AlCl_3$ (100 mg/kg body weight) for 60 days (Justin Thenmozhi et al. 2017; 2015). The dose of Al used to induce AD in rats was higher than the dose (10 mg/kg i.p) used in other studies including Khan et al., (2013). The dose used in our study is correlated to humans, who are exposed to extreme levels of Al under certain conditions, e.g., occupational Al toxicity including welding, living near cement factories, and dialysis encephalopathy (Prakash and Kumar, 2013; Kumar et al., 2009a). Higher doses including 100 mg/kg b.w. (Ali et al., 2016), 150 mg/kg b.w. (Cao et al., 2017), and 200 mg/kg b.w. (Prakash and Sudhandiran, 2015) were used in previous studies reported to deposit amyloid plaques.

Group III: Rats were treated orally using intragastric tubes with hesperidin (100 mg/kg. b.w.) dissolved in saline (1 h prior to $AlCl_3$) and intra peritoneally injected with $AlCl_3$ as group II for 60 days (Justin Thenmozhi et al. 2017; 2015). To determine the dose-dependent effect of hesperidin against $AlCl_3$-induced experimental model of AD, three different doses (50, 100, and 200 mg/kg b.w.) were used. It was observed that 100 and 200 mg/kg b.w. of hesperidin showed a similar reduction in Al levels, AChE activity, memory loss and histopathological changes, but more significant than 50 mg/kg. As a consequence, hesperidin (100 mg/kg) is chosen as the optimum dose (Justin Thenmozhi et al. 2015).

Group IV: Rats were treated orally using intragastric tubes with hesperidin alone (100 mg/kg b.w.) for 60 days.

At the end of the experimental period, behavioral studies were carried out. Then the rats were fasted overnight and ketamine chloride (24 mg/kg b.w.) was injected as an anesthetic agent (intramuscular injection). The animals were sacrificed by cervical dislocation. The tissues of interest (frontal cortex and hippocampus) were dissected on ice from 2-mm-thick coronal slices obtained using an adult rat brain slicer. The location of the slices and shape of the tissue samples dissected were chosen on the basis of a stereotaxic atlas (Paxinos and Watson, 2007).

## Y-Maze Test

Y- maze consisted of three identical painted wood arms (40 cm long × 35 cm high × 12 cm width) positioned at equal angles and labeled as A, B, and C. Each and every animal was placed individually at the end of one arm and were allowed to explore freely through the maze during a 5-min session. An arm entry was scored, if the hind paws of the rat were completely placed in the arm. Maze arms were cleaned thoroughly to remove residual odors between tasks. Rats avoid most recently visited arm and tend to explore new arms between the three arms. Spontaneous alternation was defined as successive entries into the three different arms on consecutive choices (i.e., ABC, CBA, and BCA). Total arm entries and number of alternations were recorded and used to calculate the spontaneous alternation percentage (SAP) using the following equation: SAP (%) = [(number of alternations)/(total arm entries − 2)] × 100. Same arm returns (SARs) were also recorded (Hidaka et al. 2011).

## Novel Object Recognition Test

The apparatus consists of square box (100 × 100 × 100 $cm^3$) made of gray painted wood. Two familiar objects to be distinguished (A1 and A2) were placed inside the box and fixed with white cement so that rats were unable to move them. Another object which is generally consistent in height and volume, but is different in shape and appearance is used as a novel object, B. The test was performed in three phases; habituation, training, and test session. On day one in order to habituate the rats were pre-exposed to the testing chamber for 10 min. The next day, rat was positioned inside the box in-between two similar objects (A1 and A2) and was allowed to explore the objects for 5 min. After 5 min, rat was removed from the object recognition box and returned to its home cage. Objects A1 and A2 were also removed from the box. After 20 min (test phase), rat was exposed to one new object (B) and one of the old object for 3 min. The time spent exploring each object and the discrimination index percentage was recorded. The discrimination index is an index of measure of discrimination between the familiar and the novel objects corrected for exploratory activity. It is calculated as follows: time spent on novel object − time spent on familiar object/(time spent on novel object + time spent on familiar object). The discrimination index can range from − 1 to 1, with − 1 indicating complete preference for the familiar object, 0 indicating no preference for either object, and 1 indicating complete preference for the novel object (Okuda et al. 2004).

## Rotarod Test

The animals were forced to adjust their posture in response to a moving surface at the speed starting from 0 to 20 rpm (rotation per minute). In the rotarod test, the beam revolves around its longitudinal axis and the rat must therefore walk or run forward in synchrony with it. The latency to fall in a test session of 180 s was taken as a measure of motor coordination (Janakiraman et al. 2016).

## Western Blot Analysis

Proteins from the mitochondrial and cytosolic fractions were extracted as described previously (Janakiraman et al. 2016). Tissues from the entire hippocampus and cortex were gently homogenized, using a Teflon homogenizer in 7 volume of cold suspension buffer (20 mM Hepes–KOH (pH 7.5), 250 mM sucrose, 10 mM KCl, 1.5 mM MgCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.1 mM PMSF, 2 mg/ml aprotinin, 10 mg/ml leupeptin, 5 mg/ml pepstatin, and 12.5 mg/ml of *N*-acetyl-Leu-Leu-Norleu-Al). The homogenates were centrifuged at 750×*g* at 4 °C for 10 min to first isolate the nuclear fraction, and then at 10,000×*g* for 20 min at 4 °C to separate the mitochondria from the soluble fraction. The 10,000*g* pellets were resuspended in cold buffer without sucrose and used as the mitochondrial fraction for the estimation of mitochondrial cyto c. The supernatant was further centrifuged at 100,000*g* for 60 min at 4 °C and was then used as cytosolic fraction. Protein concentration was measured by the method of Lowry et al. (1951).

About 50 μg of total cellular protein were loaded on 10% of SDS-PAGE and transferred onto a polyvinylidene fluoride membrane (Millipore) after separated. Membranes were incubated with the blocking buffer (with 5% non-fat dry milk powder) for 2 h to reduce non-specific binding sites and then incubated with cyto c; caspase-3, caspase-8, and caspase-9; tGSK-3β; pGSK-3β (ser 9); pAkt (ser 473); tAkt; GFAP; NF-kB; COX-2; CDK 5; tTau, pTAU; IL-1β, IL-4, IL-6; TNF-α; iNOS; Iba-1; IDE; and β-actin antibodies in TBST (5% bovine serum albumin in Tris-buffered saline and 0.05% Tween-20) and placed in a shaker at 4 °C for overnight. Then membranes were hatched with secondary antibodies (IgG conjugated to horseradish peroxidase) at room temperature for 2 h. For 30 min, membranes were washed thrice with TBST. Final results were visualized by the chemiluminescence protocol (GenScript ECL kit, Piscataway, NJ, USA). Gel image analysis program was used for the densitometry analysis. The data were

normalized using β-actin as a loading control (Dhanalakshmi et al. 2016; Prema et al. 2017).

### Data Analysis

All data were expressed as mean ± standard error (SEM) of number of experiments. The statistical significance was calculated by one-way analysis of variance (ANOVA) using SPSS version 15.0 and the individual comparisons were obtained by Duncan's multiple range test (DMRT). A value of $p < 0.05$ was considered to indicate a significant difference between groups and the values having symbols differ significantly with each other.

## Results

### Hesperidin Attenuated $AlCl_3$-Induced Memory and Behavioral Deficits

Figures 1, 2, and 3 revealed the memory and movement coordination functions of control and experimental rats. Chronic $AlCl_3$ induction significantly reduced the SAP in Y-maze test (Fig. 1), sniffing time for new object, discrimination index in NOR test (Fig. 2a, b), and retention time in rotarod test (Fig. 3) and increased the SARs (Fig. 2c) in Y-maze test as compared to control rats. Co-administration of hesperidin significantly attenuated these memory and behavioral deficits as compared to $AlCl_3$-alone treated rats. Moreover, no significant changes were observed between control and hesperidin-alone treated rats.

### Hesperidin Ameliorated $AlCl_3$-Induced Tau and Aβ Pathologies

We have performed western blot to investigate the changes in the expression of amyloid β and Tau protein accumulation related markers in $AlCl_3$ model with or without the hesperidin treatment. Enhanced protein expressions of IDE, CDK 5 and pTau were found in hippocampus and cortex of $AlCl_3$ treated rats as compared to control rats, on the other hand, hesperidin co-treatment down regulated their expressions significantly as compared to $AlCl_3$ treated rats. Hesperidin alone treatment did not cause significant changes as compared to control animals. (Fig. 4 A-C).

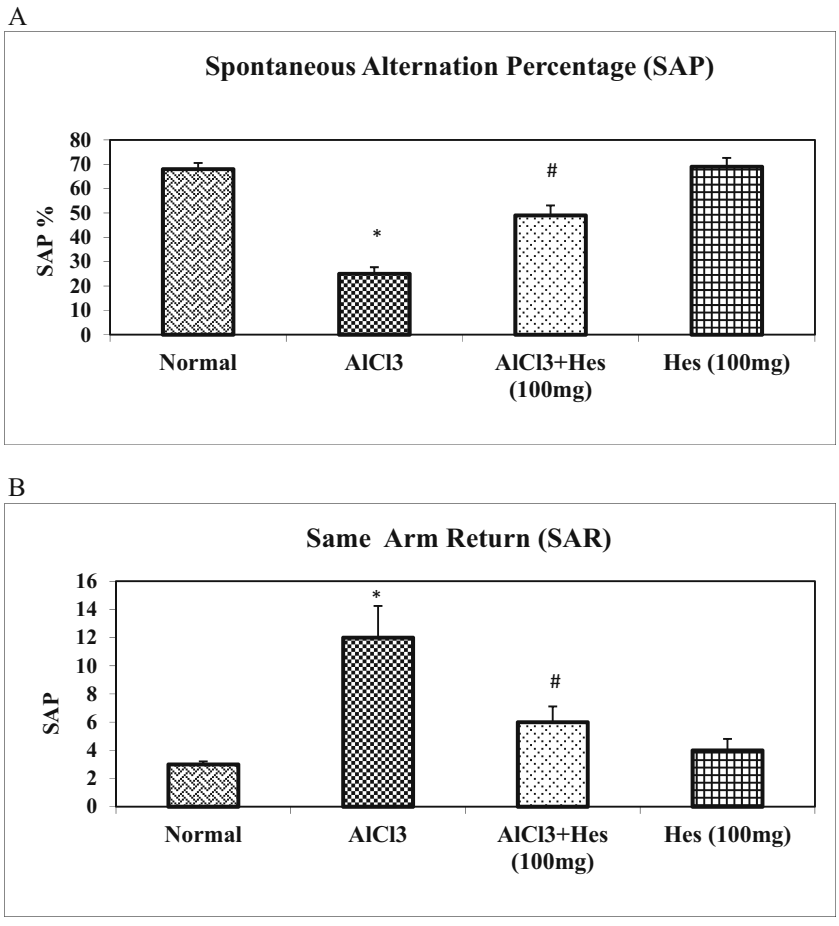
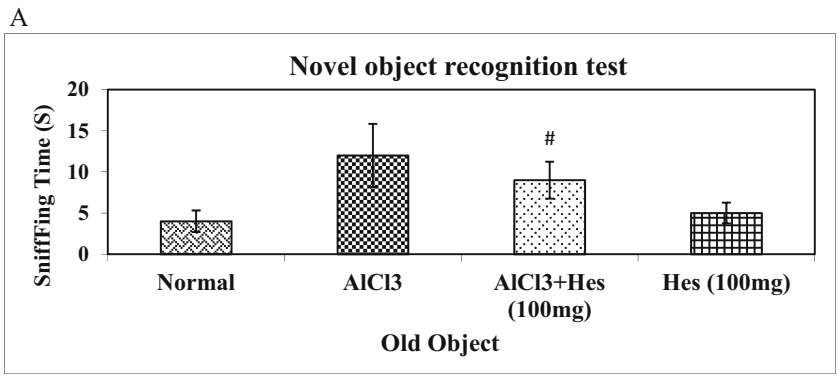
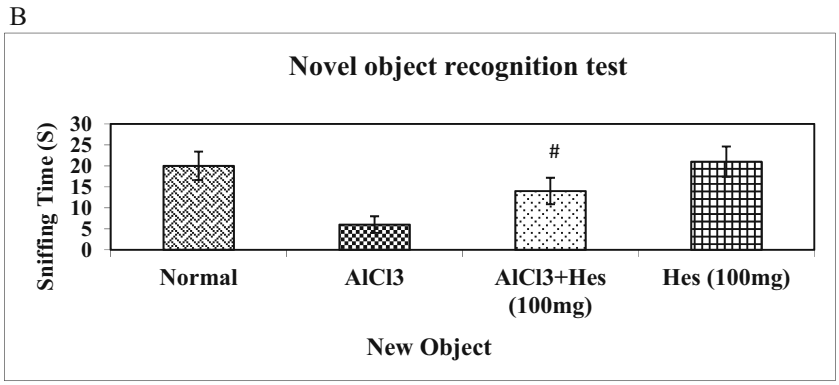



**Fig. 1** Rats treated with $AlCl_3$ exhibited reduced SAP (**a**) and enhanced SAR (**b**), whereas co-administration of hesperidin significantly attenuated memory deficits induced by $AlCl_3$. Results given are mean ± SD ($n = 6$); values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$-treated rats, one-way ANOVA followed by DMRT



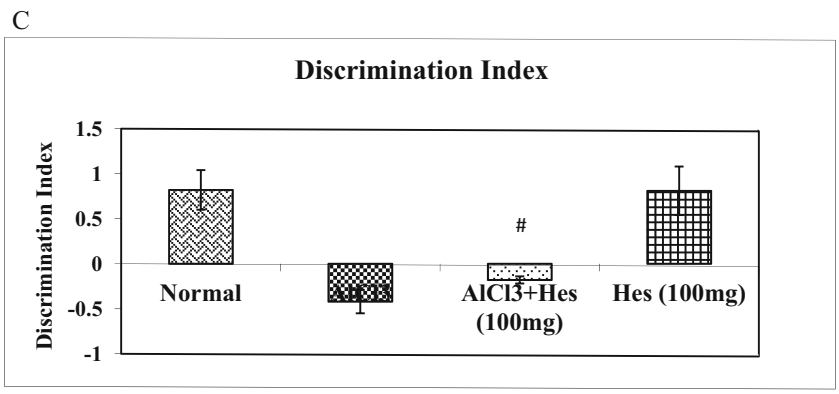




**Fig. 2** $AlCl_3$ treatment enhanced the time spent on old object (**a**), diminished sniffing time on new object (**b**) and discrimination ratio (**c**), in novel object recognition test; meanwhile, treatment with hesperidin significantly attenuated neurocognitive impairment of $AlCl_3$-induced rats. Results given are mean ± SD ($n$ = 6); values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$ treated rats, one-way ANOVA followed by DMRT

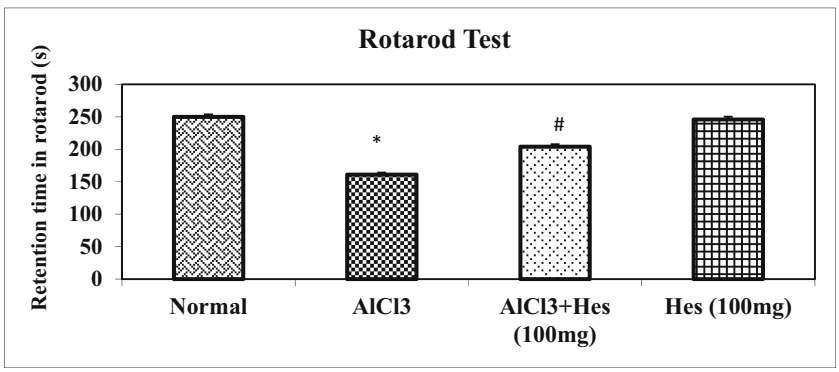



**Fig. 3** Administration of $AlCl_3$ led to a significant reduction in the retention activities in rotarod test, however, these reductions were attenuated by administration of hesperidin. Results given are mean ± SD ($n = 6$); values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$-treated rats, one-way ANOVA followed by DMRT

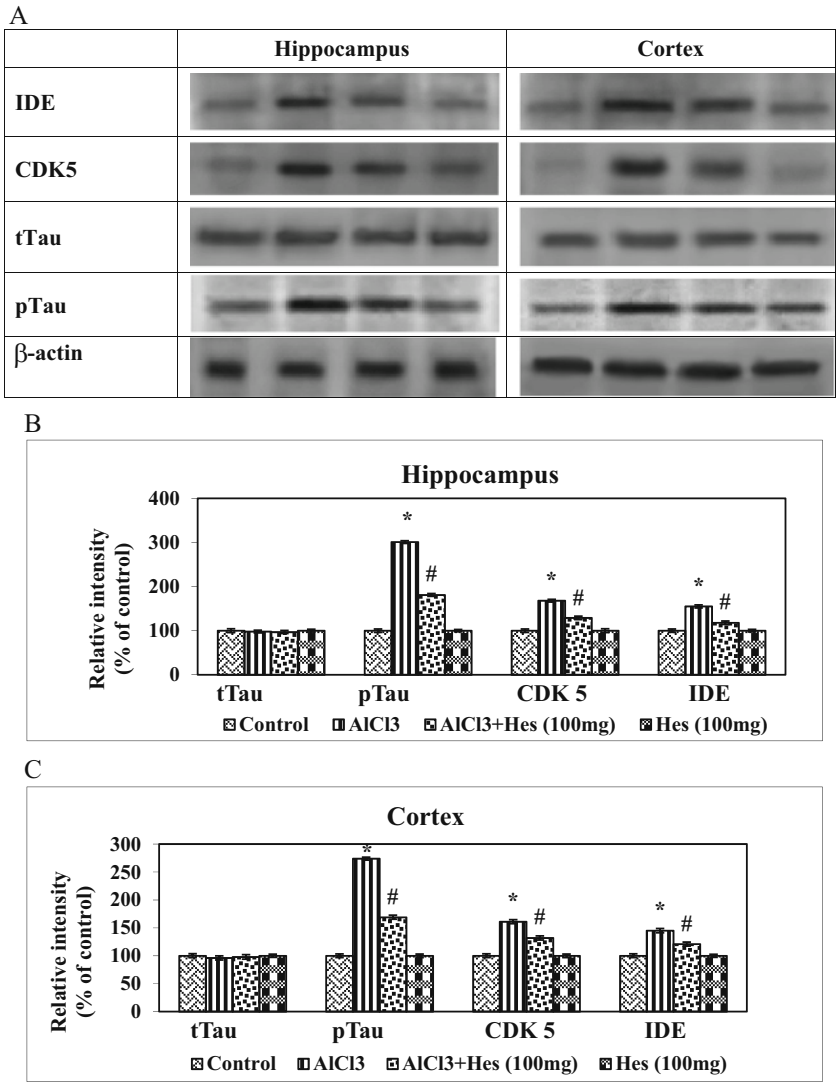



**Fig. 4** **a** $AlCl_3$-injected rats showed significantly enhanced the expressions of pTau, CDK 5, and IDE as compared to control rats, while hesperidin co-treatment significantly diminished their expressions as compared to $AlCl_3$ treated rats. **b, c** Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as mean ± SD ($n = 3$). Lane 1: control, lane 2: $AlCl_3$, lane 3: hesperidin + $AlCl_3$, lane 4: hesperidin. Values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$ treated rats, one-way ANOVA followed by DMRT

## $AlCl_3$?-Induced Apoptosis Is Blocked by Hesperidin

Apoptosis is the major form of neuronal death in all the NDDs. Elevated protein expressions of cytosolic cyto c and caspase-3, caspase-8, and caspase-9 and diminished expression of mitochondrial cyto c were found in the hippocampus and cortex of $AlCl_3$-treated rats as compared to control rats; on the other hand, hesperidin co-treatment attenuated their expressions significantly as compared to $AlCl_3$-treated rats. Hesperidin-alone treatment did not cause significant changes in the control animals (Figs. 5 and 6a–c).

## $AlCl_3$?-Induced Neuroinflammation Is Attenuated by Hesperidin

The expression of GFAP and Iba 1, markers of astroglial and microglial activation respectively were found to be enhanced in the $AlCl_3$-treated rats, whereas their protein expression were reduced by hesperidin treatment. From our western blot analysis, we observed the enhanced protein expression of iNOS, NF-kB, TNF-α, IL-1β, IL-4, 6, and COX-2 in $AlCl_3$-treated rats, whereas hesperidin treatment attenuated these indices through its anti-inflammatory properties (Figs. 7 and 8a–c).

## Hesperidin Activated the $AlCl_3$-Inhibited Akt/GSK3β Signaling Pathway

Animals treated with a chronic $AlCl_3$ regimen manifested significant lowered expressions of pAkt and pGSK-3β in the hippocampus and cortex, whereas their expression were elevated in animals co treated with hesperidin. $AlCl_3$-alone treatment showed no significant changes in pAkt and pGSK-3β expressions compared to control rats. No significant changes in the expression of tAkt and tGSK-3β were observed in the control and experimental rats (Fig. 9a–c).

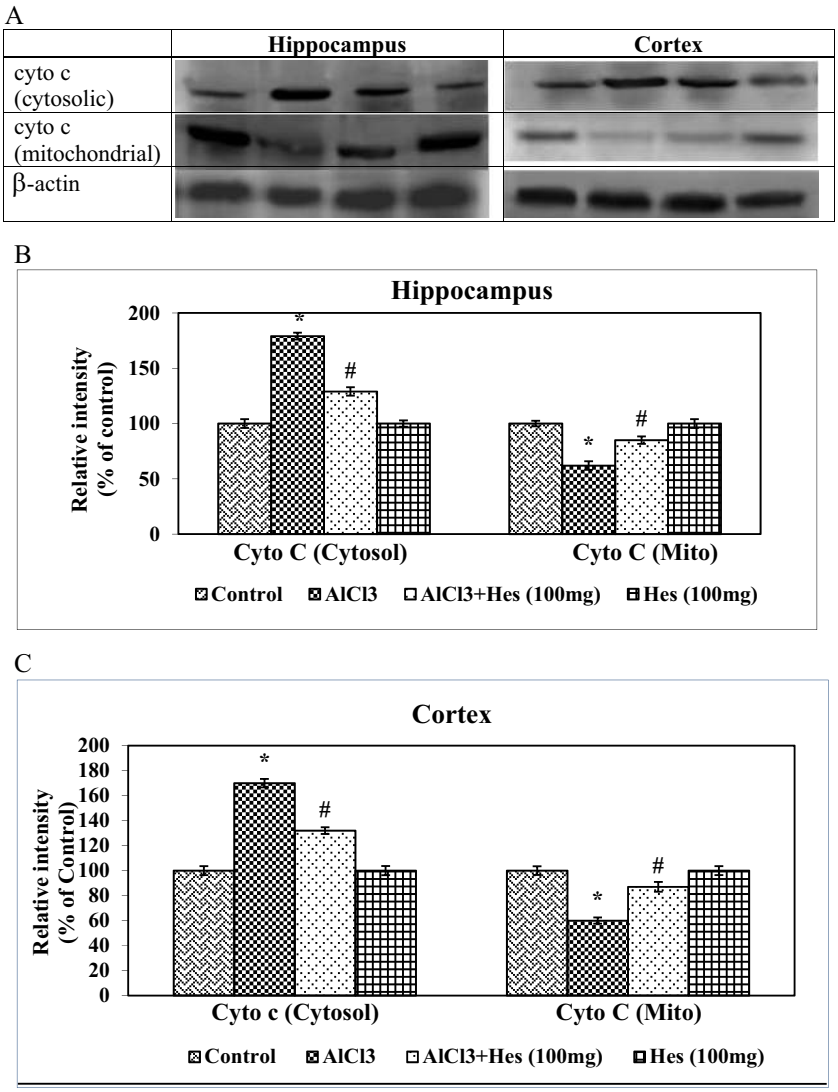



**Fig. 5** **a** $AlCl_3$-injected rats showed significantly enhanced the expressions of cytosolic cyto c and diminished the expressions of mitochondrial cyto c as compared to control rats, while hesperidin co-treatment significantly attenuated their expressions as compared to $AlCl_3$ treated rats. **b, c** Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as mean ± SD ($n = 3$). Lane 1: control, lane 2: $AlCl_3$, lane 3: hesperidin + $AlCl_3$, lane 4: hesperidin. Values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$-treated rats, one-way ANOVA followed by DMRT

## Discussion

Rats induced with $AlCl_3$ showed a significant ($p < 0.05$) decrease in body weight as compared to control rats, whereas hesperidin treatment significantly enhanced body weight (Justin Thenmozhi et al., 2015). Decreased water and food intake, transient diarrhea, and reduced efficacy in converting feed-to-body weight gain causes the reduction in body mass of Al-treated animals as compared to controls (Kowalczyk et al., 2004; Miyasaksa et al., 2016).

In the present study, $AlCl_3$-treated rats exhibited less number of SAP and more SAR as compared to control rats, whereas hesperidin co-treatment significantly attenuated these memory deficits. The Y-maze apparatus is used for the assessment of the short-term memory (Foyet et al., 2011) and diminished percentage of alternation is an indicator of an impaired spatial working memory (Hritcu et al., 2012; Rout et al., 2012). Because the rat cannot remember which arm it has just visited and thus shows decreased spontaneous alternation. Short-term memory impairment is the first clinical feature of AD and when the condition progresses, other cognitive functions such as the ability to analyze and usage of common objects and tools in human beings are impaired (Kimura and Ohno 2009). Hesperidin and its aglycone hesperetin induced the long-term potentiation in the rat hippocampus in vitro and attenuated the toxicity of various convulsive compounds and iberiotoxin (Dimpfel 2006). Our results also indicated that a reduction in retention time in rotarod test during $AlCl_3$ injection reflected reduction in muscle coordination. In novel object recognition test, rats injected with Al showed less anxiety response to an unfamiliar environment. $AlCl_3$-induced hypoactivity could affect the cognitive performance. Previous studies from our lab (Justin Thenmozhi et al., 2015) and others (Borsini and Meli, 1998) reported that the impaired physiological functions or reduced motor

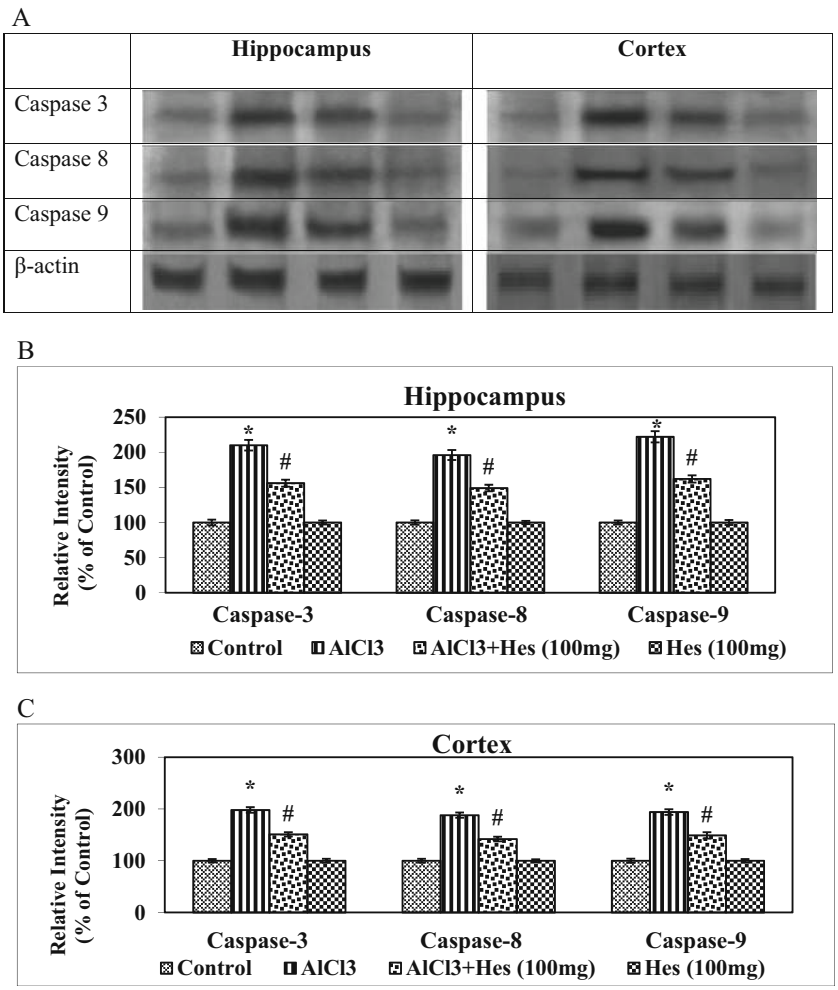



**Fig. 6** **a** Administration of $AlCl_3$ markedly enhanced the expressions of caspase-3, caspase-8, and caspase-9 in the hippocampus and cortex as compared to control rats, while hesperidin co-treatment significantly diminished their expressions as compared to $AlCl_3$ treated rats. **b, c** Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as mean ± SD ($n$ = 3). Lane 1: control, lane 2: $AlCl_3$, lane 3: hesperidin + $AlCl_3$, lane 4: hesperidin. Values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$-treated rats, one-way ANOVA followed by DMRT

performance diminished the memory in learning and memory tests. The loss of motor coordination is a common characteristic of many neurological disorders and difficulties in motor performance can confound behavioral assays of learning and memory, exploration, and motivation (Rustay et al., 2003). In addition to motor impairments, Al induced memory impairment by enhancing the levels of AChE thereby reducing the acetylcholine, a neurotransmitter responsible for memory (Justin Thenmozhi et al., 2016a). Moreover, Al accumulates predominantly in hippocampus and cortex, the regions responsible for learning and memory and induces neurodegeneration (Justin Thenmozhi et al., 2016b). So the decreased performance in NORT and Y-maze might be due to impaired memory and learning ability. Hesperidin treatment improved the muscle coordination in rotarod test and the capacity to recognize new objects in novel object recognition (NOR) test, which might be due to its metal chelation property (Justin Thenmozhi et al. 2015).

In the present study, $AlCl_3$ treatment upregulated the IDE and CDK 5 expression with higher phosphorylated Tau levels than control rats, whereas hesperidin co-treatment downregulated these expressions. Many evidence supported the impact of IDE in the breakdown of Aβ (Selkoe 2001) whereas IDE knockout transgenic mice showed enhanced endogenous Aβ levels (Farris et al. 2003). Previous studies indicated that the flavanoids such as pratensein (Wei et al. 2015) and icariin (Li et al. 2015a, b) markedly diminished the level and deposition of β-amyloid peptide by regulating the expressions of Aβ-related genes including IDE. It is needed to compare the hesperidin effects with those of pretensein or icariin to understand where hesperidin positions in the neuroprotective flavonoids. Hesperidin co-exposure attenuated the Tau phosphorylation thereby favoring cognitive improvement. As CDK 5 is initiated the paired helical filament formation (Takahashi et al. 2000), their levels and activity were found to be increased in the AD patients (Pei et al. 1998). Elevated activity of the CDK 5 has been reported to play a key function in neuronal death

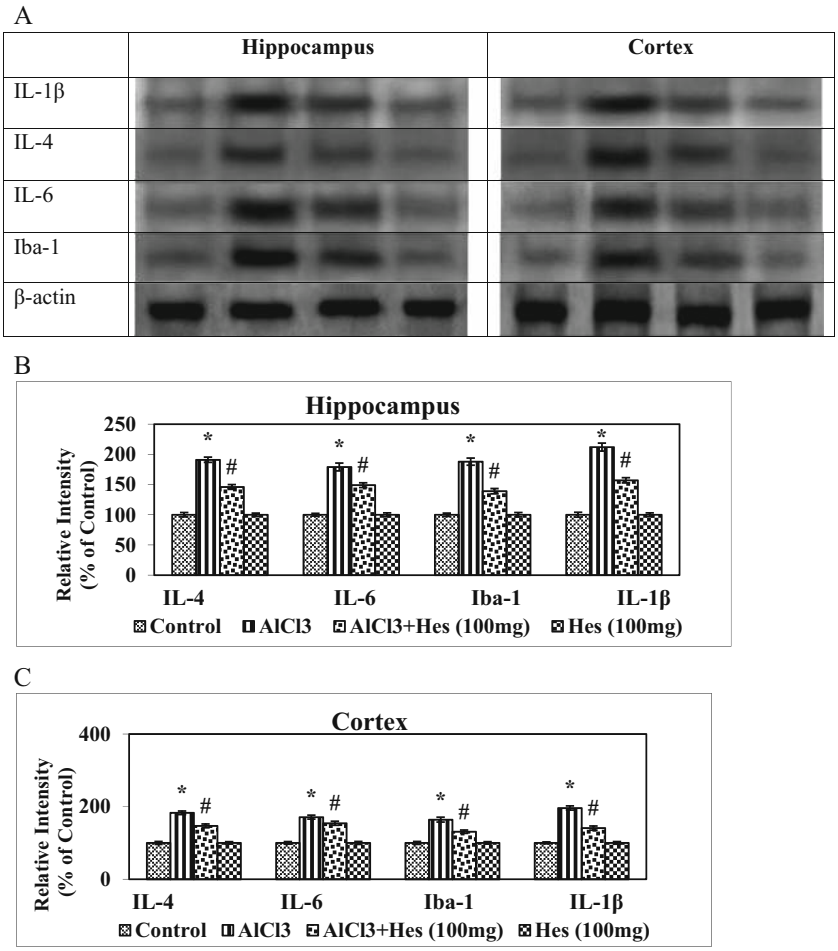



**Fig. 7** **a** $AlCl_3$ injected rats showed significantly enhanced the expressions of IL-1β, IL-4, IL-6, and Iba-1 as compared to control rats, while hesperidin co-treatment significantly diminished their expressions as compared to $AlCl_3$ treated rats. **b, c** Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as mean ± SD ($n = 3$). Lane 1: control, lane 2: $AlCl_3$, lane 3: hesperidin + $AlCl_3$, lane 4: hesperidin. Values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$ treated rats, one-way ANOVA followed by DMRT

triggered by excito-toxicity, oxidative stress, and ischemia and in experimental models of neurodegenerative diseases including AD and PD (Cheung and Ip 2004).

Several experiments stated that apoptosis (Su et al. 1994) have been acting as a principal cause for death of neurons in AD. In our previous report (Justin Thenmozhi et al. 2017), the occurrence of neuronal degeneration in $AlCl_3$-induced rats were shown by histochemical and immunohistological studies. About 13 to 50% of the deteriorated neurons are present within or near Aβ deposits, but these are 5.7-fold more than neurons without contact to plaques. NFTs involve about 41% of all degenerating neurons which means an about 3-fold increase in the apoptosis risk than tangle-free neurons (Lassman et al. 1995). The existence of cleaved and active caspases around SPs, NFTs, and postsynaptic densities are confirmed by various biochemical studies and immunohistochemical experiments (Shimohama 2000; Behl 2000; Louneva et al. 2008). The cyto c reached the cytoplasm from mitochondria during the MTP opening or succeeding Bax translocation into mitochondria (Gupta et al. 2009). Cyto c consequently activates caspases, which eventually results in cell death. Caspases such as caspase-9 act as an upstream "initiator" that combines apoptotic stimuli to the downstream "effector" caspases like caspase-3. In our study, $AlCl_3$ administration augmented the expressions of cytosolic cyto c, caspase-3, and caspase-9 and diminished the expression of mitochondrial cyto c. Hesperidin treatment attenuated the apoptosis by inhibiting cyto c release and diminishing the caspase activation in various toxin-induced models of brain injury (Kiasalari et al., 2016; Ciftci et al. 2015; Banji et al. 2014), which strengthen our present results.

Astrocyte and microglia are activated by toxins that are having an important role in the neuroinflammation and neurodegeneration (Kaushik et al. 2012). Severe astrocytic activation involved in the AD pathogenesis leading to the release of various neurotoxic agents with increased GFAP expression, a marker for astrogliosis (Eng and Ghirnikar 1994). Microglia responds quickly to any injury or insult than astrocytes (Gonzalez-Scarano and Baltuch 1999) and Iba-1 synthesis occurs only in microglial cells. Al carries

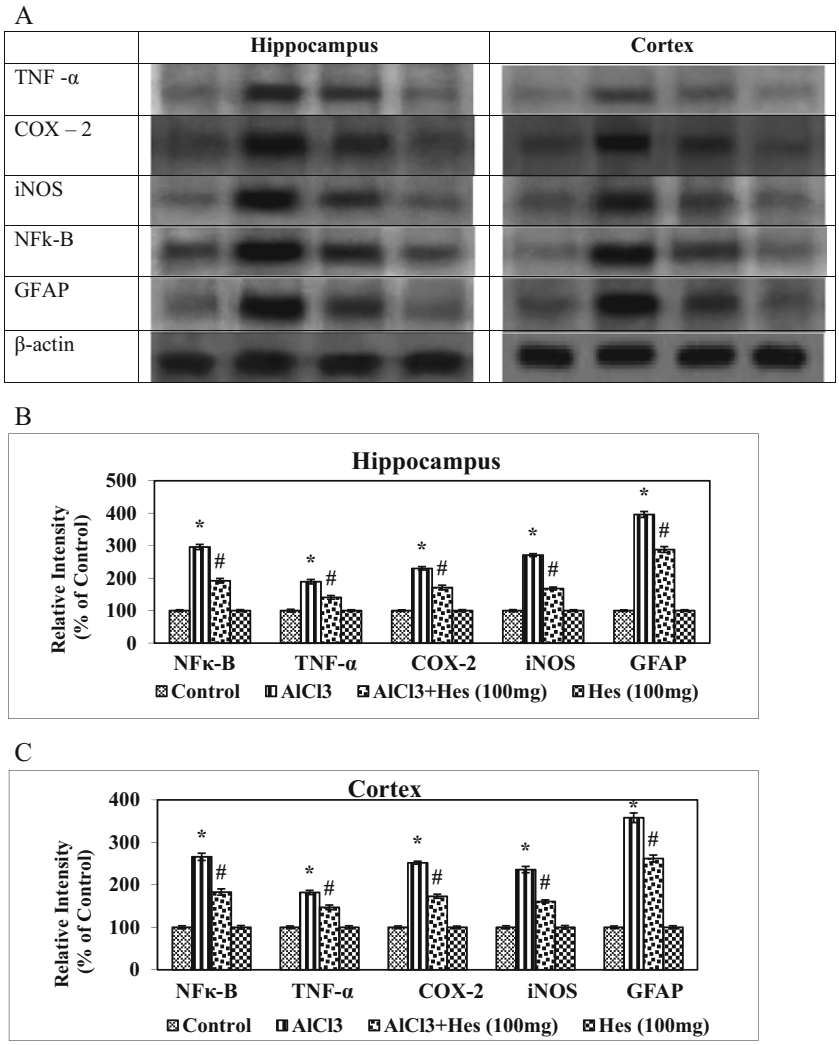



**Fig. 8** **a** $AlCl_3$ injected rats showed significantly enhanced the expressions of NFK-B, TNF-α, COX-2, iNOS, and GFAP as compared to control rats, while hesperidin co-treatment significantly diminished their expressions as compared to $AlCl_3$ -treated rats. **b, c** Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as mean ± SD ($n = 3$). Lane 1: control, lane 2: $AlCl_3$, lane 3: hesperidin + $AlCl_3$, lane 4: hesperidin. Values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$-treated rats, one-way ANOVA followed by DMRT

out AD pathogenesis by activating the inflammatory responses. The elevated expression of Iba-1 and GFAP, observed in our study, clearly demonstrated the activation of macro- and microglial cells after $AlCl_3$ treatment, but hesperidin administration nullifies the active gliosis by diminishing the Iba-1 and GFAP expressions. In this study, the increased GFAP expression during the Al-alone treatment, might have induced the edema. Stefanovich and Joo (1990) suggested that Al administration increases the permeability of the blood-brain barrier and results in the development of brain edema of the vasogenic type. Their study also demonstrated that Al gluconate increased the permeability for Evans blue which indicates the opening of the blood-brain barrier to serum albumin, after both acute and chronic treatments. Sumathi et al., (2013) indicated that the administration of Al showed diffused gliosis and pericellular edema in the cerebral cortex of brain. However in this study, the edematous conditions were not analyzed.

Multiple pro-inflammatory agents like cytokines (TNF-α), interleukins (IL-1β and IL-6), chemokines, ROS, and COX-2 (Akiyama et al. 2000; Heneka and O-Banion 2007) were secreted by the active form of microglial cells and astrocytes. Chronic Al accumulation might lead to neuroinflammation through activation of microglia and astrocytes, which is one of the key event occuring during AD (Cao et al. 2016). Our results indicated that $AlCl_3$ co-administration alters the expressions of pro-inflammatory factors like IL-1β, IL-6, COX-2, TNF- α, and NFk-B, which is corroborated with the earlier study (Singla and Dhawan 2017). Hesperidin showed potent anti-inflammatory activity against 6-hydroxydopamine-induced PD (Kiasalari et al., 2016), transgenic APP/PS1 AD (Li et al., 2015a, b), streptozotocin-induced cognitive impairment (Javed et al. 2015), quinolinic acid-induced Huntington's disease (Kumar et al. 2013), and middle cerebral artery occlusion-induced stroke (Raza et al. 2011) models.

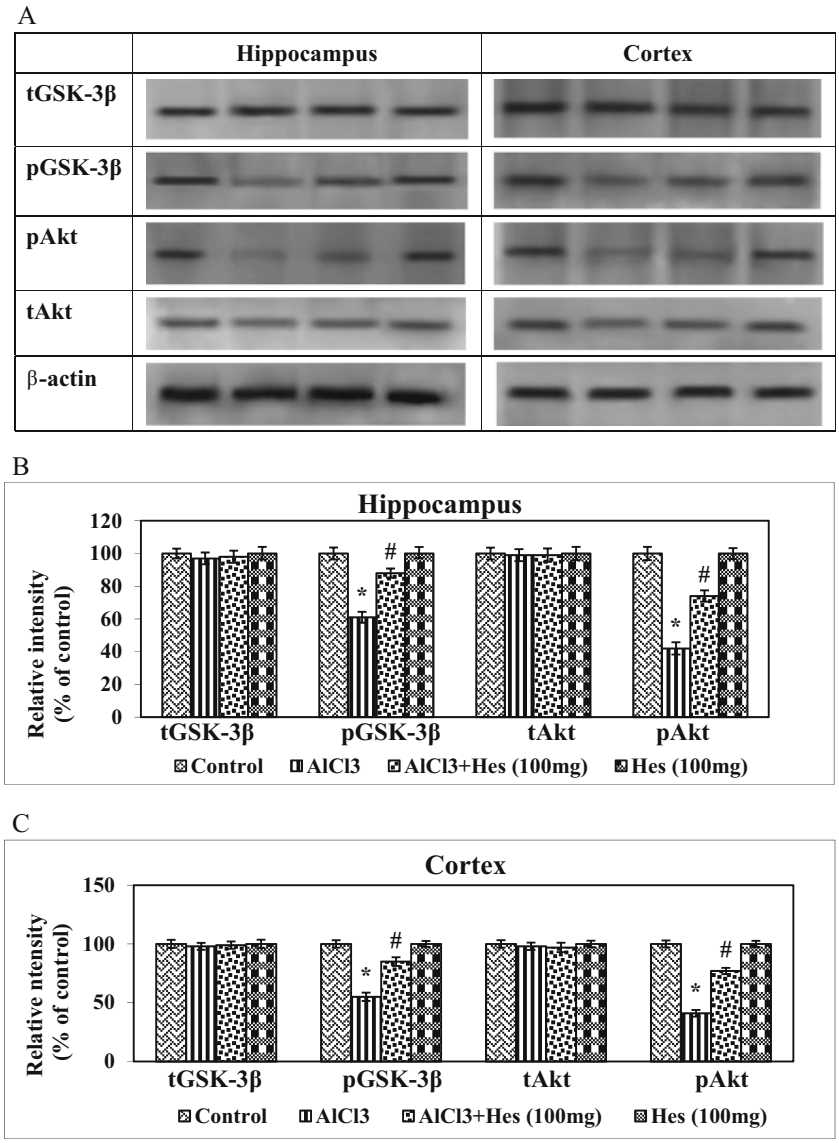



**Fig. 9** **a** $AlCl_3$-injected rats showed significantly reduced the expressions of pAKT and pGSK-3β as compared to control rats, while hesperidin co-treatment significantly enhanced their expressions as compared to $AlCl_3$ treated rats. **b, c** Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as mean ± SD ($n$ = 3). Lane 1: control, lane 2: $AlCl_3$, lane 3: hesperidin + $AlCl_3$, lane 4: hesperidin. Values not sharing common symbols differ significantly—$^{*}p < 0.05$ compared to the control, $^{\#}p < 0.05$ compared to the $AlCl_3$-treated rats, one-way ANOVA followed by DMRT

Neuronal apoptosis are regulated by three major kinase pathways: Akt pathway, JNK pathway, and GSK-3β pathway (Borsello and Forloni 2007). Activated Akt regulates the caspase-9 (Cardone et al. 1998), Bad (Datta et al. 1997), and GSK-3β (Pap and Cooper 1998), thereby believed to suppress apoptosis. In the present study, $AlCl_3$ injection downregulated the pAkt expression, but hesperidin co-administration improved its expression. Akt activation downregulates GSK-3β expression by enhancing the Ser 9 phosphorylation (Hu et al. 2002), which in turn suppresses its kinase activity. In this study, $AlCl_3$ exposure diminished the Ser 9 pGSK3β expressions, thereby enhancing the kinase activity of GSK-3β and Tau hyperphosphorylation, which coincides with the recent report (Zhang et al. 2016). Hesperidin co-treatment enhanced Ser9 pGSK-3β expressions, thereby lowering the GSK-3β kinase activity and Tau hyperphosphorylation. From our results, it is suggested that hesperidin suppresses the apoptosis and Tau pathology by activating the Akt signaling pathway. It is needed to block the AKT/GSK-3β pathway with inhibitors, siRNA or knockouts, to prove our claim. However, hesperidin and its derivative hesperitin offered neuroprotective effect against hypoxia-ischemic brain injury (Rong et al. 2013) and cerebral ischemia-reperfusion injury (Wang and Cui 2013) by regulating the AKT/GSK-3β pathway which supports our findings.

## Conclusions

This current study demonstrated that hesperidin exhibited a strong memory-enhancing property and provided an

understanding of the mechanisms involved in the modes of action of hesperidin against Al-induced neurotoxicity. It can be concluded that hesperidin could be a promising candidate for preventing or treating inflammatory and apoptotic conditions in neurodegenerative diseases including AD.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

## References

Ahmed SS, Santosh W (2010) Metallomic profiling and linkage map analysis of early Parkinson's disease: a new insight to aluminium marker for the possible diagnosis. PLoS One 5:e11252

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM et al (2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21:383–321

Ali HA, Afifi M, Abdelazim AM, Mosleh YY (2014) Quercetin and omega 3 ameliorate oxidative stress induced by aluminium chloride in the brain. J Mol Neurosci 53:654–660

Ali AA, Ahmed HI, Elfotuh KA (2016) Modeling stages mimic alzheimer's disease induced by different doses of aluminum in rats: focus on progression of the disease in response to time. J Alzheimers Parkinsonism Dementia 1:1–11

Amador FC, Henriques AG, Da Cruz E Silva OAB, Da Cruz E Silva EF (2014) Monitoring protein phosphatase 1 isoform levels as a marker for cellular stress. Neurotoxicol Teratol 26:387–395

Banji OJ, Banji D, Ch K (2014) Curcumin and hesperidin improve cognition by suppressing mitochondrial dysfunction and apoptosis induced by D-galactose in rat brain. Food Chem Toxicol 74:51–59

Behl C (2000) Apoptosis and Alzheimer's disease. J Neural Transm 107: 1325–1344

Bhullar KS, Rupasinghe HP (2013) Polyphenols: multipotent therapeutic agents in neurodegenerative diseases. Oxid Med Cell Longev 891748

Borsello T, Forloni G (2007) JNK signalling: a possible target to prevent neurodegeneration. Curr Pharm Des 13:1875–1886

Borsini F, Meli A (1998) Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl) 94: 147–160

Cao Z, Yang X, Zhang H, Wang H, Huang W, Xu F, Zhuang C, Wang X, Li Y (2016) Aluminum chloride induces neuroinflammation, loss of neuronal dendritic spine and cognition impairment in developing rat. Chemosphere 151:289–295

Cao Z, Wang F, Xiu C, Zhang J, Li Y (2017) Hypericum perforatum extract attenuates behavioral, biochemical, and neurochemical abnormalities in Aluminum chloride-induced Alzheimer's disease rats. Biomed Pharmacother 91:931–937

Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC (1998) Regulation of cell death protease caspase-9 by phosphorylation. Science 282:1318–1321

Cheung ZH, Ip NY (2004) CDK5: mediator of neuronal death and survival. Neurosci Lett 361:47–51

Ciftci O, Ozcan C, Kamisli O, Cetin A, Basak N, Aytac B (2015) Hesperidin, a citrus flavonoid, has the ameliorative effects against experimental autoimmune encephalomyelitis (EAE) in a C57BL/J6 mouse model. Neurochem Res 40:1111–1120

Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231–241

Dhanalakshmi C, Janakiraman U, Manivasagam T, Justin Thenmozhi A, Essa MM, Kalandar A et al (2016) Vanillin attenuated behavioural impairments, neurochemical deficts, oxidative stress and apoptosis against rotenone induced rat model of Parkinson's disease. Neurochem Res 41:1899–1910

Dhivya Bharathi M, Justin Thenmozhi A, Manivasagam T (2015) Protective effect of black tea extract against aluminium chloride-induced Alzheimer's disease in rats: a behavioural, biochemical and molecular approach. J Funct Foods 16:423–435

Dimpfel W (2006) Different anticonvulsive effects of hesperidin and its aglycone hesperetin on electrical activity in the rat hippocampus in-vitro. J Pharm Pharmacol 58:375–379

Eng LF, Ghirnikar RS (1994) GFAP and astrogliosis. Brain Pathol 4:229–237

Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe D, Guenette S (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid betaprotein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci 100:4162–4167

Foyet HS, Hritcu L, Ciobica A, Stefan M, Kamtchouing P, Cojocaru D (2011) Methanolic extract of Hibiscus asper leaves improves spatial memory deficits in the 6-hydroxydopamine-lesion rodent model of Parkinson's disease. J Ethnopharmacol 133:773–779

Gonzalez-Scarano F, Baltuch G (1999) Microglia as mediators of inflammatory and degenerative diseases. Annu Rev Neurosci 22:219–240

Gupta S, George EN, Kass, Szegezdi E, Josephc B (2009) The mitochondrial death pathway: a promising therapeutic target in diseases. J Cell Mol Med 13:1004–1033

Heneka MT, O-Banion MK (2007) Inflammatory processes in Alzheimer's disease. J Neuroimmunol 184:69–91

Hidaka N, Suemaru K, Takechi K, Li B, Araki H (2011) Inhibitory effects of valproate on impairment of Y-maze alternation behavior induced by repeated electroconvulsive seizures and c-Fos protein levels in rat brains. Acta Med Okayama 65:269–277

Hritcu L, Cioanca O, Hancianu M (2012) Effects of lavender oil inhalation on improving scopolamine-induced spatial memory impairment in laboratory rats. Phytomedicine 19:529–534

Hu YY, He SS, Wang XC, Duan QH, Khatoon S, Iqbal K, Grundke-Iqbal I, Wang JZ (2002) Elevated levels of phosphorylated neurofilament protein in cerebrospinal fluid of Alzheimer disease patients. Neurosci Lett 320:156–160

Janakiraman U, Manivasagam T, Justin Thenmozhi A, Essa MM, Barathidasan R, Saravana Babu C et al (2016) Influences of chronic mild stress exposure on motor, non-motor impairments and neurochemical variables in specific brain areas of MPTP/probenecid induced neurotoxicity in mice. PLoS One 11:e0146671

Javed H, Vaibhav K, Ahmed ME, Khan A, Tabassum R, Islam F, Safhi MM, Islam F (2015) Effect of hesperidin on neurobehavioral, neuroinflammation, oxidative stress and lipid alteration in intracerebroventricular streptozotocin induced cognitive impairment in mice. J Neurol Sci 348:51–59

Justin Thenmozhi A, Raja TR, Janakiraman U, Manivasagam T (2015) Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats. Neurochem Res 40:767–776

Justin Thenmozhi A, Dhivya Bharathi M, William Raja T, Manivasagam T, Essa MM (2016a) Tannoid principles of *Emblica officinalis* renovate cognitive deficits and attenuate amyloid pathologies against aluminium chloride induced rat model of Alzheimer's Disease. Nutr Neurosci 19:269–278

Justin Thenmozhi A, Dhivya Bharathi M, Manivasagam T, Essa MM (2016b) Tannoid principles of *Emblica officinalis* attenuated

aluminium chloride induced apoptosis by suppressing oxidative stress and tau pathology via Akt/GSK-3β signaling pathway. J Ethnopharmacol 194:20–29

Justin Thenmozhi A, William Raja TR, Manivasagam T, Janakiraman U, Essa MM (2017) Hesperidin ameliorates cognitive dysfunction, oxidative stress and apoptosis against aluminium chloride induced rat model of Alzheimer's disease. Nutr Neurosci 20:360–368

Kaushik DK, Mukhopadhyay R, Kumawat KL, Gupta M, Basu A (2012) Therapeutic targeting of Kruppel-like factor 4 abrogates microglial activation. J Neuroinflammation 9:57

Kawahara M, Kato Negishi M (2011) Link between aluminum and the pathogenesis of Alzheimer's disease: the integration of the aluminum and amyloid cascade hypotheses. Int J Alzheimers Dis: 276393

Kean RJ, Lamport DJ, Dodd GF, Freeman JE, Williams CM, Ellis JA, Butler LT, Spencer JP (2015) Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: an 8-wk, randomized, double-blind, placebo-controlled trial in healthy older adults. Am J Clin Nutr 101:506–514

Khan KA, Kumar N, Nayak PG, Nampoothiri M, Shenoy RR, Krishnadas N, Rao CM, Mudgal J (2013) Impact of caffeic acid on aluminium chloride-induced dementia in rats. J Pharm Pharmacol 65:1745–1752

Kiasalari Z, Khalili M, Baluchnejadmojarad T, Roghani M (2016) Protective effect of oral hesperetin against unilateral striatal 6-hydroxydopamine damage in the rat. Neurochem Res 41:1065–1072

Kimura R, Ohno M (2009) Impairments in remote memory stabilization precede hippocampal synaptic and cognitive failures in 5XFAD Alzheimer mouse model. Neurobiol Dis 33:229–235

Kitagishi Y, Nakanishi A, Ogura Y, Matsuda S (2014) Dietary regulation of PI3K/Akt/GSK-3β pathway in Alzheimer's disease. Alzheimers Res Ther 6(35):1–7

Kowalczyk E, Kopffl A, Kedzioral J, Blaszczyk J, Kopffl M, Niedworok J, Fijałkowski P (2004) Effect of long-term aluminum chloride intoxication on selected biochemical parameters and oxidative-antioxidative balance in experimental animals. Pol J Environ Stu 13:41–43

Kumar A, Dogra S, Prakash A (2009) Protective effect of curcumin (Curcuma longa), against aluminum toxicity: possible behavioral and biochemical alterations in rats. Behav Brain Res 205:384–390

Kumar A, Chaudhary T, Mishra J (2013) Minocycline modulates neuroprotective effect of hesperidinagainst quinolinic acid induced Huntington's disease like symptoms in rats: behavioral, biochemical, cellular and histological evidences. Eur J Pharmacol 720:16–28

Lassman H, Bancher C, Breitschopf H, Wegiel J, Bobinski M, Jellinger K, Wisniewski HM (1995) Cell death in Alzheimer's disease evaluated by DNA fragmentation in situ. Acta Neuropathol 89:35–41

Li WX, Deng YY, Li F, Liu B, Liu HY et al (2015a) Icariin, a major constituent of flavonoids from Epimedium brevicornum, protects against cognitive deficits induced by chronic brain hypoperfusion via its anti-amyloidogenic effect in rats. Pharmacol Biochem Behav 138:40–48

Li C, Zug C, Qu H, Schluesener H, Zhang Z (2015b) Hesperidin ameliorates behavioral impairments and neuropathology of transgenic APP/PS1 mice. Behav Brain Res 281:32–42

Louneva N, Cohen JW, Han LY, Talbot K, Wilson RS, Bennett DA, Trojanowski JQ, Arnold SE (2008) Caspase-3 is enriched in post-synaptic densities and increased in Alzheimer's disease. Am J Pathol 173:1488–1495

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Menze ET, Tadros MG, Abdel-Tawab AM, Khalifa AE (2012) Potential neuroprotective effects of hesperidin on 3-nitropropionic acid-induced neurotoxicity in rats. Neurotoxicology 33:1265–1275

Miyasaksa SC, Hue NV, Dunn MA (2016) Aluminium. In: Barker AV, Pilbeam DJ, editors. Hand Book of Plant Nutrition. Second edition: CRC Press. Science 441–481

Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74:418–425

Okuda S, Roozendaal B, McGaugh JL (2004) Glucocorticoid effects on object recognition memory require training-associated emotional arousal. Proc Natl Acad Sci 101:853–858

Pap M, Cooper GM (1998) Role of glycogen synthase kinase-3 in the phosphatidylinositol 3-Kinase/Akt cell survival pathway. J Biol Chem 273:19929–19932

Paxinos G, Watson C (2007) The rat brain in sterotaxic coordinates. Academic Press, San Diego

Pei JJ, Grundke-Iqbal I, Iqbal K, Bogdanovic N, Winblad B, Cowburn RF (1998) Accumulation of cyclin-dependent kinase 5 (cdk5) in neurons with early stages of Alzheimer's disease neurofibrillary degeneration. Brain Res 797:267–277

Prakash A, Kumar A (2013) Mitoprotective effect of Centella asiatica against aluminum-induced neurotoxicity in rats: possible relevance to its anti-oxidant and anti-apoptosis mechanism. Neurol Sci 34:1403–1409

Prakash D, Sudhandiran G (2015) Dietary flavonoid fisetin regulates aluminium chloride induced neuronal apoptosis in cortex and hippocampus of mice brain. J Nutr Biochem 26:1527–1539

Prakash D, Gopinath K, Sudhandiran G (2013) Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminium chloride-induced neurotoxicity. NeuroMolecular Med 15:192–208

Prema A, Justin Thenmozhi A, Manivasagam T, Mohamed Essa M, Guillemin GJ (2017) Fenugreek seed powder attenuated aluminum chloride-induced tau pathology, oxidative stress, and inflammation in a rat model of Alzheimer's disease. J Alzheimer's Dis 60:209–220

Raza SS, Khan MM, Ahmad A, Ashafaq M, Khuwaja G, Tabassum R, Javed H, Siddiqui MS, Safhi MM, Islam F (2011) Hesperidin ameliorates functional and histological outcome and reduces neuroinflammation in experimental stroke. Brain Res 1420:93–105

Reale M, Larlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M (2009) Peripheral cytokines profile in Parkinson's disease. Brain Behav Immun 23:55–63

Rong Z, Pan R, Xu Y, Zhang C, Cao Y, Liu D (2013) Hesperidin pretreatment protects hypoxia-ischemic brain injury in neonatal rat. Neuroscience 255:292–299

Rout SK, Kar DM, Rout AB (2012) Study of central nervous system activity of leaf extracts of nerium oleanderin experimental animal models. Int J Pharm Pharm Sci 4:378–382

Rustay NR, Wahlsten D, Crabbe JC (2003) Influence of task parameters on rotarod performance and sensitivity to ethanol in mice. Behav Brain Res 141:237–249

Selkoe DJ (2001) Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. J Alzheimers Dis 3:75–80

Shimohama S (2000) Apoptosis in Alzheimer's disease–an update. Apoptosis 5:9–16

Singla N, Dhawan DK (2017) Zinc improves cognitive and neuronal dysfunction during aluminium-induced neurodegeneration. Mol Neurobiol 54:406–422

Stefanovich V, Joo F (1990) Effect of propentofylline on the biochemical lesion of the rat brain in aluminium-induced neurotoxicity. Metabol Brain Dis 5:7–17

Su JH, Anderson AJ, Cumming BJ, Cotman CW (1994) Immunocytochemical evidence for apoptosis in Alzheimer's disease. Neuroreport 5:2529–2533

Sumathi T, Shobana C, Mahalakshmi V, Sureka R, Subathra M, Vishali A, Rekha K (2013) Oxidative stress in brains of male rats

intoxicated with aluminium and neuromodulating effect of celastrus paniculatus alcoholic seed extract. Asian J Pharm Clin Res 6:80–90

Takahashi M, Iseki E, Kosaka K (2000) Cdk5 and munc-18/p67 co-localization in early stage neurofibrillary tangles-bearing neurons in Alzheimer type dementia brains. J Neurol Sci 172:63–69

Tamilselvam K, Nataraj J, Janakiraman U, Manivasagam T, Essa MM (2013) Antioxidant and anti-inflammatory potential of hesperidin against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced experimental Parkinson's disease in mice. Int J Nutr Pharmacol Neurol Dis (3):294–302

Teismann P, Schulz JB (2004) Cellular pathology of Parkinson's disease: astrocytes, microglia and inflammation. Cell Tissue Res 318:149–161

Use of Animals in Scientific Research. (2000) Available from: http://icmr.nic.in/bioethics/animals_biomedical%20research.pdf/. [Last accessed on 2012 July 13].

Wang JJ, Cui P (2013) Neohesperidin attenuates cerebral ischemia-reperfusion injury via inhibiting the apoptotic pathway and activating the Akt/Nrf2/HO-1 pathway. J Asian Nat Prod Res 15:1023–1037

Wei L, Lv S, Huang Q, Wei J, Zhang S, Huang R, Lu Z, Lin X (2015) Pratensein attenuates Aβ-induced cognitive deficits in rats: enhancement of synaptic plasticity and cholinergic function. Fitoterapia 101:208–217

Zhang B, Li Q, Chu X, Sun S, Chen S (2016) Salidroside reduces tau hyperphosphorylation via up-regulating GSK-3β phosphorylation in a tau transgenic Drosophila model of Alzheimer's disease. Transl Neurodegener 5:21