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ORIGINAL ARTICLE

Cortico-hippocampal memory enhancing activity of hesperetin on scopolamine-induced amnesia in mice: role of antioxidant defense system, cholinergic neurotransmission and expression of BDNF

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Abstract

Alzheimer disease (AD) is an age related neurodegenerative disease causing severe cognitive and memory decline in elderly people. Flavonoids play neuroprotective role by inhibiting and/or modifying the self-assembly of the amyloid-β (Aβ) or tau peptide into oligomers and fibrils. This study sought to investigate the effect of hesperetin (HPT) on scopolamine-induced memory impairments in mice. Mice were orally pretreated with HPT (1, 5 or 50 mg/kg) or vehicle (normal saline; 10 ml/kg) for 3 consecutive days. One hour post-treatment on day 3, scopolamine (3 mg/kg, i.p.) was administered 5 min before locomotor activity (open field test) and memory function (novel object recognition test (NORT) for 2 consecutive days and Morris water maze task (MWM) for 5 consecutive days). Levels of oxidative stress markers / brain derived neurotrophic factors (BDNF) and acetylcholinesterase activity were determined in the hippocampus and prefrontal cortex after completion of MWM task. Scopolamine caused no significant change in mice exploration of the familiar or novel object in the test session whereas the HPT-treated mice spent more time exploring the novel object more than familiar object in NORT. Scopolamine also increased the escape latency in acquisition phase and decreases time spent in target quadrant in probe phase which were ameliorated by the pretreatment with HPT. Scopolamine-induced alteration of oxidant-antioxidant balance, acetylcholinesterase activity and neurogenesis in the hippocampus and prefrontal cortex were attenuated by HPT treatment. This study showed that HPT ameliorated non-spatial/spatial learning and memory impairment by scopolamine possibly through enhancement of antioxidant defense, cholinergic and BDNF signaling.

Keywords Brain derived neurotrophic factors · Cholinergic neurotransmission · Novel object recognition test · Oxidative stress · Neurogenesis · Morris water maze task

Introduction

Alzheimer disease (AD) is an age-related neurodegenerative disease causing severe cognitive and memory decline. It accounts for more than 50% of the overall cases of dementia among persons over 65 years of age (Francis et al. 1999). The pathological hallmarks of AD are the appearance of β-Amyloid plaques, neurofibrillary tangles and the deficiency in cholinergic neurotransmission. AD brain is characterized microscopically by the combined presence of extracellular amyloid plaques and intraneuronal neurofibrillary tangles, both of which comprise highly insoluble, densely packed filaments. The soluble building blocks of these structures are amyloid-β (Aβ) peptides for plaques and tau for tangles. Amyloid-β peptides are proteolytic fragments of the transmembrane amyloid precursor protein, whereas tau is a brain-specific, axon-enriched microtubule-associated protein (Seward et al. 2013; Bloom 2014). Studies have shown that β-amyloid or tau protein tangles disrupts neurotropic growth factors (NGF) metabolism causing degeneration of the cholinergic neurons which depend on NGF for their survival (Turnbull and Coulson 2017, Pepeu and Grazia Giovannini 2017).

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In AD brain, there is an extensive loss of forebrain cholinergic neurons accompanied by a reduction of the cholinergic fibre network of the cortical mantel and hippocampus (Pepeu and Grazia Giovannini 2017). Drugs currently used in the management of AD includes; acetyl cholinesterase inhibitors and N-methyl-D-aspartate receptor agonist which produces symptomatic treatment without affecting the underlying cause of the disease. Hence, the need for the discovery of a possible disease modifying drug for the management of AD. Flavonoids are naturally occurring phytochemicals found in a variety of fruits and vegetables and offer nutritional and health benefits. Flavonoids have been found to play a neuroprotective role by inhibiting and/or modifying the self-assembly of the amyloid-β (Aβ) or tau peptide into oligomers and fibrils, which are linked to the pathogenesis of AD (Thapa and Chi 2015).

Hesperetin is an aglycone of hesperidin, abundantly present in the skins of oranges (Fig. 1), have been shown to possess antioxidant, anti-inflammatory (Hirata et al. 2005), neuroprotective (Rainey-Smith et al. 2008; Hwang and Yen 2009; Hwang et al. 2012), and anti-neoplastic (Zarebczan et al. 2011) effects. It has also been shown to prevent diabetes-induced gliosis and vascular permeability in the retina (Kumar et al. 2013). This study sought to evaluate the protective effect of hesperetin (HPT) on scopolamine-induced memory deficit in mice.

Materials and methods

Drugs and chemicals

Hesperetin, glacial acetic acid, trichloroacetic acid, scopolamine hydrobromide, sodium hydroxide, thiobarbituric acid, dimethylsulfoxide, chloral hydrate, 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), 2-(1-Naphthylamino)ethylamine dihydrochloride, acetylthiocholine, and others of analytical grades were purchased from Sigma Aldrich St. Loius MO, USA, normal Saline (Unique Pharmaceutical Lagos, Nigeria).

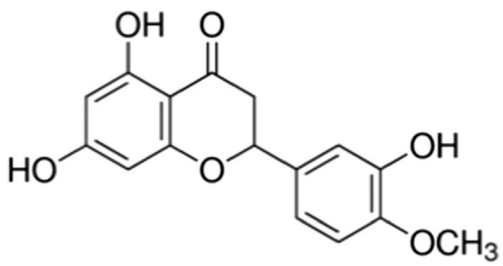

Fig. 1 Chemical structure of hesperitin

Laboratory animals

Male albino mice (20–25 g) used in this study were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos state, Nigeria. The mice were housed in plastic acrylic cage at room temperature (23 ± 2 °C) under standard environmental conditions (12 h light /dark cycle). They were fed with pelleted feed (Livestock feeds, Lagos, Nigeria) and water ad libitum. The experimental procedures adopted in this study was approved by Health Research Ethics Committee of the College of Medicine, University of Lagos, Nigeria (CMUL/HREC/11/17/305) and in agreement with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals in Biomedical Research (2011).

Behavioural test

Treatment regimen

Male albino mice (20–25 g) were randomly divided into 5 groups ($n = 6$) and treated as follows for 3 consecutive days: Group 1- vehicle (10 ml/kg; *p.o.*; normal control), Group 2- vehicle (10 ml/kg, *p.o.,* treatment control), Group 3–5: HPT (1, 5, or 50 mg/kg; *p.o.,* respectively). One hour after vehicle or drug administration on day 3, scopolamine (3 mg/kg, i.p.) was given to mice in all the groups except animals in Group 1. Dose selection was based on our preliminary studies and findings from previous studies (Choi 2008; Bodduluru et al. 2015; Wang et al. 2017).

Open field test (OFT)

OFT provides simultaneous measures of locomotion, exploration and anxiety. The apparatus consist of plywood 72 × 72 cm wide and 36 cm high divided into 16 squares (18 × 18 cm) and a central square (18 cm × 18 cm) in the middle of the open field (Brown et al., 1999; Ishola et al. 2017). Mice were placed in the center and allowed to explore the apparatus for 5 min after 1 min of habituation, the number of line crosses, rearing, grooming and time spent at the central square were recorded. On completion of the study, mice were returned to their home cages and the apparatus was clean with 10% ethanol to prevent auditory cue in between tests (Ishola et al. 2017).

Novel object recognition test (NORT)

The apparatus is made up of a plywood measuring 72 cm × 72 cm with 36 cm high. During acquisition test, mouse was placed at the centre of the apparatus and allowed to explore 3 objects (3 square boxes (familiar objects (F)) placed in three opposite corners of the apparatus for 5 min. Twenty four hours

later, mice were given HPT or vehicle, I h later, scopolamine (3 mg/kg, i.p.), was administered. Five minutes post scopolamine injection, the mice were allowed to explore the objects (2 familiar objects, square objects (F) + 1 new object, circular object (N)), for five minutes. Exploration was defined as sniffing or touching the object with the nose and /or forepaws and sitting on the object. The total time spent in exploring the three objects, F1, F2, and N were recorded. The Discrimination Index (DI) represents the difference in exploration time expressed as a proportion of the total time spent exploring the two familiar objects (F) to the new (N) object in T2 (Ishola et al. 2017).

$$\text{Discrimination Index (DI)} = \frac{\text{Novel object exploration time}}{\text{Total exploration time of both objects}} \times 100$$

Morris water maze test

Morris water maze (MWM) is widely used to test hippocampal-dependent learning, including acquisition of spatial memory and long-term spatial memory (Bromley-Brits et al. 2011). The apparatus consisted of a circular water tank (110 cm diameter and 60 cm height) filled with water (26 ± 2) to a depth of 30 cm. The pool was divided into four hypothetical quadrants, designated as: N (North), E (East), W (West), S (South). Black 10 cm diameter platform was placed in the SW quadrant 1 cm below the water pool (Ishola et al. 2013). On third day, 5 min post scopolamine (3 mg/kg, i.p.), the animal was subjected to MWM test. The MWM tasks were given for five consecutive days (day 3–7) with 3 trials per day. The mice were given a maximum of 60 s (cut-off time) to locate the hidden platform and were allowed to stay on it for 30 s. The time taken for the mouse to locate the hidden platform was recorded. Escape latency time (ELT) (time to locate the hidden platform in the water maze) was used as an index of learning (Ishola et al. 2013). On day 8, the platform was removed from the tank, and the mice underwent a spatial probe trial in which they were given 45 s to search for the platform. The time spent in a 2× (40 cm diameter) concentric area surrounding the platform (time in platform *annulus*) was recorded (Ishola et al. 2017).

Biochemical assay

Brain tissue preparation

Forty-five minutes after the MWM test on day 8, the mice were anaesthetized with chloral hydrate (300 mg/kg, i.p.) and perfused with cold saline. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed, the prefrontal cortex (PFC) and hippocampus (HIPPO) were isolated on an ice-cold plate. The isolated brain areas were weighed and homogenized in 0.03 M sodium phosphate buffer, *p*H -7.4 with an Ultra-Turrax T25 (USA) homogenizer at a speed of 9500 rpm. The homogenate was used to assay for malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and nitrite (Ishola et al. 2017).

Estimation of oxidative and nitrosative stress markers

MDA an indicator of lipid peroxidation, was spectrophotometrically measured using the thiobarbituric acid assay procedure (Ohkawa et al., 1979), GSH (endogenous antioxidant) was determined by its reaction with 5, 5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore (Rahman et al. 2006). The activity of superoxide dismutase (SOD) was assayed according to the method described by Winterbourn et al. (1975) while nitrite level (indicator of nitric oxide production) was estimated using the method of Green et al. (1982).

Acetylcholinesterase activity in the hippocampus and prefrontal cortex

The brain homogenate (500 μl) was mixed with 1% Triton X-100, centrifuged at 100,000×*g* at 4 °C in a Beckman Ultracentrifuge (LE 80, USA) for 1 h. The kinetic profile of enzyme activity was measured spectrophotometrically (Shimadzu, USA) at 412 nm with an interval of 15 s. One unit of acetylcholinesterase activity was defined as the number of micromoles (μmol) of acetylthiocholine iodide hydrolyzed per minute (min) per milligram (mg) of protein (Ishola et al. 2017).

Protein estimation

Protein was measured by the method of Lowry et al. (1951). Bovine serum albumin (BSA) (1 mg/ml) was used as standard and measured in the range of 0.01–0.10 mg/ml.

Measurement of brain derived neurotrophic factor (BDNF)

The concentration of brain derived neurotrophic factor was determined using Promega BDNF E_{max}® ImmunoAssay System kit (CAT No: G7610; Promega, Madison, WI 53711 USA) according to the manufacturer's protocol. Briefly, the hippocampus and prefrontal cortex were weighed and homogenized in lysis buffer, then centrifuged at 12,000×*g* at 4 °C in a Beckman Ultracentrifuge (LE 80, USA) for 5 min. The supernatants were collected for determination of BDNF levels. Total protein concentrations in supernatants were determined using a Pierce BCA Protein assay kit (Rockford, IL, USA).

The relative content of BDNF levels was depicted as per milligram total protein in the tissue.

Statistical analysis

Results obtained were expressed as mean ± SEM ($n = 6$). The statistical level of significance was determined by one- or two-way ANOVA followed by Tukey post hoc multiple comparison test using Graphpad prism version 6 (Graphpad prism Inc., CA, USA).

Results

Effect of hesperetin on spontaneous motor activity

Post hoc analysis showed that neither the pretreatment of mice with HPT or vehicle nor intraperitoneal injection of scopolamine produced no significant change locomotor activity, exploratory behaviors and anxiolytic activity (Fig. 2a and b). Although, HPT (50 mg/kg) showed possible anxiolysis evidenced in dose dependent and significant increase in centre square exploration. One way ANOVA revealed no significant effect of treatment [$F(4,25) = 0.03, P = 0.998$] (Fig. 2a). Similarly, Two way ANOVA revealed non-significant differences of HPT pretreatment [$F(4,75) = 1.22, P = 0.31$], scopolamine treatment [$F(2,75) = 20.83, P < 0.001$] and HPT pretreatment × scopolamine treatment interaction [$F(8,75) = 0.96, P = 0.48$] (Fig. 2b).

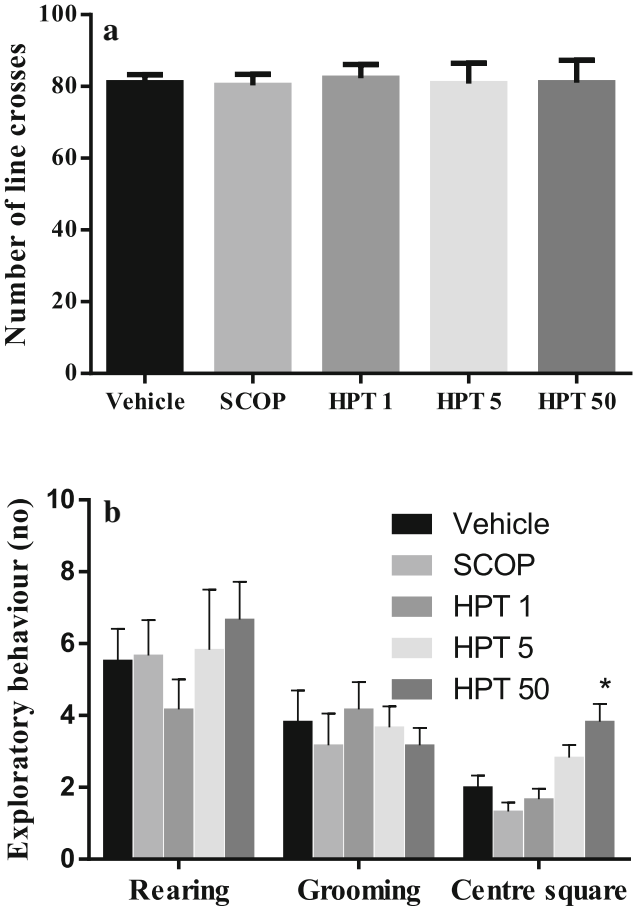

Fig. 2 **a**, **b** Effect of hesperitin and scopolamine on (a) number of line crosses and (b) number of rearing, grooming and centre square entries in open field test in mice. Values are expressed as mean ± SEM ($n = 6$), $^{*}P < 0.05$ versus SCOP-vehicle control. Statistical level of significance analysis by one or two way ANOVA followed by Tukey post hoc multiple comparison test

Effect of hesperetin on the novel object recognition test in mice

Two way ANOVA revealed a significant interaction between treatment and time spent exploring familiar or novel objects [$F(4,50) = 6.51, P = 0.0003$], treatment groups [$F(1,50) = 4.14, P = 0.04$] and HPT pretreatment × scopolamine treatment interaction [$F(4,50) = 2.67, P = 0.04$] (Fig. 3a). Tukey post hoc test revealed that HPT treated mice significantly more time exploring the novel object compared to familiar objects. Moreover, one way ANOVA revealed significant effect of discrimination between novel and familiar objects [$F(4,25) = 10.15, P < 0.001$] (Fig. 3b). Post hoc analysis showed a significant decrease in discrimination index in scopolamine treated mice compared with vehicle treated. However, the pretreatment of mice with HPT (5 and 50 mg/kg) produced significant increase discriminatory index compared with scopolamine-vehicle treated (Fig. 3b).

Effect of hesperetin on Morris water maze task

Post hoc analysis showed that the vehicle treated mice quickly acquired the task evidenced in time course and significant decrease in escape latency. In contrast, vehicle-scopolamine treated failed to acquire the task shown in non-significant change in escape latency. However, the pretreatment of mice with HPT 5 or 50 mg/kg before scopolamine administration produced time course decrease in escape latency. Two way ANOVA revealed a significant effect of treatment on escape latency [$F(4,100) = 13.15, P < 0.0001$], treatment groups [$F(3,100) = 44.21$, $P < 0.001$] and HPT pretreatment × scopolamine treatment interaction [$F(12,100) = 11.93, P < 0.0001$] (Fig. 4a). In probe test, there was a significant difference in the time spent in platform area crossings between the groups (one-way ANOVA post-hoc Tukey's test; F (4, 25) = 32.39; $p < 0.001$) (Fig. 4b). Scopolamine injection reduced time spent in quadrant location while the pretreatment of mice with HPT 5 or 50 mg/kg significantly increased time spent in platform location.

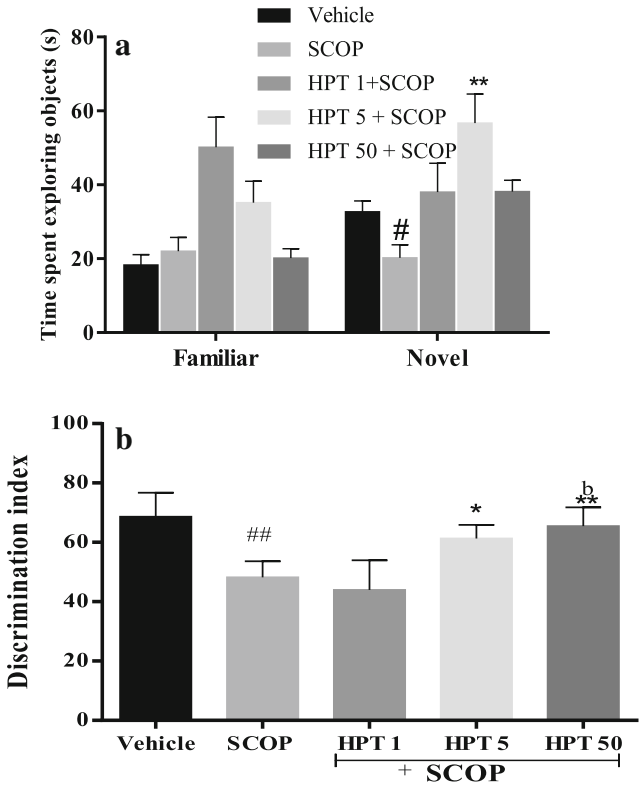

Fig. 3 **a**, **b** Effect of hesperitin on scopolamine-induced memory deficit (a) time spent exploring both familiar and novel objects, (b) discrimination index in novel object recognition test in mice. Values are expressed as mean ± SEM ($n = 6$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus vehicle treated control; $^{*}P < 0.05$, $^{**}P < 0.01$ versus SCOP-vehicle treated control, $^{b}P < 0.001$ versus HPT 1 mg/kg. Statistical level of significance was assessed by one-way ANOVA followed by Tukey's post hoc multiple comparison test

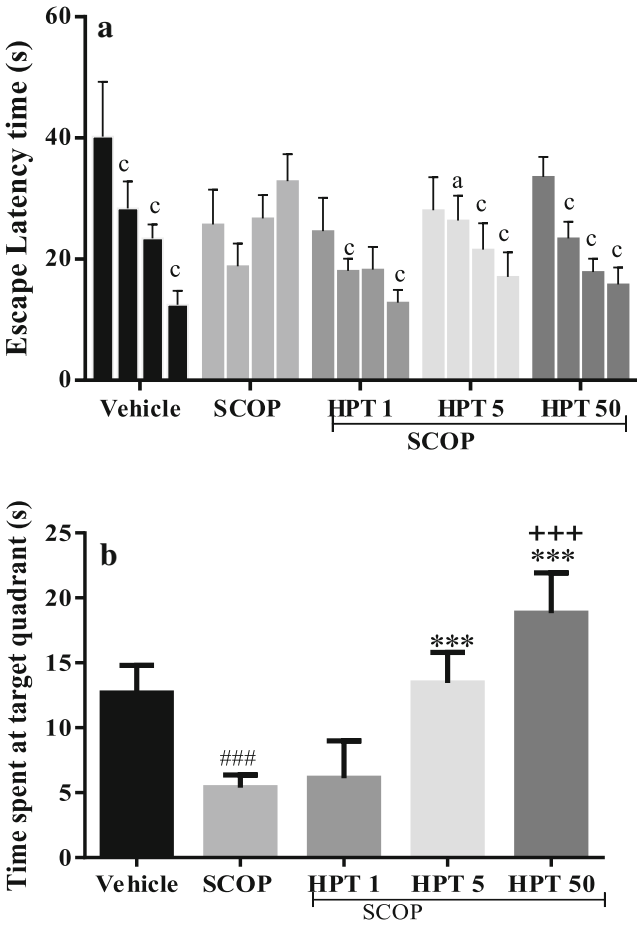

Fig. 4 **a**, **b** Effect of hesperitin on (a) escape latency (b) probe trial in Morris Water Maze task in mice. $^{\#\#\#}P < 0.001$ versus vehicle treated control; $^{***}P < 0.001$ versus SCOP-vehicle treated control, $^{+++}P < 0.001$ versus HPT 1 mg/kg; $^{c}P < 0.05$; $^{C}P < 0.0001$ versus day 1 trial. Statistical level of significance was assessed by one- or two-way ANOVA followed by Tukey's post hoc multiple comparison tests

Effect of hesperetin treatment on scopolamine-induced oxidative stress

One way ANOVA revealed significant effect of HPT and scopolamine treatment [$F(4,25) = 7.62, P < 0.01$] in the hippocampus (Fig. 5a) and [$F(4,25) = 14.61, P < 0.001$] in the prefrontal cortex (Fig. 5b). Post hoc analysis showed that scopolamine induced a significant increase MDA level (1.82 and 1.54 folds; in the hippocampus and prefrontal cortex, respectively, compared vehicle control treated. However, the pretreatment of mice with HPT (50 mg/kg) attenuated the level of MDA generation by 1.36 and 2.05 folds, in the hippocampus and prefrontal cortex, respectively, compared with scopolamine-vehicle treated.

One way ANOVA revealed significant effect of HPT and scopolamine treatment on GSH levels [$F(4,25) = 55.98, P < 0.001$] in the hippocampus (Fig. 6a) and [$F(4,25) = 13.69, P < 0.001$] in the prefrontal cortex (Fig. 6b). Post hoc analysis showed that scopolamine induced a significant deficit GSH level (2.21 and 1.66 folds; in the hippocampus and prefrontal cortex, respectively, compared vehicle control treated. However, the pretreatment of mice with HPT (50 mg/kg) increased the level of GSH by 4.27 and 2.23 folds, in the hippocampus and prefrontal cortex, respectively, compared with scopolamine-vehicle treated.

One way ANOVA revealed significant effect of HPT and scopolamine treatment on SOD activity [$F(4,25) = 5.49, P = 0.006$] in the hippocampus (Fig. 7a) and [$F(4,25) = 9.44, P = 0.008$] in the prefrontal cortex (Fig. 7b). Post hoc analysis showed that scopolamine induced a significant deficit SOD activity (1.80 and 1.65 folds decrease; in the hippocampus and prefrontal cortex, respectively, compared vehicle control treated. However, the pretreatment of mice with HPT (50 mg/kg) increased the activity of SOD by 1.73 and 1.34 folds, in the hippocampus and prefrontal cortex, respectively, compared with scopolamine-vehicle treated.

One way ANOVA revealed significant effect of HPT and scopolamine treatment on catalase activity [$F(4,25) = 6.41, P < 0.01$] in the hippocampus (Fig. 8a) and [$F(4,25) =$

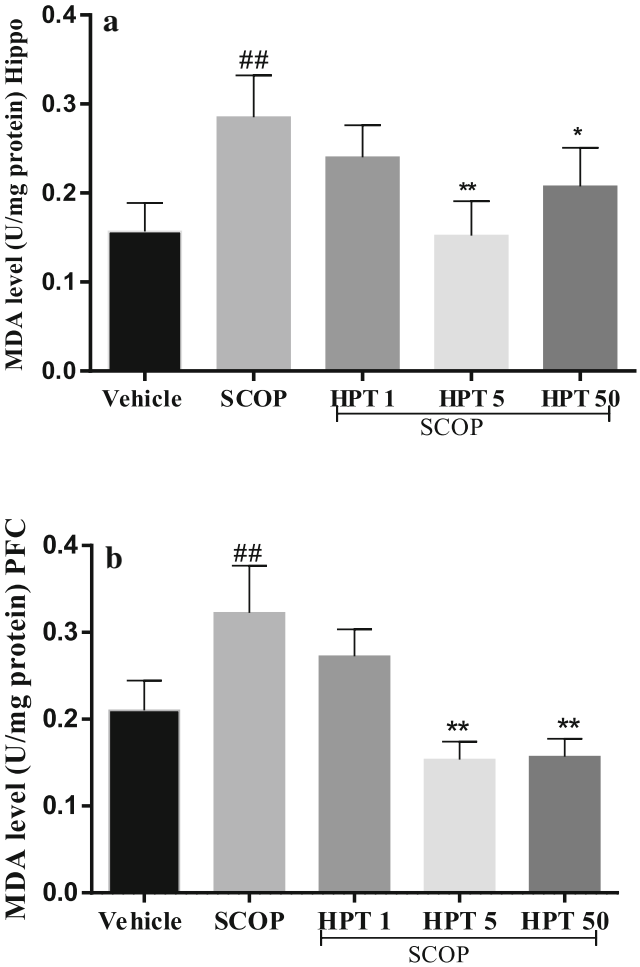

Fig. 5 **a**, **b** Effect of hesperitin on scopolamine-induced malondialdehyde in (a) hippocampus (b) prefrontal cortex in mice. $^{\#\#}P < 0.01$ versus vehicle treated, $^{*}P < 0.05$; $^{**}P < 0.01$ versus scopolamine-vehicle treated. Statistical level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test

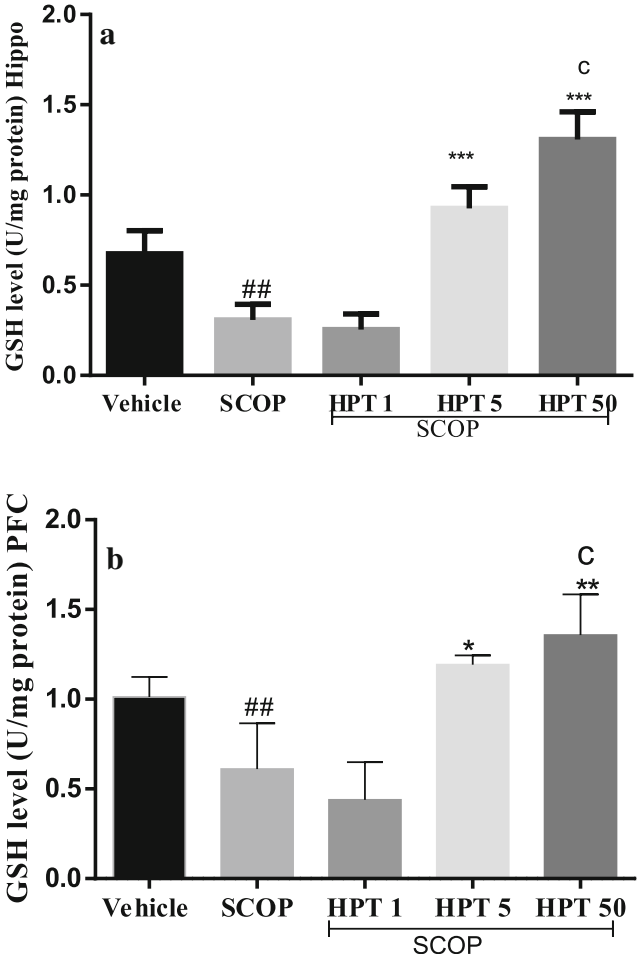

Fig. 6 **a**, **b** Effect of hesperitin on scopolamine-induced GSH deficits in the (a) hippocampus (b) prefrontal cortex in mice. $^{\#\#}P < 0.01$ versus vehicle treated, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus scopolamine-vehicle treated, $^{c}P < 0.001$ versus HPT (1 mg/kg) treated. Statistical level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test

8.06, $P < 0.01$] in the prefrontal cortex (Fig. 8b). Post hoc analysis showed that scopolamine induced a significant deficit catalase activity (1.47 and 1.70 folds decrease; in the hippocampus and prefrontal cortex, respectively, compared vehicle control treated. However, the pretreatment of mice with HPT (50 mg/kg) increased the activity of catalase by 1.52 and 1.78 folds, in the hippocampus and prefrontal cortex, respectively, compared with scopolamine-vehicle treated. Post hoc analysis revealed that scopolamine injection caused significant increase in nitrite generation in the hippocampus but not in the prefrontal cortex. Moreover, one way ANOVA revealed significant effect of treatment in the hippocampus [$F(4,25) = 6.04$, $P = 0.002$] (Fig. 9a) but not in the prefrontal cortex [$F(4,25) = 2.68$, $P = 0.054$] (Fig. 9b).

Effect of hesperetin on brain acetylcholinesterase activity

One way ANOVA revealed significant effect of HPT and scopolamine treatment on acetylcholinesterase activity [$F(4,25) = 13.05$, $P < 0.0001$] in the hippocampus (Fig. 10a) and [$F(4,25) = 9.45$, $P < 0.001$] in the prefrontal cortex (Fig. 10b). Post hoc analysis showed that scopolamine induced a significant increase in acetylcholinesterase activity (1.33 and 1.44 folds; in the hippocampus and prefrontal cortex, respectively, compared vehicle control treated. However, the pretreatment of mice with HPT (50 mg/kg) decreased the activity of acetylcholinesterase by 1.47 and 1.50 folds, in the hippocampus and prefrontal cortex, respectively, compared with scopolamine-vehicle treated.

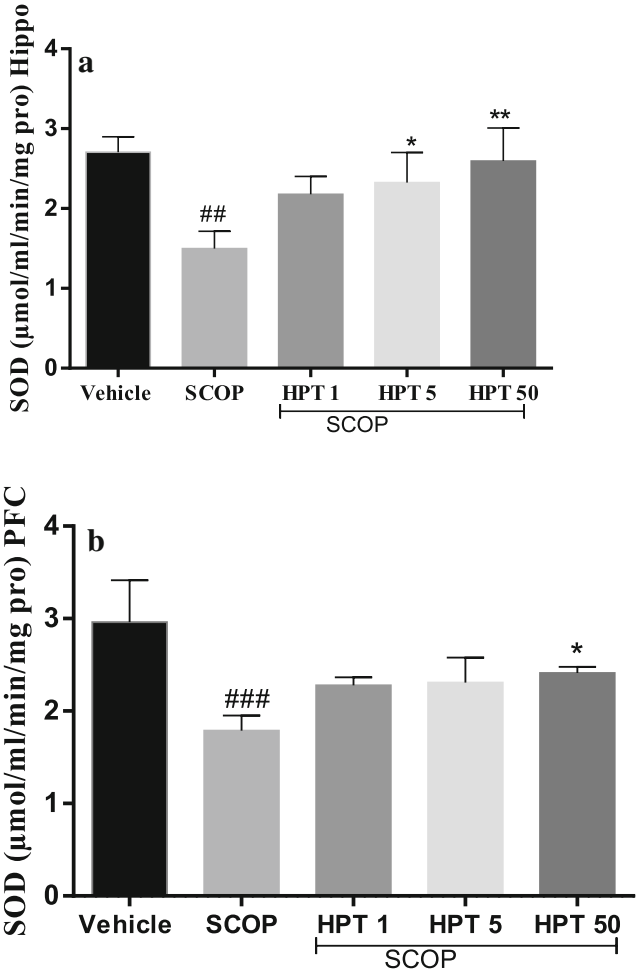

Fig. 7 **a**, **b** Effect of hesperitin on scopolamine-induced SOD deficits in the (a) hippocampus (b) prefrontal cortex in mice. $^{\#\#}P < 0.01$,$^{\#\#\#}P < 0.001$ versus vehicle treated, $^{*}P < 0.05$;$^{**}P < 0.01$ versus scopolamine-vehicle treated. Statistical level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test

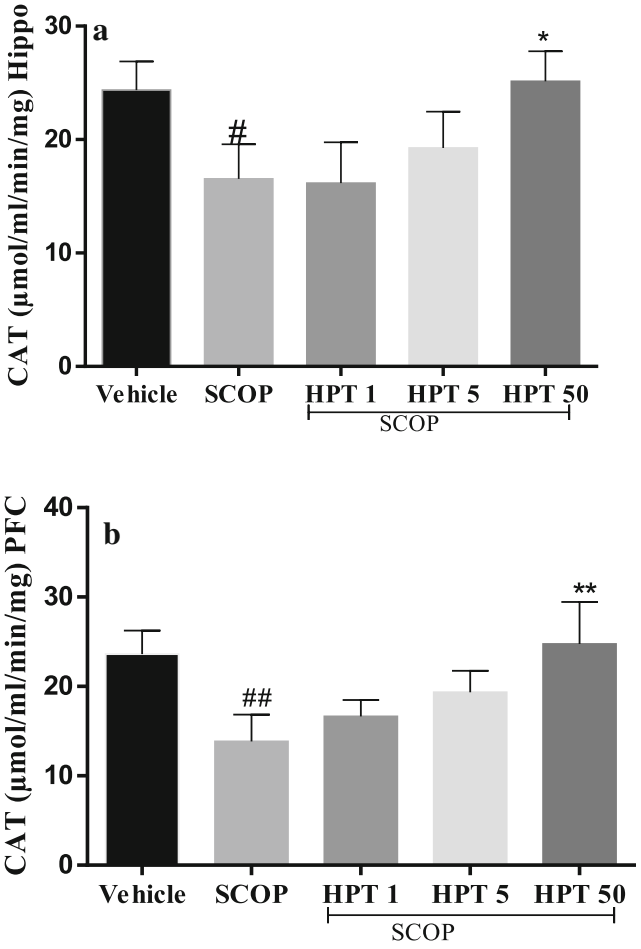

Fig. 8 **a**, **b** Effect of hesperitin on scopolamine-induced catalase deficits in the (a) hippocampus (b) prefrontal cortex in mice. $^{\#}P < 0.05$,$^{\#\#}P < 0.01$ versus vehicle treated, $^{*}P < 0.05$;$^{**}P < 0.01$ versus scopolamine-vehicle treated. Statistical level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test

Effect of hesperetin on BDNF level in brain homogenates

Two way ANOVA revealed a significant interaction between treatment and BDNF levels in brain homogenates [$F(4,50) = 62.32, P = 0.0003$], treatment groups [$F(1,50) = 10.59, P = 0.002$] and HPT pretreatment × scopolamine treatment interaction [$F(4,50) = 0.51, P = 0.73$] (Fig. 11). Tukey post hoc test revealed that scopolamine significantly reduced BDNF levels in the hippocampus (1.78 fold) and prefrontal cortex (1.57 folds). However, the pretreatment of mice with HPT caused dose dependent and significant increase in BDNF level in the hippocampus (1.50 folds) and prefrontal cortex (1.64 folds).

Discussion

Findings from this study showed that the intraperitoneal injection of scopolamine induced recognition memory and spatial learning deficits in novel object recognition and Morris water maze tests, respectively, without affecting spontaneous motor activity ruling out psychostimulant/ depressant effect of scopolamine. However, the pretreatment of mice with hesperetin prevented scopolamine-induced spatial learning and reference memory declines. In addition, scopolamine induced deficits in antioxidant enzymes activities, cholinergic systems and BDNF levels in the hippocampus and prefrontal cortex was ameliorated by hesperetin administration.

Scopolamine (muscarinic acetylcholine receptor antagonist) permeate the blood brain barrier and widely used to

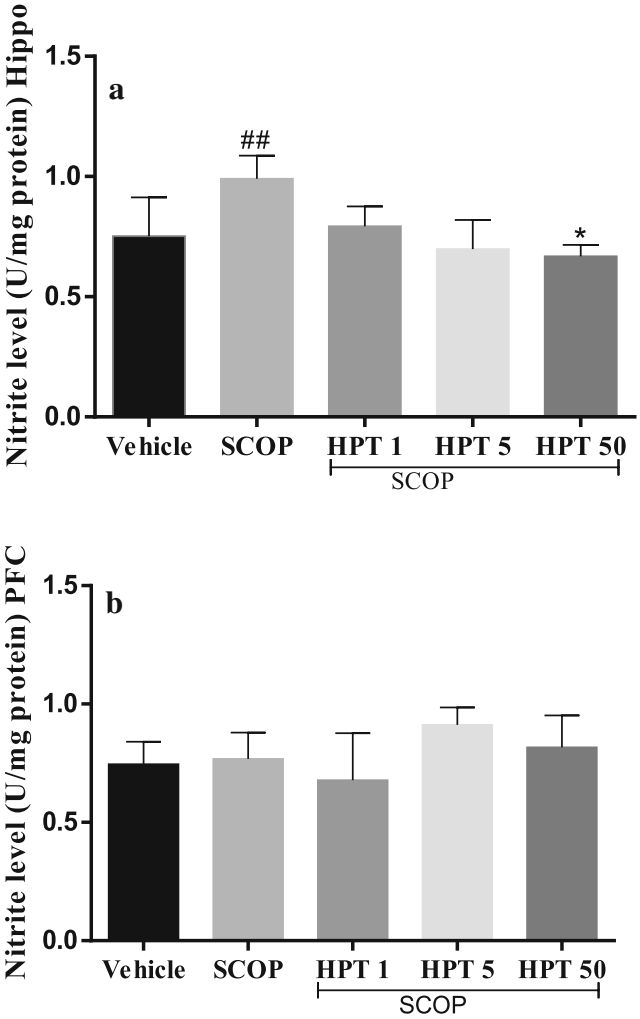

Fig. 9 **a**, **b** Effect of hesperitin on scopolamine-induced nitrite generation in the (a) hippocampus (b) prefrontal cortex in mice. $^{\#\#}P < 0.01$ versus vehicle treated, $^{*}P < 0.05$ versus scopolamine-vehicle treated. Statistical level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test

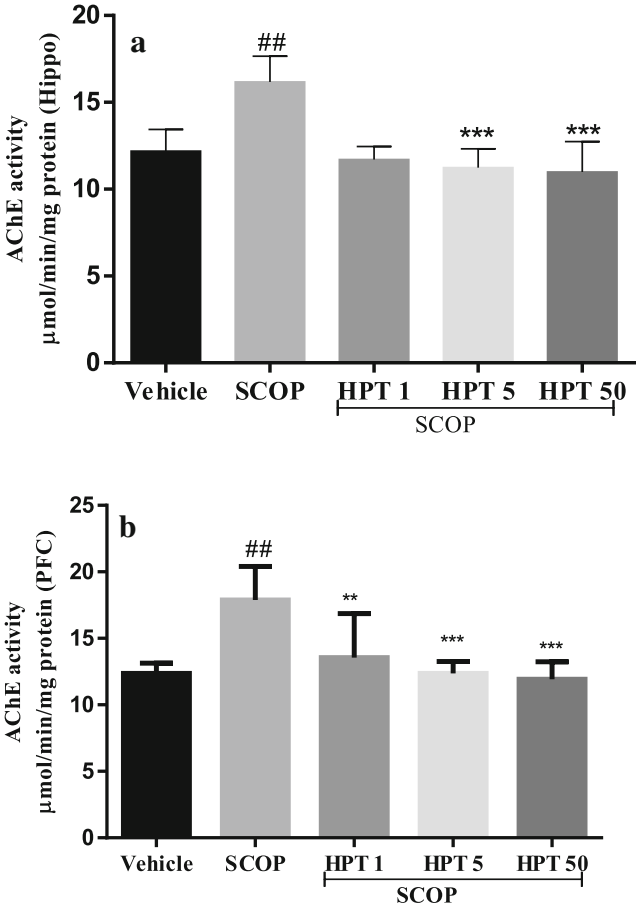

Fig. 10 **a**, **b** Effect of hesperitin and scopolamine on acetylcholinesterase activity in the (a) hippocampus (b) prefrontal cortex in mice. $^{\#\#}P < 0.01$ versus vehicle treated, $^{**}P < 0.01$; $^{***}P < 0.001$ versus scopolamine-vehicle treated. Statistical level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test

model amnesia in rodents and human. In addition, scopolamine is synonymous with the appearance of cognitive amnesia and electrophysiological changes which resembles AD features (Ramos Reis et al. 2013; Bajo et al. 2015). Scopolamine has been shown to affects delta, theta, alpha and beta activity in EEG in patients with senile dementia (Ebert and Kirch 1998). Thus, in preclinical studies, the reversal of scopolamine mediated amnesia is a viable model for predicting cognitive enhancing properties of a new drug and pharmacodynamics effect. The novel object recognition (NOR) task is widely used to investigate the neurobiology of non-spatial memory in rodents (Cohen and Stackman 2015). In mammals, spatial memory and non-spatial memory depend upon the hippocampus and associated medial temporal lobe (MTL) structures (Cohen et al. 2013). In this study, scopolamine administration impaired non-spatial memory in object

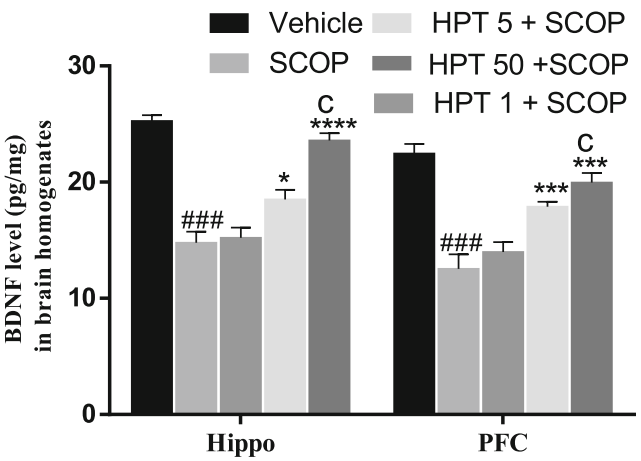

Fig. 11 Effect of hesperitin on scopolamine-induced BDNF deficit in the hippocampus and prefrontal cortex of mice. $^{\#\#\#}P < 0.001$ versus vehicle treated, $^{*}P < 0.05$; $^{***}P < 0.001$; $^{****}P < 0.0001$ versus scopolamine-vehicle treated; $^{c}P < 0.01$ versus HPT 1 + SCOP. Statistical level of significance analysis by two-way ANOVA followed by Tukey's post hoc multiple comparison test

recognition test evidenced in non-significant change in the exploration of the familiar or novel object in the test session performed 24 h after the training session, whereas the vehicle-treated mice spent more time exploring the novel object more than familiar object indicative of non-spatial memory deficit. Interestingly, hesperetin administration prevented the non-spatial working memory deficit induced by scopolamine. Thus, showed the potential of hesperetin in the maintenance of cortical and hippocampal cholinergic neurotransmission (Winters et al. 2008).

To assay the effect of hesperetin on scopolamine-induced spatial learning and memory deficits, the MWM task was carried out in mice. The MWM test is widely used to investigate spatial learning and memory in mice, thus, provides a reliable form of cognition evaluation (Morris, 1984). The hippocampal dentate gyrus plays essential role in learning and memory function (Ning et al. 2017). Moreso, hippocampal-dependent spatial learning in MWM task has been shown to increase hippocampal neurogenesis while reduction of adult neurogenesis causes impairment of spatial learning (Drapeau et al. 2003; Epp et al. 2007). In this study, scopolamine increased the escape latency in acquisition phase as well as decrease in time spent in platform area during probe phase indicative of impaired spatial learning and memory (Ishola et al. 2013, 2017; Ning et al. 2017). However, hesperetin administration produce time course decrease in escape latency in acquisition phase and increased time spent in quadrant location indicative of enhancement in spatial learning and memory (Ishola et al. 2016; Suganthy et al. 2016).

It is well known that oxidative stress (OS) plays an important role in neuronal degeneration in AD patients (Chauhan and Chauhan 2006; Suganthy et al. 2016). Soluble Aβ, Aβ fibrils, neurofibrillary tangles, mitochondrial abnormalities and aging are contributory factors to increased OS in AD. Oxidative damage to cellular components leads to impairment in membrane integrity/properties such as fluidity, ion transport, enzyme activities which eventually results in cell death (Chauhan and Chauhan 2006). In agreement with previous studies, administration of scopolamine increased malondialdehyde and nitrite production as well as decrease in antioxidant enzyme activities (glutathione, superoxide dismutase and catalase) in the hippocampus and prefrontal cortex indicative of oxidative stress (Ishola et al. 2013, 2016). However, the pretreatment of mice with hesperetin attenuated scopolamine induced lipid peroxidation and nitrosative stress through enhancement of glutathione, superoxide dismutase and catalase activities in the hippocampus and prefrontal cortex, thus, prevented the impairment of membrane properties through enhancement of antioxidative defense system. Findings from this study corroborated the free radical scavenging activity of hesperetin (Hirata et al. 2005; Parhiz et al. 2015). Moreover, previous studies showed that the antioxidant activity of hesperetin was not only limited to its radical scavenging activity, but it augmented the antioxidant cellular defenses via the ERK/Nrf2 signaling pathway as well (Parhiz et al. 2015).

The basal forebrain cholinergic complex provides the major cholinergic projections to the cerebral cortex and hippocampus. The cholinergic neurons of this complex have been assumed to undergo moderate degenerative changes during aging, resulting in cholinergic hypofunction that has been related to the progressing memory deficits with aging (Schliebs and Arendt 2011). In this study, scopolamine administration obstructed cholinergic neurotransmission leading to increased acetylcholinesterase activity in the hippocampus and prefrontal cortex. This increase in acetylcholinesterase activity leads to a decrease in acetylcholine level responsible for the impaired non-spatial and spatial memory. However, hesperetin treatment caused significant inhibition of acetylcholinesterase activity in the hippocampus and prefrontal cortex, thus, enhanced cortico-hippocampal cholinergic neurotransmission.

The growth factor brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase receptor type B (TRKB) are actively produced and trafficked in multiple regions in the adult brain, where they influence neuronal activity, function and survival throughout life (Nagahara and Tuszynski 2011). Hence, BDNF plays potential role in the pathogenesis and treatment of AD. AD affects the entorhinal cortex (primary source of inputs to the hippocampus, exerting a key influence in learning and memory) profoundly and early in the disease, contributing to cardinal symptom of amnesia (Nagahara et al. 2009). BDNF is widely expressed in entorhinal cortex and is anterogradely trafficked into hippocampus (Gómez-Isla et al. 1996). Moreso, BDNF is involved in synaptic plasticity mechanism underlying learning. It is of interest to state that the level of BDNF is reduced in the entorhinal cortex and hippocampus of AD patients (Hock et al. 2000). Findings from this study, showed that scopolamine reduced the level of BDNF in the hippocampus and prefrontal cortex. However, hesperetin treatment ameliorate the deficits in BDNF level in the hippocampus and prefrontal cortex suggesting possible role in neurogenesis and plasticity (Hwang and Yen, 2011). However, further study will be carried out to understand the cellular mechanism through which hesperetin enhanced antioxidant defense system, neurogenesis and synaptic plasticity in scopolamine-induced cognitive deficit.

Conclusion

Findings from this study showed that hesperetin prevented non-spatial/ spatial learning and memory decline induced by scopolamine possibly through enhancement of cortico-hippocampal antioxidant defense, cholinergic and BDNF signaling.

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Compliance with ethical standards

Conflict of interest We do not have any conflict of interest to declare.

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