Metabolic Brain Disease (2019) 34:505–518
https://doi.org/10.1007/s11011-018-0332-1

ORIGINAL ARTICLE

Bacopa monnieri prevents colchicine-induced dementia by anti-inflammatory action

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Received: 16 June 2018 / Accepted: 18 October 2018 / Published online: 2 January 2019

Abstract

Inflammation is considered as an early event in the development of Alzheimer's disease (AD) that precedes the formation of Aβ plaques and neurofibrillary tangles. Therefore, strategies aimed at attenuating inflammation by phytochemicals may be a potential therapeutic intervention against AD. The present study was designed to evaluate if colchicine-induced inflammation and Aβ production could be prevented by *Bacopa monnieri* (BM) supplementation. Dementia was induced by a single intracerebroventicular injection of colchicine (15 μg/5 μl), whereas, BM extract was administered orally (50 mg/kg body weight, daily) for 15 days. Assessment of cognitive functions using Morris water maze revealed deficits in colchicine administered animals. This was accompanied by significant increase in oxidative stress in terms of accentuated ROS and NO production. Expression of pro-inflammatory cytokines (IL-6, TNF-α) and chemokine (MCP-1) increased in the brain regions. Furthermore, COX-2 and iNOS expression also increased significantly in the brain regions of colchicine-administered animals. In addition, BACE-1 activity increased in the colchicine treated animals, which was accompanied by enhanced Aβ production. On the other hand, BM supplementation was able to improve cognitive functions, suppress Aβ formation by reducing BACE-1 activity. Inflammatory and oxidative stress markers were attenuated in the brain regions of BM supplemented animals. Taken together, the findings reveal that BM reverses colchicine-induced dementia by its anti-inflammatory and anti-oxidant action suggesting that it may be an effective therapeutic intervention to ameliorate progression of AD.

Keywords Alzheimer's disease · Amyloid β · *Bacopa monnieri* · BACE-1 · Cholchicine · Oxidative stress · Inflammation

Abbreviations

AD	Alzheimer's disease
Aβ	β-Amyloid peptide
APP	Amyloid precursor protein
BM	*Bacopa monnieri*
BACE-1	β-site amyloid precursor protein cleaving enzyme 1
COX-2	Cyclooxygenase-2
GSK-3β	Glycogen synthase kinase-3β
iNOS	Inducible Nitric oxide synthase
NO	Nitric Oxide
ROS	Reactive Oxygen species
RNS	Reactive Nitrogen Species

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Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly that affects memory and cognitive functions (Joachim and Selkoe 1992). AD is pathologically characterized by the presence of extracellular plaques and intracellular tangles. In particular, the senile plaques are extracellular deposits of the amyloid β-peptide (Aβ) that are produced by cleavage of amyloid precursor protein (APP) (Hahr 2015). Recent reports suggest that chronic inflammation plays a key role in pathogenesis of AD (Spangenberg and Green 2017). Neuroinflammation involves activation of microglia as well as increase in pro-inflammatory cytokine and chemokine levels (Krstic et al. 2012; McGeer and McGeer 2002). Clinical reports have shown that individuals with increased levels of pro-inflammatory molecules in brain exhibit poor cognitive functions and have increased tendency to develop age associated neurodegenerative pathologies like AD (Nagga et al. 2014). Aβ peptide can trigger microglial activation by inducing release of pro-inflammatory molecules (Khandelwal et al. 2011). Sutinen et al. (2012) have shown that cytokines

upregulate APP expression along with induction in BACE-1 that results in enhanced Aβ production. The ability of cytokines to induce glycogen synthase kinase-3β (GSK-3β) expression may contribute to disease pathology by phosphorylating tau protein (Medina and Avila 2010). Chronic activation of microglia also has the potential to generate cytotoxic reactive oxygen and nitrogen (ROS and RNS) species, which in turn accentuates inflammation resulting in loss of neurons (Yuste et al. 2015). Available evidence suggests that oxidative stress increases expression of APP thereby leading to increased Aβ production (Andorn and Kalaria 2000). It is believed that neuroinflammation may exacerbate brain lesions leading to synaptic dysfunctions and neuronal degeneration that may contribute to progression of AD.

Oxidative stress and inflammation are the two most critical events in development of neurodegenerative diseases (Fischer and Maier 2015). A possible intervention in preventing neurodegeneration is to break the events involved in inflammation and oxidative stress. Many investigators have focused on modulating key components that regulate oxidative stress and inflammation with the aim to develop rational drugs. In this context, Indian system of medicine 'Ayurveda' deals with revitalization of metabolism and immunity by use of plant extracts alone or in combination, which may be a promising strategy in preventing neuron loss in neurological conditions (Howes and Houghton 2003). Medicinal properties of various plants that are effective in preventing memory disorders have been documented for many centuries (Howes and Houghton 2012). Clinical and experimental studies have shown beneficial effects of plant products in AD (Anekonda and Reddy 2005; Dos Santos-Neto et al. 2006). *Bacopa monnieri* (BM) is a medicinal herb used in Ayurvedic system of medicine as a 'memory enhancer' for centuries (Kumar 2006). The commercial preparations of BM have been shown to improve cognitive functions in young as well as in aged population (Russo and Borrelli 2005). The ability of BM to prevent cognitive deficits has been attributed to steroidal saponins, commonly known as bacosides. Apart from its ability to enhance cognitive functions and antioxidant potential (Kumar 2006; Russo and Borrelli 2005), triterpenoid and bacoside fractions of BM have been reported to exert anti-inflammatory effect on LPS-induced inflammation under in vitro conditions (Viji and Helen 2011). Recent work demonstrates that beneficial effect of BM may involve anti-inflammatory and antioxidant action. Nemetchek et al. (2017) have shown that BM inhibits release of pro-inflammatory cytokines from microglia and inhibits enzymes associated with inflammation in the brain. Simpson et al. (2015) have reported that BM has the potential to reduce oxidative stress and improve cognitive functions. Therefore, the present study aims to investigate the efficacy of BM in colchicine-induced neurodegeneration through its potential anti-inflammatory and antioxidant action. Colchicine-induced dementia was used as a model as it emulates AD like phenotype (Saini et al. 2012).

Material and methods

Chemicals

Colchicine and *Bacopa monnieri* (crude extract) were procured from Sisco Research Laboratory, Mumbai (India) and Himalaya Drug Company, Bangalore (India) respectively. SYBR Green was procured from Roche Diagnostics (Mannheim, Germany). cDNA synthesis kit was obtained from Thermo Scientific (Waltham, MA, USA). Polyvinylidene difluoride (PVDF) membrane was obtained from Millipore (Temecula, CA, USA). Primary antibodies: iNOS, COX-2 and β-actin were procured from Santa Cruz Biotechnology Inc., (Santacruz, CA, USA). Secondary antibodies: anti-mouse IgG and anti-rabbit IgG were procured from Bangalore Genei (India). ELISA kits (IL-6, TNF-α and MCP-1) were obtained from BD Biosciences, (Franklin Lakes, NJ, USA). β-Secretase (BACE1) activity detection kit (Fluorescent) and Thioflavin-T were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from local suppliers.

Experimental design

Male Wistar rats, weighing between 200 and 250 g were obtained from the Central Animal facility of University and were acclimatized to the local vivarium conditions for 7 days. The protocols used for the study were approved by the Institutional Animal Ethics Committee and were in accordance with the guidelines for humane use and care of laboratory animals (IAEC/346-356 dt. 11-02-13). The rats were randomly segregated into following four groups with each group having 6 animals.

Control group: Animals received the vehicle.
Colchicine treated Group: Animals were infused with colchicine solution (15 μg colchicine dissolved in 5 μl artificial cerebrospinal fluid) intracerebroventricularly (icv) under anesthesia.
***Bacopa monnieri* treated group:** BM was given orally as suspension dissolved in water, a day after surgery at a dosage of 50 mg/kg body weight/day for a period of 15 days.
Colchicine + *Bacopa monnieri* group: Water suspension of BM was administered to the colchicine treated group at the dose of 50 mg/kg body weight /day for 15 days a day post-surgery.

The dose of colchicine used in study was based on those reported in earlier studies (Nakagawa et al. 1987). BM was administered at a dose of 50 mg/kg body weight, as it is the most effective dose that reversed aluminum-induced neurotoxicity (Jyoti et al. 2007).

Colchicine administration

Colchicine was intracerebroventricularly (i.c.v.) infused into the brain of rats under chloral hydrate anesthesia (300 mg/kg intraperitoneally) using stereotaxic set-up according to the method described by Khurana et al. (2012). The head was held in a frame for midline incision and two holes were drilled through the skull for positioning of injection cannula into the lateral ventricles. Animals were infused bilaterally with 15 μg of colchicine dissolved in aCSF (147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$ and 2.2 mM dextrose). The coordinates for colchicine administration were 3.5 mm posterior to bregma, 2.0 mm lateral to sagittal suture and 2.7 mm beneath the cortical surface as per the rat atlas (Paxinos et al. 1985). The microsyringe was left for 2 min following injection for free diffusion of colchicine and the scalp was closed with sutures. The animals were administered gentamicin (5 mg/kg body weight, i.p.) to prevent sepsis and were allowed to recover. The animals were returned to home cages after complete recovery.

Morris water maze

Morris water maze was performed to assess spatial memory according to the method described by Morris (1984). The animals were trained to escape from drowning by swimming to a hidden escape platform by using visuospatial navigation cues. The position of the platform remained fixed on each day but the starting point of the animals varied in the each trial. The acquisition test is a measure of spatial reference memory, whereas, retrieval test is considered as a measure of strength of spatial memory.

Acquisition test: A water tank (140 cm in diameter and 55 cm in high) was filled with water up to 25 cm from the top. A platform (11 cm diameter) was placed at the center of one of the quadrant and submerged about 1 cm below the surface of water. All animals were subjected to four trials (acquisition) from day 1 to 4. During each trial, the animals were placed in one of the four randomly selected quadrants facing the wall of the tank. On day 5, time spent and distance covered to reach the platform was recorded using ANY-maze tracking software (Stoelting Co., Wood Dale, IL, USA).

Retrieval test: After 24 h of acquisition, the platform was removed and animals were allowed to swim for 180 s. The memory was assessed by measuring the time spent in the target quadrant (that earlier had the hidden platform) along with latency and number of entries to the target quadrant.

Biochemical estimations

After 15 days of respective treatments, animals in various groups were fasted overnight and sacrificed by cervical dislocation under light ether anesthesia. Their brains were removed and rinsed in ice-cold isotonic saline. The brains were dissected to separate cerebral cortex and hippocampus and stored at −80 °C for further analysis.

Preparation of homogenate

10% (*w*/*v*) tissue homogenate was prepared in 50 mM phosphate buffered saline (pH 7.4) using Potter-Elvehjam-type glass homogenizer. The homogenates were centrifuged at 1000 g for 10 min at 4 °C to remove nuclei, unbroken cells and cell debris.

Reactive oxygen species (ROS)

ROS that includes hydroxyl, peroxyl and other reactive oxygen radicals were quantitated by using 2′, 7′-Dichlorofluorescin diacetate (DCFH-DA) as described by LeBel et al. (1992). The samples containing requisite amount of protein were added to reaction buffer containing 10 μM DCFH-DA and 10 mM succinate. The samples were incubated at 37 °C for 30 min and the fluorescence emission was determined at 530 nm after excitation at 488 nm. Results were expressed as pmoles DCF/min/mg protein.

Nitric oxide (NO)

The concentration of nitrite was quantitated by method described by Halejcio-Delophont et al. (2001). Briefly, Griess reagent (1:1 mix of 0.1% naphthyl ethylenediamine dihydrochloride and sulfanilamide in 5% (*v*/v) orthophosphoric acid) was added to the samples followed by incubation at room temperature for 30 min in dark. The absorbance of chromogen formed was measured at 548 nm and the results were expressed as nmoles/mg protein.

Real-time RT-PCR

Animals were anesthetized, decapitated and their brains dissected to isolate cortex and hippocampus. The tissue samples were stored in RNA later. Total RNA was isolated using Tri Reagent (Sigma-Aldrich Co, St Louis, MO, USA). RNA samples were treated with DNase to remove genomic DNA contamination. Agarose gel electrophoresis and Nanodrop were used to check the quality and concentration of RNA in the samples. cDNA was synthesized from 1 μg of total RNA using cDNA synthesis kit (#K1632; Thermo Fischer Scientific, Waltham, MA, USA). RT-PCR was performed using Roche LightCycler® 480 Real-Time PCR System.

Quantitative PCR for IL-6, TNF-α, MCP-1, COX-2, iNOS and BACE-1 was performed based on SYBR Green fluorescence. cDNA (10 ng) and gene-specific primers (Table 1) were added to SYBR Green master mix and subjected to PCR amplification with one cycle at 95 °C for 5 min followed by 40 cycles of 10 s at 95 °C; 20 s at 57/58 °C and 10 s at 72 °C. The resulting amplicons were visualized on agarose gels to confirm the size and specificity of PCR product. β-actin was used as a housekeeping gene and relative mRNA expression was determined using $2^{-\Delta\Delta Ct}$ method (Pfaffl et al. 2002).

Cytokine and chemokine levels

IL-6, TNF-α and MCP-1 levels in cortex and hippocampus were determined using commercially available ELISA kits (BD OptEIA), which are based on using validated set of monoclonal antibodies. The assay was performed as per manufacturer-defined protocol. Standards, samples and controls were added in duplicates to the appropriate wells in 96-well ELISA plates. After 2 h, the plates were washed, incubated with streptavidin-HRP for 30 min at room temperature followed by addition of 100 μl of chromogen to each well. The reaction was terminated by addition of stop solution. The absorbance was read at 450 nm within 30 min of stopping the reaction. The wavelength was corrected by subtracting the absorbance at 570 nm from the absorbance at 450 nm. The concentration of each molecule was determined from the regression curve of respective standards (concentration range from 7.8- 10,000 pg/ml). The results were expressed as pg/mg protein.

Western blotting

The immuno blot for various protein were performed by the method described by Towbin et al. (1979). Cortex and hippocampus were homogenized in lysis buffer, centrifuged at 20,000 g, and the supernatant collected. The cytosolic fraction containing 50 μg of protein was separated at 100 V on SDS-PAGE along with pre-stained protein markers (Bio-Rad, Hercules, CA, USA). The proteins from the gel were transferred to PVDF membrane in transfer medium at a constant current of 100 V for a period of 2 h. Transfer of pre-stained marker proteins confirmed transfer of proteins to the membrane. The non-specific binding of proteins to PVDF membrane was prevented by blocking the membranes with 5% (*w*/*v*) non-fat dry milk in PBS for 3 h at room temperature. After three washes in PBST (10 min), the membranes were probed by incubating overnight with primary antibodies (rabbit polyclonal COX-2, iNOS and mouse polyclonal β-actin) diluted 1:750 in 2% (w/v) non-fat dry milk in PBS. This was followed by washing the membrane three times with PBST (10 min) and incubation for 3 h with corresponding horseradish peroxidase (HRP) conjugated secondary antibodies diluted 1:2000 in 2% nonfat dry milk PBS. The membranes were subsequently washed five times in PBST (10 min) and the bands were visualized by adding 5 ml of substrate solution containing di-aminobenzidine (DAB) and H_2O_2. The membranes were washed with PBS to stop the reaction and were then photographed for analysis of band intensities.

BACE-1 activity

Activity of BACE1 was determined using the fluorescence resonance energy transfer (FRET) assay kit (Sigma-Aldrich Co, St, Louis, MO, USA) as described by Gutierrez et al. (2017). The BACE-1 activity was measured in cortex and hippocampus according to the manufacturer described procedure. Briefly, positive (with BACE-1 enzyme) and negative (no enzyme) controls included 20 μl of BACE substrate solution and the final reaction volume was made to 100 μl with assay buffer. The activity in the test samples was determined by adding 20 μl samples (5 mg/ml), 20 μl of BACE substrate in a final volume of 100 μl made with assay buffer. The fluorescence was measured immediately at excitation and emission wavelength of 320 nm and 405 nm respectively. The plate was covered with paraffin and incubated at 37 °C for 2 h and fluorescence was

Table 1 Sequence of primers and conditions for real time RT-PCR

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Tm (°C)	Product Size (bp)
β-actin	CTTCTTGCAGCTCCTCCGTCG	TCACACCCTGGTGCCTAGGGC	58	178
IL-6	CCTACCCCAACTTCCAATGCTC	TTGGATGGTCTTGGTCCTTAGCC	58	78
TNF-α	CCCAGACCCTCACACTCAGAT	TTGTCCCTTGAAGAGAACCTG	58	215
MCP-1	AGCACCTTTGAATGTGAACT	AGAAGTGCTTGAGGTGGTTG	58	82
COX-2	CTTCCTCCTGTGGCTGATGA	CCGGGATGAACTCTCTCCTC	57	115
iNOS	CGGTGCGGTCTTTTCCTATG	TCTCCAAACCCCTCACTGTC	57	275
BACE-1	GGCCGGAGTGGTATTATGAA	TGCCTTGATGGACTTGACTG	58	135

again measured at the above-mentioned wavelengths. The percentage of substrate cleavage was based on following equation:

$$\%\text{Cleavage} = \frac{\text{S (pmol)} \times 50}{500\ \text{(pmol)}}$$

S= amount of fluorescent product in the test samples obtained from the standard curve.

Aβ levels

Aβ aggregation was visualized in the tissue sections using Thioflavin-T staining as described by Schmidt et al. (1995). Briefly, Samples were de-paraffinized in xylene and graded ethanol as follows: 100% xylene for 5 min, 50%/50% xylene/100% ethanol for 3 min, 100% ethanol for 3 min, 90% ethanol for 3 min, 70% ethanol for 3 min, 50% ethanol for 3 min, twice with distilled water for 3 min. The samples were incubated with 1% Thioflavin-T for 8 min at room temperature. After incubation, the sections were washed twice for 3 min with 80% ethanol, 3 min with 95% ethanol and finally three washings with distilled water. The sections were mounted in aqueous mounting media and allowed to dry overnight in dark. The sections were visualized using fluorescent microscope.

Estimation of protein

The protein content in the samples was estimated according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

Values are expressed as mean ± SD of six animals per group. The analysis of data was done using one way analysis of variance (ANOVA) followed by Newman–Keuls test for multiple pairwise comparisons between the various treated groups. The values with $p < 0.05$ were considered as statistically significant.

Results

Effect of BM on colchicine induced cognitive impairment

Spatial learning and memory of animals was assessed by Morris water maze test (Barnes et al. 1996; Morris 1984). During the acquisition test (learning), escape latency was significantly increased in colchicine treated animals as compared to the controls (Fig. 1c). However, colchicine treated group supplemented with BM showed significant reduction in escape latency in comparison to the colchicine treated group. Representative track plots of the animals from different groups are shown in Fig. 1a. Track plots clearly show that colchicine treated animals had impaired learning ability as revealed by longer path length, while colchicine treated rats supplemented to BM performed significantly better.

In the retrieval test (memory), latency for first entry to the platform zone was significantly increased in colchicine treated animals as compared to the controls, which was significantly decreased in animals supplementation with BM (Fig. 1d). Distance travelled in the quadrant containing platform and time spent in the platform zone was also significantly decreased in cocchicine treated animals, which was reversed on BM supplementation. Track plots also revealed significant memory impairment in colchicine treated animals, whereas, BM administration was found to be effective in restoring memory in colchicine treated animals (Fig. 1b). Thus, the results reveal that colchicine treated animals had significant impairment in spatial learning and memory as compared to controls, which was reversed following BM supplementation.

Effect of BM on colchicine induced oxidative stress

The data for ROS levels is presented in Table 2. There was a significant increase in ROS generation in cortex and hippocampus following colchicine treatment, whereas BM administration decreased ROS generation. The levels of nitrite, an indicator of NO production were increased in colchicine treated animals as compared to control (Table 3). Interestingly, BM administration to colchicine treated animals resulted in significant reduction in NO levels. However, there was no significant change in ROS and NO levels in control animals supplemented with BM.

Effect of BM on cytokine and chemokine levels in colchicine-induced dementia

Colchicine administration increased pro-inflammatory cytokine and chemokine levels in cortex and hippocampus. IL-6 mRNA and protein expression (Fig. 2a, b) was significantly increased in the brain regions of colchicine treated animals. BM, on the other hand, significantly reduced the expression of IL-6 to near normal values. Increased TNF-α mRNA expression was observed in colchicine treated animals, which was normalized on BM supplementation (Fig. 3a). There was a significant increase in TNF-α protein levels in the colchicine treated animals as compared to the control animals (Fig. 3b). However, BM administration to colchicine treated animals reduced TNF-α expression. Similarly, MCP-1 mRNA and protein (Fig. 4a, b) expression were markedly higher

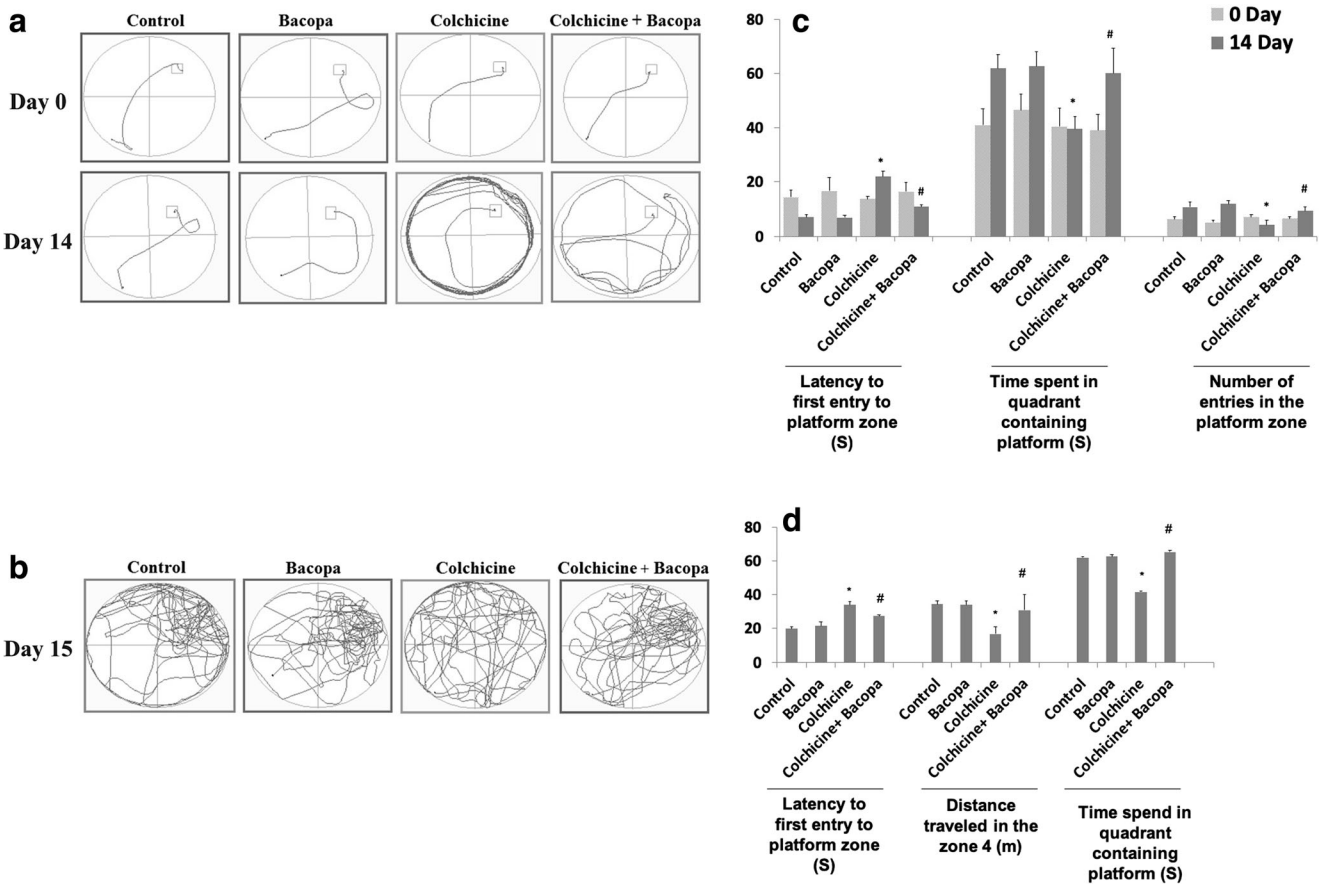

Fig. 1 Effect of *Bacopa monnieri* administration on colchicine-induced spatial learning impairment assessed by Morris water maze. Representative track plots for acquisition test (**a**) and retrieval test using ANY-maze video tracking software (**b**); Histogram representing parameters studied for acquisition test (**c**); and retrieval test (**d**). Values are mean ± SD; $n = 6$. *$p < 0.05$ compared with control animals. #$p < 0.05$ compared to colchicine treated animals

in both regions following colchicine treatment. BM supplementation was observed to significantly lower the MCP-1 expression in colchicine treated animals.

Effect of BM on COX-2 and iNOS expression in colchicine-induced dementia

The effect of BM on COX-2 and iNOS mRNA expression was analyzed by real time RT-PCR analysis. A significant increase in COX-2 mRNA was observed in colchicine treated animals, which was attenuated on BM administration (Fig. 5a). In addition, colchicine treated animals revealed higher expression of COX-2 protein as analyzed by Western blot analysis (Fig. 5b, c). This enhanced expression of COX-2 was markedly attenuated on BM supplementation. Concomitantly, increased iNOS mRNA expression was observed in colchicine treated rats, which was significantly prevented on BM supplementation (Fig. 6a). Western blot analysis showed a significant elevation in iNOS protein levels following colchicine administration in comparison to controls (Fig. 6b, c). On the other hand, BM supplementation was able to significantly suppress iNOS protein levels near to control levels.

Table 2 Effect of *Bacopa monnieri* administration on ROS production in brain regions of colchicine treated rats

ROS (pmoles DCF/mg protein)		
Groups	Cortex	Hippocampus
Control	27.97 ± 1.64	22.69 ± 1.97
Bacopa	26.98 ± 1.34	22.67 ± 1.66
Colchicine	39.24 ± 2.40*	37.52 ± 3.10*
Colchicine + Bacopa	29.57 ± 2.16#	23.34 ± 3.21#

Values are mean ± SD; n = 6. * $p < 0.05$ compared with control animals. #$p < 0.05$ compared to colchicine treated animals

Effect of BM on BACE-1 activity and Aβ deposits in colchicine induced dementia

There was a significant increase in β-site APP-cleaving enzyme (BACE1) mRNA in brain regions of colchicine treated animals in comparison to controls. BACE1 mRNA

Table 3 Effect of *Bacopa monnieri* administration on nitrite levels in brain regions of colchicine treated rats

Nitrite levels (nmoles/mg protein)		
Groups	Cortex	Hippocampus
Control	30.67 ± 1.25	25.32 ± 1.21
Bacopa	29.35 ± 1.24	25.43 ± 1.35
Colchicine	45.23 ± 2.21*	41.39 ± 2.95*
Colchicine + Bacopa	32.37 ± 2.11#	27.23 ± 2.85#

Values are mean ± SD; n = 6. * $p < 0.05$ compared with control animals. #$p < 0.05$ compared to colchicine treated animals

expression was significantly decreased following BM supplementation. However, there was no significant difference in BACE1 mRNA expression between control and BM treated groups (Fig. 7a). BACE1 activity measured by FRET showed a significant elevation in colchicine treated animals, whereas BM supplementation was able to lower the BACE1 activity to near control levels (Fig. 7b). Furthermore, there was no significant difference in BACE1 activity between control and BM treated animals.

In order to assess amyloid plaque formation in colchicine treated animals, the brain sections were stained with thioflavin-T. Fig. 8 revealed that control and BM treated animals had no plaque formation in the brain regions, whereas, in colchicine treated animals, thioflavin-T fluorescence was increased in comparison to control animals (Fig. 8c) indicating amyloid deposits. Thioflavin-T fluorescence was reduced on supplementation of BM to colchicine treated animals indicating that BM is effective in reducing amyloid formation.

Discussion

Evidence suggests that inflammatory processes in the CNS involves release of factors such as cytokines, chemokines, NO and ROS (Krstic et al. 2012) that contributes to accumulation of Aβ thereby leading to development of AD (Holmes et al. 2009). The present study demonstrates that the beneficial effect of BM involves modulation of inflammatory responses. The results show that administration of colchicine, at a micromolar doses, induces oxidative/nitrosative stress that is associated with cognitive decline. Cognitive deficits are mediated by increase in oxidative stress in terms of ROS and NO levels (Bhattacharya et al. 2000; Dringen et al. 2000). It has already been reported that colchicine-induced oxidative damage was positively correlated with extent of cognitive impairment (Kumar et al. 2009). However, colchicine treated animals supplemented with BM showed better performance in Morris water maze indicating its ability to improve cognitive functions. These findings are in agreement with earlier studies,

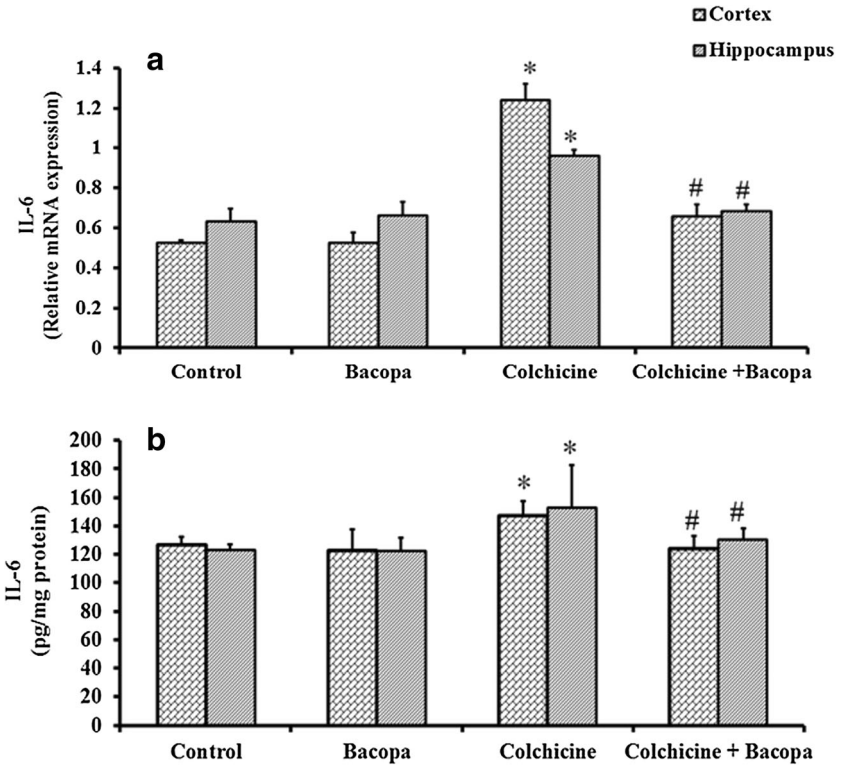

Fig. 2 Effect of *Bacopa monnieri* administration on IL-6 mRNA (**a**) and protein (**b**) in brain regions of colchicine treated rats. Values are mean ± SD; n = 6. *$p < 0.05$ compared with control animals. #$p < 0.05$ compared with colchicine treated animals

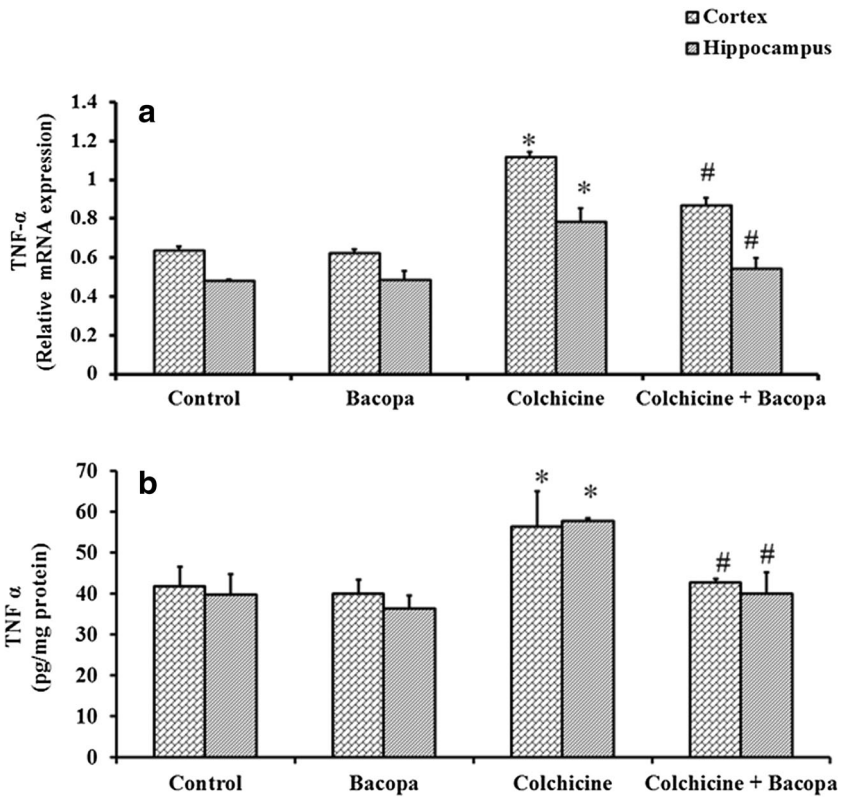

Fig. 3 Effect of *Bacopa monnieri* administration on TNF-α mRNA (**a**) and protein (**b**) in brain regions of colchicine treated rats. Values are mean ± SD; n = 6. *$p < 0.05$ compared with control animals. #$p < 0.05$ compared with colchicine treated animals

where administration of BM was able to improve cognitive deficits by modulation of endogenous oxidative stress (Shinomol and Muralidhara 2011).

Excessive free radicals trigger inflammatory response mediated by microglia resulting in increase in pro-inflammatory cytokines and chemokines that leads to neuronal injury

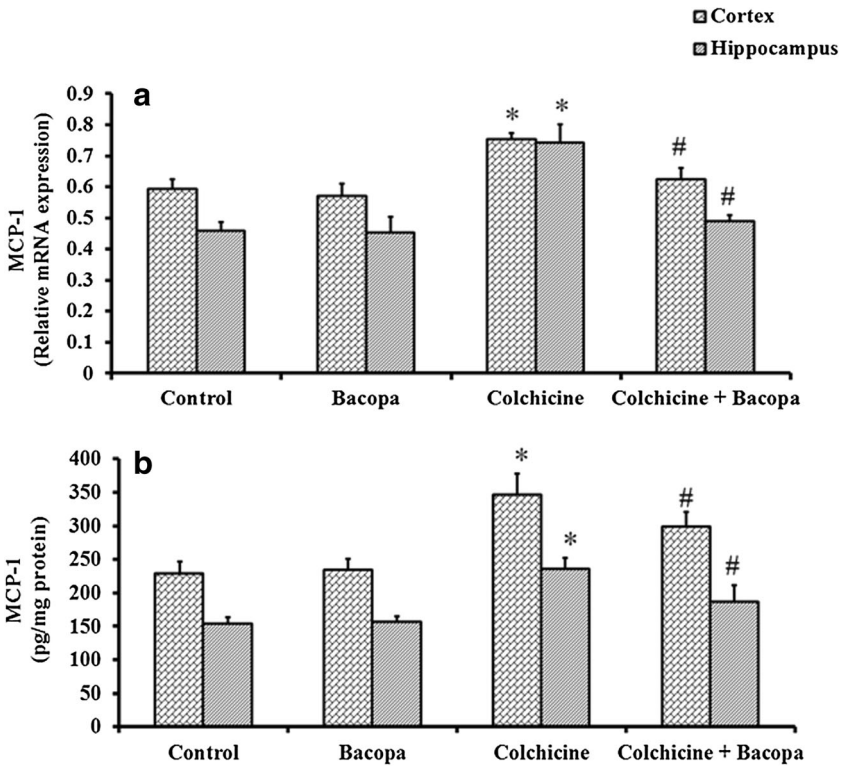

Fig. 4 Effect of *Bacopa monnieri* administration on MCP-1 mRNA (**a**) and protein (**b**) in brain regions of colchicine treated rats. Values are mean ± SD; n = 6. *$p < 0.05$ compared with control animals. #$p < 0.05$ compared with colchicine treated animals

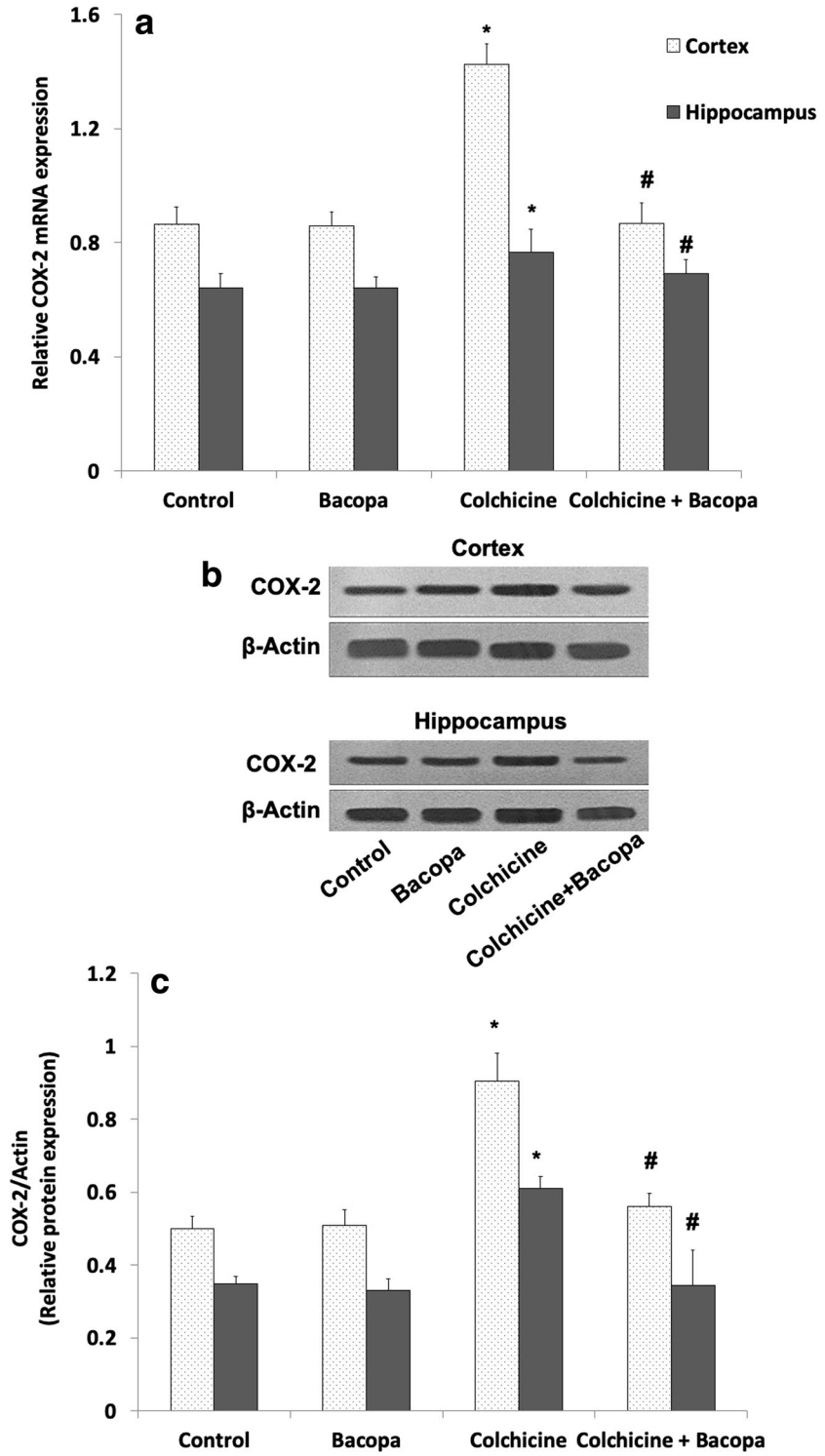

Fig. 5 Effect of *Bacopa monnieri* administration on COX-2 mRNA (**a**) in brain regions of colchicine treated rats. Western blots of COX-2 (**b**) and Densitometric analysis of Western blots (**c**). Values are mean ± SD; n = 6. *$p < 0.05$ compared with control animals. #$p < 0.05$ compared with colchicine treated animals

(Bhattacharya et al. 2000; Dringen et al. 2000). Pro-inflammatory cytokines (IL-6, TNF-α) were significantly increased in colchicine treated animals, suggesting inflammatory state (Reuter et al. 2010). IL-6 belongs to neuropoietin family of cytokines that plays an important role in host defense with either direct or indirect neurotrophic effects on neurons. TNF-α plays a central role in initiating and regulating cytokine cascade during an inflammatory response (Benveniste 1998). BM administration to colchicine treated animals showed decrease in IL-6 and TNF-α mRNA and protein levels. Viji and Helen (2011) suggested that triterpenoid and bacoside fractions of BM possess anti-inflammatory activity as evident from its ability to decrease pro-inflammatory cytokine (IL-6 and TNF-α) levels and NO

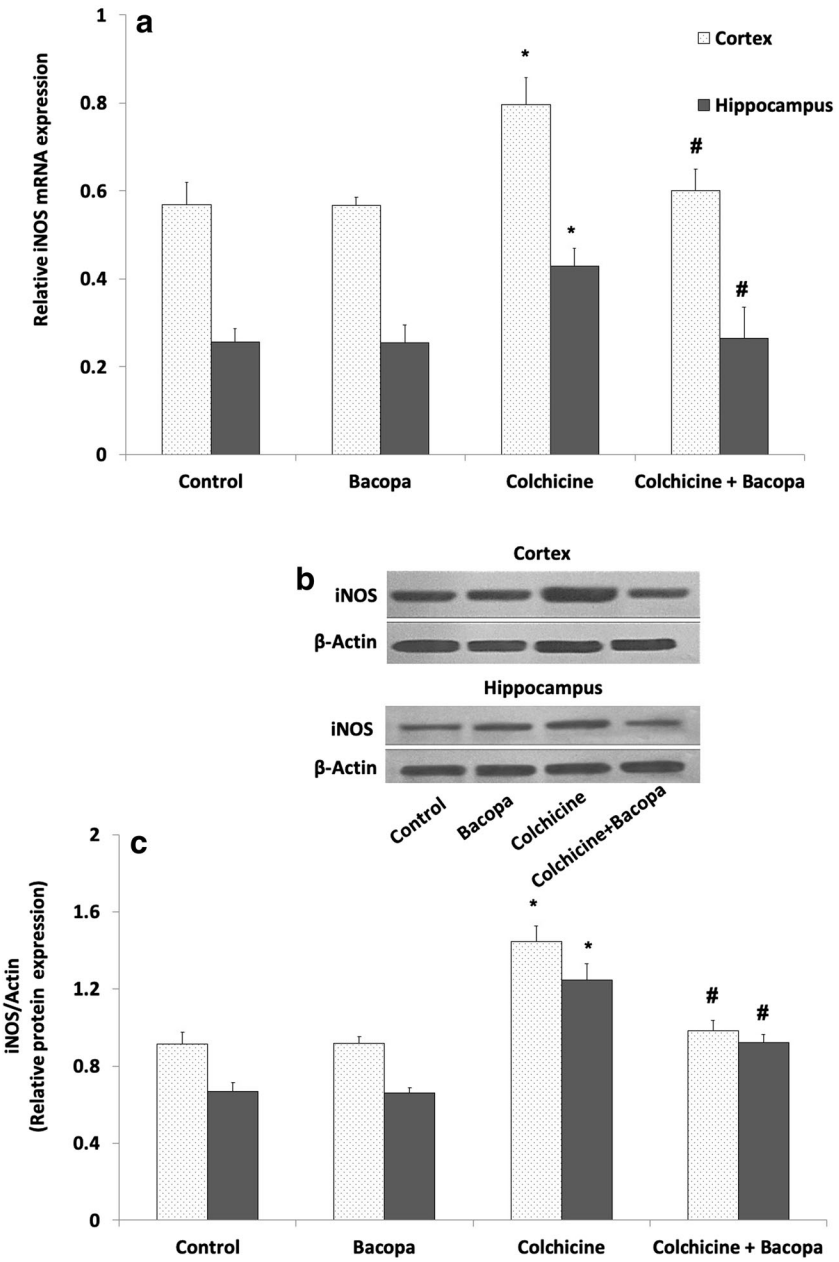

Fig. 6 Effect of *Bacopa monnieri* administration on iNOS mRNA (**a**) in brain regions of colchicine treated rats. Western blot of iNOS (**b**) and Densitometric analysis of Western blots (**c**). Values are mean ± SD; n = 6. * $p < 0.05$ compared with control animals. #$p < 0.05$ compared with colchicine treated animals

production following lipopolysachride induced inflammation under in-vitro conditions. In another study, triterpenoid and bacoside enriched fraction of BM was found to inhibit the lipopolysaccharide induced TNF- α production in a concentration dependent manner in mononuclear cells via modulation of NF-kB function (Chung et al. 2007).

MCP-1, a chemokine, was also elevated in cortex and hippocampus of colchicine treated animals. Chemokines play an important role in the inflammatory processes because of their chemotactic properties (Galimberti et al. 2006). In the present study, MCP-1 levels increased in colchicine treated animals that were suppressed on BM supplementation. BM may mediate inhibitory effect on MCP-1 levels through the suppression of oxidative stress (Viji and Helen 2011). Triterpenoids, flavonoids and saponins present in BM have been reported to exhibit anti-inflammatory properties (Fan et al. 2004; Channa et al. 2006).

It is well documented that pro-inflammatory cytokines up-regulate the expression of enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) (Graeber and Streit 2010). Studies have shown that increased COX-2 expression was related to dementia and decrease in severity of dementia was correlated with reduced number of COX-2 positive neurons (Hoozemans et al. 2002; Yermakova and O'Banion 2001). A few studies have shown increased COX-2 expression with increased Aβ levels, suggesting role of COX-2 in AD pathogenesis (Akiyama et al. 2000; Minghetti 2004). In the present study increase in COX-2

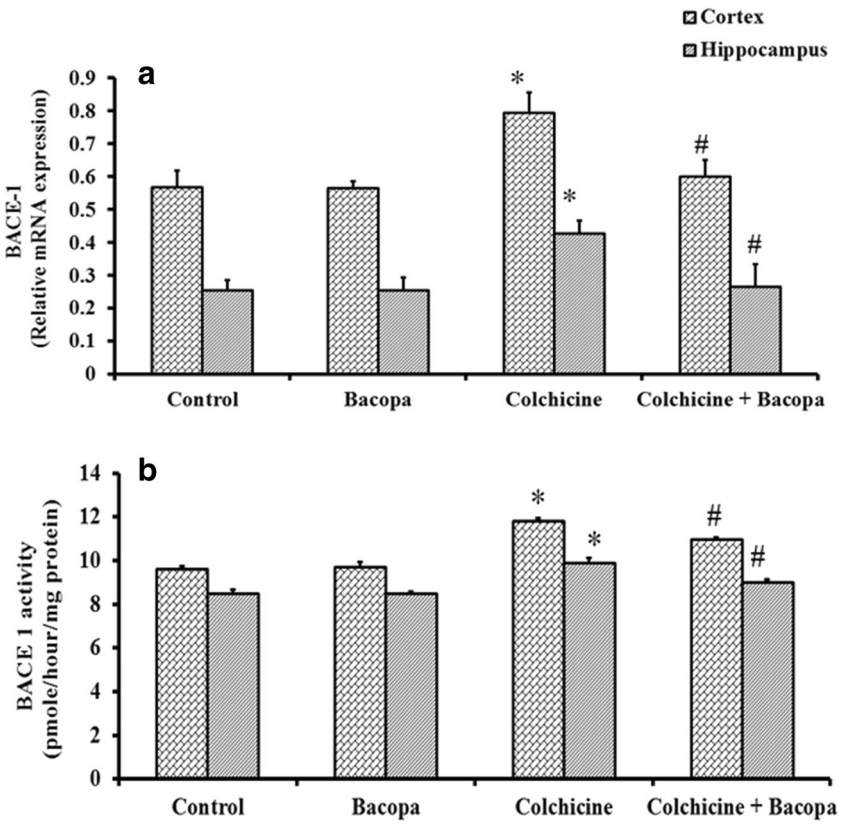

Fig. 7 Effect of *Bacopa monnieri* administration on BACE-1 mRNA (**a**) and activity (**b**) in brain regions of colchicine treated *rats*. Values are mean ± SD; n = 6. * $p < 0.05$ compared with control animals. #$p < 0.05$ compared with colchicine treated animals

expression was observed in cortex and hippocampus following colchicine administration. However, this increase was markedly attenuated by BM supplementation. PGE_2, an important proinflammatory mediator, synthesized by COX-2 is attenuated in vitro by BM extract suggesting beneficial effect of BM involves COX-2 suppression (Debnath et al.

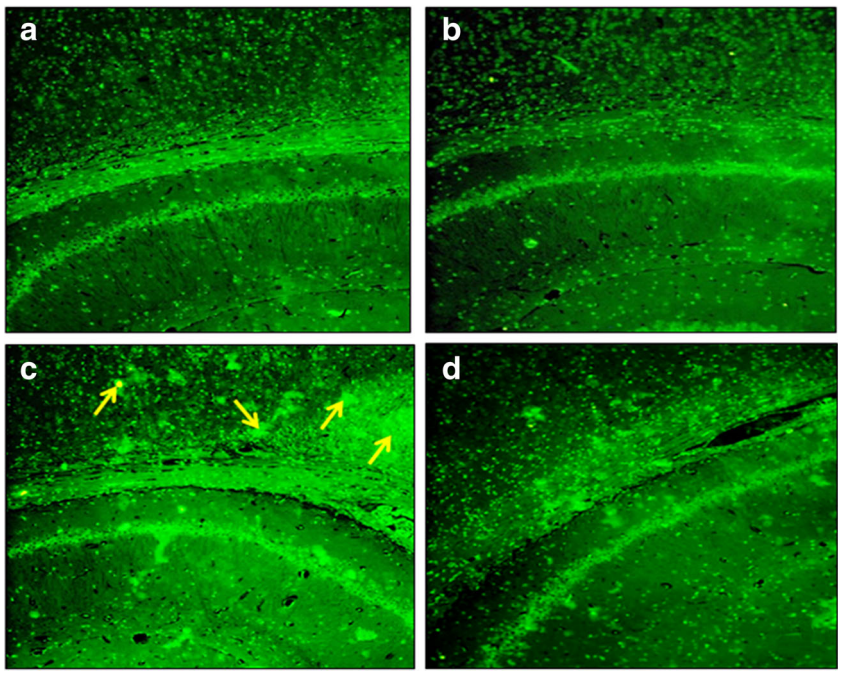

Fig. 8 Thioflavin-T staining for amyloid deposits in brain sections (**a**-control; **b**-Bacopa; **c**-Colchicine; **d**- Colchicine + Bacopa). Arrows show amyloid plaques. Magnification 40 ×

2013). It has also been reported that pretreatment with BM significantly suppressed bromic acid induced COX-2 expression (Rehman et al. 2012).

Clinical studies have shown increased iNOS levels in neuron and glial cells from AD brain (McCann 1997) as well as in various animal models with elevated APP expression (Combs et al. 2001; Heneka and Feinstein 2001). These reports are in agreement with our findings showing enhanced iNOS expression in the brain regions of colchicine treated animals. Increased NO production may damage cellular components such as DNA, protein and lipids. In addition, NO may directly interfere with energy metabolism by reacting with active thiol groups of mitochondrial proteins (Luth et al. 2002). BM supplementation was able to prevent increase in iNOS expression in colchicine treated animals. Previously, it has been suggested that triterpenoids, a basic constituent of BM, inhibits NO production by inhibiting protein synthesis de novo and decreasing protein stability via post-transcriptional mechanisms (Anand et al. 2010; Chiou et al. 2000). Another study reported that up-regulation of iNOS in aged rat brain was attenuated by administration of bacosides (Holcomb et al. 2006). These studies suggest that beneficial effect of BM may either involve suppression of iNOS expression or attenuation in pro-inflammatory cytokine expression.

Neuroinflammation has been suggested to contribute to increase in Aβ concentrations in AD brains (Krstic et al. 2012). APP, a transmembrane polypeptide, is initially cleaved by β-secretase, which is subsequently cleaved to APP fragments by γ-secretase (Kahn et al. 2012). Therefore, BACE1 (secretase) activity is crucial for the amyloidogenic processing of APP (Mouton-Liger et al. 2012). Therefore, it appears that increase in BACE1 expression in brain regions of colchicine treated animals leads to increased Aβ production. Various studies have shown increase in BACE1 protein levels and activity in AD brains (Mouton-Liger et al. 2012; O'Connor et al. 2008). Moreover, a study has shown that cytokines influence APP processing in differentiated SH-SY5Y cells by up-regulating BACE1 expression (Sutinen et al. 2012). In addition, various studies have demonstrated that oxidative stress increases production and intracellular accumulation of Aβ in human neuroblastoma cells (Mouton-Liger et al. 2012; Zhang et al. 1997). However, mechanism through which oxidative stress affects Aβ production still remains elusive. In the present study BM supplementation to colchicine treated animals was able to reduce BACE activity and consequently decrease Aβ production. Kalvodova et al. (2005) has reported that BACE1, being an integral membrane protein, is likely to be influenced by membrane lipid environment. The results of present study demonstrate that oxidative stress facilitates Aβ generation via upregulation of BACE1 activity. The antioxidant activity of BM appears to modulate the activity of BACE1. Rastogi et al. (2012) have demonstrated neuroprotective effect of bacosides against age-associated neurodegeneration involve modulation of chronic inflammation. The anti-inflammatory action of BM may also involve modulation of Ca^{2+} homeostasis as calcium channel blockers are known to suppress inflammation-induced by carrageenan (Sanchez et al. 1998), prostaglandin E1, bradykinin and serotonin (Aditya et al. 1997). This is also supported by the study suggesting calcium channel antagonism by ethanolic extract of BM and its fractions (Channa et al. 2003). Therefore, these targets may also contribute to the beneficial effects of BM. Epidemiological and experimental studies suggest that long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) are effective in reducing risk from AD via inhibition of inflammatory responses (Cole and Frautschy 2010; Kurumbail et al. 1996).

Taken together, the results from the present study provide evidence that BM supplementation is effective in preventing colchicine-induced dementia through anti-inflammatory and anti-oxidant action. The findings clearly suggest therapeutic potential of BM in ameliorating cognitive deficits in neurodegenerative conditions wherein inflammation and oxidative stress are key mediators in pathophysiology.

Acknowledgements The financial assistance provided to Dr. Neetu Saini by the Department of Science and Technology (DST), New Delhi, under women scientist fellowship scheme is acknowledged.

Compliance with ethical standards

Conflicts of interest The authors have no conflicts of interest to declare.

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