Neurochemical Research (2019) 44:1665–1677
https://doi.org/10.1007/s11064-019-02792-7

ORIGINAL PAPER

Molecular Docking and Cognitive Impairment Attenuating Effect of Phenolic Compound Rich Fraction of *Trianthema portulacastrum* in Scopolamine Induced Alzheimer's Disease Like Condition

Ekta Yadav[1] · Deepika Singh[1] · Biplab Debnath[2] · Parth Rathee[3] · Pankajkumar Yadav[4] · Amita Verma[1]

Received: 16 January 2019 / Revised: 28 March 2019 / Accepted: 1 April 2019 / Published online: 4 April 2019

Abstract

Dementia is considered as the frequent cause of neurodegenerative mental disorder such as Alzheimer's disease (AD) amongst elderly people. Free radicals as well as cholinergic deficit neurons within nucleus basalis magnocellularis demonstrated to attribute with aggregation of β amyloid which further acts as an essential hallmark in AD. Various phenolic phytoconstituents exists in *Trianthema portulastrum* (TP) leaves have been reported as active against various neurological disorders. The current investigation was undertaken to evaluate the antiamnesic potential of butanol fraction of TP hydroethanolic extract (BFTP) by utilizing rodent models of elevated plus maze (EPM) and Hebbs William Maze (HWM) along with in vitro and in vivo antioxidant as well as acetylcholinesterase (AChE) inhibition studies. Molecular docking studies were also performed for evaluation of molecular interaction of existed phenolic compounds in BFTP. In vitro antioxidant study revealed concentration dependant strong ability of BFTP to inhibit free radicals. In vitro AChE inhibition study showed competitive type of inhibition kinetics. BFTP significantly reversed ($p < 0.005$ versus scopolamine) the damaging effect of scopolamine by reducing TL (Transfer Latency) and TRC (Time taken to recognize the reward chamber) in the EPM and HWM, respectively. BFTP also contributed towards increased ($p < 0.005$ versus scopolamine) enzymatic antioxidant as well as hippocampal acetylcholine (ACh) levels. Histological studies also supported the results as BFTP pretreated mice significantly reversed the scopolamine induced histological changes in hippocampal region. Docking studies confirmed chlorogenic acid has the most significant binding affinity towards AChE. This research finding concludes that BFTP could be a beneficial agent for management of cognition and behavioral disorders associated with AD.

Keywords Learning and memory · Alzheimer's disease · Cognitive impairment · Oxidative stress · Chlorogenic acid · Molecular docking

Introduction

Alzheimer's disease (AD) is the commonest neurodegenerative mental disorder amongst aging people [1]. All over the world 33.9 million population is suffering with AD [2]. Clinicopathologically, AD is fundamentally linked with the impairment of cognitive function as well as inability to perform day to day activities [3, 4]. Onset and progression of neurodegenerative disorders such as AD are characterized by hyperphosphorylation as well as aggregation of tau protein, amyloid plaques deposition, inflammation of neurons, cholinergic hypofunction and oxidative stress accompanied by pathophysiological and psychological problem including depression, anxiety, motor dysfunction [5].

Free radicals called as reactive oxygen species (ROS), generated as a result of various bio-metabolic functions,

✉ Pankajkumar Yadav
pypharm@gmail.com

✉ Amita Verma
amitaverma.dr@gmail.com

[1] Bioorganic & Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences (SHUATS), Allahabad 211007, India

[2] Bharat Technology, Uluberia, Howrah 711316, India

[3] Department of Pharmaceutical Sciences & Technology, Birla Institute of Technology (BIT), Mesra, Ranchi 835215, India

[4] Pharmaceuics Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences (SHUATS), Allahabad 211007, India

leads to onset of oxidative stress condition which causes oxidation of lead bio-molecules and progression of neurodegenerative disorder such as AD and Parkinson's disease (PD) [6, 7]. It is believed that cholinergic deficit neuron within nucleus basalis magnocellularis, further responsible for aggregation of β amyloid, also contributes as an essential hallmark in AD which is vitally subjected to memory impairment. Down regulation of cholinesterase enzymes implicates the amelioration of cholinergic neurotransmission [8, 9]. Therefore, antioxidant and anticholinesterase approaches might act as beneficial tool for the treatment of AD.

There are numerous synthetic medications in the market to ameliorate the memory deficit associated with AD, PD and Lewy Body's dementia by accelerating the cholinergic neurotransmission [10, 11]. Presently existing acetyl cholinesterase (AChE) inhibitors (donepezil, rivastigmine etc.) for the treatment of AD are beneficial in alleviating the behavioral and cognitive impairment during early phase of disease and remains effective only with the continuation of therapy as well as having inevitable side effects grabs the attention of researchers to explore the better treatment option with sustain therapeutic effect and with minimum or no side effects [12, 13]. These neurodegenerative diseases are properly managed in developed countries but in developing as well as underdeveloped countries, due to higher medicinal expenses, most of the population is still dependant on folklore medicines.

For current experimental work we have selected *Trianthema portulacastrum* Linn. (TP) which is an important herb of Indian traditional medicinal system belongs to the Aizoaceae family. It grows as a weed in heavy rainfall as well as in irrigated regions including India and its neighboring countries such as Sri Lanka, Pakistan and Bangladesh [14]. Due to high nutritional values in Asian countries TP leaves are utilized for preparing various types of vegetable dishes. In African countries, its young leaves are used to prepare soups and as a vegetable. Traditionally, TP possess therapeutic activeness against various diseases such as inflammation, migraine, rheumatic disorders, asthma, beri–beri and bronchitis [15]. TP has been utilized as hepatoprotective, analgesic, alterative, laxative and stomachic. On the basis of scientific research, different solvent extracts with isolated active constituent from TP have demonstrated to exhibit broad spectrum of pharmacological activities, i.e., analgesic, antibacterial, antifungal, anti-inflammatory, antioxidant, antipyretic, hypoglycemic, heptoprotective, nephroprotective and anticancer [16]. TP has been investigated to consist a variety of organic bioactive compounds such as alkaloids, flavonoids, terpenes, steroids, saponins, benzoic acid as well as cinnamic acid derivatives [17]. Our previous study described the presence of various phenolic compounds in *n*-butanol fraction of TP analyzed by HPLC–DAD method. It exhibited the rich existence of protocatechuic acid, chlorogenic acid, caffeic acid and ferulic acid (34.45 ± 0.02, 20.74 ± 0.03, 4.31 ± 0.03 and 1.43 ± 0.01 mg/g of extract) in BFTP [14].

Despite many uses of TP, there have been no reports about its neuropharmacological potency as of now. Thus, in the current study we explored the anti-amnesic effect of BFTP on learning and memory by in silico studies as well as rodent model by using scopolamine induced cognitive impairment and possible biochemical estimation.

Materials and Methods

Chemicals and Reagents

Acetylthiocholine iodide (ATCI), 5,5′-dithio-bis-(2-nitro) benzoic acid (DTNB), Scopolamine hydrobromide, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), AChE were procured from Sigma-Aldrich (St. Louis, Missouri, United States). Ascorbic acid, berberine chloride, ethylene di amine tetra acetic acid (EDTA) and nitro blue tetrazolium (NBT) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. All reagents and solvents utilized during whole experimental work were of analytical grade.

Collection and Authentication of Plant Material

Fresh leaves of TP were procured from rural region of Rewari district of Haryana (India). The plant was identified by botanist Dr R. M. Kadam, Department of Botany, Mahatma Gandhi Mahavidyalaya, Latur, Maharashtra, India. The voucher specimen of plant is preserved in the herbarium of Mahatma Gandhi Mahavidyalaya.

Plants Extract Preparation and Liquid–Liquid Fractionation

Carefully collected leaves of TP were thoroughly washed with running water, dried in shade and coarsely powdered. Coarse plant powder was extracted with petroleum ether (60–80 °C) and then subjected to macerate with 70% ethanol for 9 days. Maceration process was repeated thrice. After filtration all three resultant filtrates were combined and vacuum dried to obtain hydroethanolic extract. Dried extract was subjected to fractionation by liquid–liquid partition in increasing order of polarity. Hydroethanolic extract was dissolved in water, transferred into a separating funnel and partitioned with ethyl acetate, chloroform and n-butanol to get less polar compounds from aqueous extract. After filtration and concentration, fractionated extract were attributed as

EFTP, CFTP and BFTP, respectively. They were preserved at − 20 °C till further use [14].

Antioxidant Activity

All three fraction (EFTP,CFTP, BFTP) were employed to determine free radicals scavenging ability with ABTS radical cation decolorization method with slight modification [18]. Optical density was determined at 734 nm by using spectrophotometer and BHT was utilized as a reference.

$$\text{Activity of radical scavenging}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} value of each fraction was measured by using log (dose) vs % antioxidant activity graph.

In Vitro AChE Inhibitory Activity

All three fractions of TP were subjected to in vitro AChE inhibition by using Ellman's assay with slight modifications [19, 20]. Assay reaction mixture was started with addition of sodium phosphate buffer (140 µl, 0.1 M), 20 µl of sample prepared in DMSO and 20 µl of AChE solution of 0.25 IU/ml strength. Resultant mixtures were kept aside for 15 min and then DTNB (10 µl, 2.5 mM) and ATCI (10 µl, 2.0 mM) were mixed and incubated at room temperature for 10 min. Each sample mixture was measured at 412 nm for absorbance. Berberine chloride was used as a positive control and inhibition of AChE (%) was considered by following formula:

$$\text{Inhibition of AChE}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

AChE Inhibition Kinetics

Strongest in vitro AChE inhibitor fraction, BFTP, was further utilized to analyze AChE inhibition kinetics according to previously described method [21]. Sodium phosphate buffer (140 µl, 0.1 M, pH 8.0) was mixed in 20 µl of different concentrations (0, 20, 40 and 80 µg/ml) of BFTP and then mixed AChE (20 µl, 0.25 IU/ml) into it with constant stirring. After incubation for 15 min at room temperature DTNB (10 µl, 2.5 mM) and 10 µl of ATCI at different concentrations (1.0, 2.0 and 4.0 mM) were introduced in it and scanned at 412 nm within 5 min. To detect type of inhibition kinetics of AChE, Lineweaver–Burk graph was plotted between 1/[ATCI] and 1/(Absorbance/min). Inhibition constant (K_i) was taken into account from the intercept in Dixon–Dixon plot {concentration of BFTP vs. 1/(Absorbance/min)}.

Animals

Male swiss albino mice of 25–30 g weight were utilized for the experiment and they were kept within polypropylene cages in a room with standard laboratory conditions of temperature (25 ± 2 °C), relative humidity (55–60%), and 12:12 light and dark cycle, during the whole experimental work. Mice were freely accessible to water and balanced rodent pellet food ad libitum. Animals were allowed to acclimatize to laboratory conditions for 7 days. Experimental protocol, approved by Institutional Animal Ethics Committee (IAEC), was followed.

Animal Experimental Protocol

All mice were randomly divided into six groups comprising six animals in each group.

Group I (Control)	Normal saline (10 ml/kg/day p.o.)
Group II (SCO)	Vehicle for 21 days and on 21st day after 1 h of vehicle treatment scopolamine (0.6 mg/kg/day i.p.)
Group III (DON)	Donepezil (1 mg/kg/day p.o.)
Group IV (BFTP200)	BFTP (200 mg/kg/day p.o.)
Group V (BFTP400)	BFTP (400 mg/kg/day p.o.)
Group VI (BFTP600)	BFTP (600 mg/kg/day p.o)

Animals were administered with above said drug protocol for 21 days. On day 21, after 1 h of respective treatment, groups 2–6 were injected with scopolamine (0.6 mg/kg i.p.) for induction of amnesia.

Acquisition trials via behavioral model were performed on all groups after 30 min of scopolamine treatment. Memory retention was evaluated after 24 h, on day 22. Mice were allotted washout period of 15 days for testing by another model.

Behavioral Models

Elevated Plus Maze (EPM)

EPM (exteroceptive behavioral model) is extensively employed for assessing the learning and memory ability in rodents. It is constructed with two open arms and two closed arms with dimension of 16 cm × 5 cm and 16 cm × 5 cm × 12 cm, respectively attached with a middle platform (5 cm × 5 cm). The maze was extended from the floor on a height of 25 cm [22]. On day 21 (acquisition trial), scopolamine was injected and 30 min later, each animal from all groups was placed at the open arm marginal end, taking care mouse facing opposite from central platform. Transfer latency (TL), considered as the indicator for learning and memory function and known as the time taken

by mouse to reach in one of closed arm with its four limbs from the open arm, and was determined. TL of 90 s was assigned to each animal but if it was unable to find one of the covered arms within 90 s of its placement, it was gently directed towards the covered arm. Additional 2 min were provided to each animal to look at the maze area and then they were put back into their respective cage. Further retention of memory in terms of TL was analyzed after 24 h of trial period. Significant decrease in TL exhibited antiamnesic effect of test drug. To ascertain experimental stability, mice were acclimatized for 1 h to the test room environment before behavioral testing [23].

Hebbs William Maze (HWM)

HWM (exteroceptive behavioral model) was also utilized to ascertain the spatial cognition of mice pretreated with test drug. HWM fabricated with three components, (a) animal compartment (C1), attached to (b) the middle compartment (C2) (exploratory area) and (c) a reward compartment (C3) at other side of maze, food was kept as a reward. All three chambers were interconnected with each other by Guillotine removable doors. On day 21 (Trial day), after drug treatment, each animal was located individually in C1, keeping the door open for easy the access into C2. As the mouse entered in C2, door of the start chamber was instantly closed to stop back entry. Time utilized by the mouse to reach in reward chamber (TRC) from C1 on trial day, considered as learning index, was noted down. Each mouse was allowed to explore the whole maze for another 3 min by keeping the doors open and then shifted back to its home cage. 24 h later, memory retrieval of the training task was taken into account. Significant decrease in retrieval TRC value than trial TRC defines the condition of improvement in memory [24].

Collection of Brain Tissue and Tissue Homogenate Preparation

On day 22, after behavioral experiment, animals were sacrificed by cervical dislocation. With immense care, hippocampal region from brain was swiftly removed and gently cleaned with cold buffer solution (pH 7.4). The hippocampus was kept in a glass homogenizer with 10 volumes of phosphate buffer (0.1 M, pH 8) and then centrifuged at 14,500 rpm for 15 min at a temperature of 4 °C. The resulted supernatant was utilized for determination of lipid peroxidation index, enzymatic antioxidant profile and AChE activity. Histological changes of hippocampus region were also evaluated.

Estimation of Brain AChE Activity

Brain hippocampal AChE activity was determined according to Ellman's method with slight modification. Assay reaction was started with addition of sodium phosphate buffer (0.1 M), DTNB (2.5 mM) and ATCI (2 mM) in a volumetric flask followed by introduction of supernatant from hippocampus homogenate into the resultant assay mixture and incubated at 30 °C for 10 min. This lead to formation of yellow color due to reduction of DTNB by certain brain homogenate substrates as well as hydrolysis of other substrate non-enzymatically. The sample was scanned at 412 nm spectrophotometrically to measure the change in absorbance. AChE activity was expressed as nM/min/mg protein [25].

Determination of Antioxidant Enzyme Activity

Superoxide Dismutase (SOD) Activity

SOD activity in brain hippocampus was measured on the basis of photoreduction of NBT dye by SOD enzyme [26]. Reaction mixture was prepared by adding Tris–HCl buffer (100 mM, 1.5 ml, pH 7.8), NBT (75 mM), riboflavin (2 µM) and EDTA (6 mM). Carefully measured 700 µl of prepared assay mixture and poured into 100 µl of supernatant. Color intensity was analyzed by scanning the solution at 560 nm and enzymatic activity is expressed as units/mg protein.

Catalase (CAT) Activity

Assay was initiated by mixing 100 µl of hippocampal supernatant into 150 µl of 0.01 M phosphate buffer (pH 7.0) followed by pouring of 250 µl of 0.16 M H_2O_2. Assay reaction mixture was incubated at 37 °C for 1 min and then assay was concluded by mixing of 1 ml of reagent solution (dichromate: acetic acid). Appearance of green color was obtained upon boiling the solution for 15 min and scanned at 570 nm [27]. CAT activity expressed as µM of H_2O_2 consumed/min/mg protein.

Glutathione Peroxidase (GPx) Activity

Assay was performed by DTNB method given by [28]. Assay mixture was prepared with 1 ml of 0.4 M phosphate buffer (pH 7.0), 0.4 mM EDTA, 1 ml of 5 mM NaN_3 and 1 ml of 4 mM glutathione and then added 200 µl of supernatant. The assay mixture was incubated for 5 min at 37 °C and mixed 1 ml of 4 mM H_2O_2. Glutathione amount was analyzed by measuring the absorbance of resultant solution at 412 nm spectrophotometrically.

Estimation of Lipid Peroxidation Index

Thiobarbituric acid reactive substance (TBARS) is known as a marker of lipid peroxidation. The amount of lipid peroxidation is attributed with of thiobarbituric acid concentration in brain [29]. Reaction mixture consisting 700 µl of 9% phosphoric acid and 250 µl of thiobarbituric acid as well as hippocampus supernatant was gently mixed for 2 min. Mixture was boiled for 1 h and butanol (1250 µl) was added after cooling. The assay mixture was mixed with the help of vortex mixer for 20 s and then centrifugation was performed at 25 °C for 20 min. Absorbance was measured at 534 nm. Results are represented as nM/mg protein.

Histological Studies

Carefully isolated brain hippocampus tissues were instantly stored in formalin solution (10%). After 24 h, tissues were dehydrated with ethanol followed by cleaning with xylene and fixed in paraffin wax block. Sections of 5 µm thickness were dyed with hematoxylin and eosin for the purpose of photomicroscopic examination [30].

Statistical Analysis

All results were expressed as mean ± standard deviation. Data were statistically analyzed by one-way ANOVA followed by Newman-Keuls multiple comparison test. Value of $p < 0.05$ was considered statistically significant.

Molecular Docking Studies

In order to understand molecular level interaction of protocatechuic acid, cholorogenic acid, caffeic acid and ferulic acid towards AChE, molecular docking studies were carried out using Autodock 4.2 (The Scripps Research Institute), using RHEL-5.0 Operating system (Dell Precision workstation with Intel core 2 quad processor and 8 GB RAM). Docking protocol was simulated on X-ray crystallographic structure of AChE (1EVE), downloaded from Protein Data Bank (http://www.rcsb.org). Protein was prepared by removing water molecules, merging non-polar hydrogen atoms, computing gasteiger charge with assigning AD4 atom type. Ligands were prepared through PRODRG webserver (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg). Grid parameter file (.gpf) and docking parameter file (.dpf) were written using MGLTools-1.5.6. Receptor grid was generated using Grid points in XYZ co-ordinate 60X60X60 with grid spacing of 0.375 Å. Grid box was centralized on the co-crystallized ligand E2020 (Aricept). Map types were generated using autogrid-4.2. Docking was carried out with following parameters: number of runs: 50, population size: 150, number of evaluations: 2,500,000 and number of generations: 27,000, using AutoDock4.2. Analysis of docking results was done using MGL-Tools-1.5.6. All molecules in their respective largest cluster were further analyzed for its interaction with protein.

Results

In Vitro Antioxidant Activity of BFTP

Results showed promising antioxidant activity, compared to other fractions, by BFTP with IC_{50} value of 39.64 ± 1.23 µg/ml (Table 1) (Fig. 1a) and strongest free radical scavenging activity was exhibited by ascorbic acid with IC_{50} value of 0.74 ± 0.08 µg/ml (Fig. 1b). TP fractions as well as ascorbic acid showed antioxidant activity in concentration dependant manner.

In Vitro AChE Inhibition by BFTP

IC_{50} values of TP fractions and berberine chloride on AChE inhibition is shown in Table 2. Figure 2a indicates concentration dependant effect of all fractions of TP on inhibition of AChE. Berberine chloride (Fig. 2b) showed the highest inhibitory activities followed by BFTP with IC_{50} value of 0.30 ± 0.03 µg/ml and 13.62 ± 2.31 µg/ml, respectively.

Kinetics of AChE Inhibition

AChE inhibition by BFTP was confirmed with Lineweaver–Burk plots [slope = K_m/V_{max} and intercept with X axis = (− 1/Km)] which signified mixed kinetics and competitive inhibition as shown in Fig. 3a. K_i constant (represents enzyme and inhibitor's binding affinity) was established at the point of intersection of three lines towards the negative values of the X-axis in Dixon plots. From Fig. 3b, value of K_i was observed to be 21.5 ± 1.22 µg/ml.

Effect on TL in EPM

Newman Keuls multiple comparison test showed that on day 22, during memory retention trial, scopolamine

Table 1 Antioxidant activity of BFTP and ascorbic acid

Sample	Log IC_{50} (µg/ml)	IC_{50} (µg/ml)
EFTP	1.9922	98.23 ± 1.09^{a}
CFTP	1.8303	67.66 ± 1.11^{b}
BFTP	1.5981	39.64 ± 1.23^{c}
Ascorbic acid	− 0.1258	0.74 ± 0.08^{d}

Each value is reported as mean ± SD (n = 3). Statistically significant at $p < 0.05$, where $^{a>b>c>d}$ in each column

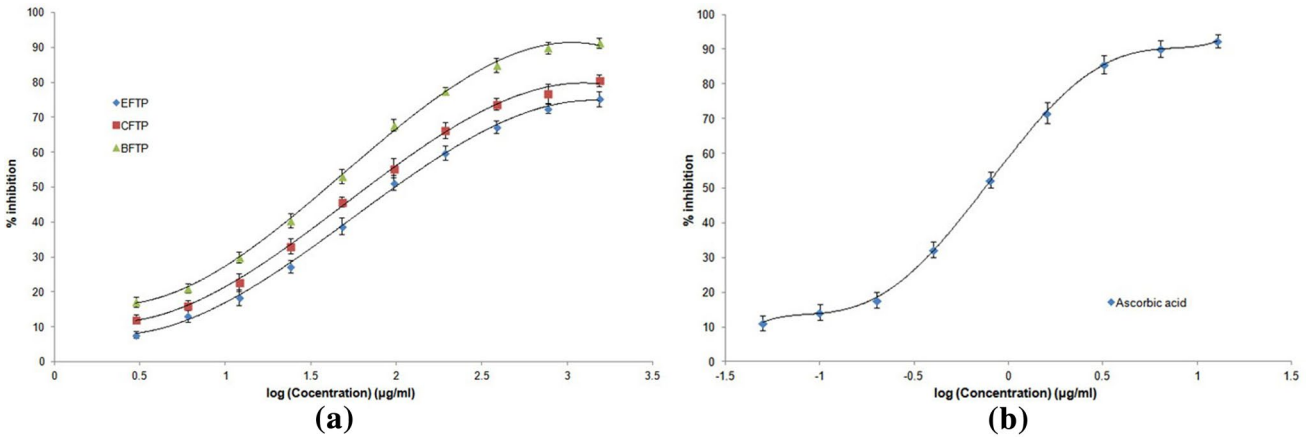

Fig. 1 In vitro antioxidant activity by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) method for **a** different fractions of *Trianthema portulastrum* (TP) hydroethanolic extract and **b** ascorbic acid. Data represented as mean (n = 3) ± SD. *BFTP n*-butanol fraction of TP hydroethanolic extract, *CFTP* chloroform fraction of TP hydroethanolic extract, *EFTP* ethanol fraction of TP hydroethanolic extract

Table 2 Inhibition of AChE by different fractions of TP extract and berberine chloride

Sample	Log IC_{50} (µg/ml)	IC_{50} (µg/ml)
EFTP	1.178	59.97 ± 1.21^{a}
CFTP	1.369	23.44 ± 2.18^{b}
BFTP	1.134	13.62 ± 2.31^{c}
Berberine chloride	−0.508	0.30 ± 0.03

Each value is reported as mean ± SD (n = 3). Statistically significant at $p < 0.05$, where $^{a>b>c}$ in each column

($p < 0.001$) treated group exhibited significant elevation in TL than control group (Fig. 4a). Strongest effect on reversal of scopolamine induced amnesia was observed in DON group ($p < 0.001$) which is more or less similar to BFTP600 ($p < 0.001$). Whereas BFTP400 group ($p < 0.01$) exhibited moderate effect and least effect was shown by BFTP200 group ($p < 0.05$) in reduction of TL as compared to SCO group.

Effect on TRC in HWM

To analyze the efficacy of test drug on reversal of memory, on 22nd day each group was subjected to observe the effect on TRC (Fig. 4b). Scopolamine injected animals exhibited significant increase in TRC ($p < 0.001$) as compared to

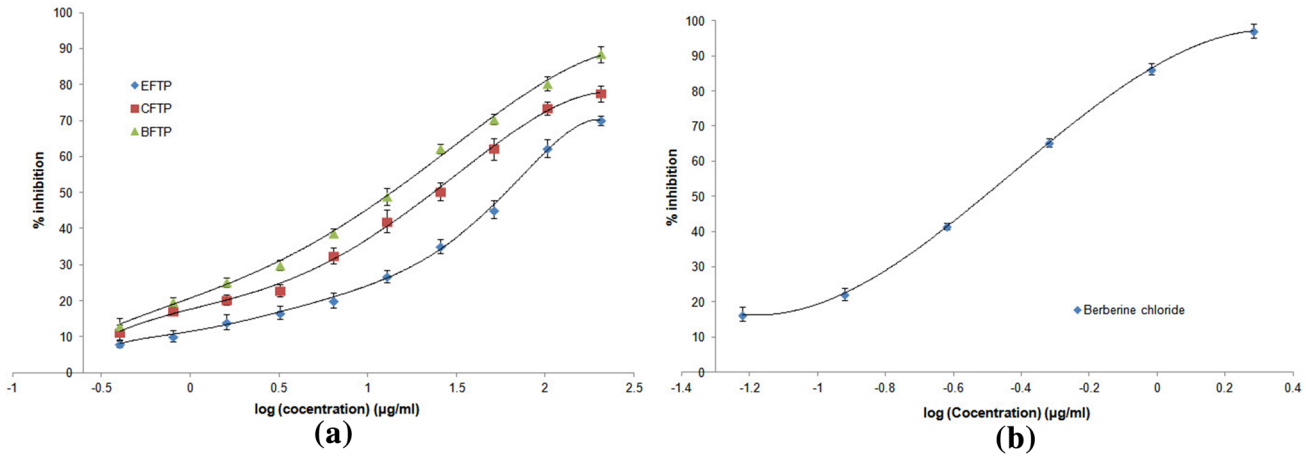

Fig. 2 In vitro acetylcholinesterase (AChE) inhibitory activity by Ellman's method for **a** different fractions of *Trianthema portulastrum* (TP) hydroethanolic extract and **b** berberine chloride. Data represented as mean (n = 3) ± SD. *BFTP n*-butanol fraction of TP hydroethanolic extract, *CFTP* chloroform fraction of TP hydroethanolic extract, *EFTP* ethanol fraction of TP hydroethanolic extract

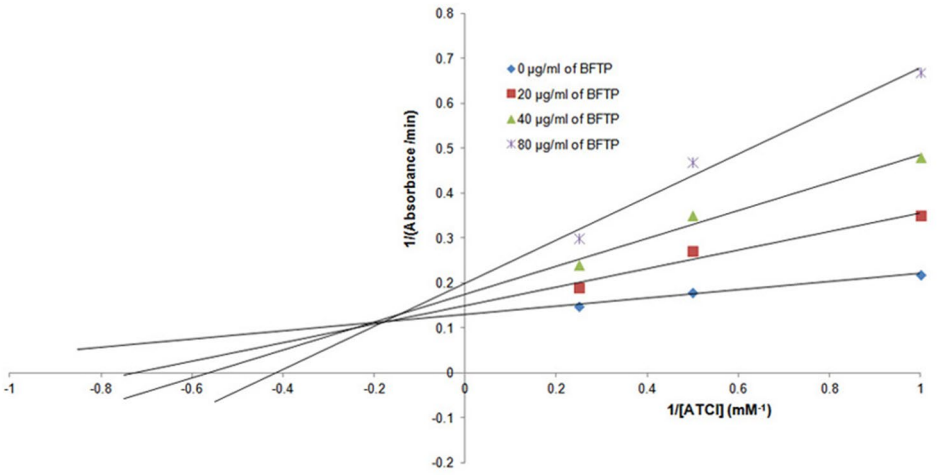

Fig. 3 Graphical determination of kinetic (inhibition) type for n-butanol fraction of *Trianthema portulastrum* hydroethanolic extract (BFTP) by **a** Lineweaver–Burk plots and **b** Dixon plots. Data represented as mean (n = 3) ± SD. *ATCI* acetylthiocholine iodide

control group. Among all groups, highest reduction in TRC was observed in DON group ($p < 0.001$). BFTP (200,400 and 600 mg/kg; $p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively) was also observed as effective in decreasing the scopolamine induced elevated TRC as compared to scopolamine treated group.

Biochemical Estimation

Effect on Brain AChE Activity

Scopolamine ($p < 0.001$) injected group showed significant increase in AChE activity in hippocampus homogenate, as compared to control group, established distinct damage in memory function as shown in Fig. 5. From the results of Newman Keuls multiple comparison test it was observed that DON group ($p < 0.001$) showed the strongest ameliorative effect on scopolamine elevated AChE activity followed by BFTP (600 mg/kg, $p < 0.01$) and lower dose of BFTP (400 and 200 mg/kg; $p < 0.05$) by restoration of cholinergic deficit induced by scopolamine.

Effect on SOD, CAT and GPx Activity

Results (Fig. 6a–c) showed SCO group significantly reduced ($p < 0.001$) SOD, CAT and GPx activity as compared to control group. However, BFTP pretreated animals (400 and 600 mg/kg; $p < 0.05$ and $p < 0.01$, respectively) exhibited significant increase in the antioxidant enzyme activity (SOD, CAT and GPx) within brain homogenate. DON group ($p < 0.001$) was most effective in repairing the scopolamine induced antioxidant enzyme deficit.

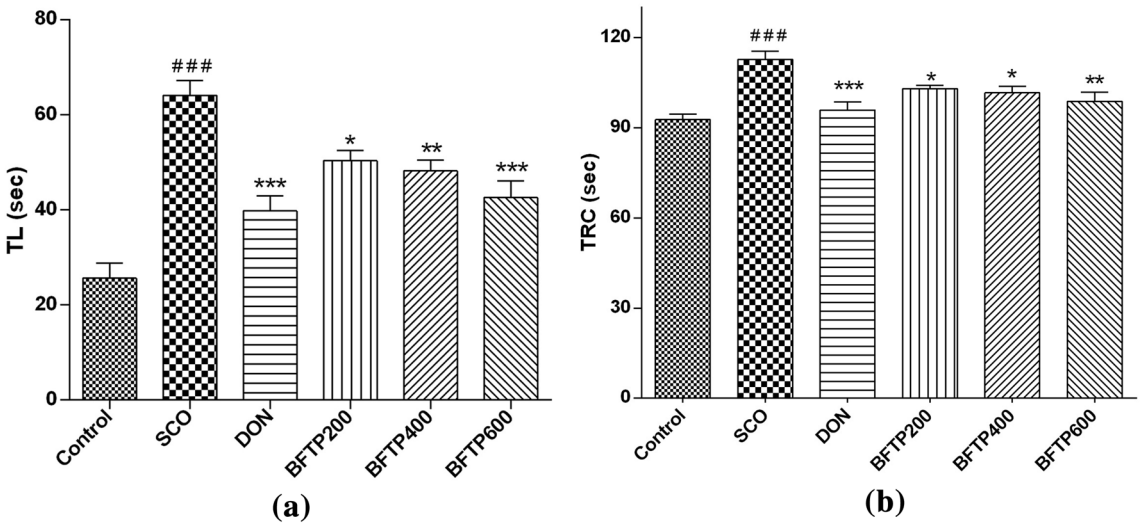

Fig. 4 Effect of *n*-butanol fraction of *Trianthema portulastrum* hydroethanolic extract (BFTP) on scopolamine induced memory impairment in mice using **a** elevated plus maze (EPM) model, **b** Hebbs William maze (HWM) model. Data represented as mean ± SEM, analyzed by one way ANOVA followed by Newman-Keuls multiple comparison test. Significant difference $^{###}p < 0.001$ in comparison to control group. Significant difference $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ in comparison to scopolamine treated group. BFTP200, 400, 600: BFTP administered groups at dose level of 200, 400 and 600 mg/kg, respectively; *DON* donepezil administered group, *SCO* Scopolamine administered group, *TL* transfer latency, *TRC* time taken to recognize the reward chamber

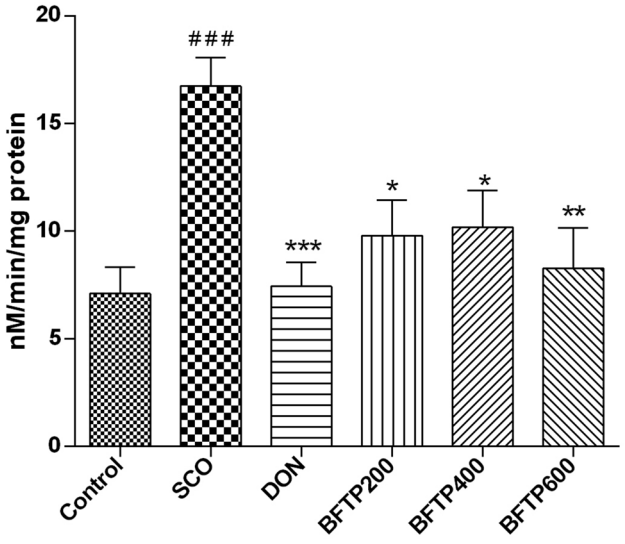

Fig. 5 Effect of n-butanol fraction of *Trianthema portulastrum* hydroethanolic extract (BFTP) on brain hippocampal acetylcholinesterase (AChE) activity. Data represented as mean ± SEM, analyzed by one way ANOVA followed by Newman-Keuls multiple comparison test. Significant difference $^{###}p < 0.001$ in comparison to control group. Significant difference $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ in comparison to scopolamine treated group

Effect on Lipid Peroxidation

Scopolamine treated mice exhibited significantly ($p < 0.001$) elevated level of TBARS in hippocampus homogenate as compared to control group. DON group ($p < 0.001$) showed the strong reducing effect on scopolamine-induced elevated lipid peroxidation level compared to SCO group (Fig. 6d). Pretreatment with BFTP (200, 400 and 600 mg/kg; $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively) for 21 days exhibited considerable reduction in TBARS level in a concentration dependant manner.

Histological Studies of Hippocampal Brain Tissue

Photomicrographs of histopathological examination of brain hippocampus demonstrated scopolamine mediated deleterious effects supported by decrease in pyramidal cells, increase in vacuolated cytoplasm and focal gliosis as compared to control group. Slides of BFTP groups exhibited significant reversal of scopolamine induced alterations in brain tissue in a dose dependent manner (Fig. 7a–f).

Molecular Docking Studies

Protocatechuic acid showed hydrogen bond formation with Ser 124 and Asn85 residues of AChE.Chlorogenic acid formed hydrogen bonds with Asp72, Tyr70, Gln69, Tyr130 and Glu199. Caffeic acid exhibited hydrogen bond interaction with Asp72, Val71, Gln69, Ser124 and Tyr130. Ferulic acid displayed hydrogen bond formation with Val71 and Tyr130 residue.

The free energy of binding with most clusters in the clustering histogram interpreted as its docking score.

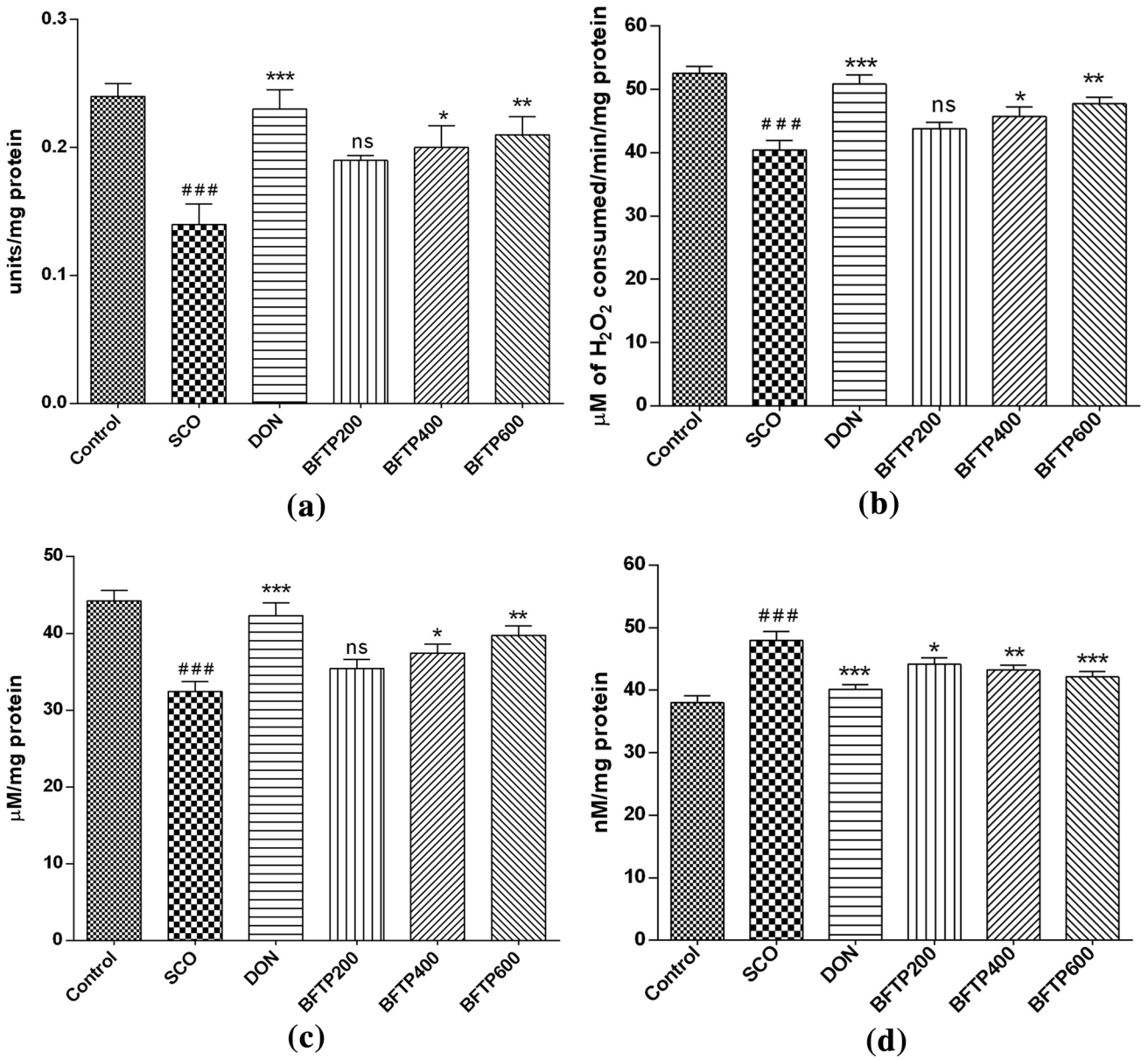

Fig. 6 Effect of n-butanol fraction of *Trianthema portulastrum* hydroethanolic extract (BFTP) on brain hippocampal **a** superoxide dismutase (SOD) level, **b** catalase (CAT) level, **c** glutathione peroxidase (GPx) level, **d** thiobarbituric acid reactive substance (TBARS) level. Data represented as mean ± SEM, analyzed by one way ANOVA followed by Newman-Keuls multiple comparison test. Significant difference $^{###}p < 0.001$ in comparison to control group. Significant difference $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ in comparison to scopolamine treated group. ns: non-significant

Chlorogenic acid found to have the most negative docking score which shows highest binding affinity towards AChE followed by caffeic acid, ferulic acid and protocatechuic acid (Fig. 8a–d; Table 3).

Discussion

In current study, the antiamnesic activity of BFTP was explored by in vitro, in silico as well as in vivo methods. Etiology of cognitive disorders is attributed to central cholinergic pathway since these are prophetic to the hippocampus as well as cerebral cortex [11, 31]. Cholinomimetic agents or inhibitors of AChE have been greatly demonstrated to correct the memory impairment of scopolamine in test objects through alleviating the ACh level of synaptic cleft

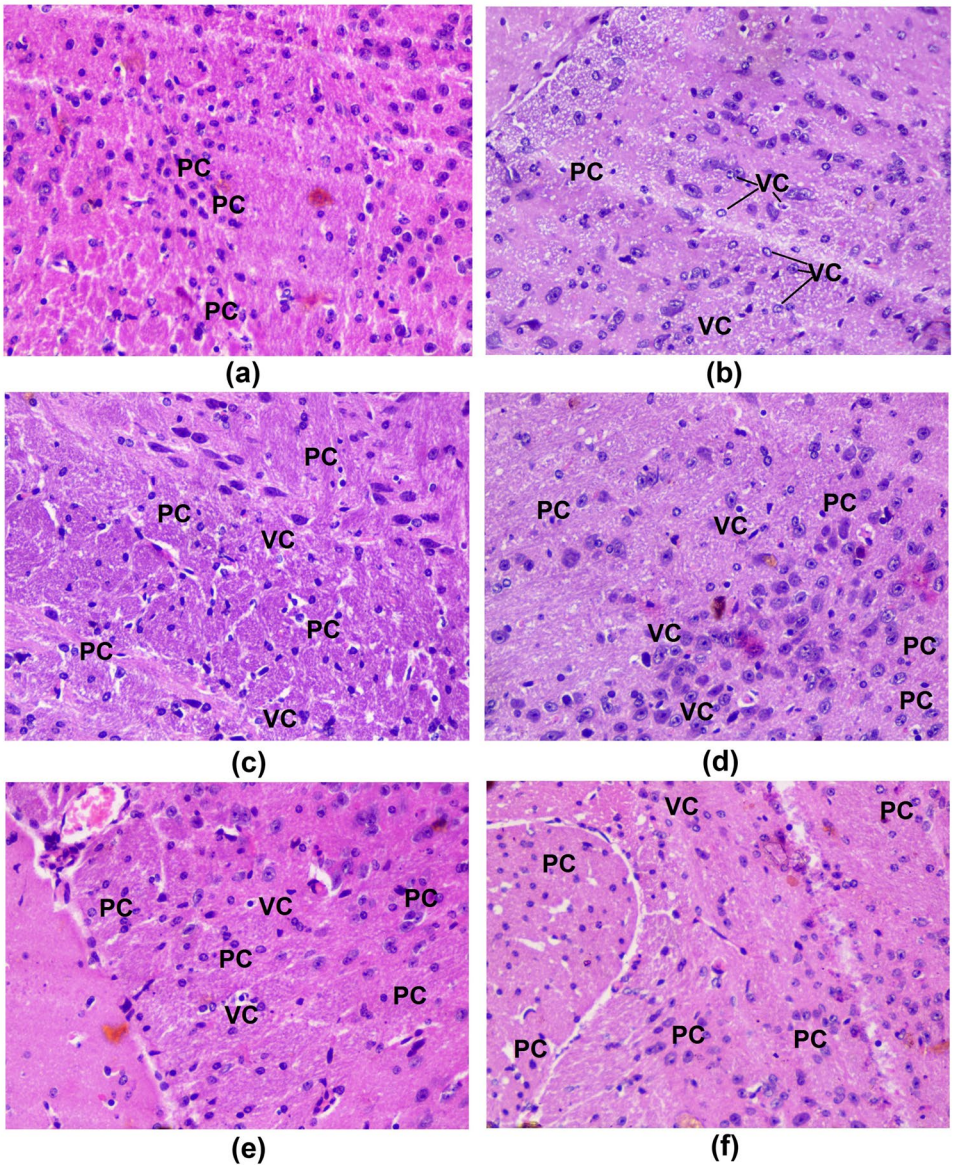

Fig. 7 Photomicrographs of brain hippocampal tissue stained with haematoxylin–eosin dye at 20× magnification **a** control group, **b** scopolamine administered group, **c** donepezil administered group, **d** *n*-butanol fraction of *Trianthema portulastrum* hydroethanolic extract (BFTP) 200 mg/kg administered group, **e** BFTP 400 mg/kg administered group and **f** BFTP 600 mg/kg administered group. *PC* pyramidal cells, *VC* vacuolated cytoplasms (index of neurodegeneration)

[32]. BFTP exhibited the strong in vitro anticholinesterase activity in a concentration dependent manner with IC_{50} value 13.62 ± 2.31 µg/ml. Lineweaver–Burk plot employed to determine the AChE inhibition study and confirmed the characteristic mixed type of kinetics. Additionally, Dixon plot (K_i = 21.5 ± 1.22 µg/ml) demonstrated the BFTP competitive binding with ATCI as it might conjugate at substrate site or another site of AChE as well as it might have coupled with AChE-ATCI complex [19]. Strong antioxidant effect of BFTP (IC_{50} = 9.64 ± 1.23) among other TP fractions might be attributed to presence of bioactive phenolic compounds, i.e., protocatechuic acid, chlorogenic acid, caffeic acid and ferulic acid, which acts as antioxidant and utilized to improve memory function in neurodegenerative disease by ceasing the attack of free radicals [7, 14].

Considering the results of in vitro anticholinesterase and antioxidant activity, the study was further designed to evaluate probable in vivo antiamnesic effect of BFTP. Oral toxicity study performed by Shivhare et al. [33] reported the safety of TP up to 4000 mg/kg, as there was no mortality and any change in behavioral response was recorded in swiss albino mice.

Cognitive behavioral models, i.e., EPM and HWM, are trustworthy tool to assess the antiamnesic potential of test substances in scopolamine induced memory deficits via estimation of exploration performance in rodents [34]. Test animals with short term memory impairment induced by scopolamine were not able to remember the compartment or arm they had just walked through therefore they exhibited significant elevation in TL and TRC, while the results of current study revealed that BFTP (400 and 600 mg/kg)

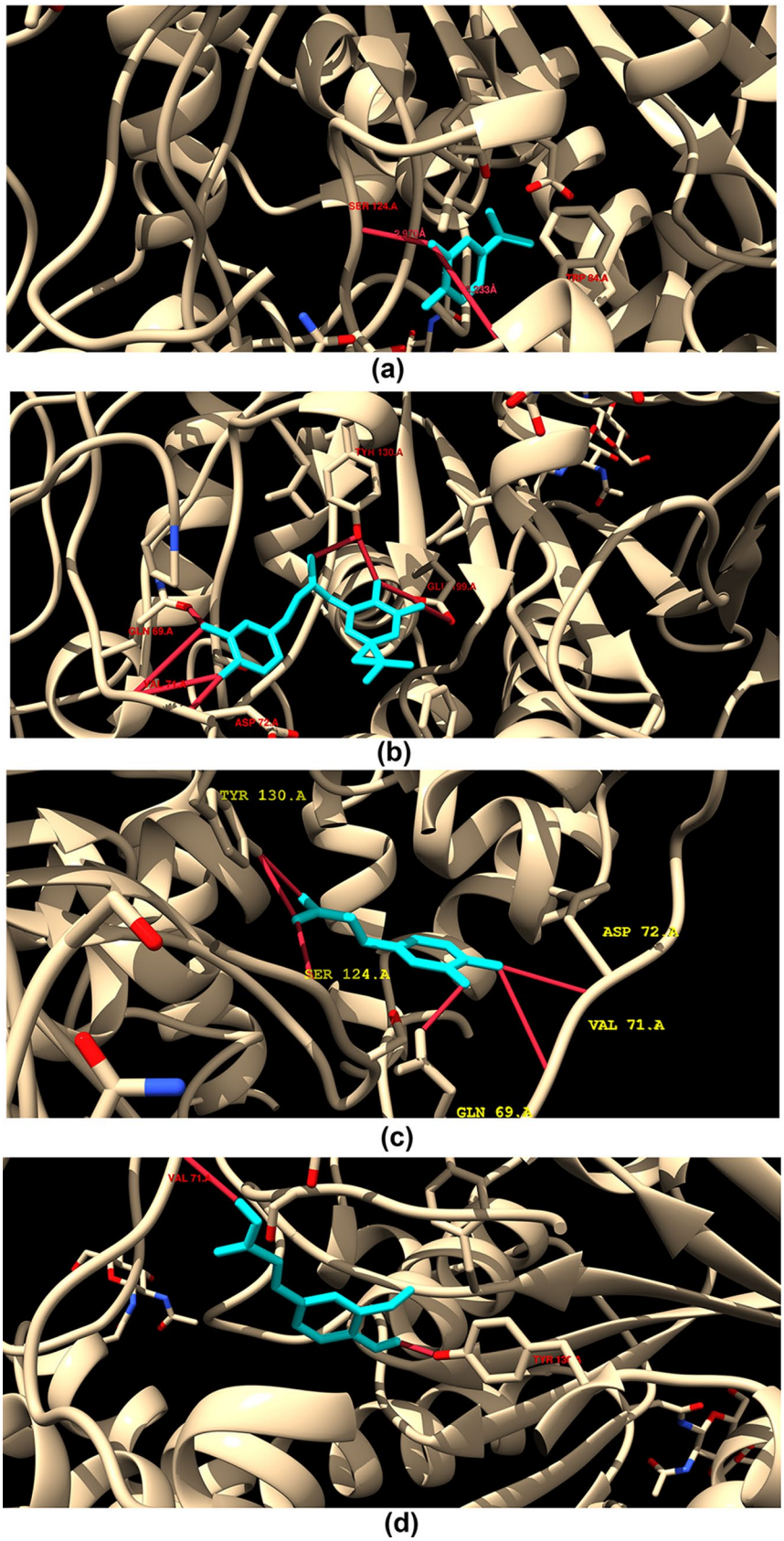

Fig. 8 Binding of **a** protocatechuic acid, **b** chlorogenic acid, **c** caffeic acid and **d** ferulic acid with active site of acetylcholinesterase (AChE) (1EVE). Hydrogen bonds are shown by red lines

Table 3 Molecular interaction of AChE active site with phenolic compounds

Compound	Docking score
Protocatechuic acid	−4.75
Chlorogenic acid	−9.48
Caffeic acid	−6.14
Ferullic acid	−5.50

pre administered mice significantly reversed scopolamine induced memory impairment. Donepezil (standard antiamnesic drug) showed the highest attenuating effect against scopolamine accelerated cognitive deficit as compared to other groups as it acts by anticholinesterase means, demonstrating a comparable underlying mechanism for the BFTP. Significant ($p < 0.01$, $p < 0.05$) reduction in brain hippocampal AChE by BFTP (600 and 400 mg/kg, respectively), in scopolamine induced increased AChE, led to increase in ACh level. It might be associated with phenolic compounds existence in a abundant amount which owes the ability to safeguard the brain cholinergic function after crossing BBB [35]. Oxidative damage due to ROS is endorsed with memory impairment in elderly patients suffering from neurodegenerative disease [36]. Utilization of antioxidants is implicated with impediment of generation of ROS as well as converting them into hydrogen peroxide and di-oxygen molecules with the help of CAT and GPx [1]. In current study, BFTP exhibited significant in vitro antioxidant effect with IC_{50} value of 39.64 ± 1.23 µg/ml. It was further established by in vivo antioxidant activity which revealed significant increase ($p < 0.05$) in various antioxidant enzyme levels (SOD, CAT and GPx) in BFTP (400 and 600 mg/kg) treated groups against scopolamine induced decrease in these enzymatic antioxidant levels. Results of current study are in well agreement with the past reports demonstrating AChE inhibitory and antioxidant potential bearing substance can be recommended as a potent therapeutic agent for treatment of diseases linked with neurodegeneration [37].

Molecular docking is a well established tool for the detection, improvement and development of new as well as existing AChE inhibitory agents. Results of docking study are also in great favor of the strong AChE inhibition by chlorogenic acid, caffeic acid, ferulic acid as well as protocatechuic acid. Cholorogenic acid exhibited the highest AChE binding affinity with −9.48 docking score and then followed by caffeic acid, ferullic acid and protocatechuic acid. Their docking scores advocate AChE inhibitory potential. It was observed that increase in the number of aromatic rings and aromatic substitutions in the ligand such as chlorogenic acid led to an increase in the number of hydrogen bonds with residues. Presence of acetyl group in chlorogenic acid, similar to that in ACh, might be responsible for high binding affinity with AChE.

Conclusion

BFTP has been proved to have in vitro as well as in vivo antioxidant and anti AChE activities and significant antiamnesic potential in rodents. Thus, BFTP could be a beneficial means for the preparation of effective therapeutic agents for the management of short and long term memory deficits in AD.

Acknowledgements Authors are grateful to Dr Vikas Kumar, Department of Pharmaceutical Sciences, SHUATS for his generous help in histological studies. Authors extend their thanks to Dr Puhspraj Gupta, Department of Pharmaceutical Sciences, SHUATS for his support during in vivo studies. They also express their gratitude to Prof. R. M. Kadam, Head of Department, Department of Botany, Mahatma Gandhi Mahavidyalaya, Latur, Maharastra, India for his kind help in identification of plant material.

References

1. Yadav E, Singh D, Yadav P, Verma A (2018) Comparative evaluation of *Prosopis cineraria* (L.) druce and its ZnO nanoparticles on scopolamine induced amnesia. Front Pharmacol 9:549. https://doi.org/10.3389/fphar.2018.00549
2. Lee J-S, Kim H-G, Lee H-W et al (2015) Hippocampal memory enhancing activity of pine needle extract against scopolamine-induced amnesia in a mouse model. Sci Rep 5:9651. https://doi.org/10.1038/srep09651
3. Mufson EJ, Counts SE, Perez SE, Ginsberg SD (2008) Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. Expert Rev Neurother 8:1703–1718. https://doi.org/10.1586/14737175.8.11.1703
4. Dharmarajan TS, Gunturu SG (2009) Alzheimer's disease: a healthcare burden of epidemic proportion. Am Health Drug Benefits 2:39–47
5. Martin JB (1999) Molecular basis of the neurodegenerative disorders. N Engl J Med 340:1970–1980. https://doi.org/10.1056/NEJM199906243402507
6. Lee B, Cao R, Choi Y-S et al (2009) The CREB/CRE transcriptional pathway: protection against oxidative stress-mediated neuronal cell death. J Neurochem 108:1251–1265. https://doi.org/10.1111/j.1471-4159.2008.05864.x
7. Karim N, Khan I, Abdelhalim A et al (2017) Molecular docking and antiamnesic effects of nepitrin isolated from *Rosmarinus officinalis* on scopolamine-induced memory impairment in mice. Biomed Pharmacother 96:700–709. https://doi.org/10.1016/j.biopha.2017.09.121
8. Candy JM, Perry RH, Perry EK et al (1983) Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinson's diseases. J Neurol Sci 59:277–289
9. Loizzo MR, Tundis R, Menichini F, Menichini F (2008) Natural products and their derivatives as cholinesterase inhibitors in the treatment of neurodegenerative disorders: an update. Curr Med Chem 15:1209–1228

10. Husain M, Mehta MA (2011) Cognitive enhancement by drugs in health and disease. Trends Cogn Sci 15:28–36. https://doi.org/10.1016/j.tics.2010.11.002
11. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM (2016) Alzheimer's disease: targeting the cholinergic system. Curr Neuropharmacol 14:101–115
12. Williams RJ, Spencer JPE (2012) Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med 52:35–45. https://doi.org/10.1016/j.freeradbiomed.2011.09.010
13. Cho N, Choi JH, Yang H et al (2012) Neuroprotective and anti-inflammatory effects of flavonoids isolated from *Rhus verniciflua* in neuronal HT22 and microglial BV2 cell lines. Food Chem Toxicol 50:1940–1945. https://doi.org/10.1016/j.fct.2012.03.052
14. Yadav E, Singh D, Yadav P, Verma A (2017) Attenuation of dermal wounds via downregulating oxidative stress and inflammatory markers by protocatechuic acid rich n-butanol fraction of *Trianthema portulacastrum* Linn. in wistar albino rats. Biomed Pharmacother 96:86–97. https://doi.org/10.1016/j.biopha.2017.09.125
15. Mandal A, Bishayee A (2015) *Trianthema portulacastrum* Linn. displays anti-inflammatory responses during chemically induced rat mammary tumorigenesis through simultaneous and differential regulation of NF-κB and Nrf2 signaling pathways. Int J Mol Sci 16:2426–2445. https://doi.org/10.3390/ijms16022426
16. Yamaki J, Nagulapalli Venkata KC, Mandal A et al (2016) Health-promoting and disease-preventive potential of *Trianthema portulacastrum* Linn. (Gadabani)—an Indian medicinal and dietary plant. J Integr Med 14:84–99. https://doi.org/10.1016/S2095-4964(16)60247-9
17. Sukalingam K, Ganesan K, Xu B (2017) *Trianthema portulacastrum* L. (giant pigweed): phytochemistry and pharmacological properties. Phytochem Rev 16:461–478. https://doi.org/10.1007/s11101-017-9493-5
18. Re R, Pellegrini N, Proteggente A et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237
19. Tung BT, Hai NT, Thu DK (2017) Antioxidant and acetylcholinesterase inhibitory activities in vitro of different fraction of *Huperzia squarrosa* (Forst.) Trevis extract and attenuation of scopolamine-induced cognitive impairment in mice. J Ethnopharmacol 198:24–32. https://doi.org/10.1016/j.jep.2016.12.037
20. Sugimoto H, Iimura Y, Yamanishi Y, Yamatsu K (1995) Synthesis and structure-activity relationships of acetylcholinesterase inhibitors: 1-Benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl] piperidine hydrochloride and related compounds. J Med Chem 38:4821–4829. https://doi.org/10.1021/jm00024a009
21. Kamal MA, Greig NH, Alhomida AS, Al-Jafari AA (2000) Kinetics of human acetylcholinesterase inhibition by the novel experimental alzheimer therapeutic agent, tolserine. Biochem Pharmacol 60:561–570. https://doi.org/10.1016/S0006-2952(00)00330-0
22. Itoh J, Nabeshima T, Kameyama T (1990) Utility of an elevated plus-maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology 101:27–33. https://doi.org/10.1007/BF02253713
23. Mani V, Parle M, Ramasamy K, Abdul Majeed AB (2011) Reversal of memory deficits by *Coriandrum sativum* leaves in mice. J Sci Food Agric 91:186–192. https://doi.org/10.1002/jsfa.4171
24. Parle M, Bansal N (2011) Antiamnesic activity of an ayurvedic formulation Chyawanprash in mice. Evid Based Complement Alternat Med. https://doi.org/10.1093/ecam/neq021
25. Hritcu L, Bagci E, Aydin E, Mihasan M (2015) Antiamnesic and antioxidants effects of *Ferulago angulata* essential oil against scopolamine-induced memory impairment in laboratory rats. Neurochem Res 40:1799–1809. https://doi.org/10.1007/s11064-015-1662-6
26. Winterbourn CC, Hawkins RE, Brian M, Carrell RW (1975) The estimation of red cell superoxide dismutase activity. J Lab Clin Med 85:337–341
27. Sinha AK (1972) Colorimetric assay of catalase. Anal Biochem 47:389–394. https://doi.org/10.1016/0003-2697(72)90132-7
28. Sharma M, Gupta YK (2002) Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. Life Sci 71:2489–2498. https://doi.org/10.1016/S0024-3205(02)02083-0
29. Yagi K (1976) A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 15:212–216. https://doi.org/10.1016/0006-2944(76)90049-1
30. Drury R (1983) Theory and practice of histological techniques. J Clin Pathol 36:609. https://doi.org/10.1136/jcp.36.5.609-d
31. Muir JL (1997) Acetylcholine, aging, and Alzheimer's disease. Pharmacol Biochem Behav 56:687–696
32. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M et al (2015) Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. Psychopharmacology 232:931–942. https://doi.org/10.1007/s00213-014-3728-6
33. Shivhare MK, Singour PK, Chaurasiya PK, Pawar RS (2012) *Trianthema portulacastrum* Linn. (Bishkhapra). Pharmacogn Rev 6:132–140. https://doi.org/10.4103/0973-7847.99947
34. Sarter M, Bodewitz G, Stephens DN (1988) Attenuation of scopolamine-induced impairment of spontaneous alteration behaviour by antagonist but not inverse agonist and agonist beta-carbolines. Psychopharmacology 94:491–495
35. Youdim KA, Shukitt-Hale B, Joseph JA (2004) Flavonoids and the brain: interactions at the blood-brain barrier and their physiological effects on the central nervous system. Free Radic Biol Med 37:1683–1693. https://doi.org/10.1016/j.freeradbiomed.2004.08.002
36. Liguori I, Russo G, Curcio F et al (2018) Oxidative stress, aging, and diseases. Clin Interv Aging 13:757–772. https://doi.org/10.2147/CIA.S158513
37. Sadiq A, Mahmood F, Ullah F et al (2015) Synthesis, anticholinesterase and antioxidant potentials of ketoesters derivatives of succinimides: a possible role in the management of Alzheimer's. Chem Cent J. https://doi.org/10.1186/s13065-015-0107-2