Neuroprotective effects of oleuropein against cognitive dysfunction induced by colchicine in hippocampal CA1 area in rats

Soheila Pourkhodadad1 · Masoud Alirezaei2 · Mehrnoush Moghaddasi3 · Hassan Ahmadadvand4 · Manizheh Karami5 · Bahram Delfan3 · Zahra Khanipour1

Abstract Alzheimer’s disease is a progressive neurodegenerative disorder with decline in memory. The role of oxidative stress is well known in the pathogenesis of the disease. The purpose of this study was to evaluate pretreatment effects of oleuropein on oxidative status and cognitive dysfunction induced by colchicine in the hippocampal CA1 area. Male Wistar rats were pretreated orally once daily for 10 days with oleuropein at doses of 10, 15 and 20 mg/kg. Thereafter, colchicine (15 μg/rat) was administered into the CA1 area of the hippocampus to induce cognitive dysfunction. The Morris water maze was used to assess learning and memory. Biochemical parameters such as glutathione peroxidase and catalase activities, nitric oxide and malondialdehyde concentrations were measured to evaluate the antioxidant status in the rat hippocampus. Our results indicated that colchicine significantly impaired spatial memory and induced oxidative stress; in contrast, oleuropein pretreatment significantly improved learning and memory retention, and attenuated the oxidative damage. The results clearly indicate that oleuropein has neuroprotective effects against colchicine-induced cognitive dysfunction and oxidative damage in rats.

Keywords Oleuropein · Colchicine · Alzheimer’s disease · Oxidative stress · Hippocampus

Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that induces decline in memory and other cognitive functions in the elderly [8]. The neuropathological signs of AD include deposits of amyloid β fibrils in senile plaques and the presence of abnormal tau protein filaments in the form of neurofibrillary tangles [7]. It has been recognized that β-amyloid aggregates and iron accumulation both synergistically cause oxidative damage by free radical generation [41]. Also, it has been proposed that oxidative stress has an important role in the pathogenesis of AD [27, 41]. The cortex, the limbic system and the hippocampus are parts of the brain which are destroyed in Alzheimer’s disease [28].

Alzheimer’s disease is shown to be associated with microtubule dysfunction and characterized by the appearance of specific cytoskeletal cellular abnormalities, which are associated with cognitive impairment [17]. Colchicine is a plant alkaloid which binds to its site on tubulin and destroys microtubule assembly. This microtubule-destroying agent affects the shape and development of the cell and neuronal function like axoplasmic transport, and also increases free radical generation and oxidative damage [15, 40]. It also reduces cholinergic function and is known as a potent inhibitor of behavioral processes, and impairs cognitive function [40]. In this regard, central administration...
of colchicine to induce cognitive dysfunction is an animal model of senile dementia of Alzheimer’s type [17, 23].

The beneficial effects of antioxidants against oxidative stress are well known, which fortify the immune system [35]. There are various antioxidants such as curcumin and rosmarinic acid with protective effects against Alzheimer’s disease [6, 22]. In this context, olive leaf extract contains antioxidant polyphenols such as oleuropein that has been reported to be a free radical scavenger [42, 43]. Recent studies indicate that oleuropein is a phenolic compound which possesses diverse pharmacological characteristics, e.g., it has vasodilatory, hypotensive, anti-inflammatory, neuroprotective and antioxidant effects [9, 14, 18, 43]. Also, it has been shown that the administration of oleuropein before ischemia–reperfusion in the CA1 area reduces oxidative stress [12]. Therefore, the purpose of this study was to evaluate the pretreatment effects of oleuropein on oxidative status and cognitive dysfunction induced by colchicine in the hippocampal CA1 area in rats.

Materials and methods

Animals

This study was carried out on 35 adult male Wistar rats (weighing 200–220 g). The animals were housed under standard laboratory conditions with a natural 12:12 h light/dark photocycle with food and tap water supplied ad libitum. All rats were treated humanely and in compliance with the recommendations of the Animal Care Committee of the Lorestan University of Medical Sciences.

Drugs and treatments

Colchicine (SDFCL, India) and oleuropein (Dana Kasian, Iran) solutions were made fresh at the beginning of each experiment. Colchicine was prepared in physiological saline as 15 μg/μl and injected intra CA1 hippocampus. Oleuropein was administered in doses of 10, 15 and 20 mg/kg orally once daily for 10 consecutive days before the administration of colchicine. The rats were divided randomly into five groups (n = 7 in each group), as follows: (1) physiological saline + colchicine (colchicine group), (2) oleuropein (10 mg/kg, p.o.) + colchicine, (3) oleuropein (15 mg/kg, p.o.) + colchicine, (4) oleuropein (20 mg/kg, p.o.) + colchicine, and (5) the control group which received equivalent volumes of vehicle for oleuropein (physiological saline) + vehicle for colchicine (physiological saline). All groups except the control group received colchicine with a dosage of 15 μg/rat, once.

Surgery and intra-hippocampal CA1 administration of colchicine

On the 10th day of oleuropein treatment, all animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) (Merck company, Germany) and positioned in a stereotaxic apparatus (Narishige Company, Japan); their skulls were exposed. Two holes were drilled in the skull for bilateral placement of the guide cannula into the hippocampus. Coordinates for the intra hippocampal CA1 cannula implantation were 3.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 2.2 mm ventral to outer skull surface, according to the atlas of Paxinos and Watson [32]. The intracerebral injection was administered through the guide cannula (21-gauge) using injection needles (27-gauge) connected by polyethylene tubing to a 5.0-μl Hamilton microsyringe. The injection needle was inserted 0.5 mm beyond the tip of the cannula. Then 0.5 μl of vehicle or colchicine was injected into each side of CA1 region during 1–2 min and the needle was left in place for an additional 60 s to allow for diffusion of the solution away from the needle tip. Then, the animals were allowed to recover for 1 week before behavioral testing.

Spatial memory assessment

To assess spatial memory, the Morris water maze (MWM) apparatus was used. The MWM consists of a black circular tank with a diameter of 140 cm and a height of 80 cm that is placed in the center of the room with visual cues on the walls and filled with water (temperature around 23 °C) [30, 38]. A hidden black platform, 10 cm in diameter, was located in the water (2 cm below the water surface) and the tank was conceptually divided into four quadrants and had four points designed as starting positions (N, S, W or E). The rats performed four trials per day for four consecutive days. In the swimming trials, each individual rat was placed into the water at a randomly chosen quadrant. During each trial, each rat was given 60 s to find the hidden platform. If a rat found the platform it was allowed to stay on the platform for 15 s and then taken back into the cage. If they could not escape to the platform within 60 s by themselves, the rats were placed on the platform by hand for 15 s and their escape latency was accepted as 60 s. A semi-automatic video tracking system (Radiab-1 software, made by Nomirei, Iran) was used to measure the time to reach the platform (latency), the length of swim path (distance) and the swim speed. On the 5th day, subjects were tested on a probe trial, during which the escape platform was removed and the time spent in the correct quadrant was measured for a 60-s trial. All testing began at 13:00 h.
Biochemical tests

Tissue preparation for assessment of antioxidant enzyme activities and MDA concentration

At the end of the behavioral experiments, the animals were decapitated and the hippocampi were removed quickly, rinsed with saline, and then frozen in a freezer (−80 °C) for 2 months. Then the samples were thawed and homogenized in a cold phosphate buffer (0.1 M, pH 7.4, containing 5 mmol/l EDTA) on liquid nitrogen [5], and debris were removed by centrifugation (Centrifuge 5415 R; Rotofix 32A, Germany). Supernatants were used for the measurement of protein concentration, antioxidant enzyme activities and MDA concentration as lipid peroxidation marker. Protein contents of brain tissue homogenates were determined using a colorimetric method as described by Lowry [24].

The measurement of glutathione peroxidase (GPx) activity

GPx activity was measured by the method described by Rotruck et al. [36]. Briefly, the reaction mixture contained 0.2 ml 0.4 M phosphate buffer (pH = 7), 0.1 ml 10 mmol/l sodium azide, 0.2 ml tissue homogenized in 0.4 M phosphate buffer (pH = 7), 0.2 ml glutathione, and 0.1 ml 0.2 mmol/l H₂O₂. The contents were incubated for 10 min at 37 °C, 0.4 ml 10% TCA was added to stop the reaction, and centrifuged at 3200 rpm for 20 min. The supernatant was assayed for glutathione content using Ellman’s reagent [19.5 g 5, 5'-dithiobisnitrobenzoic acid (DTNB) in 100 ml 0.1 % sodium citrate]. The activity was expressed as U/mg protein.

Measurement of catalase (CAT) activity

Catalase activity was estimated using the colorimetric method. The reaction was started by the addition of 20 μl of the sample in 2 ml of 30 mmol/l hydrogen peroxide (H₂O₂) in 50 mmol/l potassium phosphate buffer (pH 7.0). Each unit of CAT is mmol/l of consumed H₂O₂ per milligram protein or milliliter solution. The results are expressed as units per mg of protein (U/mg of protein) [1].

NO assay (nitrite content)

Tissue levels of NO were measured by measuring the accumulation of nitrite in tissue using the Griess reaction with sodium nitrate as the standard. Briefly, 50-μl samples of tissue were mixed with equal volumes of 1 % sulphanilamide and 0.1 % N-1-naphthylethylenediaminedichloride in 0.5 % H₃PO₄. After 10 min, at room temperature, the absorbance at 540 nm was measured in a microplate reader. Nitrite concentrations were calculated through comparison with a standard calibration curve with sodium nitrite (NaNO₂: 0–110 μmol/l) [33].

Levels of malondialdehyde (MDA)

The amount of lipid peroxidation was indicated by the content of thiobarbituric acid reactive substances (TBARS) in the brain homogenates as shown by MDA. Tissue MDA was determined by following the production of thiobarbituric acid reactive substances as described previously [39], which was reported by Alirezaei et al. [5]. In short, 40 μl of homogenate was added to 40 μl of 0.9 % NaCl and 40 μl of deionized H₂O, resulting in a total reaction volume of 120 μl. The reaction was incubated at 37 °C for 20 min and stopped by the addition of 600 μl of cold 0.8 M hydrochloric acid, containing 12.5 % trichloroacetic acid. Following the addition of 780 μl of 1 % TBA, the reaction was boiled for 20 min and then cooled at 4 °C for 1 h. In order to measure the amount of TBARS produced by the homogenate, the cooled reaction was spun at 1500×g in a microcentrifuge for 20 min and the absorbance of the supernatant was spectrophotometrically (S2000 UV model; WPA, Cambridge, UK) read at 532 nm, using an extinction coefficient of 1.56 × 10⁵/M cm. The blanks for all of the TBARS assays contained an additional 40 μl of 0.9 % NaCl, instead of homogenate as just described. MDA results were expressed as nanomoles per milligram of tissue protein (nmol/mg protein).

Determination of caspase-3 activity

Caspase-3 activity was measured using a caspase-3 colorimetric assay kit according to the manufacturer’s instructions by a spectrophotometer (Jenway, UV/Vis, Staffordshire, UK). The caspase-3 colorimetric assay is based on the hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) by caspase-3, resulting in the release of the p-nitroanilide (pNA) moiety. p-Nitroaniline absorbance is at 405 nm (εnmol/l = 10.5). The concentration of the pNA released from the substrate is calculated from the absorbance values at 405 nm. Enzyme activity was defined as produced micromole pNA/min/milligram tissue protein and expressed as U/mg protein.

Histological verification

Histological verification of the injection sites was carried out in five rats that were randomly chosen from the five groups, one from each. After behavioral testing, those animals were killed with an overdose of chloroform. Each
animal received 0.5 μl/site injection of ink (methylene blue solution). The experimental animals’ brains were removed and fixed in formalin solution 10 % for 48 h before sectioning. Sections were examined to determine location of the cannula aimed for the CA1. The cannula placements were verified using the atlas of Paxinos and Watson [32].

Statistics

Results were expressed as mean ± SEM. The behavioral assessment data in the MWM test were determined by one way ANOVA. The biochemical estimations were separately analyzed by one way ANOVA. Post-hoc comparisons between groups were made using an LSD test. The value *p < 0.05 was considered significant.

Results

Histology

Figure 1a shows location of cannula position and drug injection sites in the CA1 regions of the hippocampus. The approximate spread of drug assessed by dye (methylene blue solution) injection was verified histologically. Figure 1b also shows a representative section taken from the rat brain atlas of Paxinos and Watson [32].

Effect of oleuropein on spatial navigation task in colchicine-injected rats

Figure 2 illustrates the reduction of latency time and traveled distance to find the hidden platform in all groups during the 4-day training trial. Group comparisons revealed that colchicine-lesioned animals presented a higher latency time and traveled distance than the control group (*p < 0.001), showing a poorer learning performance due to CA1-colchicine infusion. Pretreatment with oleuropein significantly improved learning performance in the

Fig. 1 Histological verification of cannula position and drug injection sites in the CA1 area of the hippocampus (a) and verified section taken from the atlas of Paxinos and Watson (b). Open circle saline microinjection, filled circle colchicine microinjection.

Fig. 2 Effects of oleuropein on escape latency (a) and traveled distance (b) in MWM test. Oleuropein at the dosages of 10, 15, and 20 mg/kg significantly decreased the escape latency and traveled distance. Data are the mean ± SEM (n = 7). *p < 0.05 and ***p < 0.001 indicate significant difference versus colchicine group. ###p < 0.001 indicates significant difference versus control group.
oleuropein group as compared to the colchicine group (10, 15 mg/kg, \( p < 0.05 \), and 20 mg/kg, \( p < 0.001 \)).

According to Fig. 3, which illustrates the probe trial test, colchicine-lesioned animals failed to remember the location of the platform, spending less time in the target quadrant than the control group (\( p < 0.01 \)). However, the time spent in the target quadrant was significantly increased as a result of the administration of oleuropein as compared to the colchicine group (10 mg/kg, \( p < 0.05 \); 15, 20 mg/kg, \( p < 0.01 \)). In addition, there was no significant difference among the swim speeds of all five groups (Fig. 3, \( p > 0.05 \)).

**Antioxidant activities estimation**

**MDA**

Free radical damage following colchicine injection was assessed using lipid peroxidation (LPO), which was measured as MDA levels. According to Fig. 4, there was an increase in MDA levels of the colchicine group (\( p < 0.001 \)) as compared to the control group. The oral administration of oleuropein 10, 15, 20 mg/kg resulted in a significant reduction of MDA levels in the brain tissue of colchicine + oleuropein animals as compared to the colchicine group (\( p < 0.05 \), \( p < 0.01 \)).

**Glutathione peroxidase (GPx) activity**

According to Fig. 5, the GPx activity was significantly (\( p < 0.001 \)) decreased in the colchicine group as compared to the control group. The oral administration of oleuropein 10, 15, 20 mg/kg resulted in a significant reduction of MDA levels in the brain tissue of colchicine + oleuropein animals as compared to the colchicine group (\( p < 0.05 \), \( p < 0.01 \)).
Catalase (CAT) activity

According to Fig. 6, the CAT activity was significantly decreased in the colchicine group as compared to the control group. Administration of oleuropein 20 mg/kg resulted in a significant increase in catalase activity as compared to the colchicine group.

Nitrite estimation

According to Fig. 7, administration of colchicine significantly increased tissue nitrite level when compared to the control group. Treatment with oleuropein 20 mg/kg significantly attenuated the increase in tissue nitrite induced by colchicine.

Discussion

Alzheimer’s disease (AD) is a fatal neurodegenerative disorder manifested by early cognitive dysfunction associated later by behavioral and social deterioration. The hippocampus is the primary neuronal injury region involved in the pathophysiology of the disease [28]. Earlier studies indicated that centrally administered colchicine induced an animal model of senile dementia of Alzheimer’s type in humans [17]. Hence, the goal of the present study was to clarify the possible neuroprotective role of oleuropein in an animal model of AD induced by colchicine. The present study showed that the injection of colchicine into CA1 hippocampus resulted in significant memory deficits in MWM tasks, increased the escape
latency time and traveled distance, indicating that colchicine could diminish memory in rats. The results of the current study also showed that injection of colchicine causes an increase in free radical generation leading to oxidative stress that induces cognitive dysfunction. The effects of colchicine on cognitive function have been investigated in previous studies [40, 43]. These findings are in agreement with our previously described harmful effects of colchicine on hippocampal CA1 in treated rats [34].

Colchicine is also a well-known alkaloid derivative which binds tightly to microtubules, causing their depolymerization and inhibiting their assembly. This leads to reduced intracellular trafficking, and in turn leads to the death of nerve cells [26]. It induces neurofibrillary degeneration which is associated with loss of cholinergic neurons in the brain, resulting in impairment of learning function [23]. These effects of colchicine can be induced by its ability to cause apoptosis in neuronal populations by activating the c-Jun N-terminal kinase (JNK) pathway [31].

Experimentally, it has been shown that central administration of colchicine increases the expression of inducible nitric oxide synthase which increases nitric oxide (NO) levels, the peroxynitrite precursor, that results in neuronal damage. Colchicine also elevates the glutamate/GABA ratio in the cerebral cortex of mice [14, 43]; an increase in glutamate leads to free radical-induced neurotoxicity [9]. Thus, nitrite content measured in this study indicates nitrosative stress in the colchicine group when compared to the oleuropein-pretreated rats.

On the other hand, the present study revealed that pretreatment with oleuropein led to a significant improvement of the retention performance of the spatial navigation task in MWM, as evident by the significant decrease in latency time and increase in the time spent in the target quadrant (Figs. 2, 3) and attenuated colchicine-induced injuries in the hippocampus. Further investigation to elucidate the neurochemical and molecular mechanisms involved in the neuroprotective effects of oleuropein is recommended.

Acknowledgments This research was financially supported by Razi Herbal Medicines Research Center. We are most grateful to Dr. B. Rasoulian for his kind cooperation in Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences and to Mr. M. Almasian for proofreading the manuscript.

References

1. Ahmadvand H, Tavafi M, Khosrowbeygi A (2012) Amelioration of altered antioxidant enzymes activity and glomerulosclerosis by coenzyme Q10 in alloxan-induced diabetic rats. J Diabetes Complicat 26(6):476–482
2. Alirezaei M, Dezfoulian O, Kheradmand A, Neamati S, Khonsari A, Pirzadeh A (2012) Hepatoprotective effects of purified oleuropein from olive leaf extract against ethanol-induced damages in the rat. Iran J Vet Res 13:218–226
3. Alirezaei M, Dezfoulian O, Neamati S, Rashidipour M, Tanideh N, Kheradmand A (2012) Oleuropein prevents ethanol-induced
gastric ulcers via elevation of antioxidant enzyme activities in rats. J Physiol Biochem 68(4):583–592.
4. Alirezaei M, Dezfoulian O, Sookhthezari A, Asadian P, Khoshdel Z (2014) Antioxidant effects of oleuropein versus oxidative stress induced by ethanol in the rat intestine. Comp Clin Pathol 23:1359–1365.
5. Alirezaei M, Khoshdel Z, Dezfoulian O, Rashidpour M, Taghadosi V (2015) Beneficial antioxidant properties of betaine against oxidative stress mediated by levodopa/benserazide in the brain of rats. J Physiol Sci 65(3):243–252.
6. Alkan T, Nitta A, Mizoguchi H, Itoh A, Nabeshima T (2007) A natural scavenger of peroxynitrites, rosmarinic acid, protects against impairment of memory induced by Aβ 25–35. Behav Brain Res 180(2):139–145.
7. Anand R, Gill KD, Madhi AA (2014) Therapeutics of Alzheimer’s disease: past, present and future. Neuropharmacology 76:27–50.
8. Auld DS, Kornecook TJ, Bastianetto S, Quirion R (2002) Alzheimer’s disease and the basal forebrain cholinergic system: relations to β-amyloid peptides, cognition, and treatment strategies. Prog Neurobiol 68(3):209–245.
9. Bondy SC (1995) The relation of oxidative stress and hyperexcretion to neurological disease. Proc Soc Exp Biol Med 208(4):337–345.
10. Čoban J, Öztezcan S, Doğru-Abbasoglu S, Binguöl I, Yesil-Mizrak Z (2014) Antioxidant effects of oleuropein versus oxidative stress in rats. J Physiol Sci 65(3):243–252.
11. Daccache A, Lion C, Sibille N, Gerard M, Slomianny C, Lippens L (2000) Differential effects of nitric oxide on morphine-induced place conditioning in Wistar rats treated by colchicine intra-hippocampal CA1. J Clin Toxicol. 40:193:265–275.
12. Dekanski D, Selakovic’ V, Piperski V, Radulovic’ Zˇ, Korenic’ A, Iqbal K, Zaidi T, Wen GY, Grundke-Iqbal I, Merz PA, Shaikh SS, Wisniewski HM, Alafuzoff I, Winblad B (1986) Defective phosphorylation at Thr(214) of the tau protein in Alzheimer’s disease. Free Radical Biol Med 23:134–147.
13. Diomedev L, Rigacci S, Romeo M, Stefani M, Salomna M (2013) Oleuropein aglycone protects transgenic C. elegans strains expressing Aβ42 by reducing plaque load and motor deficit. PLoS One 8(3):e58993.
14. Dufourny L, Leroy D, Warembourg M (2000) Differential effects of colchicine on the induction of nitric oxide synthase in neurons containing progesterone receptors of the guinea pig hypothalamus. Brain Res Bull 52(5):435–443.
15. Goldschmidt RB, Steward O (1982) Neurotoxic effects of colchicine: differential susceptibility of CNS neuronal populations. Neuroscience 7(3):695–714.
16. Gorman AM, Bonfoco E, Zhivotovskv B, Orrenius S, Ceccatelli S (1999) Cytochrome c release and caspase-3 activation during colchicine-induced apoptosis of cerebellar granule cells. Eur J Neurosci 11(3):1067–1072.
17. Iqbal K, Zaidi T, Wen GY, Grundke-Iqbal I, Merz PA, Shaikh SS, Wisniewski HM, Alafuzoff I, Winblad B (1986) Defective brain microtubule assembly in Alzheimer’s disease. Lancet 328:421–426.
18. Khalatbari AR, Ahmadvand H (2012) Neuroprotective effect of oleuropein following spinal cord injury in rats. Neurol Res 34(1):44–51.
19. Kim JA, Mitsukawa K, Yamada MK, Nishiyama N, Matsuki N, Ikegaya Y (2002) Cytoskeleton disruption causes apoptotic degeneration of dentate granule cells in hippocampal slice cultures. Neuropharmacology 42(8):1109–1118.
20. Kostomoiri M, Fragkouli A, Sagnou M, Skalsousnis LA, Pelecanou M, Tsilibary EC, Tzinia AK (2013) Oleuropein, an antioxidant polyphenol constituent of olive promotes α-secretase cleavage of the amyloid precursor protein (APP). Cell Mol Neurobiol 33(1):147–154.
21. Kroemer G, Dallaporta B, Resche-Rigon M (1998) The mitochondrial death/life regulator in apoptosis and necrosis. Annu Rev Physiol 60:619–642.
22. Kumar A, Naidu PS, Seghal N, Padi SS (2007) Effect of curcumin on intracerebroventricular colchicine-induced cognitive impairment and oxidative stress in rats. J Med Food 10(3):486–494.
23. Kumar A, Seghal N, Naidu PS, Goyal R (2007) Colchicine-induced neurotoxicity as an animal model of sporadic dementia of Alzheimer’s type. Pharmaco Rep 59(3):274–283.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275.
25. Lucchinri I, Dami T, Grossi C, Rigacci S, Stefani M, Casamenti F (2014) Oleuropein aglycone counteracts Aβ42 toxicity in the rat brain. Neurosci Lett 558:67–72.
26. Mandelkow EM, Stamer K, Vogel R, Thies E, Mandelkow E (2003) Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. Neurobiol Aging 24(8):1079–1085.
27. Markesbery WR (1997) Oxidative stress hypothesis in Alzheimer’s disease. Free Radical Biol Med 23:134–147.
28. McKlroy S, Craig D (2004) Neurobiology and genetics of behavioural syndromes of Alzheimer’s disease. Curr Alzheimer Res 1(2):135–142.
29. Mohagheghi F, Bigdeli MR, Kasoulain B, Hashemi P, Pour MR (2011) The neuroprotective effect of olive leaf extract is related to improved blood–brain barrier permeability and brain edema in rats with experimental focal cerebral ischemia. Phytomedicine 18:170–175.
30. Morris R, Garrud P, Rawlins JN, O’Keefe J (1982) Place navigation in rats with hippocampal lesions. Nature 297(5868):681–683.
31. Mülleer GJ, Geist MA, Veng LM, Willesen MG, Johansen FF, Leist M, Vaudano E (2006) A role for mixed lineage kinases in granule cell apoptosis induced by cytoskeletal disruption. J Neurochem 96(5):1242–1252.
32. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic, New York.
33. Petrola MJ, de Castro AJ, Pitombeira MH, Barbosa MC, Quixada AT, Duarte FB, Goncalves RP (2012) Serum concentrations of nitrite and malondialdehyde as markers of oxidative stress in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors. Rev Bras Hematol Hemoter 34(5):352–355.
34. Porkhodadad S, Karami M, Jalali MR (2011) Positive effect of nitric oxide on morphine-induced place conditioning in Wistar rats treated by colchicine intra-hippocampal CA1. J Clin Toxicol. doi:10.4172/2169-0495.1000102.
35. Rajadurai M, Stanley MAINZEN Prince P (2006) Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats: biochemical and histopathological evidences. Toxicology 228:259–268.
36. Rotruck JT, Pope AL, Ganther HE, Swanson BA, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179(4073):588–590.
37. Sarbishegi M, Mehraein F, Soleimani M (2014) Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. Iran Biomed J 18(1):16–22.
38. Stackman RW, Hammond RS, Linardatos E, Gerlach A, Maylie J, Adelman JP, Tsouopoulos T (2002) Small conductance Ca2+–activated K+ channels modulate synaptic plasticity and memory encoding. J Neurosci 22(25):10163–10171.
39. Subbarao KV, Richardson JS, Ang LC (1990) Autopsy samples of Alzheimer’s cortex show increased peroxidation in vitro. J Neurochem 55(1):342–345.
40. Veerendra Kumar MH, Gupta YK (2002) Intracerebroventricular administration of colchicine produces cognitive impairment associated with oxidative stress in rats. Pharmacol Biochem Behav 73(3):565–571

41. Vina J, Lloret A, Orti R, Alonso D (2004) Molecular bases of the treatment of Alzheimer’s disease with antioxidants: prevention of oxidative stress. Mol Aspects Med 25:117–123

42. Waterman E, Lockwood B (2007) Active components and clinical applications of olive oil. Altern Med Rev 12(4):331–342

43. Yu Z, Cheng G, Hu B (1997) Mechanism of colchicine impairment on learning and memory, and protective effect of CGP36742 in mice. Brain Res 750:53–58