Advances in Alzheimer's Disease, 2016, 5, 87-102
Published Online September 2016 in SciRes. http://www.scirp.org/journal/aad
http://dx.doi.org/10.4236/aad.2016.53007

Analyzing Nootropic Effect of *Phyllanthus reticulatus* Poir. on Cognitive Functions, Brain Antioxidant Enzymes and Acetylcholinesterase Activity against Aluminium-Induced Alzheimer's Model in Rats: Applicable for Controlling the Risk Factors of Alzheimer's Disease

Md. Sahab Uddin[1*], Abdullah Al Mamun[1], Mohammed Ashraful Iqbal[2], Ariful Islam[3], Md. Farhad Hossain[1], Sayema Khanum[1], Mamunur Rashid[1,4]

[1]Department of Pharmacy, Southeast University, Dhaka, Bangladesh
[2]Department of Chemistry, Fareast International University, Dhaka, Bangladesh
[3]Department of Pharmacy, State University of Bangladesh, Dhaka, Bangladesh
[4]Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh
Email: *msu-neuropharma@hotmail.com, *msu_neuropharma@hotmail.com

Received 6 March 2016; accepted 7 July 2016; published 21 July 2016

Abstract

Oxidative stress is intensely linked with neurodegenerative disorders, especially Alzheimer's disease (AD). Searching for medicinal plant with the nootropic activity for controling the development and progression of AD has received extensive consideration. The plant *Phyllanthus reticulatus* (PR) Poir. is known in Bengali as Panjuli belongs to family Euphorbiaceae. Previous studies have shown the antioxidant, analgesic, anti-inflammatory, etc. activities of this plant. Therefore, the objective of this study was to examine the nootropic effect of ethanolic extracts of *Phyllanthus reticulatus* (EEPR) on cognitive functions, brain antioxidatant enzymes and acetylcholinesterase activity in aluminium-induced rats of cognitive impairment and oxidative stress. The effects of EEPR fruit (*i.e.*, 100 and 200 mg/kg b.w.) were examined for 30 days and its nootropic effect was determined in aluminium treated Swiss albino male rats by behavioral studies such as Passive Avoidance (PA) test, Rewarded Alternation (RA) test and biochemical studies such as superoxide

*Corresponding author.
How to cite this paper: Uddin, Md.S., *et al*. (2016) Analyzing Nootropic Effect of *Phyllanthus reticulatus* Poir. on Cognitive Functions, Brain Antioxidant Enzymes and Acetylcholinesterase Activity against Aluminium-Induced Alzheimer's Model in Rats: Applicable for Controlling the Risk Factors of Alzheimer's Disease. *Advances in Alzheimer's Disease*, **5**, 87-102.
http://dx.doi.org/10.4236/aad.2016.53007

dismutase (SOD), catalase (CAT), contents of thiobarbituric acid reactive substances (TBARS) and acetylcholinesterase (AChE) activity in rats brain tissue homogenates. In PA test, administration of EEPR fruit (*i.e.,* 100 and 200 mg/kg, b.w.) significantly ($P < 0.05$, $P < 0.01$) increased step-through latency (STL) in rats on 30th day with respect to disease control group. The percentage of memory retention (MR) for this test was pointedly ($P < 0.05$) increased in rats treated with EEPR fruit (*i.e.,* 200 mg/kg b.w.) as compared with disease control group. For RA test, EEPR fruit (*i.e.,* 200 mg/kg b.w.) markedly ($P < 0.01$) increased the correct responses (CR) in rats on 30th day related to disease control group. In case of this test the percentage of MR was significantly ($P < 0.05$, $P < 0.01$) increased in rats treated with EEPR fruit (*i.e.,* 100 and 200 mg/kg b.w.) with respect to disease control group. Administration of EEPR fruit (*i.e.,* 100 and 200 mg/kg b.w.) considerably ($P < 0.05$, $P < 0.01$) increased the level of SOD, CAT and expressively ($P < 0.05$) decreased TBARS level compared to disease control group. Treatment with EEPR fruit (*i.e.,* 100 and 200 mg/kg b.w.) markedly ($P < 0.05$, $P < 0.01$) decreased the level of AChE activity to that of disease control group. The present study shows that EEPR fruit has excellent nootropıc effect on cognitive performance and brain antioxidant markers in aluminium-induced rats of cognitive impairment and oxidative stress which could be developed in the management of neurodegenerative diseases especially AD.

Keywords

Nootropic, *Phyllanthus reticulatus*, Cognitive Functions, Brain Antioxidant Enzymes, Acetylcholinesterase Activity, Alzheimer's Disease

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative brain disease that causes problems with memory, thinking, behavior and finally lead to death [1]. It is the most common form of dementia (60%) which is not a normal part of aging [2]. AD is characterized by the presence of excessive amounts of neuritic plaques containing amyloid-β (Aβ) and abnormal tau protein filaments in the form of neurofibrillary tangles (NFTs) in the cerebral cortex and subcortical gray matter [3]. The brain of Alzheimer patient's has increased levels of acetylcholinesterase (AChE), which is accountable for the breakdown of acetylcholine (ACh) [4]. ACh is a neurotransmitter which plays a pivotal role for the appropriate functioning of the central cholinergic system (CCS) [5]. A diminution of ACh in the brain of patients with AD appears to be a foremost reason in producing dementia [6]. Worldwide at present about 35 million people are affected by AD and it is the 6th foremost cause of death in the United States [7]. Patients with Alzheimer's may live an average of 8 years after their symptoms become considerable to others. However, survival range can be diverse from 4 to 20 years which is depending on age and other health conditions. The current Alzheimer's treatments cannot discontinue the progression of this neurodegenerative disease, but these can temporarily slow down the worsening of dementia symptoms and ameliorate the quality of life in Alzheimer's patients [8]. Today the scientists have been working relentlessly and providing their maximum effort to find better ways to treat the disease, delay its onset and prevent it from developing [9].

Oxidative stress occurs when free radicals and their by-products are produced in excessive amount compare to antioxidant defense mechanisms [10]-[12]. The pathogenesis of AD leading to neuronal dysfunction and cell death, mainly due to imbalance between free radical production and antioxidant defenses [13] [14]. Research has recently displayed that brain tissue in patients with AD is exposed to protein oxidation, DNA oxidation, lipid oxidation, glycoxidation etc. during the period of the disease [15] [16]. The oxidation of proteins by free radicals plays a significant role in AD [17]. Several studies exhibit an augment in protein carbonyls in multiple brain areas in subjects with AD [18]. Enzyme mainly glutamine synthetase and creatine kinase are sensitive to oxidative modification and noticeably reduced in the brains of Alzheimer's patients [19]. Higher levels of lipid peroxidation take place in the brain in AD and are most well-known where degenerative changes are most noticeable [20]. Polyunsaturated fatty acids (PUFAs) of the brain membrane phospholipids are particularly susceptible to free radical attack because their double bonds permit easy withdrawal of hydrogen ions [21]. Oxidation of PUFAs, principally arachidonic and docosahexaenoic convey lipid peroxidation in AD [22] by generating alde-

hydes most importantly 4-hydroxynonenal (HNE), a highly reactive cytotoxic agent able to inhibit glycolysis, nucleic acid and protein synthesis and degrading proteins [23]. Oxidation of DNA can generate strand breaks, sister chromatid exchange, DNA-protein crosslinking and base alterations [24]. Numerous studies exhibit an increase in oxidative DNA damage in the brain's of subjects with AD [25]. The greatest marked DNA adduct defined is 8-hydroxy-2-deoxyguanosine (8-OHdG) [26]. On the other hand advanced glycation end products are produced due to posttranslational modifications of proteins and may play a role in AD that is connected to oxidative modifications of Aβ peptides and tau [27]. In addition to this, the brain is largely composed of easily oxidized lipids, has a high oxygen consumption rate, high metabolic rate of transitional metals and lacks strong antioxidant defenses, that's why it is quite vulnerable to oxidative injury [28].

Aluminium is a generally manifested neurotoxin and possesses diverse mode of action on the central nervous system (CNS) [29]. It is able to rise the permeability and crossing of the blood-brain barrier (BBB) [30] [31], which plays a significant role to increase the concentration of aluminium in the hippocampus [32], cortex, singulated bundles and corpus callosum [33]. Various neuropathological, biochemical and epidemiological studies have recommended a potential connection between the pathogenesis of AD and neurotoxicity of aluminium [34]. Aluminium develops accumulation of insoluble Aβ, aggregation of hyper phosphorylated tau protein which contains NFTs [35] and causes harmful alteration to cholinergic neurotransmission [36]. Furthermore, aluminium promotes oxidation triggered by various transition metals such as chromium (Cr) and copper (Cu) [37]. In this study to created cognitive dysfunction and oxidative stress aluminium maltolate was used. The advancement of drugs for the treatment of AD that breaks the vicious cycles of oxidative stress and neurodegeneration recommends new prospects.

Antioxidants are agents that are the vital part of most favorable health and able to prevent or delay some kinds of cell damage [38]. Several scientific researchers recommended that antioxidants play a central role in the management of AD. Naturally occurring antioxidants are extremely useful for AD in order to reduce risk connected with synthetic antioxidants [39]. The greatest origin of natural antioxidant is medicinal plants. The neuroprotective effects of natural antioxidants and nootrpics, such as *Ginkgo biloba*, [40] *Bacopa monnieri* [41] and *Huperzia serrata* has [42] attained considerable attention in the management of AD.

The plant *Phyllanthus reticulatus* (PR) Poir. is known in Bengali as Panjuli belongs to Euphorbiaceae family [43]. This plant is extensively distributed throughout the tropical areas of India, China, Malay Islands and fallow lands of Bangladesh [44]. The fruit of this plant is roundish berry with a diameter of about 4 to 6 mm, green in color at first and becomes purplish black [45]. In the traditional system of medicine different parts of this plant are used for curing various diseases. Leaves are used as antidiarrheal, diuretic, cooling medicine, roots are used for treating malaria, asthma and bark is used as astringent and diuretic. The fruit of this plant shows astringent properties to the bowels and used in inflammation [43]. The important therapeutic uses of this plant are analgesic, anti-inflammatory, hypocholesterolemic, cytotoxic, immunostimulant, antidiabetic, antiplasmodial, antimicrobial, hepatoprotective activities etc. [46]. The chemical studies of this plant ensured the presence of following phytoconstituents including tannic acid, octacosanol, sitosterol, scopoletin, lupeol acetate, teraxerone, betulin, teraxerol acetate, friedeline, stigamasterol and lupeol [47] [48].

Previous preliminary studies suggested *in vitro* antioxidant activity of this plant [49]. Consequently, the intention of this study was to investigate the neuroprotective effect of ethanolic extract of PR (EEPR) fruits on aluminium-induced rats of cognitive impairment and oxidative stress by behavioral tests such as Passive Avoidance (PA) test, Rewarded Alternation (RA) test and the activity of brain antioxidant enzymes by biochemical tests such as superoxide dismutase (SOD), catalase (CAT), estimation of contents of thiobarbituric acid reactive substances (TBARS) and acetylcholinesterase (AChE) activity in rat brain tissue homogenates.

2. Materials and Methods

2.1. Chemicals and Drugs

Aluminium maltolate, phenazinemethosulphate, sodium pyrophosphate, nicotinamide adenine dinucleotide phosphate (NADPH), acetyl thiocholine iodide (ATCI), 5,5-dithiobis-2-nitrobenzoate ion (DTNB), trisamino methane hydrochloride (Tris-HCl), bovine serum albumin (BSA), trichloroacetic acid (TCA) and thiobarbituric acid (TBA) all were purchased from Sigma-Aldrich, USA. All other chemicals were of analytical grade, unless otherwise specified and purchased from indigenous sources. Donepezil hydrochloride powder was obtained as gift from Incepta Pharmaceuticals Ltd., Bangladesh.

2.2. Collection and Identification of Plant Materials

The fruits of PR were collected from the Kasba, Brahmanbaria, Bangladesh, in June, 2015. The identification of the plant was done by the expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. Accession number: DACB-41509 for PR.

2.3. Drying and Grinding of Plant Materials

The fruits of PR weighing of about 6 kg were washed appropriately to remove dirty materials and shade dried for 30 min. Then the fruits were permitted to shade dried for several days with irregular sun drying. Afterward, these were dried in an oven for 24 hrs at considerably lower temperatures for the purpose of better grinding. By using a suitable grinder the dried fruits were ground into coarse powder.

2.4. Extraction of Plant Materials

Powdered plant material (fruits) weighing of about 450 g was taken in an amber colored glass bottle and soaked in 2 liter of 98% ethanol. The bottle with its contents was sealed and allowed to occasional shaking and stirring at room temperature (25˚C) over a period of 7 days. After 7 days ethanol containing the extract was filtered through cotton and then through Whatman No. 1 filter paper. After finishing the filtration the obtained liquid filtrates of the extract was permitted to concentrate and evaporate by drying at 45˚C temperature using a rotary evaporator under reduced pressure to become the crude extract (11.47 g). Lastly dried crude ethanolic fruit extracts were kept in refrigerator at 4˚C until further experiments.

2.5. Animals

In this experiment 50 healthy adult male Swiss albino rats of about 200 - 230 g was acquired from ICDDR,B, Dhaka, Bangladesh. The rats were kept in 6 per animal polypropylene cage and located under standard environmental conditions (25˚C ± 2˚C temperature, 60% ± 5% relative humidity) with lighting (light/dark 12:12 hrs) and sufficient supply of food and water. The animals use and care was maintained as per guidelines for laboratory animals of the National Institutes of Health (NIH) [50]. The protocol of the experiment was approved by the animal ethics committee of the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

2.6. Administration of Drugs and Test Compounds

A solution of donepezil hydrochloride was prepared by using 0.9% saline solution (pH 7.4) and allowed to administer orally to experimental rats at 1 mg/kg body weight (b.w.). Aluminium maltolate was dissolved in 0.9% saline solution (pH 7.4) and administered orally at the dose of 10 mg/kg b.w. for one month to rats. The suspension of EEPR was made by using 0.9% saline solution (pH 7.4) and orally administered at 100 and 200 mg/kg for one month to rats. The duration of this study and doses of the donepezil, aluminium maltolate and EEPR we reselected according to the literature review [46] [51] [52]. Suspension of the extract, donepezil and aluminium maltolate were freshly prepared everyday and administered once daily at 10:00 am.

2.7. Experimental Design

In this experiment rats were divided into six groups and each group contains 6 rats as follows:

Group 1: Standard food and water were administered for one month to rats (Con).

Group 2: Aluminium maltolate at a dose of 10 mg/kg b.w. was administered orally for one month to rats (Alu).

Group 3: Donepezil hydrochloride at a dose of 1 mg/kg b.w. was administered orally for one month to rats (Don).

Group 4: Aluminium maltolate at a dose of 10 mg/kg b.w. + Plant extract at a dose of 100 mg/kg b.w. were administered orally for one month to rats (Alu + EEPR 100).

Group 5: Aluminium maltolate at a dose of 10 mg/kg b.w. + Plant extract at a dose of 200 mg/kg b.w. were administered orally for one month to rats (Alu + EEPR 200).

Group 6: Aluminium maltolate at a dose of 10 mg/kg b.w. + Donepezil hydrochloride at a dose of 1 mg/kg b.w. were administered orally for one month to rats (Alu + Don).

2.8. Acute Toxicity Study

Acute toxicity study was accomplished as per guidelines of the Organisation for Economic Cooperation and Development (OECD) [53]. For this test rats were separated into 6 groups, with 6 rats per groups. The fruit extract was administered orally to rats only once at a dose of 100, 200, 500, 1000, 1500 and 2000 mg/kg b.w. by using intragastric tube. Before administration of the extracts rats were fasted for 3 to 4 hrs and after administration food was withdraw for 1 to 2 hrs. But only water was supplied continuously. The rats were closely observed for next 24 hrs for any behavioral, neurological toxicity and 14 days for possible mortality.

2.9. Behavioral Study

Before treatment the rats were trained for 1 week to familiarize with the apparatus and in this period they did not receive any plant extract or drug. Experiments were performed in the light period between 10:00 am and 03:00 pm in a soundproof room.

2.9.1. Passive Avoidance (PA) Test

The PA test was performed for the determination of the sensitive memory of rats depends on contextual fear conditioning learning and instrumental learning [54]. The apparatus of PA test was made up of light and dark compartment, each measuring 270 (depth) × 370 (width) × 360 (height) mm. In the middle part of the two compartments of this apparatus were linked by a sliding door having 90 mm of diameter. The floor of this apparatus was made up of a metal grid with spaced 0.9 cm separately and joined to a shock generator able to generate shock in the range of 0.5 mA. Fluorescent lamp was used to provide lighting in the light compartment [55]. Each test comprises of two distinct trials including acquisition trial and retention trial. For the acquisition trial, each rat was positioned in the light compartment fronting the wall opposed to the sliding door. The sliding door was opened and when the rat entered into the dark compartment an electrical foot shock was provided for 3 sec after adaptation period of 15 sec [56]. The latency times, once the rat had entered the dark compartment was recorded as initial transfer latency (ITL) with the help of stopwatch. Then the rat was returned to its home cage. A retention trial was carried out after 24 hrs of the acquisition trial, in which no shock was given when the rat entered the dark compartment and latency times to re-enter the dark chamber was considered as step-through latency (STL) up to 300 sec [45]. In this study the number of ITL and STL were determined on 29th and 30th day respectively. Based on ITL and STL the percentage of memory retention (MR) was calculated by using the formula given below:

$$\% \text{ MR} = (\text{STL} - \text{ITL})/\text{ITL} \times 100$$

An increase in percentage of MR indicated improved retention of memory [57]. The apparatus was cleaned after each test with 70% ethanol to remove any olfactory clue [58].

2.9.2. Rewarded Alternation (RA) Test

The RA test was carried out for the determination of the spatial working memory of rats [59]. The apparatus of RA test was made up of three identical arms, each measuring 500 (length) × 100 (width) × 100 (height of the side walls) mm. The arms were linked by a central square in the middle of the maze so as to form a T shape. These three arms were denoted as start arm, force arm and novel arm. During the test, each rat was subjected to 6 trials and each test comprises of two separate trials including forced run trial and a choice run trial. For the forced run trial, novel arm was blocked and each rat was positioned in the start arm facing toward the central square and forced to the force arm owing to consume the pellet located previously. After that the rat was returned to its home cage. A choice run trial was carried out after 60 sec of the forced run trial, in which novel arm was opened (*i.e.*, both the arms were free for the rat to choose). In this choice run trial, force arm was kept empty and pellets were positioned in the novel arm. During the choice run trial, if the rat entered into the novel arm, then the response was measured as correct response (CR). If the rats entered into the force arm, then it was considered as a wrong response (WR) [60] [61]. In this study the number of CR and WR were determined on 30th day. Based on CR and WR the percentage of MR (*i.e.*, learned task) was calculated by using the formula given below:

$$\% \text{ MR} = \text{TCRs} \times 100/\text{TTs}$$

where, TCRs = Total number of correct responses, TTs = Total number of trials. An increase in percentage of MR was considered as an index of improved cognition [60] [61]. The apparatus was cleaned after each test with

70% ethanol to remove any olfactory clue [58].

2.10. Biochemical Study

After 30th days of treatment period on the next day, with the help of anesthesia the rats from all the experimental groups were sacrificed. The entire brain was detached from the skull and then cerebellum was separated afterward remaining brain portion (*i.e.*, brain portion without cerebellum) was washed with ice-cold 0.9% NaCl and finally each hemisphere was separated. Then by using one of the two hemispheres, a 10% brain homogenate was made by using ice-cold 30 mM phosphate buffer (pH 7.6) in a homogenizer. The homogenates were permitted to centrifuge at 20000 RPM for 30 min at 4˚C to get homogenates which were free from any types of cell debris and the resultant supernatant was used for the estimation of SOD and CAT. Residual hemispheres were homogenized (10% w/v) by using a glass homogenizer in ice-cold 30 mM phosphate buffer (pH 7.6) and allowed to centrifuge at 20000 RPM for 2 hrs at 4˚C to get the salt soluble (SS) portion. The pellets were re-extracted with an equivalent volume of ice-cold phosphate buffer comprising 1% Triton X-100 and permitted to centrifuge at 20000 RPM for 2 hrs at 4˚C to get the detergent soluble (DS) portion [62]. For determining the AChE activity supernatant obtaining from both extraction processes were stored at −20˚C. The protein concentration was measured with the help of bovine serum albumin (BSA) [63].

2.10.1. Super Oxide Dismutase (SOD) Assay

The SOD activity was determined according to the method of Kakkar *et al*., [64]. The total volume of the reaction mixture for this test was 1.6 ml, contained 0.1 ml of 186 µM phenazinemethosulphate, 1.2 ml of 0.052 mM sodium pyrophosphate buffer (pH 7.0) and 0.3 ml of supernatant after centrifugation (1500 × g, 10 min followed by 10000 × g, 15 min) of 10% brain tissue homogenate. In order to start enzyme reaction 0.2 ml of 780 µM NADH was added to the reaction mixture. The enzyme reaction was stopped by adding 1 ml of glacial acetic acid after 1 min incubation period. The changes in absorbance of the reaction mixture were determined at 560 nm by the help of spectrophotometer and represented as U/mg protein.

2.10.2. Catalase (CAT) Assay

The CAT activity was determined according to the method of Chance and Maehly with slight modification [65]. For this test the total volume of the reaction mixture was 3.0 ml, contained 2.5 ml of 50 mM phosphate buffer (pH 5.0), 0.4 ml of 5.9 mM hydrogen peroxide and 0.1 ml of 10% brain tissue homogenate. Then the reaction mixture was allowed to incubate for 1 min and subsequently by the help of spectrophotometer the changes in absorbance of the reaction mixture was measured at 240 nm. Here one unit of CAT activity was denoted as an absorbance change of 0.01 as U/min.

2.10.3. Lipid Peroxidation (TBARS) Assay

The TBARS activity was determined according to the method of Iqbal *et al*., [66]. The total volume of the reaction mixture was 1.0 ml, made up of 0.58 ml of 0.1 M phosphate buffer (pH 7.4), 0.2 ml of 100 mM ascorbic acid, 0.02 ml of 100 mM ferric chloride and 0.2 ml of 10% brain tissue homogenate. The reaction mixture was permitted to incubate at 37˚C in a shaking water bath for 1 hrs. Then 1.0 ml of 10% TCA was added to discontinue the reaction. Subsequently the addition of 1.0 ml 0.67% TBA, all the test tubes was boiled in a water-bath for 20 min. Then the test tubes were transferred to crushed ice-bath before centrifuging (2500 × g for 10 min). The quantity of TBARS formed in each of the samples was measured by determining the optical density of the supernatant at 535 nm by the help of spectrophotometer against a reagent blank and represented as nM TBARS/min/mg protein at 37˚C using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1} \cdot cm^{-1}$.

2.10.4. Acetylcholinesterase (AChE) Assay

The AChE activity was determined according to the method of Ellman *et al*., [67]. For this test, 25 µl of 15 mM ATCI, 75 µl of 3 mM DTNB and 75 µl of 50 mM Tris-HCl (pH 8.0), containing 0.1% BSA were added in the 96 well plates and incubated for 5 min at 25˚C. Then the absorbance was measured at 405 nm by using spectrophotometer. Any increase in the absorbance on account of the regular hydrolysis of the substrate was regulated by deducting the rate of the reaction prior to adding the enzyme. Afterward 25 µl of brain tissue homogenates (*i.e.*, SS and DS portion) was added and the absorbance was measured again after incubation period of 5 min at 25˚C. The AChE activity was represented as M/min/g protein.

2.11. Statistical Analysis

The results were expressed as mean ± SEM and analyzed with one-way analysis of variance (ANOVA). Tukey's post hoc test were performed for behavioral studies and in case of biochemical studies the least significant difference (LSD) was determined using post hoc testing for inter group comparisons at a probability level of 0.05% and 0.01%. SPSS 14.0 (Chicago, IL, USA) and Microsoft Excel 2010 (Roselle, IL, USA) was used for the statistical and graphical evaluations. The results were considered as statistically significant at $P < 0.05$ compared to disease control group.

3. Results

3.1. Determination of Acute Toxicity

EEPR up to a dose level of 2000 mg/kg b.w. had no harmful effect on the behavioral, motor and neuronal reactions of the experimental rats up to 14 days of observation. Diverse doses of EEPR exhibited that there were no signs of alters in the skin, eyes, fur and body weight therefore the extracts were considered safe.

3.2. Nootropic Effect of EEPR on Learning and Memory of Rats Using PA Test

In PA test the ITL was measured on 29th day and STL was measured on 30th day (after 24 hrs of ITL) specified in **Figure 1**. The rats treated with EEPR fruit (*i.e.*, 100 and 200 mg/kg, b.w.) significantly ($P < 0.05$, $P < 0.01$) increased the STL in rats on 30th day as compared to disease control group. Treatment with donepezil suggestively ($P < 0.01$) increased the STL of rats on 30th day with respect to disease control group. Percentage of MR of rats is given in **Figure 2** in which an increase in MR indicated improved learning and memory of rats. The percentage of MR was pointedly ($P < 0.05$) increased in rats treated with EEPR fruit (*i.e.*, 200 mg/kg b.w.) as compared with disease control group.

3.3. Nootropic Effect of EEPR on Learning and Memory of Rats Using RA Test

In RA test, the number of WR and CR were measured on 30th day given in **Figure 3**. Administration of donepezil considerably ($P < 0.01$) increased the number of CR on 30th day related to disease control group. EEPR fruit

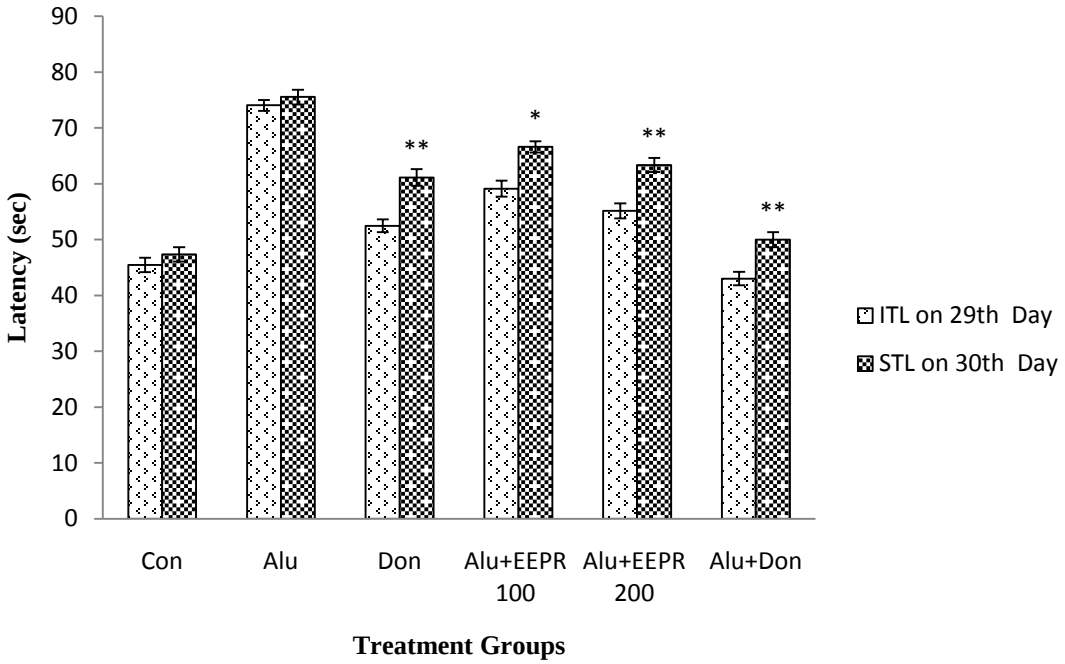

Figure 1. Nootropic Effect of EEPR on ITL and STL of rats on 29th and 30th day using PA test. Values were expressed as mean ± SEM (n = 6/group). $^{*}P < 0.05$, $^{**}P < 0.01$ significant difference from the disease control group.

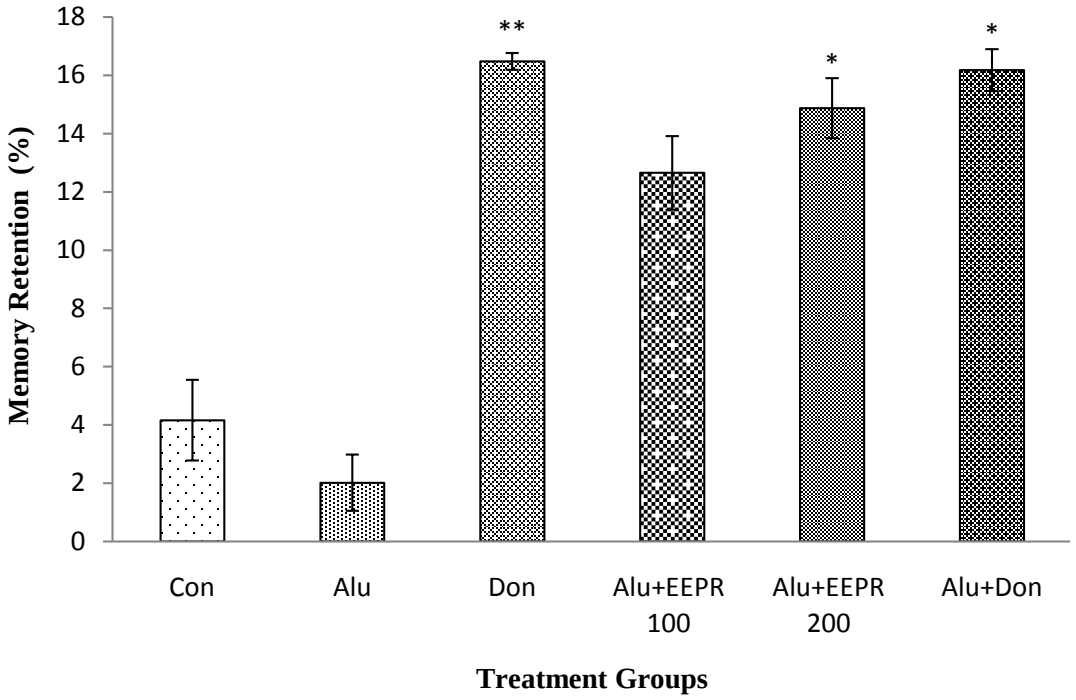

Figure 2. Nootropic Effect of EEPR on percentage of MR of rats using PA test. Values were expressed as mean ± SEM (n = 6/group). $^{*}P < 0.05$, $^{**}P < 0.01$ significant difference from the disease control group.

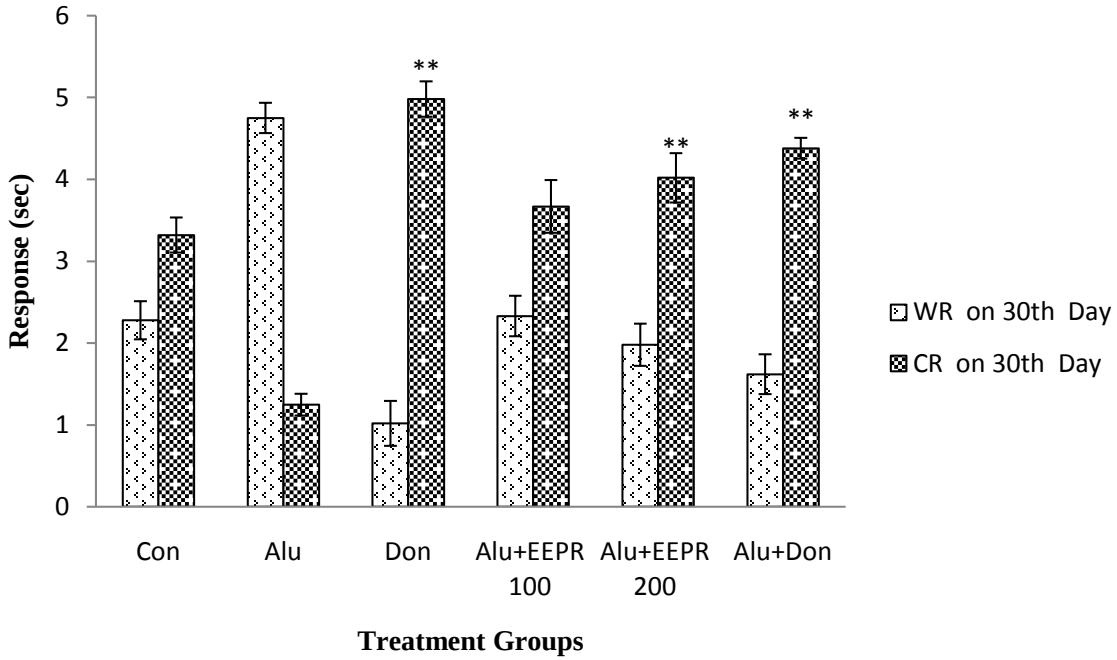

Figure 3. Nootropic Effect of EEPR on WR and CR of rats on 30th day using RA test. Values were expressed as mean ± SEM (n = 6/group). $^{*}P < 0.05$, $^{**}P < 0.01$ significant difference from the disease control group.

(*i.e.*, 200 mg/kg b.w.) considerably ($P < 0.01$) increased the number of CR on 30th day with respect to disease control group. **Figure 4** presented the percentage of MR of rats in which EEPR fruit (*i.e.*, 100 and 200 mg/kg b.w.) treated rats showed considerably ($P < 0.05$, $P < 0.01$) increased in the percentage of MR as compared to disease control group.

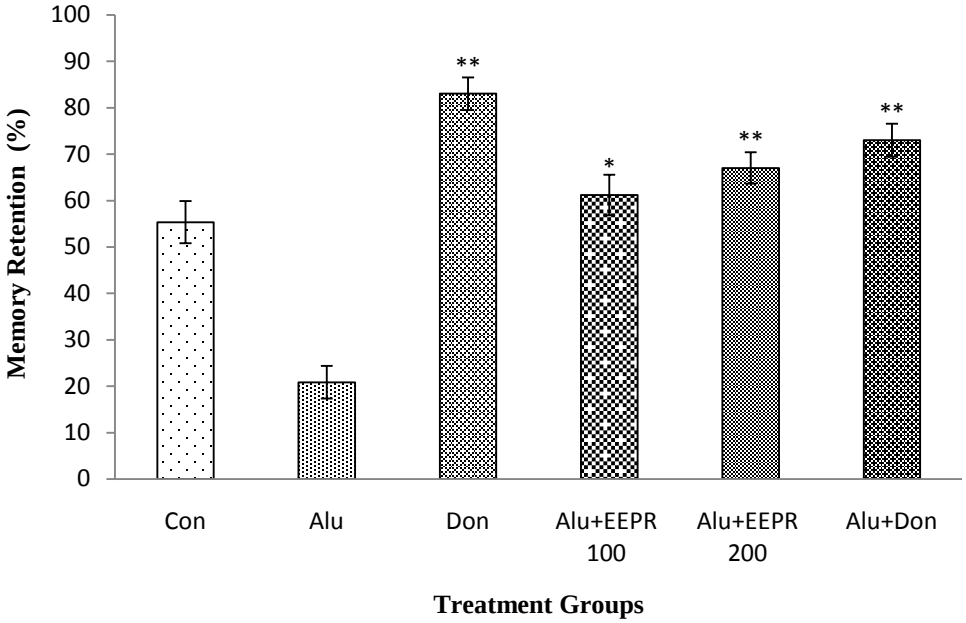

Figure 4. Nootropic Effect of EEPR on percentage of MR of rats using RA test. Values were expressed as mean ± SEM (n = 6/group). $^{*}P < 0.05$, $^{**}P < 0.01$ significant difference from the disease control group.

3.4. Nootropic Effect of EEPR on Brain Oxidative Status

Table 1 represents the alteration of antioxidant enzyme activities in rat's brain tissue homogenates. The rats treated with EEPR fruit (*i.e.*, 100 and 200 mg/kg b.w.) significantly ($P < 0.05$, $P < 0.01$) increased the level of SOD and CAT but markedly ($P < 0.05$) reduced the concentration of TBARS to that of the disease control group. Treatment with donepezil considerably ($P < 0.05$, $P < 0.01$) increased the level of SOD and CAT as well as meaningfully ($P < 0.05$, $P < 0.01$) decreased the level of TBARS with respect to the disease control.

3.5. Nootropic Effect of EEPR on Brain AChE Activity

The activity of AChE in SS and DS portion of rat brain tissue homogenate is given in **Table 2**. Donepezil treated group showed significantly ($P < 0.05$, $P < 0.01$) decreased in the brain AChE activity in both SS and DS portions of brain tissue homogenate with respect to disease control group. Administration of EEPR fruit (*i.e.*, 100 and 200 mg/kg b.w.) suggestively ($P < 0.05$, $P < 0.01$) decreases the AChE activity in both SS and DS portions of brain tissue homogenate of rats as compared to disease control group.

4. Discussion

Natural nootropics are increasing in popularity owing to preference of the people [68]. Worldwide at present there is an incredible urge to investigate medicinal plants for improving cognitive function in order to their less adverse effects [69]. This is the first study showing neuroprotective activity of EEPR in aluminium-induced rats of cognitive impairment and oxidative stress by using various behavioral and biochemical studies. In this study, EEPR administration for 30 days showed significant neuroprotective effect by improving various types of memory, learning, antioxidant enzymes and anti-acetylcholinesterase activity in rats.

The PA test is commonly known as fear-aggravated test which is used to assess learning and memory [70]. In PA test, rats learn to avoid an environment in which an aversive stimulus (*i.e.*, foot-shock) was previously provided. In this test the measured parameters were ITL and STL. The latency times, once the rat had entered the dark compartment was recorded as ITL as definite earlier. The STL is a measure of the memory of the aversive experience [45]. The mean STL of rats treated with the EEPR were ominously higher than those of the other groups. In the study of effect of *Aronia melanocarpa* fruits juice on memory in rats, Valcheva-Kuzmanova *et al.*, also reported analogous findings [71]. The RA test is used to assess learning and memory based on alteration of

Table 1. Nootropic Effect of EEPR on biochemical parameters of rat brain antioxidant defense system.

Treatment	SOD (U/mg protein)	Cat (U/min)	TBARS (nM/min/mg protein)
Con	9.57 ± 1.62	10.25 ± 1.13	204.58 ± 4.03
Alu	6.89 ± 2.24	7.32 ± 2.05	294.76 ± 5.64
Don	26.57 ± 1.30**	23.98 ± 3.58*	132.59 ± 4.37*
Alu + EEPR 100	12.67 ± 2.96*	11.04 ± 1.39*	198.62 ± 6.79*
Alu + EEPR 200	14.54 ± 3.45*	14.58 ± 1.42**	182.98 ± 6.97*
Alu + Don	18.95 ± 1.28*	19.07 ± 2.71**	174.8 ± 5.74**

The rats brain biochemical parameters were expressed as mean ± SEM values (n = 6/group). *P < 0.05, **P < 0.01 significant difference from the disease control group.

Table 2. Nootropic Effect of EEPR on AChE activity in rat brain.

Treatment	SS AChE (M/min/g protein)	DS AChE (M/min/g protein)
Con	0.195 ± 0.052	0.772 ± 0.016
Alu	0.303 ± 0.013	1.216 ± 0.031
Don	0.085 ± 0.020*	0.296 ± 0.027*
Alu + EEPR 100	0.188 ± 0.042*	0.812 ± 0.067*
Alu + EEPR 200	0.153 ± 0.021**	0.595 ± 0.064*
Alu + Don	0.106 ± 0.047*	0.406 ± 0.076**

The AChE activity for each group were expressed as mean ± SEM values (n = 6/group). *P < 0.05, **P < 0.01 significant difference from the disease control group.

the arms entry [72]. In RA test the measured parameters were WR and CR. Increased the number of CRs indicated that the improvement of the learning and memory of rats. In the current study, improvement of learning and memory was reported by EEPR. Sharma *et al.*, in the study of neuroenhancing potentiality of *Acacia auriculiformis* leaves, monitored better learning and memory enhancing potentiality in rats [73].

Metabolism of oxygen is greatly responsible for producing superoxide and if not controlled causes many types of cell damage [74]. SOD is a metalloenzyme that exert protection in cells exposed to oxygen. It catalyzes the dismutation or partitioning of the superoxide ($O^{2-}\cdot$) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) [75]. Hydrogen peroxide is also harmful, but not more so and is degraded by other enzymes including CAT. CAT is a haem-based enzyme in defensive the cell from oxidative damage by reactive oxygen species (ROS) [76]. It catalyzes the transformation of H_2O_2 to H_2O and O_2, thus shield cells from the noxious effects of H_2O_2 [77]. Study suggested that in each minute one molecule of CAT can convert approximately 5 million H_2O_2 to H_2O and O_2 [78]. Presently it has become evident that the oxidation of lipids, or lipid peroxidation, is a critical step in the pathogenesis of numerous disease states in all age's patients [79]. Lipid peroxidation is the by-product of oxidative degradation of lipids by the effect of various ROS (hydroxyl radical, hydrogen peroxide etc.) [80]. It is the process in which free radicals steal electrons from the lipids in cell membranes and finally causes cell damage. This process continues by a free radical chain reaction mechanism. It most often affects PUFAs and initiating a self-propagating chain reaction [81]. Since lipid peroxidation is a self-propagating chain-reaction, the initial oxidation of only a few lipid molecules can result in significant tissue damage [82]. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues [83]. The current study showed that administration of EEPR pointedly increases the level of brain antioxidant enzymes and decrease the levels of TBARS. In the study of nootropic activity of aerial parts of *Persicaria flaccida* on brain antioxidant markers and cognitive performance of rats by Uddin *et al.*, also reported equivalent results [84].

AChE is the major cholinesterase in the body [85]. It is an exzyme of carboxylesterase family that catalyzes the breakdown of ACh and of some other choline esters that function as neurotransmitters. AChE is available in primarily neuromuscular junctions and in chemical synapses of the cholinergic type [86]. The results of this study exposed that AChE activity was significantly decreased in the EEPR treated rats. In the study of neuro-

protective effect of *Phyllanthus acidus* fruits on learning and memory impairment in scopolamine-induced animal model of dementia and oxidative stress, Uddin *et al.*, disclosed increases brain acetylcholine levels and enhances cognitive function in rats [87].

The consequence of this study suggested that administration of EEPR for 30 days produced superior nootropic effect and reversed aluminium-induced cognitive dysfunction and oxidative stress in rats.

5. Conclusion

This study concludes that EEPR fruit has a potential nootropic effect on altering the aluminium-induced cognitive dysfunction and oxidative stress in rats brain by improving cognitive functions, brain antioxidant enzymes and anti-acetylcholinesterase activity. Therefore, this fruit extract can be used in controlling neurodegenerative diseases more precisely AD. Despite these outcomes, further studies are required for isolation and identification of promising nootropic compound(s) and disclose the possible mechanism of action.

Acknowledgements

The authors wish to thank the Department of Pharmacy, Southeast University, Dhaka, Bangladesh for providing research facilities.

Ethical Approval

The study protocol was approved by the ethics committee of the Department of Pharmacy, Southeast University, Dhaka, Bangladesh. The care and use of the animals were followed in accordance with the principles of NIH.

Authors' Contributions

This work was carried out in collaboration among all authors. MSU designed the study, wrote the protocol and managed the analyses of the study. MSU, AAM and MAI performed the laboratory experiments and prepared the draft of the manuscript. AI and MFH prepared the plant extract and performed literature review. SK performed the statistical analysis. MR reviewed the scientific contents of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors proclaim that they have no competing interests.

References

[1] Rouch, S., Dorey, J.-M., Boublay, N., Henaff, M.-A., Dibie-Racoupeau, F., Makaroff, Z., *et al*. (2014) Personality, Alzheimer's Disease and Behavioural and Cognitive Symptoms of Dementia: The PACO Prospective Cohort Study Protocol. *BMC Geriatrics*, **14**, 1-10.

[2] Gaugler, J.E., Ascher-Svanum, H., Roth, D.L., Fafowora, T., Siderowf, A. and Beach, T.G. (2013) Characteristics of Patients Misdiagnosed with Alzheimer's Disease and their Medication Use: An Analysis of the NACC-UDS Database. *BMC Geriatrics*, **13**, 1-10. http://dx.doi.org/10.1186/1471-2318-13-137

[3] Serrano-Pozo, A., Frosch, M.P., Masliah, E. and Hyman, B.T. (2011) Neuropathological Alterations in Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*, **1**, Article ID: a006189. http://dx.doi.org/10.1101/cshperspect.a006189

[4] McGleenon, B.M., Dynan, K.B. and Passmore, A.P. (1999) Acetylcholinesterase Inhibitors in Alzheimer's Disease. *British Journal of Clinical Pharmacology*, **48**, 471-480. http://dx.doi.org/10.1046/j.1365-2125.1999.00026.x

[5] Francis P.T., Palmer, A.M., Snape, M. and Wilcock, G.K. (1999) The Cholinergic Hypothesis of Alzheimer's Disease: A Review of Progress. *Journal of Neurology, Neurosurgery & Psychiatry*, **66**, 137-147. http://dx.doi.org/10.1136/jnnp.66.2.137

[6] Goverdhan, P., Sravanthi, A. and Mamatha, T. (2012) Neuroprotective Effects of Meloxicam and Selegiline in Scopolamine-Induced Cognitive Impairment and Oxidative Stress. *International Journal of Alzheimer's Disease*, **2012**, 1-7. http://dx.doi.org/10.1155/2012/974013

[7] Hazzan, A.A., Ploeg, J., Shannon, H., Raina, P. and Oremus, M. (2015) Caregiver Perceptions Regarding the Measurement of Level and Quality of Care in Alzheimer's Disease. *BMC Nursing*, **14**, 1-7.

[8] Yang, H.-Q., Sun, Z.-K. and Chen, S.-D. (2012) Current Advances in the Treatment of Alzheimer's Disease: Focused on Considerations Targeting Aβ and Tau. *Translational Neurodegeneration*, **1**, 1-10. http://dx.doi.org/10.1186/2047-9158-1-21

[9] Bindu, A.H., Siddiqui, A. and Jeevani, T. (2011) Genetic and Degenerative Neurological Disorders—An Emphasis on Alzheimer's, the Mystery. *Journal of Genetic Syndromes & Gene Therapy*, **2**, 1-4.

[10] Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010) Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. *Pharmacognosy Reviews*, **4**, 118-126. http://dx.doi.org/10.4103/0973-7847.70902

[11] Markesbery, W.R. (1999) The Role of Oxidative Stress in Alzheimer Disease. *Archives of Neurology*, **56**, 1449-1452. http://dx.doi.org/10.1001/archneur.56.12.1449

[12] Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., *et al*. (2014) Oxidative Stress, Prooxidants, and Antioxidants: The Interplay. *BioMed Research International*, 1-12. http://dx.doi.org/10.1155/2014/761264

[13] Zhao, Y. and Zhao, B. (2013) Oxidative Stress and the Pathogenesis of Alzheimer's Disease. *Oxidative Medicine and Cellular Longevity*, 1-9. http://dx.doi.org/10.1155/2013/316523

[14] Cui, H., Kong, Y. and Zhang, H. (2012) Oxidative Stress, Mitochondrial Dysfunction, and Aging. *Journal of Signal Transduction*, 1-6. http://dx.doi.org/10.1155/2012/646354

[15] Perry, G., Cash, A.D., Mark, A. and Smith, M.A. (2002) Alzheimer Disease and Oxidative Stress. *Journal of Biomedicine and Biotechnology*, **2**, 120-123. http://dx.doi.org/10.1155/S1110724302203010

[16] Feng, Y. and Wang, X. (2012) Antioxidant Therapies for Alzheimer's Disease. *Oxidative Medicine and Cellular Longevity*, **2012**, Article ID: 472932. http://dx.doi.org/10.1155/2012/472932

[17] Lovell, M.A. and Markesbery, W.R. (2007) Oxidative DNA Damage in Mild Cognitive Impairment and Late-Stage Alzheimer's Disease. *Nucleic Acids Research*, **35**, 7497-7504. http://dx.doi.org/10.1093/nar/gkm821

[18] Hamilton, R.T., Bhattacharya, A., Walsh, M.E., Shi, Y., Wei, R., Zhang, Y., *et al*. (2013) Elevated Protein Carbonylation, and Misfolding in Sciatic Nerve from *db*/*db* and *Sod1*$^{-/-}$ Mice: Plausible Link between Oxidative Stress and Demyelination. *PLoS ONE*, **8**, e65725. http://dx.doi.org/10.1371/journal.pone.0065725

[19] Nuss, J.E., Amaning, J.K., Bailey, C.E., DeFord, J.H., Dimayuga, V.L., Rabek, J.P., *et al*. (2009) Oxidative Modification and Aggregation of Creatine Kinase from Aged Mouse Skeletal Muscle. *Aging*, **1**, 557-572. http://dx.doi.org/10.18632/aging.100055

[20] Huang, W.-J., Zhang, X. and Chen, W.-W. (2016) Role of Oxidative Stress in Alzheimer's Disease. *Biomedical Reports*, **4**, 519-522. http://dx.doi.org/10.3892/br.2016.630

[21] Ayala, A., Muñoz, M.F. and Argüelles, S. (2014) Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Medicine and Cellular Longevity*, **2014**, Article ID: 360438. http://dx.doi.org/10.1155/2014/360438

[22] Ozias, M.K., Carlson, S.E. and Levant, B. (2007) Maternal Parity and Diet (n-3) Polyunsaturated Fatty Acid Concentration Influence Accretion of Brain Phospholipid Docosahexaenoic Acid in Developing Rats. *Journal of Nutrition*, **137**, 125-129.

[23] Milkovic, L., Gasparovic, A.C. and Zarkovic, N. (2015) Overview on Major Lipid Peroxidation Bioactive Factor 4-Hydroxynonenal as Pluripotent Growth-Regulating Factor. *Free Radical Research*, **49**, 850-860. http://dx.doi.org/10.3109/10715762.2014.999056

[24] Leonora, J.L., Hoehr, N., Mazur, S.J., Dianov, G.L., Sentürker, S., Dizdaroglu, M., *et al*. (1999) Repair of Oxidative DNA Base Lesions Induced by Fluorescent Light Is Defective in Xeroderma Pigmentosum Group A Cells. *Nucleic Acids Research*, **27**, 3153-3158. http://dx.doi.org/10.1093/nar/27.15.3153

[25] Rahman, K. (2007) Studies on Free Radicals, Antioxidants, and Co-Factors. *Clinical Interventions in Aging*, **2**, 219-236.

[26] Shao, C., Xiong, S., Li, G.-M., Gu, L., Mao, G. and Markesbery, W.R. (2008) Altered 8-Oxoguanine Glycosylase in Mild Cognitive Impairment and Late-Stage Alzheimer's Disease Brain. *Free Radical Biology & Medicine*, **45**, 813-819. http://dx.doi.org/10.1016/j.freeradbiomed.2008.06.003

[27] Gella, A. and Durany, N. (2009) Oxidative Stress in Alzheimer Disease. *Cell Adhesion & Migration*, **3**, 88-93. http://dx.doi.org/10.4161/cam.3.1.7402

[28] Gandhi, S. and Abramov, A.Y. (2012) Mechanism of Oxidative Stress in Neurodegeneration. *Oxidative Medicine and Cellular Longevity*, **2012**, Article ID: 428010. http://dx.doi.org/10.1155/2012/428010

[29] Miu, A.C. and Benga, O. (2006) Aluminum and Alzheimer's Disease: A New Look. *Journal of Alzheimer's Disease*, **10**, 179-201.

[30] Exley, C. (2001) Aluminum and Alzheimer's Disease. *Journal of Alzheimer's Disease*, **3**, 551-552.

[31] Banks, W.A. and Kastin, A.J. (1985) Aluminum Alters the Permeability of the Blood-Brain Barrier to Some Non-Pep-

tides. *Neuropharmacology*, **24**, 407-412. http://dx.doi.org/10.1016/0028-3908(85)90025-5

[32] Struys-Ponsar, C., Kerkhofs, A., Gauthier, A., Soffie, M. and Van den Bosch de Aguilar, P. (1997) Effects of Aluminum Exposure on Behavioral Parameters in the Rat. *Pharmacology Biochemistry and Behavior*, **56**, 643-648. http://dx.doi.org/10.1016/S0091-3057(96)00515-1

[33] Platt, B., Fiddler, G., Riedel, G. and Henderson, Z. (2001) Aluminium Toxicity in the Rat Brain: Histochemical and Immunocytochemical Evidence. *Brain Research Bulletin*, **55**, 257-267. http://dx.doi.org/10.1016/S0361-9230(01)00511-1

[34] Kawahara, M., Kato, M. and Kuroda, Y. (2001) Effects of Aluminum on the Neurotoxicity of Primary Cultured Neurons and on the Aggregation of Beta-Amyloid Protein. *Brain Research Bulletin*, **55**, 211-217. http://dx.doi.org/10.1016/S0361-9230(01)00475-0

[35] Kawahara, M. (2005) Effects of Aluminum on the Nervous System and Its Possible Link with Neurodegenerative Diseases. *Journal of Alzheimer's Disease*, **8**, 171-182.

[36] Johnson, G.V. and Jope, R.S. (1986) Aluminum Impairs Glucose Utilization and Cholinergic Activity in Rat Brain *in Vitro*. *Toxicology*, **40**, 93-102. http://dx.doi.org/10.1016/0300-483X(86)90049-1

[37] Bondy, S.C., Guo-Ross, S.X. and Pien, J. (1998) Mechanisms Underlying the Aluminum-Induced Potentiation of the Pro-Oxidant Properties of Transition Metals. *Neurotoxicology*, **19**, 65-71.

[38] Pham-Huy, L.A., He, H. and Pham-Huy, C. (2008) Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science*, **4**, 89-96.

[39] Satyanarayana, U., Kumar, A.N., Naidu, J.N. and Prasad, D.K.V. (2014) Antioxidant Supplementation for Health—A Boon or a Bane? *Journal of Dr. NTR University of Health Sciences*, **3**, 221-230. http://dx.doi.org/10.4103/2277-8632.146595

[40] Chen, L.-E., Wu, F., Zhao, A., Ge, H. and Zhan, H. (2016) Protection Efficacy of the Extract of *Ginkgo biloba* against the Learning and Memory Damage of Rats under Repeated High Sustained +Gz Exposure. *Evidence-Based Complementary and Alternative Medicine*, **2016**, Article ID: 6320586.

[41] Aguiar, S. and Borowski, T. (2013) Neuropharmacological Review of the Nootropic Herb *Bacopa monnieri*. *Rejuvenation Research*, **16**, 313-326. http://dx.doi.org/10.1089/rej.2013.1431

[42] Singhal, A.K., Naithani, V. and Bangar, O.P. (2012) Medicinal Plants with a Potential to Treat Alzheimer and Associated Symptoms. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, **2**, 84-91. http://dx.doi.org/10.4103/2231-0738.95927

[43] Shalini, S. and Sunil, K. (2013) *Phyllanthus reticulatus* Poir—An Important Medicinal Plant: A Review of Its Phytochemistry, Traditional Uses and Pharmacological Properties. *International Journal of Pharmaceutical Sciences & Research*, **4**, 2528.

[44] Shruthi, S.D., Ramachandra, Y.L., Rai, S.P. and Jha, P.K. (2010) Pharmacognostic Evaluation of the Leaves of *Kirganelia reticulata* Baill. (Euphorbiaceae). *Asian and Australasian Journal of Plant Science and Biotechnology*, **4**, 62-65.

[45] Mandisa Kondlo Walter Sisulu National Botanical Garden (2010) *Phyllanthus reticulatus* Poir. http://www.plantzafrica.com/plantnop/phyllanthusret.htm

[46] Shalini, S., Sunil, K., Kumar, S., Sharma, S., Kumar, D., Kumar, T., *et al*. (2012) Pharmacognostic Study and Anti-Inflammatory Activity of *Phyllanthus reticulatus* Poir. Fruit. *Asian Pacific Journal of Tropical Disease*, **2**, S332-S335.

[47] Jamal, A.K., Yaacob, W.A. and Din, L.B. (2008) A Chemical Study on *Phyllanthus reticulatus*. *Journal of Physical Science*, **19**, 45-50.

[48] Begum, T., Rahman, S.M. and Rashid, A.M. (2006) Phytochemical and Biological Investigations of *Phyllanthus reticulatus*. *Dhaka University Journal of Pharmaceutical Sciences*, **5**, 21-23.

[49] Aswatha Ram, H.N., Shreedhara, C.S., Gajera, F.P. and Zanwar, S.B. (2008) *In Vitro* Free Radical Scavenging Potential of Methanol Extract of Entire Plant of *Phyllanthus reticulates* Poir. *Pharmacologyonline*, **2**, 440-451.

[50] National Research Council (2011) Guide for the Care and Use of Laboratory Animals. National Academies Press, Washington DC.

[51] Weon, J.B., Lee, J., Eom, M.R., Jung, Y.S. and Ma, C.J. (2014) The Effects of *Loranthus parasiticus* on Scopolamine-Induced Memory Impairment in Mice. *Journal of Evidence-Based Complementary & Alternative Medicine*, **2014**, Article ID: 860180.

[52] Nannepaga, J.S., Korivi, M., Tirumanyam, M., Bommavaram, M. and Kuo, C.-H. (2014) Neuroprotective Effects of *Bacopa monniera* Whole-Plant Extract against Aluminum-Induced Hippocampus Damage in Rats: Evidence from Electron Microscopic Images. *The Chinese Journal of Physiology*, **57**, 279-285. http://dx.doi.org/10.4077/CJP.2014.BAC221

[53] Organization for Economic cooperation and Development (2002) OECD Guidelines for the Testing of Chemicals: Acute Oral Toxicity—Acute Toxic Class Method. OECD Environment, Health and Safety Publications, Paris.

[54] Akar, F., Mutlu, O., Celikyurt, I.K., Ulak, G., Erden, F., Bektas, E. and Tanyeri, P. (2014) Zaprinast and Rolipram Enhances Spatial and Emotional Memory in the Elevated Plus Maze and Passive Avoidance Tests and Diminishes Exploratory Activity in Naive Mice. *Medical Science Monitor Basic Research*, **20**, 105-111. http://dx.doi.org/10.12659/MSMBR.891149

[55] Van der Staay, F.J., Schuurman, T., van Reenen, C.G. and Korte, S.M. (2009) Emotional Reactivity and Cognitive Performance in Aversively Motivated Tasks: A Comparison between Four Rat Strains. *Behavioral and Brain Functions*, **5**, 50. http://dx.doi.org/10.1186/1744-9081-5-50

[56] Wang, J., Wang, X., Lv, B., Yuan, W., Feng, Z., Weidong, M.I., *et al*. (2014) Effects of *Fructus akebiae* on Learning and Memory Impairment in a Scopolamine-Induced Animal Model of Dementia. *Experimental and Therapeutic Medicine*, **8**, 671-675. http://dx.doi.org/10.3892/etm.2014.1775

[57] Saadipour, K., Sarkaki, A., Alaei, H., Badavi, M. and Rahim, F. (2009) Forced Exercise Improves Passive Avoidance Memory in Morphine-Exposed Rats. *Pakistan Journal of Biological Sciences*, **12**, 1206-1211. http://dx.doi.org/10.3923/pjbs.2009.1206.1211

[58] Benchenane, K., Castel, H., Boulouard, M., Bluthe, R., Fernandez-Monreal, M., Roussel, B.D., *et al*. (2007) Anti-NR1 N-Terminal-Domain Vaccination Unmasks the Crucial Action of tPA on NMDA-Receptor-Mediated Toxicity and Spatial Memory. *Journal of Cell Science*, **120**, 578-585. http://dx.doi.org/10.1242/jcs.03354

[59] Sossin, W.S., Lacaille, J.-C., Castellucci, V.F. and Belleville, S. (2008) Progress in Brain Research: Essence of Memory. Elsevier, Amsterdam.

[60] Rao, M.K., Rao, M.S. and Rao, G.S. (2007) Treatment with *Centella asiatica* (Linn) Fresh Leaf Extract Enhances Learning Ability and Memory Retention Power in Rats. *Neurosciences*, **12**, 236-241.

[61] Deacon, R.M. and Rawlins, J.N. (2006) T-Maze Alternation in the Rodent. *Nature Protocols*, **1**, 7-12. http://dx.doi.org/10.1038/nprot.2006.2

[62] Morris, R. (1984) Developments of a Water-Maze Procedure for Studying Spatial Learning in the Rat. *Journal of Neuroscience Methods*, **11**, 47-60. http://dx.doi.org/10.1016/0165-0270(84)90007-4

[63] Kameyama, T., Nabeshima, T. and Kozawa, T. (1986) Step-down-Type Passive Avoidance- and Escape-Learning Method: Suitability for Experimental Amnesia Models. *Journal of Pharmacological Methods*, **16**, 39-52. http://dx.doi.org/10.1016/0160-5402(86)90027-6

[64] Bhaskar, M. and Chintamaneni, M. (2014) Investigating the role of *Eclipta alba* on Brain Antioxidant Markers, Cognitive Performance and Acetylcholinesterase Activity of Rats. *International Journal of Pharmaceutical and Phytopharmacological Research* (*eIJPPR*), **3**, 390-394.

[65] Chance, B. and Maehly, A.C. (1955) Assay of Catalase and Peroxidases. *Methods in Enzymology*, **11**, 764-775. http://dx.doi.org/10.1016/S0076-6879(55)02300-8

[66] Iqbal, M., Sharma, M.D., Zadeh, H.R., Hasan, N., Abdulla, M., Athar, M., *et al*. (1996) Glutathione Metabolizing Enzymes and Oxidative Stress in Ferric Nitrilotriacetate (Fe-NTA) Mediated Hepatic Injury. *Redox Report*, **2**, 385-391.

[67] Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. (1961) A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochemical Pharmacology*, **7**, 88-95. http://dx.doi.org/10.1016/0006-2952(61)90145-9

[68] Kumar, G.P. and Khanum, F. (2012) Neuroprotective Potential of Phytochemicals. *Pharmacognosy Reviews*, **6**, 81-90. http://dx.doi.org/10.4103/0973-7847.99898

[69] Kulkarni, R., Girish, K.J. and Kumar, A. (2012) Nootropic Herbs (Medhya Rasayana) in Ayurveda: An Update. *Pharmacognosy Reviews*, **6**, 147-153. http://dx.doi.org/10.4103/0973-7847.99949

[70] Ogren, S.O., Stone, W.S. and Altman, H.J. (1987) Evidence for a Functional Interaction between Serotonergic and Cholinergic Mechanisms in Memory Retrieval. *Behavioral and Neural Biology*, **48**, 49-62. http://dx.doi.org/10.1016/S0163-1047(87)90574-7

[71] Valcheva-Kuzmanova, S.V., Eftimov, M.T., Tashev, R.E., Belcheva, I.P. and Belcheva, S.P. (2014) Memory Effects of *Aronia melanocarpa* Fruit Juice in a Passive Avoidance Test in Rats. *Folia Medica*, **56**, 199-203. http://dx.doi.org/10.2478/folmed-2014-0029

[72] Savage, L.M., Hall, J.M. and Vetreno, R.P. (2011) Anterior Thalamic Lesions Alter both Hippocampal-Dependent Behavior and Hippocampal Acetylcholine Release in the Rat. *Learning & Memory*, **18**, 751-758. http://dx.doi.org/10.1101/lm.023887.111

[73] Sharma, A., Shetty, M., Parida, A., Adiga, S., Kamath, S. and Sowjanya (2014) Effect of Ethanolic Extract of *Acacia auriculiformis* Leaves on Learning and Memory in Rats. *Pharmacognosy Research*, **6**, 246-250.

http://dx.doi.org/10.4103/0974-8490.132605

[74] Salin, M. and McCord, J. (1975) Free Radicals and Inflammation. Protection of Phagocytosing Leukocytes by Superoxide Dismutase. *The Journal of Clinical Investigation*, **56**, 1319-1323. http://dx.doi.org/10.1172/JCI108208

[75] Ansari, M.A. and Scheff, S.W. (2010) Oxidative Stress in the Progression of Alzheimer Disease in the Frontal Cortex. *Journal of Neuropathology & Experimental Neurology*, **69**, 155-167. http://dx.doi.org/10.1097/NEN.0b013e3181cb5af4

[76] Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M. (2012) Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany*, **2012**, Article ID: 217037. http://dx.doi.org/10.1155/2012/217037

[77] Spitz, D.R., Adams, D.T., Sherman, C.M. and Roberts, R.J. (1992) Mechanisms of Cellular Resistance to Hydrogen Peroxide, Hyperoxia, and 4-Hydroxy-2-nonenal Toxicity: The Significance of Increased Catalase Activity in H_2O_2-Resistant Fibroblasts. *Archives of Biochemistry and Biophysics*, **292**, 221-227. http://dx.doi.org/10.1016/0003-9861(92)90071-4

[78] Aoyama, K. and Nakaki, T. (2015) Glutathione in Cellular Redox Homeostasis: Association with the Excitatory Amino Acid Carrier 1 (EAAC1). *Molecules*, **20**, 8742-8758. http://dx.doi.org/10.3390/molecules20058742

[79] Gammone, M.A., Riccioni, G. and D'Orazio, N. (2015) Marine Carotenoids against Oxidative Stress: Effects on Human Health. *Marine Drugs*, **13**, 6226-6246. http://dx.doi.org/10.3390/md13106226

[80] Powers, S.K. and Jackson, M.J. (2008) Exercise-Induced Oxidative Stress: Cellular Mechanisms and Impact on Muscle Force Production. *Physiological Reviews*, **88**, 1243-1276. http://dx.doi.org/10.1152/physrev.00031.2007

[81] Madhura, T.K. (2015) Role of Oxidative Stress in the Pathogenesis of OCD. *Biochemistry & Analytical Biochemistry*, **4**, 217.

[82] Sosa, R.A., Murphey, C., Robinson, R.R. and Forsthuber, T.G. (2015) IFN-γ Ameliorates Autoimmune Encephalomyelitis by Limiting Myelin Lipid Peroxidation. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, E5038-E5047.

[83] Mylonas, C. and Kouretas, D. (1999) Lipid Peroxidation and Tissue Damage. *In Vivo*, **13**, 295-309.

[84] Uddin, M.S., Nasrullah, M., Hossain, M.S., Rahman, M.M., Sarwar, M.S., Amran, M.S., *et al*. (2016) Evaluation of Nootropic Activity of *Persicaria flaccida* on Cognitive Performance, Brain Antioxidant Markers and Acetylcholinesterase Activity in Rats: Implication for the Management of Alzheimer's Disease. *American Journal of Psychiatry and Neuroscience*, **4**, 26-37. http://dx.doi.org/10.11648/j.ajpn.20160402.12

[85] Čolović, M.B., Krstić, D.Z., Lazarević-Pašti, T.D., Bondžić, A.M. and Vasić, V.M. (2013) Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. *Current Neuropharmacology*, **11**, 315-335. http://dx.doi.org/10.2174/1570159X11311030006

[86] Oren, M., Brikner, I., Appelbaum, L. and Levy, O. (2014) Fast Neurotransmission Related Genes Are Expressed in Non Nervous Endoderm in the Sea Anemone *Nematostella vectensis*. *PLoS ONE*, **9**, e93832. http://dx.doi.org/10.1371/journal.pone.0093832

[87] Uddin, M.S., Mamun, A.A., Hossain, M.S., Ashaduzzaman, M., Noor, M.A.A., Hossain, M.S., Uddin, M.J., Sarker, J. and Asaduzzaman, M. (2016) Neuroprotective Effect of *Phyllanthus acidus* L. on Learning and Memory Impairment in Scopolamine-Induced Animal Model of Dementia and Oxidative Stress: Natural Wonder for Regulating the Development and Progression of Alzheimer's Disease. *Advances in Alzheimer's Disease*, **5**, 53-72. http://dx.doi.org/10.4236/aad.2016.52005

Abbreviations

AD: Alzheimer's disease;
PR: *Phyllanthus reticulatus*;
EEPR: Ethanolic Extract of *Phyllanthus reticulatus*;
PA: Passive avoidance;
RA: Rewarded alternation;
SOD: Superoxide dismutase;
CAT: Catalase;
TBARS: Thiobarbituric acid reactive substances;
AChE: Acetylcholinesterase;
ITL: Initial transfer latency;
STL: Step-through latency;
MR: Memory retention;
CR: Correct response;
WR: Wrong response;
ROS: Reactive oxygen species;
NFTs: Neurofibrillary tangles;
Aβ: Amyloid-β;
ACh: Acetylcholine;
CCS: Central cholinergic system;
PUFAs: Polyunsaturated fatty acids;
HNE: 4-Hydroxynonenal;
8-OHdG: 8-Hydroxy-2-deoxyguanosine;
CNS: Central nervous system;
BBB: Blood-brain barrier;
Cr: Chromium;
Cu: Copper;
NIH: National institutes of health;
NADPH: Nicotinamide adenine dinucleotide phosphate;
ATCI: Acetyl thiocholine iodide;
DTNB: 5,5-Dithiobis-2-nitrobenzoate ion;
Tris-HCl: Trisfamino methane hydrochloride;
BSA: Bovine serum albumin;
TCA: Trichloroacetic acid;
TBA: Thiobarbituric acid;
SS: Salt soluble;
DS: Detergent soluble.