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Neurodefensive Effect of *Olea europaea* L. in Alloxan-Induced Cognitive Dysfunction and Brain Tissue Oxidative Stress in Mice: Incredible Natural Nootropic

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Abstract

Background: In the controlling of Alzheimer disease (AD) plant with antioxidant activity has attained considerable attention. The plant *Olea europaea* (OE) L. belongs to family Oleaceae is a rich source of natural antioxidant. Therefore the intention of this study was to analyze the neuroprotective effects of ethanolic extract of OE (EEOE) fruits in alloxan-induced cognitive impairment and brain tissue oxidative stress in mice by using Hole Cross (HC) test, Open Field (OF) test, Free Exploration (FE) test, Y-Maze (YM) test and contents of thiobarbituric acid reactive substances (TBARS) in brain tissue homogenates of mice.

Methods: EEOE fruits (200 and 400 mg/kg b.w.) were administered to alloxan-induced mice for 21 days. The neuroprotective effect of this fruit extracts were examined by using behavioral studies such as HC test, OF test, FE test, YM test and biochemical study such as lipid peroxidation (TBARS) assay.

Results: In HC test, administration of EEOE on 14th and 21st day was remarkably ($P < 0.05$, $P < 0.01$) increased the number of hole crossed from one chamber to another by mice as compared to the disease control group. Administration of EEOE significantly ($P < 0.01$) increased the number of square travelled by mice on 21st day with respect to that of disease control group in OF test. In FE test EEOE considerably ($P < 0.05$, $P < 0.01$) increased the number of entries to the novel area and time spent in the novel area of the mice on 7th, 14th and 21st day as compared to the disease control group. The administration of EEOE significantly ($P < 0.05$, $P < 0.01$) increased the percentage of spontaneous alternation behavior of the mice on 14th and 21st day as compared to that of disease control group in YM test. EEOE administration for successive days markedly ($P < 0.05$) decreased TBARS level in the brain tissue homogenates of mice with respect to disease control group.

Conclusion: The existing study suggests that EEOE fruit shows momentous neurodefensive activity. Consequently, this fruit extract may have impending therapeutic value in the treatment of some neurological disorders alike AD.

Keywords: Neurodefensive; Nootropic; *Olea europaea*; Cognition; Oxidative stress; Alzheimer´s disease

Abbreviations: AD: Alzheimer's Disease; QOL: Quality of Life; Aβ: β-amyloid; NFTs: Neurofibrillary Tangles; DM: Diabetes Mellitus; ROS: Reactive Oxygen Species; CNS: Central Nervous System; Alx: Alloxan; WHO: World Health Organization; OE: *Olea europaea*; EEOE: Ethanolic Extract of *Olea europaea*; HC: Hole Cross; OF: Open Field; YM: Y-Maze; FE: Free Exploration; TBARS: Thiobarbituric Acid Reactive Substances; OECD: Organization for Economic Cooperation and Development; NIH: National Institutes of Health.

Introduction

Dementia primarily Alzheimer's disease (AD) is a rising public health problem that consequences from ageing of the population [1]. This progressive, fatal neurodegenerative disorder is characterized by worsening in cognition and memory, progressive impairment in the ability to carry out activities of daily living, as well as a number of neuropsychiatric and behavioral symptoms [2]. AD obliterates brain cells, triggering difficulties with memory, thinking and behavior strong enough to affect work, family plus social relationships and ultimately the most basic quality of life (QOL) [3]. Brain regions predominantly the neocortex and hippocampus that are connected with greater mental activities are most influenced by the distinctive pathology of AD [4]. This comprises the extracellular deposits of β-amyloid (Aβ) in senile

plaques, intracellular formation of neurofibrillary tangles (NFTs) and the loss of neuronal synapses and pyramidal neurons [5-11]. These pathological alterations result in the progression of the characteristic symptomatology of AD characterized by progressive damages of cognitive function and frequently associated with behavioral difficulties such as depression, aggression and wandering [12]. In the United States, there are an enumerated 5.3 million cases of dementia and by 2050 this number is projected to rise to 18.5 million [13]. Due to the ageing population the number of those distressed is rising every year [14].

Diabetic mellitus (DM) is linked with pathological changes in the central nervous system (CNS) that lead to cognitive and moving deficits and also increased risk of brain vascular difficulties [15]. Numerous brain alterations have been found in diabetes of the animal models including increased hippocampal astrocytic reactivity, vascular changes, impaired synaptic plasticity, decreased dendritic complexity and disturbed neurotransmission [16]. Alloxan is a diabetogenic agent extensively used in diabetes research to induce insulin reduction after intra-peritoneal injection [17]. Alloxan acts by aggregating the pancreatic β-cells via the Glut2 glucose transporter and demolish them through reactive oxygen species (ROS) and free radicals mechanisms [18]. It has been recognized that oxidative stress plays an important role in the enhancement of diabetes complications, including learning and memory impairments as a result of the increased generation of free radicals and diminished antioxidant defenses [19]. These free radicals lead to increased neuronal death in several brain areas including the hippocampus, DNA damage, through protein oxidation and peroxidation of membrane lipids [20]. The oxidation of mitochondrial DNA and nuclear DNA has been found in the parietal cortex of AD patients along with in aged patients without AD [21]. Moreover, free radicals contribute the oxidation of proteins may be momentous in AD as the oxidation of brain proteins can attack enzymes that are very dangerous to neuron and glial functions [22].

To cure the disease development and progression medicinal plants and their ingredients are widely used since ancient time [23]. Plants are always considered as a principal origin of drugs in complementary and alternative system of medicine in different forms such as crude form, juice, decoction, crude extracts etc [24]. According to World Health Organization (WHO) about 80% people of the world, mainly in the rural areas of developing countries, continue using traditional resources in health care because they cannot afford the high cost of pharmaceuticals and health care as well as due to traditional medicines are more acceptable from a cultural and spiritual perspective [25,26]. Most of the clinically active drugs are either natural products or pharmacophore of the natural substance [27]. The phytoconstituents of medicinal plants contribute a pivotal role in the treatment of cognitive dysfunctions connected with age and neurodegenerative difficulties. Medicinal plants including *Ginko biloba*, *Bacopa monnieri* and *Huperzia serrata* have been extensively used for the treatment of AD [28,29]. By using the plant as an origin of medicine is as old as mankind [30].

Olea europaea (OE) L. belongs to the family Oleaceae is known in Bangali as Jolpai is a plant of medium to big size with simple leaves, small flowers in alxillary racemes. The fruit of this plant is ovoid, blackish-violet when ripe, typically 1 to 2.5 cm long [31]. Oleaceae family comprises 30 genera [32] and its families numbering about 600 species [33]. OE plant have been widely used in folk medicines throughout the world like in Spain, France, Italy, Greece, Israel, Morocco, Tunisia, India, Bangladesh, Turkey and the Mediterranean islands for centuries [34,35]. It has been therapeutically recognized to treat, asthma, hemorrhoids, rheumatism, stomach and intestinal diseases, diarrhea, respiratory and urinary tract infections, mouth cleanser and as a vasodilator [36]. Several experimental studies on the fruits and leaf extracts from OE observed that they have antioxidant, anti-inflammatory, antithrombolytic, antihypertensive, anticancer, hypoglycemic, antimicrobial and antiatherogenic activities [37]. This fruit is a great source of active components of phenols like secoiridoids such as oleuropein and demethyloleuropein, phenolic glycosides such as ligstroside and hydroxycinnamic acid derivatives such as verbascoside [38].

A preliminary study suggested that the constituents of OE have neuroprotective activity [39,40]. Thus the purpose of this current study was to investigate the neuroprotective effect of ethanolic extract of OE (EEOE) on learning and memory improvement in alloxan-induced mice of cognitive impairment and brain tissue oxidative stress via different behavioral studies, including the Hole Cross (HC) test, Open Field (OF) test, Free Exploration (FE) test, Y-Maze (YM) test and biochemical study such as thiobarbituric acid reactive substances (TBARS) assay.

Materials and Methods

Drugs and chemicals

Donepezil hydrochloride powder was the generous gift from Incepta Pharmaceuticals Ltd. (Dhaka, Bangladesh). Alloxan monohydrate was bought from Explicit Chemicals, Pvt. Ltd. (Pune, India). Blood samples analyzed to determine blood glucose level by using Ez Smart 168 (Tyson Bioresearch, Inc. Chu-Nan, Taiwan) glucose test meter. All other chemicals were purchased from indigenous sources and were of analytical grade.

Collection and identification of plant materials

The leaves of OE were collected from the Kurigram Sadar, Bangladesh, in March, 2016. The plant was recognized by the expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. Accession number: DACB-43251 for OE.

Drying and grinding of plant materials

The fruits of OE weighing 3 kg were rinsed properly to remove dirt materials and shade dried for 30 min. Then seeds were detached from the fruits and shade dried for several days with irregular sun drying. For better grinding then these were

dried in an oven for 24 hrs at noticeably lower temperatures. The dried fruits were ground into a coarse powder with the help of a suitable grinder.

Plants extract preparation

The powdered fruits material having a weight of about 400 g was taken in an amber colored glass bottle and soaked in 2 liter of 98% ethanol. The bottle with its contents were sealed and kept at room temperature and allowed to stand for 7 days with occasional shaking and stirring. After 7 days the extracts thus obtained were filtered through cotton and then through Whatman No.1 filter paper. After completing the filtration, the liquid filtrates obtained of the extract were concentrated and evaporated to dry at 45°C temperature with the help of a rotary evaporator under reduced pressure to get the crude extract (10.57 g). Finally dried crude ethanolic fruit extracts were stored in refrigerator at 4°C until further tests.

Administration of drug and test compounds

Normal saline (pH 7.4) was used to prepare a solution of donepezil hydrochloride and administered orally (p.o.) to mice at a dose of 1.5 mg/kg body weight (b.w.). The suspension of EEOE was prepared by adding normal saline (pH 7.4) and administered orally to mice. Alloxan monohydrates injection dissolved in a normal saline (0.9% NaCl) solution was injected to mice intraperitoneally (i.p.) after an 18 hrs fasting period at a dose of 150 mg/kg b.w. After 72 hrs of alloxan administration, blood samples were collected from the tip of the tail of the mice and measured using a blood glucometer. In case of all alloxan treated mice the blood glucose level was identical or more than 200 mg/dl which confirmed diabetic [41]. The doses of the donepezil hydrochloride, alloxan and EEOE were selected according to the literature review [42-44]. Suspension of the extract and the standard drug were freshly prepared every day and administered 30 min before tests.

Animals

For this experiment 45 healthy adult male Swiss albino mice of about 30-35 g was purchased from ICDDR,B, Dhaka, Bangladesh. The mice were housed in 6 per animal cage and placed under standard environmental conditions. The mice were fed with standard pellet diet supplied from the same institution. The use and care of animals was done by the guidelines for laboratory animals of the National Institutes of Health (NIH) [45]. The protocol of the experiment was approved in accordance with the animal ethics committee of the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

Experimental animals and protocol

In the experiment, a total of 36 mice were used. The mice were divided into six groups and each group contains six mice as follows:

Group 1: Standard pellet diet and water were administered to mice (Con)

Group 2: Donepazil hydrochloride (1.5 mg/kg b.w.; p.o.) was administered to mice (Don)

Group 3: Alloxan (150 mg/kg b.w.; i.p.) was administered to mice (Alx)

Group 4: Alloxan (150 mg/kg b.w.; i.p.) + Plant extract (200 mg/kg b.w.; p.o.) were administered to mice (Alx + EEOE 200)

Group 5: Alloxan (150 mg/kg b.w.; i.p.) + Plant extract (400 mg/kg b.w.; p.o.) were administered to mice (Alx + EEOE 400)

Group 6: Alloxan (150 mg/kg b.w.; i.p.) + Donepazil hydrochloride (1.5 mg/kg b.w.; p.o.) were administered to mice (Alx + Don)

Acute toxicity study

The guidelines of the Organisation for Economic Cooperation and Development (OECD) were used for acute toxicity study [46]. For this test mice were divided into 4 groups, with 6 mice per groups. By using normal saline the extracts suspension were prepared and administered to mice only once at a dose of 100, 200, 500 and 2000 mg/kg of b.w. with the help of intragastric tube. Mice were fasted for 3-4 hrs before administration of the extracts, but only water was supplied. For next 24 hrs mice were closely observed for any behavioral, neurological profiles and 14 days for mortality.

Behavioral study

Before treatment the mice 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.

Hole Cross (HC) test

The hole cross test was carried out according to the method of Takagi K et al., with slight modification [47]. The apparatus of hole cross test was consisted of a wooden box having a dimension of 30 cm × 20 cm × 14 cm, designed with a fixed partition in the middle of the box having a hole of 3 cm in diameter was prepared at a height of 7.5 cm from the floor. For this test, every mouse was positioned on one side of the apparatus. The spontaneous movement of the mice from one chamber to another through the hole was observed for 3 min on 7^{th}, 14^{th} and 21^{st} day. To eliminate any olfactory clue the apparatus was cleaned with 70% ethanol after each test [48].

Open Field (OF) test

The open field test was carried out according to the method of Gupta et al., with slight modification [49]. The floor of an open field apparatus was consisting of an opaque-plexiglas box having a dimension of 60 × 60 × 35 cm. The apparatus had a wall of 40 cm height. The open field area was divided into 16 square areas with alternatively white and black colored areas. During testing each mouse was positioned in the middle of the open field. Then the number of squares visited by the mice was counted for a period of 3 min on 7^{th}, 14^{th} and 21^{st} day. To

eliminate any olfactory clue the apparatus was cleaned with 70% ethanol after each test [48].

Free Exploration (FE) test

The free exploratory test was carried out according to the method of Hughes with slight modification [50]. The apparatus of free exploratory test was consisted of a polyvinyl chloride box having a dimension of 30 × 20 × 20 cm enclosed with plexiglas. The box was divided into 6 uniform square exploration units, these were all interconnected by small doors. A temporary divider separated the apparatus in half lengthwise. Before testing nearly 24 hrs the mice were allowed to explore the one half of the apparatus with the temporary divider in place. After 24 hrs the temporary partition was removed to facilitate the mouse to explore both the familiar and novel environments for a period of 10 min on 7^{th}, 14^{th} and 21^{st} day. In this test, the number of units entered to the novel side and the time spent in the novel side was recorded. To eliminate any olfactory clue the apparatus was cleaned with 70% ethanol after each test [48].

Y-Maze (YM) test

Spontaneous alternation behavior in the Y-maze test is used to determine the assessment of short-term memory of animal model [51]. The Y-maze apparatus was consist of three uniform arms (each arm was 40 cm long, 12 cm high, 5 cm wide at the bottom and 10 cm wide at the top) in which the arms were symmetrically separated at 120° and arms were joined in the middle equilateral triangle area in order to permit the mice to spontaneously enter into any of the arms. During testing each mice was positioned in one of the arm compartments and was permitted to move spontaneously for 8 min on 7^{th}, 14^{th} and 21^{st} day. An arm entry was counted when the hind paws of the mouse were entirely within the arm. An entry into all three arms on successive choices was called spontaneous alternation behavior. Then the sequence and number of arm entries were recorded manually. The percentage of spontaneous alternation behavior was calculated according to the following formula [52]:

Spontaneous alternation behavior (%) = Na/Nabc − 2 × 100%

Where, Na is the number of alternation and Nabc is total number of arm entries. To eliminate any olfactory clue the apparatus was cleaned with 70% ethanol after each test [48].

Biochemical study

After 21 days of treatment period on 22 day, by using anesthesia the mice from all the treatment groups were sacrificed. The whole brain was removed from the skull and then cerebellum was separated followed by washed with ice-cold 0.9% NaCl. Then by using remaining brain, a 10% brain homogenate was prepared with the help of ice-cold 30 mM phosphate buffer having pH 7.6. The homogenates were allowed to centrifuge at 20000 RPM for 30 min at 4°C to get crude homogenates, which were free from any kinds of cell debris and the resultant supernatant was used for the estimation of brain lipid peroxidation [53].

Lipid peroxidation (TBARS) assay

The levels of lipid peroxidation were measured by the thiobarbituric acid-reactive substances (TBARS) assay according to the method of Ohkawa et al., with minor modification [54]. Initially, the reaction mixture was made by adding 0.2 ml brain tissue homogenate with 0.2 ml of 8.1% sodium dodecylsulfate, 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid and 0.6 ml of distilled water. Then the reaction mixture was allowed to incubate for 1 hrs at 95°C in water bath. The reaction mixtures were cooled and diluted with 1.0 ml distilled water and a mixture of butanol:pyridine (15:1 v/v). Subsequently the reaction mixture was shaken and permitted to centrifuge at 4000 RPM for 10 min. The quantities of TBARS were estimated by spectrophotometry at 532 nm and were expressed as nM TBARS/min/mg protein at 37°C using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Statistical analysis

The results were expressed as mean ± SEM. To perform statistical analysis of the results, one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was used. All statistical analysis was performed by SPSS 19 software. Differences between groups were considered significant at the level of $P < 0.05$.

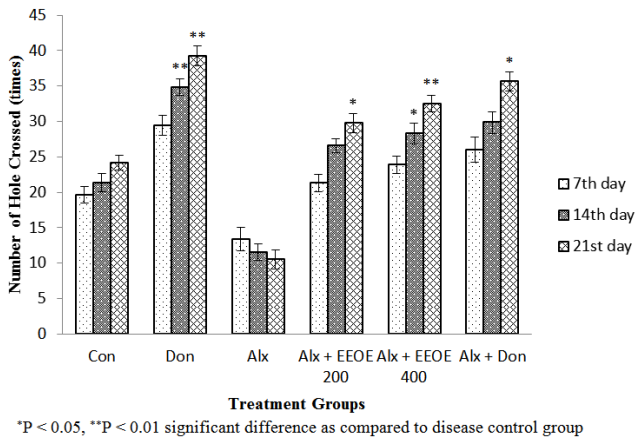
Results

Determination of acute toxicity

EEOE at a dose of 2000 mg/kg b.w. had no adverse effect on the behavioral, motor and neuronal responses of the tested mice up to 14 days of observation. Different doses of EEOE showed that there were no signs of changes in the skin, eyes, fur and body weight hence the extracts were considered safe.

Neurodefensive effect of EEOE on HC test in mice

The effect of EEOE on the movement activity of HC test is represented in **Figure 1**. The number of hole crossed from one chamber to another by mice was gradually increased on 7^{th}, 14^{th} and 21^{st} day for all treatment groups except alloxan treated group. The mice treated with donepazil remarkably ($P < 0.05$, $P < 0.01$) increased the number of hole crossed as compared to that of disease control mice on 14^{th} and 21^{st} day. Administration of EEOE significantly reversed alloxan treated cognitive dysfunctions in mice and increased ($P < 0.05$, $P < 0.01$) the number of hole crossed as compared to the disease control group on 14^{th} and 21^{st} day that indicates improvement of spatial memory and learning.

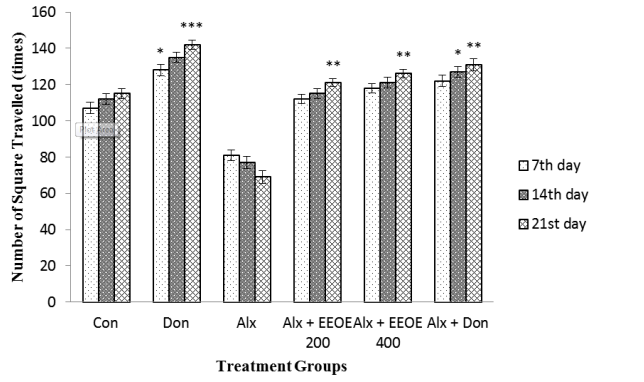


*P < 0.05, **P < 0.01 significant difference as compared to disease control group

Figure 1 Neurodefensive effect of EEOE on the number of hole crossed by mice exposed to HC test. Values were expressed as mean ± SEM (n = 6/group).

Neurodefensive effect of EEOE on OF test in mice

In **Figure 2** the effects of EEOE on the number of square travelled by mice in OF test is displayed. The number of square travelled by mice of the alloxan-induced group was lower that remaining groups on 7th, 14th and 21st day. Donepezil treated mice showed significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) increased in the number of square travelled on 7th, 14th and 21st day as compared to the disease control group. Administration of EEOE on consecutive days remarkably ($P < 0.01$) increased the number of square travelled by mice with respect to that of disease control mice on 21st day in which an increased in the number of square travelled indicates improvement of spatial memory and learning.

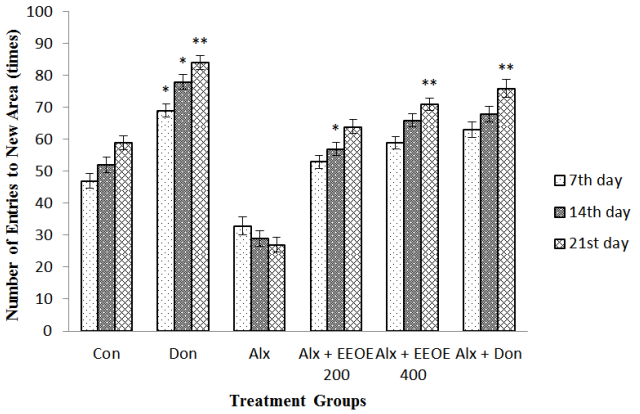


*P < 0.05, **P < 0.01, ***P < 0.001 significant difference as compared to disease control group

Figure 2 Neurodefensive effect of EEOE on the number of squares travelled by mice exposed to OF test. Values were expressed as mean ± SEM (n = 6/group).

Neurodefensive effect of EEOE on FE test in mice

The effects of EEOE on the number of entries to the novel area and time spent in the novel area by using FE test are given in **Figure 3** and **Table 1**. The number of entries to the novel area and time spent in the novel area in the aloxan treated mice was lower than existing group on 7th, 14th and 21st day. Administration of donepazil on consecutive days remarkably ($P < 0.05$, $P < 0.01$) increased the number of entries to the novel area and time spent in the novel area as compared to that of disease control mice. Administration of EEOE significantly increased ($P < 0.05$, $P < 0.01$) the number of entries to the novel area and time spent in the novel area on 7th, 14th and 21st day as compared to the disease control group. An increase in the number of entries to the novel area and time spent in the novel area indicates the improvement of cognition, memory and learning.



*P < 0.05, **P < 0.01 significant difference as compared to disease control group

Figure 3 Neurodefensive effect of EEOE on the number of entries to novel area by mice exposed to FE test. Values were expressed as mean ± SEM (n = 6/group).

Neurodefensive effect of EEOE on YM test in mice

Figure 4 represents the percentage of spontaneous alternation behavior of the mice using YM test. During the period of study the percentage of spontaneous alternation behavior of the mice was gradually increased in all treatment groups except alloxan treated group. Donepazil treated group showed significantly increased ($P < 0.05$, $P < 0.01$, $P < 0.001$) in the percentage of spontaneous alternation behavior on 14th and 21st day as compared to the disease control group. The administration of EEOE on consecutive days remarkably ($P < 0.05$, $P < 0.01$) increased the percentage of spontaneous alternation behavior of the mice as compared to that of disease control group on 14th and 21st day in which an increase in the percentage of spontaneous alternation behavior

indicates improvement of spatial short-term memory and learning.

Table 1 Neurodefensive effect of EEOE on time spent in the novel area by mice exposed to FE test.

Treatment	Time Spent in the Novel Area (min)		
	7th day	14th day	21st day
Con	7.52 ± 0.43	8.33 ± 0.39	9.24 ± 0.56
Don	8.37 ± 0.28*	8.74 ± 0.34*	9.42 ± 0.29**
Alx	4.92 ± 0.49	4.45 ± 0.43	5.13 ± 0.51
Alx + EEOE 200	6.95 ± 0.26	7.47 ± 0.39*	8.15 ± 0.34*
Alx + EEOE 400	7.33 ± 0.31*	7.86 ± 0.23*	8.20 ± 0.37**
Alx + Don	7.94 ± 0.48	8.42 ± 0.57*	8.58 ± 0.45**

Values were expressed as mean ± SEM (n = 6/group).

*P < 0.05, **P < 0.01 significant difference as compared to disease control group

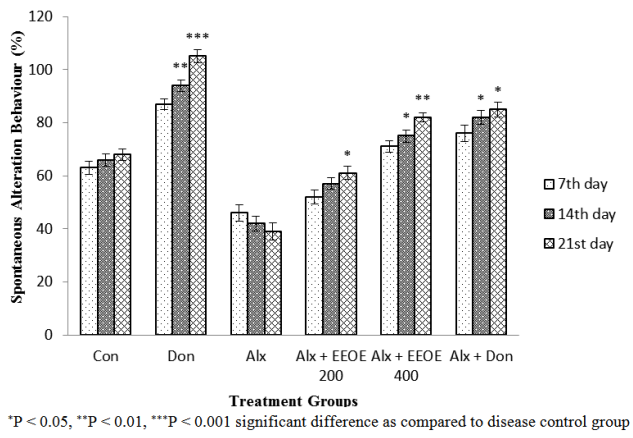


Figure 4 Neurodefensive effect of EEOE on the spontaneous alteration behavior of the mice exposed to YM test. Values were expressed as mean ± SEM (n = 6/group).

Neurodefensive effect of EEOE on TBARS status in mice brain

Table 2 displayed that the effects of EEOE in brain TBARS levels of mice by using lipid peroxidation assay. An increased in the brain TBARS levels was reported during the study period for all treatment groups except alloxan-treated group. The mice group treated with donepezil remarkably ($P < 0.05$, $P < 0.01$) decreased in brain TBARS levels as compared to disease control group. Administration of EEOE treated mice group significantly ($P < 0.05$) decreased in brain TBARS levels as compared to disease control group.

Table 2 Neurodefensive effect of EEOE on TBARS status of mice brain.

Treatment	TBARS (nM/min/mg protein)
Con	67.58 ± 5.26
Don	55.43 ± 6.72**
Alx	94.15 ± 8.43
Alx + EEOE 200	69.37 ± 7.18
Alx + EEOE 400	64.34 ± 5.89*
Alx + Don	59.53 ± 7.48*

Values were expressed as mean ± SEM (n=6/group).

*P < 0.05, **P < 0.01 significant difference as compared to disease control group

Discussion

In this current study demonstrated that administration of EEOE for 21 days revealed better memory and learning enhancing effect in alloxan induced mice of cognitive dysfunction and oxidative stress. In this experiment HC test, OF test, FE test, YM test and TBARS assay were performed for the evaluation of neurodefensive effect based on cognition, memory and learning.

The movement activity is a degree of the level of excitability of the CNS and an increment in the spontaneous motor activity could be deliberated as the nootropic effect [55]. In HC test, the extracts significantly increased the passages of a mouse through the hole from one chamber to another. An increase in the movement activity was evident on the 14th and 21st day specified improvement of spatial memory and learning of mice as compared to disease control mice. Sharmen et al., in the study on *Alpinia nigra* leaf extract in mice reported significantly decreased the motor activity and exploratory activity [56].

In OF test, the measure parameter is the number of square travelled in which an increase in the movement activity indicates upgradation of learning and memory. Considerable square travelled activity was observed at the 21st day observation period specified improvement of spatial learning and memory of mice as compared to disease control group. Kishore et al., reported in the study on *Foeniculum vulgare* fruit extract in mice model revealed significant increase in the number of square crossed [57].

In the FE test, the parameters that were investigated were the number of entries to new area and time spent in new area. Mice that enter in the novel area and spend more time in the new area is considered to improve cognition, memory and learning. EEOE treated mice showed an increased in the number of entries to the novel area and time spent in the novel area on 7th, 14th and 21st day as compared to the disease control group. In the study on zaprinast and rolipram in mice reduced exploratory activity was reported by Akar et al., in this test [58].

The YM test is one of the behavioral tests for studying short-term memory, general movement activity and stereotypic behavior [59]. It is recognized that spontaneous alternation is

a degree of spatial memory and to alternate among spatial locations [60]. In this test the percentage of spontaneous alternation behavior was investigated. In YM test an increase in the percentage of spontaneous alternation behavior of EEOE treated mice on 14th and 21st day indicated improvement of spatial long-term memory and learning of mice as compared to disease control group. Hazim et al., in the study on *Mitragyna speciosa* alkaloid extract in mice reported almost similar findings [61].

Lipid peroxidation is one of the principal causes of free radical-mediated injury that impairs neuronal membranes and higher levels of TBARS are the vital markers of various neurodegenerative diseases particularly AD [62]. Administration of EEOE for 21 days remarkably improved brain oxidative stress, as showed by significant decrease in lipid peroxidation activity in alloxan-induced fruit extract. In the study of neuroprotective effect of *Phyllanthus acidus* L. on learning and memory impairment in a scopolamine-induced animal model of dementia and oxidative stress by Uddin et al., also claimed analogous results [63].

Conclusion

The present study demonstrated that EEOE has neurodefensive effect and revealed significant improvement of cognition, spatial memory and learning in alloxan induced diabetic mice. Our findings showed the neurological justification for the traditional use of OE in the treatment of neurodegenerative disorders more specifically AD and encourage further studies to characterize the active compounds with their mechanism of action that are predominantly accountable for their beneficial effects.

Ethical Approval

The protocol of the experiment was approved by the animal 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. AAM performed the laboratory experiments and prepared the draft of the manuscript. FW collected and prepared the plant extract. MAI performed literature review. MMR participated in statistical analysis. All authors read and approved the final manuscript.

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Competing Interests

The authors proclaim that they have no competing interests regarding the publication of this article.

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