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Ocimum basilicum extract modulates Tau aggregation and improves memory function in a neurodegenerative rat model

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ABSTRACT

Alzheimer disease (AD) is a neurological condition that worsens with time. *Ocimum basilicum* is a well-known plant that has been used for centuries in many different cultures throughout the world as a treatment for lung disorders, anti-diarrhea medication, painkiller for the abdomen, and anti-inflammatory and antioxidant. The main objective of this study was evaluation of the neuroprotective potential effect of *Ocimum basilicum* on oxidative stress status in rat induced AD. Fifty rats were divided into five groups (10 rats/group) such as i) Control, ii) AlCl3-induced AD, iii) AlCl3 rats treated with rivastigmine (3 mg/kg/day), iv) AlCl3 rats treated with *Ocimum basilicum* (250 mg/kg/day), and v) AlCl3 rats treated with *Ocimum basilicum* (500 mg/kg/day). The results showed that the *Ocimum basilicum* plant leaves extract increased serum superoxide dismutase with a significant decrease of a serum MDA while the aggregation of tau protein expression was decreased. Histological changes were observed in brain tissues of AD rats. However, the high dosing of the plant leaves extract (500 mg/kg) was more powerful than the low treatment with low dose (250 mg/kg) by decreasing tau protein expression. The results suggest that *Ocimum basilicum* can relieve symptoms and prevent the progression of AD severity by improving memory function. It can be concluded that *Ocimum basilicum* leaves alleviated the memory impairment and learning abilities due to antioxidants activity of flavonoids, tannins and terpenoids.

INTRODUCTION

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by oxidative stress, neuro-inflammation, and synaptic dysfunction, which is caused in part by abnormal accumulation of senile plaques and neurofibrillary tangles [1]. AD is one of the diseases leading to enormous social and economic burdens placed on society due to its debilitating nature of patients [2]. The main neuropathologic feature underlying the symptoms of AD is neuronal loss [3]. AD is the most common dementia distinguished by the existence of senile plaques and neurofibrillary tangles (NFTs). Plaques are extracellular deposits of filamentous β-amyloid, a protease cleavage product of amyloid precursor protein [4]. Oxidative stress has been presented to be a protruding and initial feature of vulnerable neurons in AD. It is well known that oxidative stress induces the accumulation of intracellular reactive oxygen species (ROS), which in turn led to cellular damage in protein, lipid, and DNA oxidations. ROS levels are also connected with increased deposition of amyloid-β and formation of senile plaques, a hallmark of the AD brain [5].

Several studies have suggested that many organic compounds and bioactive phytochemicals might be used for the treatment of AD [6]. Phytochemicals existing in many plants display several essential properties such as DNA repair, autophagy, anti-inflammatory potential, scavenge oxygen free radicals and improve antioxidant activities [7]. Numerous natural products have used for the treatment of neurodegenerative diseases, including Salvia triloba and Piper nigrum [8]. However,

many of them are currently used for the treatment of neurodegenerative diseases which lack full therapeutic efficacy and show major side effects. The development of new drugs with higher efficacy and fewer side effects for the treatment of AD is required. Thus, there is a high demand for discovering new therapies of natural origin gained from medicinal plants for protection, slowing down or halting progression of AD disease in its primary stages [9].

Ocimum basilicum or basil is a medicinal plant and a significant essential oil crop which belongs to the Lamiaceae family [10]. It is an herbal plant wildly cultivated in regions of Central and Southeast Asia such as Iran and Pakistan [11,12]. *Ocimum basilicum* is a well-known herb traditionally used in diverse cultures across the globe for the management of lung diseases, anti-diarrhea medicine, abdominal pain reliever, anti-inflammatory and anti-oxidant [12,13]. In addition, it has been usually used for treatment of headaches, migraines, anxiety, nerve pains, inflammation and a variety of neurological disorders [14,15,16].

Neurodegenerative conditions known as tauopathies are characterized by the buildup of aggregates in the brain made of aberrant tau protein. The presence of tau aggregates is one of the key characteristics of tauopathy. As a result, tau aggregation is a hallmark of a number of neurodegenerative disorders. Thus, the present study was designed to evaluate the neuroprotective potential effect of *Ocimum basilicum* on the oxidative stress status in Alzheimer's disease induced in rats by measuring tau protein expressions. Also, *Ocimum basilicum* was used in this study as an herbal medicine which considered as harmless agents and used as alternative and complementary therapeutics.

MATERIALS AND METHODS

Aluminum chloride ($AlCl_3$) (MW, 133.34) was purchased from Sigma-Aldrich Co. (Germany). Rivastigmine of Exelon 1.5 mg was purchased from Novartis Co. (Thi-qar, Iraq). Malondialdehyde (MDA) content assay Kit (BC0020) and superoxide dismutase (SOD) activity assay Kit were purchased from Solarbio (China). While *Ocimum basilicum L* leaves were obtained from local farm in Thi-qar, Iraq.

Preparation of *Ocimum basilicum*

Leaves of *Ocimum basilicum* were collected from the local Thi-qar farm (Figure 1). The botanical identification was kindly identified by Dr. Yaas K. Abbas (Biology department, Education college for Pure Sciences, University of Thi-Qar). After collection, the plant leaves were dried in oven at 60°C and then grounded to fine powder. Preparation of aqueous plant leaves was done by soaking 100 gm of plant extract powder in 100 ml sterile boiled water for 7 days at 25°C with interim shaking. After that, the mixture was centrifuged at 5000 rpm for few minutes and the supernatant was evaporated by rotary evaporator. The 98% methanol was added to plant extract by maceration then filtered through Whatman No. 1 filter paper and was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to make crude extract [17].

Figure 1. Natural view of *Ocimum basilicum* leaves.

Induction of Alzheimer's disease

Alzheimer's disease was induced to rats by oral administration of AlCl3 (dissolved in distilled water) in a dose of 17 mg/kg b. wt. daily for 1 month [18].

Experimental animals

The current study was conducted with 50 adult male Sprague Dawley rats weighing from 200 to 250 g and obtained from the animal house colony of the animal house, College of Science, University of Thi-Qar, Iraq. The animals were maintained on standard laboratory diet and water. After one week, the animals were housed in standard cages at room temperature with artificially illuminated (12 h dark/light cycle) room free from chemical contamination. The animals used were divided into five groups (10 rats each) as follows: i) Rats (negative group) were received normal diet and tap water to end of experiment, ii) Rats (positive group) obtained 17 mg/kg of AlCl3 orally and normal diet for one month, iii) Rats obtained 17 mg/kg of AlCl3 orally and treated with 3 mg/kg rivastigmine for one month [19], iv) Rats obtained 17 mg/kg of AlCl3 orally and treated with 250 mg/ml *Ocimum basilicum* for one month, and v) Rats obtained 17 mg/kg of AlCl3 orally and treated by 500 mg/ml *Ocimum basilicum* for one month.

All animals received human care and use according to the guidelines for Animal Experiments which were approved by the Ethical Committee of Medical Research, National Research Centre, Iraq (No 63438/23/9).

Sample collection

At the end of experiment, rats were kept fasting for half a day. Blood samples were collected using the orbital sinus technique. Each blood sample was left to clot in clean dry test tubes, and then centrifuged at 3000 rpm for 10 minutes to obtain serum. The clear supernatant serum was used for MDA and SOD assays. Rats were then killed by decapitation and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic buffer, and dried for immediately frozen at –80°C to measure tau proteins.

Biochemical analysis

Serum MDA nmol/ml was measured by thiobarbituric acid colorimetric method using MDA assay kit by spectrophotometer colorimetric assay (Cat No: BC0020S, Solarbio – China). While serum SOD (u/ml) was measured by spectrophotometer colorimetric assay using SOD activity assay Kit (Cat No: BC0170, Solarbio – China).

Immunohistochemistry assay

The immunohistochemistry was performed using Dako EnVision detection immunohistochemistry kit (Envision FLEX, Dako, K8000, Denmark) as per manufacturer's instruction. Anti-Tau primary antibody (Polyclonal rabbit Anti-Tau Antibody: E-AB-60186, Elabscience, China) was used for detection of the expression of Tau protein in brain.

Paraffin embedded brain tissue sample were sectioned at 4 μm thickness. The tissue sections were the flooded with 100 μL of peroxidase block solution (EnVision FLEX Peroxidase-Blocking Reagent, SM801) as a blocking reagent and incubated in a humidity chamber for 10 minutes. The tissue sections were then applied with 100 μL of secondary antibody labeled to horseradish peroxidase (EnVision FLEX /HRP, SM802) and incubated in a humidity chamber at room temperature for 30 minutes. The tissue sections were applied with 100 μL of freshly prepared DAB+ substrate-chromogen solution (prepared by applying one drop of EnVision FLEX DAB+ Chromogen SM827) to 1 ml of EnVision FLEX Substrate Buffer (SM803)) and incubated in a humidity chamber for 10 minutes.

The tissue sections were counter stained with Mayer hematoxylin (Mayer hematoxylin, Bio-Optica, 05-06002/L, Italy) for 3 minutes. The tissue sections were immersed in two changes (10 minutes each) of xylene and mounted with mounting media (DPX) and covered with cover slips. The tissue sections were examined under a light microscope at 100x and 400x magnifications.

Statistical analysis

All data were performed at least three times and expressed as average value ± standard deviation. Multiple data were analyzed using one way analysis of variance (ANOVA) using graphed prism (version 9). Data is considered statistically significant when $p < 0.001$.

RESULTS

Effect of *Ocimum basilicum* leaves extract against oxidative stress in AD rats

Antioxidant activity as characterized by increased serum SOD levels. AlCl3 administration in AD-induced group showed significant decreasing in serum SOD level ($p < 0.001$) in comparison with control group (Figure 2). As expected, treatment of AD induced rats with rivastigmine significantly increased the serum SOD level as compared with AD-induced rats (Figure 2). Interestingly, treatment with *Ocimum basilicum* showed a significant increase in serum SOD level for both concentration 250 mg/kg and 500 mg/kg (65% and 67%, respectively) as compared to AD induced group (Figure 2).

The results in Figure 3 showed the effects of *Ocimum basilicum* on oxidative stress biomarker as represented by MDA level in serum of AD-induced rats. In comparison with control group, AlCl3 administration in AD-induced group exhibited significant increase in serum MDA level ($p < 0.001$). As expected, treatment of AD induced rats with rivastigmine produced significant reduction in serum MDA level as compared with AD-induced group (Figure 3). Consistently, treatment with *Ocimum basilicum* showed a significant decreasing in serum MDA level for both concentration 250 mg/kg and 500 mg/kg compared to AD induced group (Figure 3).

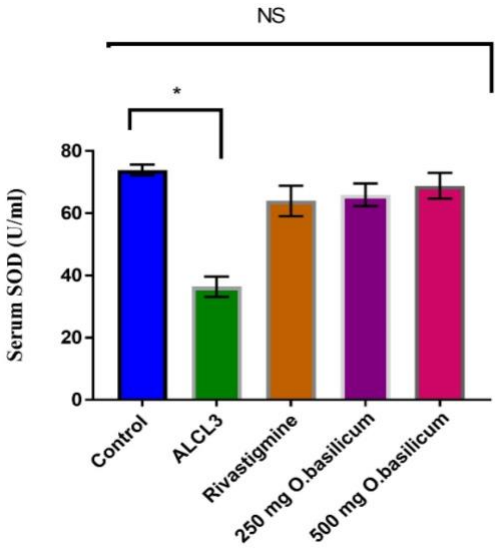

Figure 2. Effect *Ocimum basilicum* extract on antioxidant activity in AlCl3-induced AD rats (n=10 for each group). Antioxidant activity was screened SOD assay kit. The mean ± SD of SOD in U/ml in all studied groups of rats. * Symbols signify the levels of significance at $p < 0.001$ across groups using one way ANOVA.

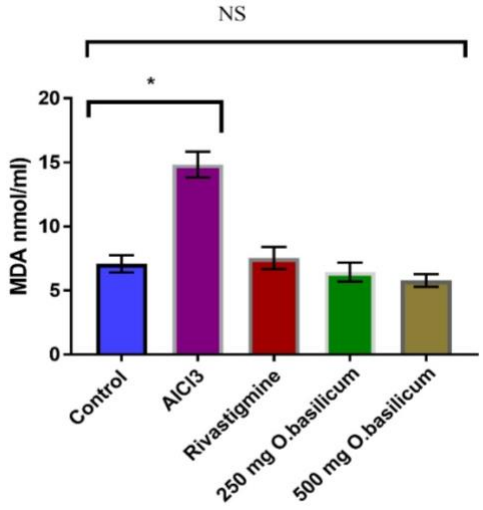

Figure 3. Effect *Ocimum basilicum* extract on oxidative stress status in AlCl3-induced AD rats (n=10 each group). Oxidative stress status was screened MDA assay kit. The mean ± SD of MDA in nmol/ml in all studied groups of rats. * Symbols signify the levels of significance at $p < 0.001$ across groups using one way ANOVA.

Effect of *Ocimum basilicum* leaves extract against Tau protein expression in AD rats

Microscopic investigation of brain section of control rat presented normal morphological structure of the hippocampus with positive expression of tau protein in Cornu Ammonis, where the expression of tau protein was observed surrounded nucleus of neurons and extended to axons of these neurons as shown in Figure 4A. As well as positive expression of tau protein in Cornu Ammonis, where the expression of tau protein was observed surrounded nucleus of neurons of Cornu Ammonis layer and deposition of tau aggregates follows a highly stereotyped pattern, starting in the hippocampus. However, tau protein expression was observed irregularly inside concavity of Cornu Ammonis layer of hippocampus as represented Figure 4B.

Interesting, results of treated rivastigmine group (Figure 4C) showed that positive expression of tau protein in Cornu Ammonis, where the expression of tau protein was observed surrounded nucleus of neurons and extended to axons of these neurons. Also, NFTs morphology was observed in one neuron of molecular layer.

AD rats-treated with 250 mg/kg *Ocimum basilicum* had a positive expression of tau protein in Cornu Ammonis, where the expression of tau protein was observed surrounded nucleus of neurons (Figure 4D). Also, positive expression of tau protein was detected in Dentate gyrus layer of hippocampus. AD rats-treated with 500 mg/kg *Ocimum basilicum* determined that there was positive expression of tau protein in Cornu Ammonis, where the expression of tau protein was observed surrounded nucleus of neurons and indicated normal histological structure of the hippocampus with normal cells arrangement as represented in Figure 4E.

Histological investigation of brain section of AD-induced rats treated with rivastigmine and/or *Ocimum basilicum* in a dose of 250 mg/kg and 500 mg/kg revealed less normal histological structure of the hippocampus and all tau aggregation disappeared in AlCl3-administrated rats (Figures 4C, D and E). The study also presented that the high dosing of the plant leaves extract (500 mg/kg) was more powerful than the treatment with low dosing (250 mg/kg). Furthermore, the number of tau aggregation was notably decreased ($p < 0.001$) in 500 mg/kg treated group compared with AD induced group (Figure 5). A study also showed that *Ocimum basilicum* decreased the depressive-like behavior and hippocampal neuron atrophy.

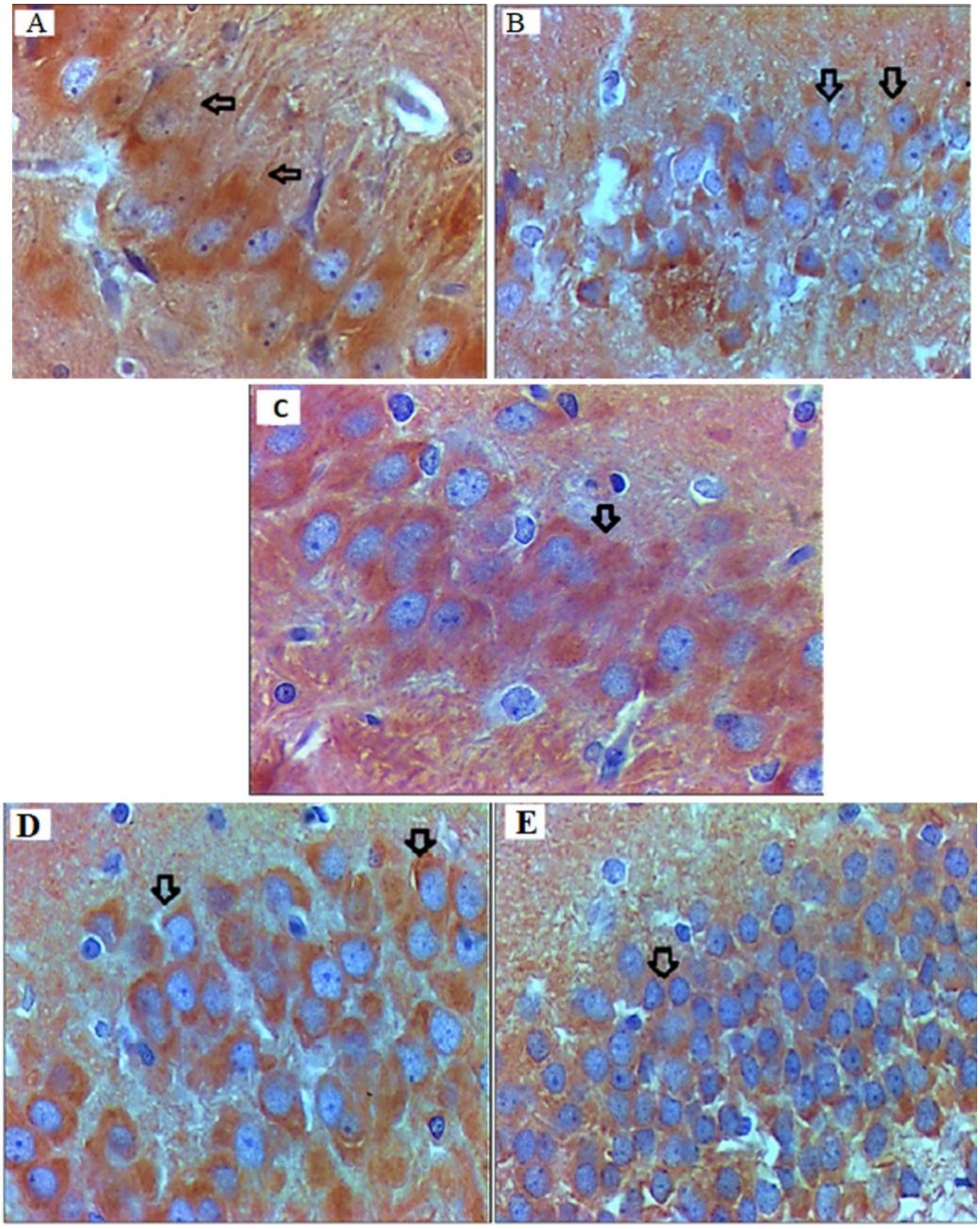

Figure 4. Expression of tau protein in brain section. Photomicrograph of hippocampus of control, AD, rivastigmine, *Ocimum basilicum* 250 mg/kg and 500mg/kg-treated group. (A) Showing normal histological structure of hippocamus. (B) Photomicrograph of brain section of AD-induced rats displaying tau protein expression was observed irregularly (black arrow) inside concavity of Cornu Ammonis layer of hippocampus. (C) Photomicrograph showing normal structure of hippocampus with neurofibrillary tangles (NFTs) (black arrow) morphology was observed in one neuron of molecular layer. (D) Photomicrograph displaying, positive expression of tau protein was observed in Dentate gyrus (black arrow) layer of hippocampus. (E) Photomicrograph of healthy hippocampus shows positive expression of Tau protein (black arrow) in Cornu Ammonis, where the expression of tau protein was observed surrounded nucleus of neurons. DAB and Hematoxylin 400x.

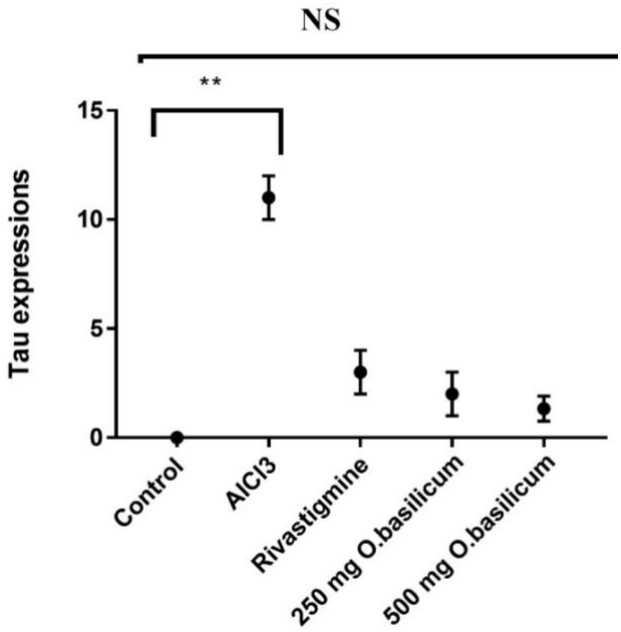

Figure 5. Effect *Ocimum basilicum* extract on tau protein expression in in AlCl3-induced AD rats (n=10 each group). The mean ± SD of tau protein expression in all studied groups of rats. *Symbols signify the levels of significance at $p < 0.001$ across groups using one way ANOVA.

DISCUSSION

AD is one of most a severe problem over the world due to the number of AD patients increasing repeatedly [20]. Several studies displayed that oxidative damage, a result imbalance between pro-oxidants and antioxidants is leading to AD pathogenesis [21]. In same context, it has been reported that the underlying mechanism of oxidative damage to critical molecules occurs early in the pathogenesis of AD and leads pronounced neuropathological modifications [22].

In the current study the AlCl3 induced significant reduction in serum SOD activities, and this could be because of accumulation of H_2O_2 and lead to protein oxidation and lipid peroxidation and eventually to cell damage. Due to producing an elevated level of pro-oxidants leads to consumption of antioxidants [23]. Therefore, it is difficult for antioxidants to supply protection against free radicals with following progress of the pathological alterations that characterize neurodegenerative illnesses [24]. While significant increase in serum MDA were reported in serum of rats. These results in agreement with the findings of [25]. They indicated that in both AD and vascular dementia increasing level of MDA in AlCl3-induced rats in addition to the microscopic investigation for brain section the existence of tau aggregation in hippocampus of rats. The mechanism of action of aluminum is not very well known. Conversely, it has been stated that aluminum potentiates the activity of ferrous (Fe2+) and ferric (Fe3+) ions to cause oxidative damage leading to AD [26]. Furthermore, aluminum promotes aggregation of tau protein in Alzheimer disease [27].

Rivastigmine was used as a drug for AD patients because it was shown to improve memory functions and stabilize the patient's life [28]. Treatment of AD group of rats with rivastigmine exhibited an improvement in oxidative stress status as represented by a significantly higher expression of serum SOD compared to AD-induced rats. Additionally, rivastigmine made a significant decrease in serum MDA. These results corresponded with the histopathological results in brain where, the tau aggregation that are formed under the influence of AlCl3 administration has been mostly not

disappeared than AD group. Our results in agreement with other studies that indicated that rivastigmine treatment recovers cognition, daily living activities by targeting a glutameric mechanism resulting in reduction of oxidative status and restore of antioxidants defense [29,30].

Ocimum basilicum is well known as a plant medicine and it has been cultivated in different regions over the world. The oil of the plant has been found to be beneficial for the alleviation of mental fatigue, colds, spasm, rhinitis. However, there is no report available for the chemical components of the *Ocimum basilicum* leaves as well as their pharmacological activities on the CNS for patients suffering from AD. Thus, the main objective of the current study was designed to evaluate neuroprotective effects of *Ocimum basilicum* in AD-induced rats. The present study showed that treatment of AD-induced rats with *Ocimum basilicum* total extracts produced a significant increase in serum antioxidant SOD and significant decrease in serum oxidant MDA levels than AD group. Our finding are in agreement with previous studies on other *Ocimum* which showed the injection of *Ocimum sanctum* water extract enhanced memory in mice by enhancing antioxidant activity [31]. This suggest that this plant may be effective in decreasing oxidative damage. Also, this study showed that *Ocimum basilicum* decreased the depressive-like behavior and hippocampal neuron atrophy. Consistently, Ali et al., results that explained that the possible mechanism for anti-depressant effects of *Ocimum basilicum* could be related to the existence of phenolic, flavonoid, tannin contents and terpenoid components [32]. However, high dosing (500 mg/kg) is more powerful than the low dose (250 mg/kg) of *Ocimum basilicum* leaves extract, as well as treatment with high dosing is more effective than low dosing in improvement the AD diseases in rats as evidenced by the biochemical markers as well as histopathological investigations.

CONCLUSION

Based on the current study, improvement of memory impairment and ameliorated neurodegeneration in hippocampus of AD-induced rats by *Ocimum basilicum* extract due to existence of antioxidants for example terpenoids, tannins and flavonoids and their property to scavenge free radicals. This study concluded that *Ocimum basilicum* has a neuroprotective role in experimental AD model. Therefore, it is suggested that *Ocimum basilicum* leave extract works as a potential antioxidant and it decreases tau protein expressions in rat brain. However, our results require further clinical studies.

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AUTHOR CONTRIBUTIONS

All authors contributed to design experiments and data analyses and writing the manuscript and approved the submitted article.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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