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ORIGINAL ARTICLE

Ocimum basilicum improve chronic stress-induced neurodegenerative changes in mice hippocampus

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Abstract

Alzheimer's disease (AD), one of the progressive neurodegenerative diseases might be associated with exposure to stress and altered living conditions. This study aimed to evaluate the effectiveness of *Ocimum basilicum* (OB) essential oils in improving the neurodegenerative-like changes induced in mice after exposed to chronic unpredictable mild stress (CUMS). Forty male Swiss albino mice divided into four groups ($n = 10$); the control, CUMS, CUMS + Fluoxetine, CUMS + OB were used. Behavioral tests, serum corticosterone level, hippocampus protein level of the glucocorticoid receptors (GRs) and brain-dreived neurotropic factor (BDNF) were determined after exposure to CUMS. Hippocampus was histopathologically examined. Data were analyzed using statistical package for the social sciences (SPSS) and P value of less than 0.05 was considered significant. OB diminished the depression manifestation as well as impaired short term memory observed in the mice after exposure to the CUMS as evidenced by the forced swimming and elevated plus maze test. OB also up-regulated the serum corticosterone level, hippocampal protein level of the glucocorticoid receptor and the brain-derived neurotropic factor and reduced the neurodegenerative and atrophic changes induced in the hippocampus after exposure to CUMS. Essential oils of OB alleviated the memory impairment and hippocampal neurodegenerative changes induced by exposure to the chronic unpredictable stress indicating that it is the time to test its effectiveness on patients suffering from Alzheimer disease.

Keywords Sweet Basil · Depression · Alzheimer · Corticosterone · GR · BDNF

Introduction

Aromatherapy is used for relieving the emotional changes associated with some clinical problems as the neurodegenerative diseases (Yoshiyama et al. 2015). It was reported to be effective in treating dementia in experimental animals (Hritcu et al. 2012) as well as in human (Bae et al. 2012). *Ocimum basilicum* (OB), also known as sweet basil, is a common popular annual herb (Grayer et al. 1996). It is widely used in perfumes and foods (Khan and Abourashed 2010). It has been traditionally used in treating of a variety of neurological disorders as anxiety, headaches and migraines, nerve pains and as carminative and antispasmodic (Bora et al. 2011). In a more recent study, the leaf extract of OB was proved to have potential to improve the neuromuscular coordination, exploratory behavior, and short-term memory in healthy male albino mice (Zahra et al. 2015). It's antidepressant-like effect was recently described (Ayuob et al. 2016). So it is efficacy in improving the neurodegenerative changes resulted from exposure to chronic stress was investigated in this study.

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One of the progressive neurodegenerative brain disorders is the Alzheimer's disease (AD) in which the person exhibits dementia associated with memory disorder and cognitive deterioration (Chung et al. 2016). About 5.4 million Americans of different ages were suffering from AD in 2016. Furthermore, the number is expected to be a triple over the next 30 years (Alzheimer's Association 2015). Chronic stress was reported to have a critical role in the etiology of sporadic AD and it was among its risk factor (Wilson et al. 2005 and Johansson et al. 2010). Stress induces atrophy and functional impairments in several brain areas such as the frontal cortex and hippocampus that play an important role in the generation and progression of depression and AD (Sotiropoulos et al. 2011; Wang et al. 2014). Stress also increases hippocampal vulnerability to neuronal atrophy that reversibly impairs cognitive function (Wingenfeld and Wolf 2014). Hence this study aimed to assess the effectiveness of OB in relieving AD-like neurodegenerative changes induced by chronic stress in mice.

Material and methods

This study design was approved by the biomedical research ethics committee at the Faculty of Medicine, King Abdulaziz University (KAU), Jeddah, Saudi Arabia (SA) (reference number 48–16). It was conducted according to the guidelines of dealing with experimental animals that are followed in the King Fahed Medical Research Center (KFMRC) and were in accordance with Helsinki Declaration (NIH publication No. 80–23, revised 1996) (World Medical Association 2013).

OB was obtained from the gardens at south of Jeddah Saudi Arabia and it was confirmed by consulting a specialist in Botany from the Faculty of Science, KAU. The essential oils of OB was extracted as described by Ismail 2006 and its constituents were identified by using gas chromatography & mass spectrometry (GC-MS; Agilent, Columbia, USA) Table 1 in order to characterize it. OB essential oils was diluted before use by Propylene glycol (5%; Sigma, St. Louis, MO, USA) as described by Chioca et al. 2013.

Fluoxetine (FLU) hydrochloride (Dar Al Dawa Pharmaceuticals Co., Ltd., Aman, Jordan), the selective serotonin reuptake inhibitor used in this study as a positive control to treat stress-induced depression, was diluted with 0.03% sodium carboxymethyl cellulose (CMC-Na) and given through the intragastric gavage at a dose of 20 mg/kg (Li et al. 2014). Amyl acetate 5% obtained from Sigma (St. Louis, MO, USA) was given to the positive control group because it has no effect on anxiety (Pavesi et al. 2011). Both OB and amyl acetate were administrated daily through inhalation for 15 min after exposure to the CUMS in an odor-isolated chamber (24 cm × 32 cm × 32 cm) according to Chioca et al. (2013). 5% amyl acetate, OB essential oil (1.0% [v/v]) in a constant volume of 1 ml were placed on cotton wool that weighed 0.7 g. The soaked cotton wool was placed in the corner of the chambers, and the lid was closed. Each cotton wool with lavender oil, amyl acetate, or distilled water was used only once and renewed each time to maintain its concentration in the apparatus.

Table 1 Chemical composition of essential oil of *Ocimum basilicum* (OB) obtained by GC-MS

Compound	Retention time (min)	Percentage
Linalool L	14.699	35.945
1,8-Cineole	13.594	11.228
Alpha-Cadinol	24.297	10.395
Farnesyl acetate	38.401	10.178
E,E-.alpha.-Farnesene	20.226	4.851
Trans-.beta.-Ocimene	13.774	3.724
Gamma cadinene	22.022	3.101
(−)-Camphor	15.539	2.331
Borneol	15.932	1.96
Alpha.-Copaene	23.882	1.75
Fenchyl acetate	16.591	1.316
L-.alpha.-bornyl acetate	17.569	1.225
Cis-.beta.-Terpineol	14.231	1.19
Alpha.-Guaiene	20.321	1.162
Alpha.-Humulene	20.864	0.931
p-Menth-1-en-8-ol	15.879	0.756
Alpha.-bisabolol	24.956	0.721
Trans-epoxy-ocimene	15.305	0.63
(−)- Cadina-1,3,5-triene	22.171	0.588
Trans-Caryophyllene	25.296	0.574
Trans-.gamma.-bisabolene	21.958	0.504
Alpha.-Thujone	14.561	0.462
Calarene	19.333	0.301
Non-identified compounds	–	4.177

Forty male Swiss albino mice, five weeks old, were obtained from the animal unit at the KFMRC, Jeddah Saudi Arabia, and left to acclimatize for two week to the lab conditions (22 ± 3 °C and relative humidity of 44%–55% with a 12 h dark/light cycle). The animals were divided into 4 groups ($n = 10$ each); the control group left without exposure to stress, the CUMS group that was exposed to chronic unpredictable mild stress (CUMS) for 4 weeks then treated with amyl acetate. The third (CUMS + FLU) and fourth (CUMS + OB) groups were exposed to CUMS then treated with FLU and OB respectively. All treatments were started after stopping the exposure to CUMS and were administrated for 2 weeks. The CUMS procedure adopted in this study was according to Doro et al. (2014). The mice were subjected, for 4 weeks, to various types of stressors at different times during the day. It was fully described in a previous study (Ayuob et al. 2016). Stressors included; social stress by placing mice in soiled cages of other

mice, inversing the light/dark cycle, placing mice in cages with wet sawdust, tilt cages at 30 degrees, restraining the mice and water stress by placing mice in an empty cage with 1 cm of water at the bottom.

Procedures

After exposure to CUMS procedure for 4 weeks, behavioral tests were performed in order to assess the depressive status of the mice. On the experimental day, the mice were habituated to the test room for 30 min before the assays, with free access to food and water. Different groups of mice were tested, such that each mouse was tested only once. All tests took place between 8:00 and 11:30 a.m. in a dimly lit room. After each test, mice were returned to their cages and eventually to the holding rooms once every animal was tested.

1. Forced swim test (FST): It was done according to Doro et al. (2014). Shortly, after being placed in a transparent plexiglas cylinder (20 cm diameter) filled with water (25°c) to a depth of 12 cm, mice were video recorded for 6 min using behavior software (Noldus Information Technology, EthoVision XT®). Total time spent immobile in the 6 min was assessed and presented in seconds. Immobility was defined as the cessation of limb movements except minor movement necessary to keep the mouse afloat.
2. Elevated plus-maze (EPM) that was done according to Chioca et al. (2013). Each mouse was placed in the center of the EPM and its behavior video recorded for 5 min, and later coded by an observer blind to the mouse treatment, using the Biobserve software. The maze was thoroughly cleaned with ethanol and allowed to dry between subjects in order to eliminate any odor cues. The numbers of closed arms entries in 6 min and time spent by each mouse inside the open and closed arms were observed and recorded and expressed in seconds. Transfer latency (TL), the time it took by the mouse to move from the open arm to either of the closed arms when it placed in the open arm, was also recorded in three successive days in order to examine whether the mice learn this scape behavior as described by Itoh et al. 1990. The TL of the 3rd day was included in the analysis.
3. Open field test (OFT) that was carried out according to Mineur et al. (2006). The mice were individually placed in the center of a dimly illuminated observation cage (109 cm × 49 cm × 49 cm). The animals were observed for 25 min directly and continuously by an observer. The number of mouse rearing (standing upright on hind legs while the forepaws are free) in 25 min was registered manually and the distance traveled by the mouse in this period was measured using a software (Columbus Instruments, OHIO 43204, USA).

The day after finishing the behavior tests, the mice were anaesthetized in the morning using light ether and blood samples was collected from retro-orbital venous plexus, centrifuged for 10 min (2200 g, 4uC) and kept at the refrigerator till the estimation of serum corticosterone levels using RIA (ELISA kits, ALPCO Diagnostics, Orangeburg, NY) in order to biochemically confirm the depressive status of the mice.

After finishing the blood sampling, the animals were scarified by cervical dislocation. The brain was immediately extracted from the skull and cut into 2 halves in the sagittal plane then the hippocampus on the left side was dissected according to Paxinos and Watson (1998). Tissue punches from the hippocampus were taken then homogenized in cold extraction buffer (Tris-buffered saline, pH 8.0, with 1% NP-40, 10% glycerol, 5 mM sodium metavanadate, 10 mM PMSF, 100 μg/ml aprotinin and 10 μg/ml leupeptin) and processed by means of sandwich enzyme-linked immunosorbent assay (ELISA) described by Doron et al. 2014 to assess BDNF and GR protein levels in order to biochemically confirm the depressive status of the mice.

The right half of the brain one was fixed for 24 h in 10% neutral buffered formalin then dehydrated, cleared, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) for histopathological examination (Bancroft and Gamble 2008). Congo red stain, an accepted histochemical marker for the β-pleated-sheet structure of amyloid, was also performed as described by Nobakht et al. 2011.

Paraffin sections were also processed as described by Makhlouf et al. (2014) for immunohistochemical studies using the peroxidase-labeled Streptavidin–Biotin Technique. Anti-caspase-3, the primary antibody used for the demonstration of apoptosis, we obtained from Santa Cruz Biotechnology, USA and utilized with the dilution of 1:1000. For demonstration of astrocytes, anti GFAP (Dako Cytomation-USA, with the dilution of 1:1000) was utilized for 1 h. Anti-Ki-67 (Abcam, Cambridge, UK, with the dilution 1:100) was utilized to determine proliferating cells. In order to examine and photograph the sections, a digital camera connected to a light microscope (Olympus, BX-61, Los Anglos) was utilized. Morphometric measurements including the thickness and the surface area of CA1 pyramidal cell layer and DG granular cell layer were performed through the Image ProPlus, (Cybernetics, USA). Adding to that counting the number of GFAP- and Caspase-3-positive cells in both CA1 and DG as well as the number of Ki67-positive cells in DG only. The counting was performed in 5 high power field (×400) in each mouse as described by Makhlouf et al. 2014.

The Statistical Program of Social Sciences (SPSS, version 16) software was used to analyze the data and the results were presented as mean and standard deviation. ANOVA (F-test) was used to compare between the studied groups followed by Bonferroni post hoc test. A p value less than 0.05 was considered significant.

Results

Effect on behavioral tests

Mice exposed to the CUMS for 4 weeks developed a depressive-like behavior evidenced by the significant ($p < 0.001$) increase in the immobility time of the FST compared to the unexposed mice. Treating the stressed mice with FLU or OB after exposure to the CUMS significantly ($p = 0.02$, $p < 0.001$) reduced it in comparison to the untreated mice respectively (Fig. 1).

The spontaneous locomotor activity of mice exposed to the CUMS recorded during the OFT was enhanced as the mice significantly ($p < 0.001$) travelled more than the unexposed mice while the mice treated with FLU ($p < 0.001$) or OB ($p = 0.004$) travelled significantly less than the untreated mice. The number of rearing induced by mice in the CUMS group was significantly ($p = 0.02$) more than that of the unexposed group, administration of FLU or OB significantly ($p < 0.001$) reduced it (Fig. 1).

Exposure to CUMS resulted also in developing an anxiety-like behavior as the mice spent a significantly shorter ($p < 0.001$) time in the open arms of the EPM and enter the closed arms more frequent compared to the unstressed mice. In addition, both the TL on the 3rd and the time spent in the closed arm were also prolonged indicating impaired memory. FLU or OB administration reduced these behaviors as they significantly increased ($p = 0.001$) the time spent in the open arms and significantly ($p < 0.001$) decreased the number of closed arm entry in comparison to the CUMS mice. FLU or OB administration also prevented the memory impairment induced after exposure to CUMS as evidenced by the significant ($p = 0.01$, $p = 0.001$) shorting in the TL on the 3rd as well as the significant ($p = 0.04$, $p = 0.03$) reduction in the time spent in the closed arm in comparison to the CUMS mice (Fig. 2).

Effect on the biochemical tests

Exposure to the CUMS procedure resulted in a significant ($p < 0.001$) increase in the serum level of corticosterone when compared to the unexposed mice while treating the mice with FLU or OB after exposure to the CUMS significantly ($p < 0.001$) reduce it (Fig. 3).

On assessing the GR and BDNF protein expression levels it was observed that exposure to the CUMS significantly ($p < 0.001$) down-regulated them compared to those of the unexposed mice while administrating FLU ($p < 0.001$, $p < 0.001$) or OB significantly ($p = 0.04$, $p = 0.01$) up-regulated it respectively compared to the CUMS group (Fig. 3).

Effect on the histological structure

The CA1 region of cornu amonis as well as the dentate gyrus (DG) of the hippocampus was selectively examined in this study as they are the affected areas in AD. In the CA1, the polymorphic, the molecular and the pyramidal layers were shown. In the latter layer of the control, the pyramidal neurons had open face nuclei and slightly basophilic cytoplasm while some of these neurons possessed dark nuclei and cytoplasm in the mice exposed to the CUMS. In addition, eosinophilic intraneural structures; Hirano's bodies (HBs) were frequently observed in these pyramidal neurons. The thickness and the surface area of the pyramidal layer was significantly ($p < 0.001$, $p = 0.01$) reduced respectively in the CUMS group. In Congo red-stained hippocampus of mice exposed to CUMS, some scattered amyloid cores or plaques were observed in the CA1 and near the pyramidal cell layer. Administration of FLU or inhalation of OB reduced these changes and significantly increased the pyramidal cell layer thickness ($p < 0.001$, $p = 0.06$) and surface area ($p = 0.004$, $p = 0.04$) compared to the CUMS group (Figs. 4 and 5).

In the DG three layers were visualized; the molecular, pleomorphic and the granular cell layers. The latter layer of the control shown polyhedral cells with vesicular nuclei and lightly basophilic cytoplasm. In the stressed mice some of these cells possessed darkly stained cytoplasm as well as apoptotic nuclei while others were vacuolated. The number of the immature cell that possessed darkly stained nuclei were also increased in this group. There was significant reduction in both thickness ($p = 0.02$) and surface area ($p < 0.001$) of the

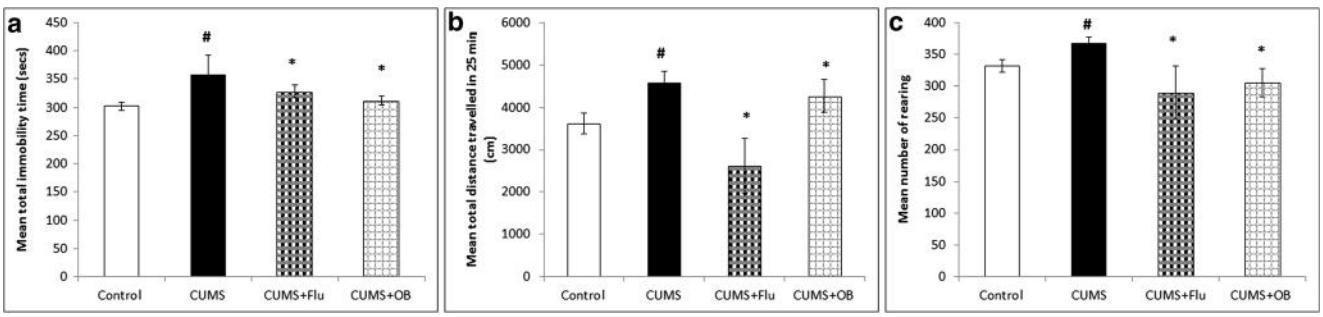

Fig. 1 Immobility time of the forced swimming test (**a**) and Open field test (**b**, **c**) of the control, chronic unpredictable mild stress (CUMS), fluoxetine-treated (FLU) and *Ocimum basilicum*-treated (OB) groups (n = 10 each). Data was shown as mean ± SD. # indicates significance compared to the control group, * indicates significance compared to the CUMS group

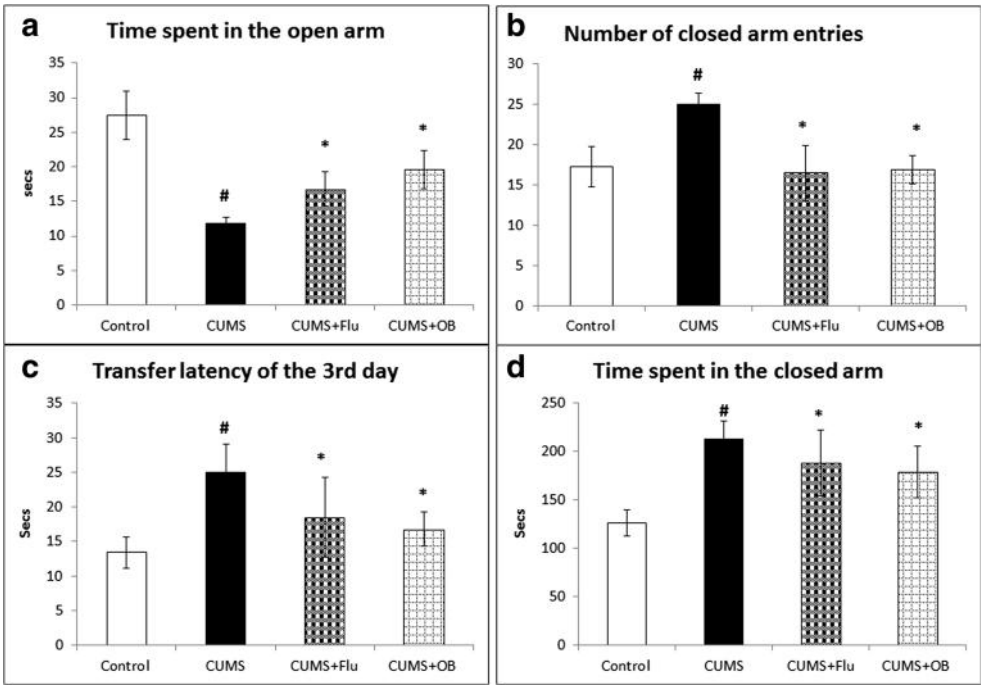

Fig. 2 Elevated plus maze test of the control, chronic unpredictable mild stress (CUMS), fluoxetine-treated (FLU) and *Ocimum basilicum*-treated (OB) groups ($n = 10$ each). Data was shown as mean ± SD. # indicates significance compared to the control group, * indicates significance compared to the CUMS group

granular cell layer of the CUMS group. Some scattered amyloid plaques were observed in the DG of the Congo red-stained hippocampus of the stressed mice. These changes were less evident in mice received FLU or OB. Both thickness and surface area of the granular cell layer increased significantly in mice received FLU ($p = 0.04$, $p = 0.02$) or OB ($p = 0.05$, $p = 0.03$) respectively when compared to the untreated group (Figs. 5 and 6).

It was found that the GFAP-positive cells decreased significantly in number, in both CA1 ($p = 0.004$) and DG ($p = 0.02$) of stressed mice compared to the control indicating a reduction in the number of glial cells (Figs. 4 and 7). On the other hand, a significant increase in the number of these cells was observed in both groups treated with FLU ($p = 0.03$, $p = 0.02$) or OB ($p = 0.01$, $p = 0.03$) (Fig. 5c). The number of Caspase-3 positive cells was significantly ($p < 0.001$) increased in both CA1 and

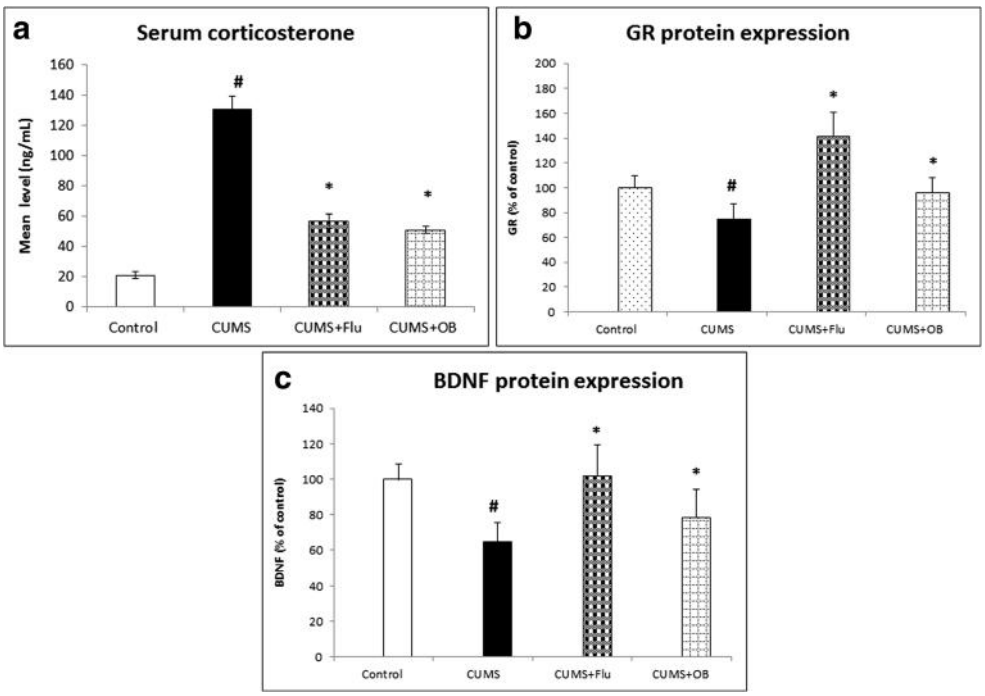

Fig. 3 **a** Serum level of corticosterone shown as mean ± SD. GR protein (**b**) and BDNF protein (**c**) expression levels in the hippocampus assessed by ELISA and expressed as percent of control value ± SD (n = 10 each). # indicates significance compared to the control group, * indicates significance compared to the CUMS group. (CUMS: chronic unpredictable mild stress, fluoxetine (FLU) and *Ocimum basilicum* (OB)

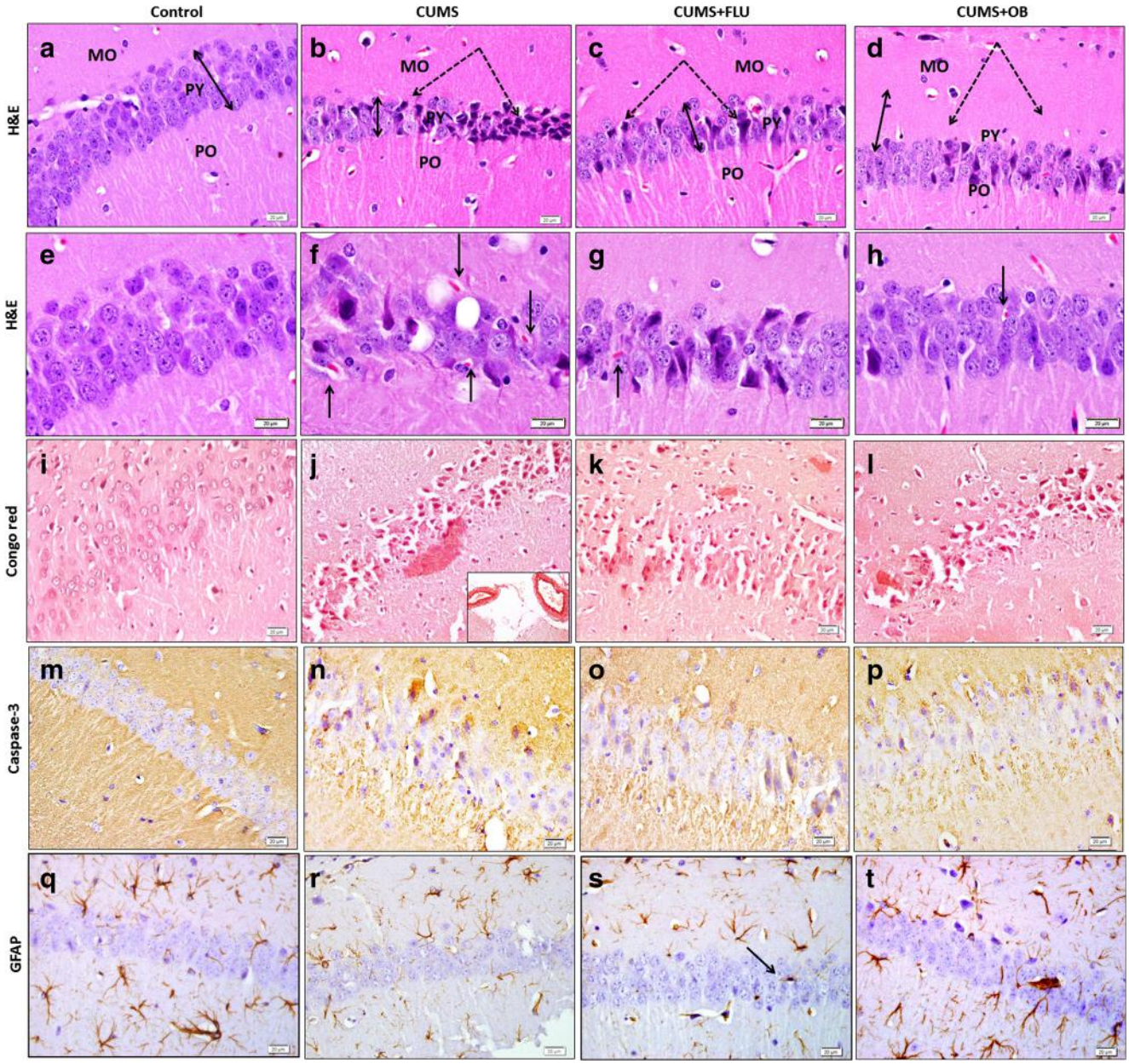

Fig. 4 The hippocampal CA1 is formed of the molecular layer (MO), the pyramidal layer (PY) and the polymorphic layer (PO). The thickness of the PY layer appears smaller in the CUMS compared to the control (black arrow). Some darkly stained cells (interrupted arrow) are observed. An eosinophilic structure (black arrow) is seen related to the pyramidal cell. Amyloid deposition with salmon red coloration observed near CA1 (insert show blood vessel with amyloid deposition in its wall). Immunoexpression of Caspase, GFAP in the hippocampal CA1 are shown (A-D, I-T × 400, E-H × 1000). ((CUMS: chronic unpredictable mild stress, FLU: fluoxetine and OB; *Ocimum basilicum)*

DG of the stressed mice in comparison to the unstressed mice indicating an increase in the apoptosis while their number was significantly reduced after administration of FLU ($p < 0.001$) and OB ($p < 0.001$) (Fig. 5d). Exposure to CUMS also resulted in a significant ($p < 0.001$) reduction in the DG neurogenesis indicated by immunohistochemical staining with Ki67 (Fig. 7). Treatment with FLU or OB resulted in a significant ($p = 0.003$, $p = 0.001$) increase in the number of Ki67-positive cells in the subgranular zone of DG compared to the stressed mice which indication proliferation of these cells (Fig. 5e).

Discussion

Huang et al. (2015) described an association between exposure to chronic stress and neurodegenerative disease although the underlying mechanism was controversial. Anyhow, the discovery of drugs that can improve chronic stress induced learning and memory impairments and neurodegeneration are of great importance for the treatment of AD (Laroche et al. 2000).

Mice exposed to the CUMS procedure, in this study, developed depression diagnosed by FST and confirmed by both

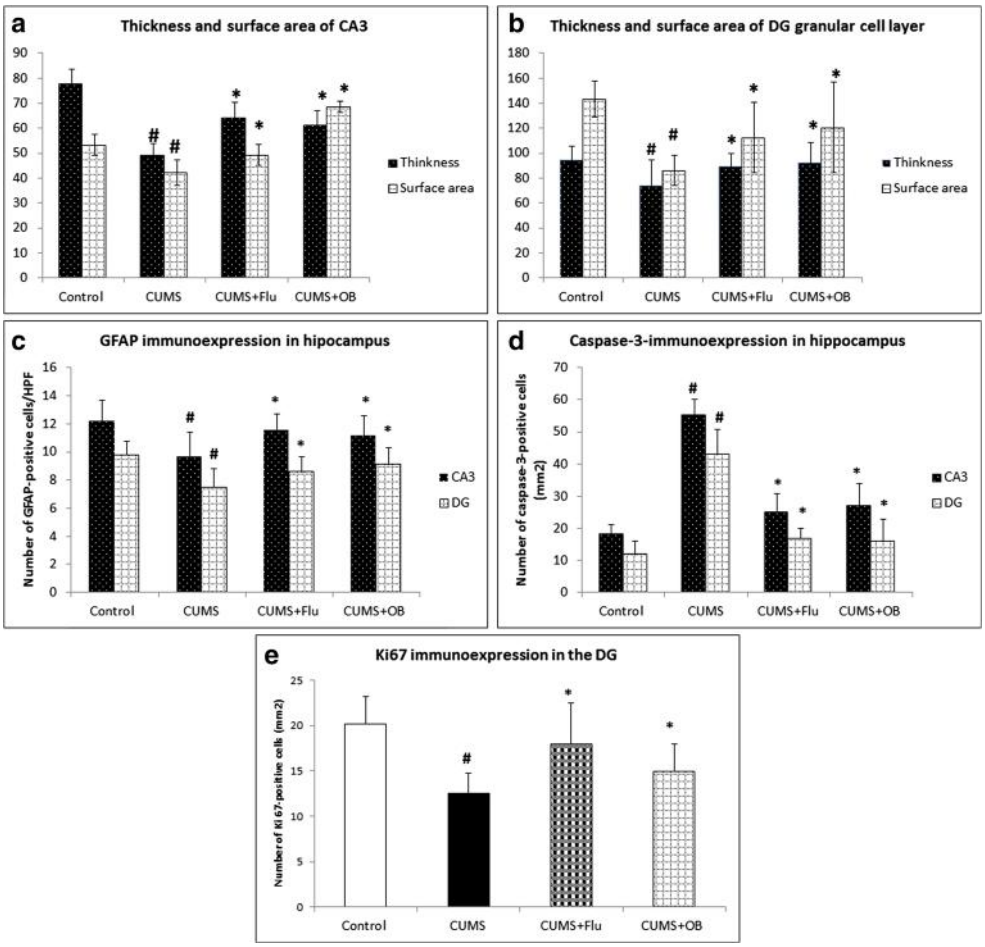

Fig. 5 Thickness and surface area of CA1 and dentate gyrus (**a**, **b**). Immunoexpression of Caspase-3 (**c**), GFAP (**d**), Ki67 (**e**), in the studied groups. Data is expressed as mean. # significance versus control, * significance versus CUMS. (CUMS: chronic unpredictable mild stress, FLU, fluoxetine, OB: *Ocimum basilicum*)

the EPM and the OFT and this finding was consistent with that of Liu et al. 2014. To evaluate the short-term memory of the mice, the EPM was utilized as recommended by Itoh et al. 1990 and Kulkarni 2007. The TL and the time spent by the mice in the closed arm were measured on the 3rd day to assess the mice learning of the escape behavior in the 1st and 2nd days. In this study, the TL on 3rd was prolonged after exposure to CUMS indicating an impairment in mice memory as was previously reported by (Itoh et al. 1990). One of the explanation of these behavior changes induced by stress was postulated by Sotiropoulos et al. 2011 as he reported that chronic stress induce abnormal hyperphosphorylation of TAU in the hippocampus and prefrontal cortex (PFC), with contemporaneous impairments of hippocampus- and PFC-dependent behaviors including; learning and memory (Laroche et al. 2000).

Previous reports on human (de Paula et al. 2015) and on animal (Chen et al. 2015) showed that hippocampus is the part of the brain responsible for cognitive and memory functions and that its impairment, especially the CA1 region, occurs early in AD and results in development of dementia, the early sign of AD. In the present study, exposure to the CUMS for 4 weeks resulted in many histopathological changes included apoptosis and vacuolation of many neurons in both CA1 and DG, reduced thickness of both CA1 and DG and reduced number of the GFAP-positive astrocytes. These changes were consistent with those reported by McEwen 2007 and Wang et al. 2014 in response to stress as well as those described by Okasha 2012 as neurodegenerative changes induced in an Alzheimer model in adult male albino rats. Both Hirano's bodies (HBs), eosinophilic intraneural structures in the hippocampal pyramidal cells, and the amyloid plaques, observed in Congo red-stained hippocampus in this study were among the neuropathological hallmarks of Alzheimer described by Cvetković-Dožić et al. 2001. The DG is one of the few regions of the adult brain where neurogenesis takes place. Neurogenesis is thought to play a role in the formation of new memories (Saab et al. 2009). Differentiation, not only the birth, of the newborn cells into functional neurons is required for maintenance of cognitive abilities and normal learning and memory. This differentiation is compromised in AD although neuroproliferation is increased (Li et al. 2008). This explained the presence of increased number of the new born cells, which possessed dark nuclei, in the SGZ of the DG after exposure to CUMS in this study. The presence of large number of these cells meant they remained immature and did not differentiate into mature neurons. These

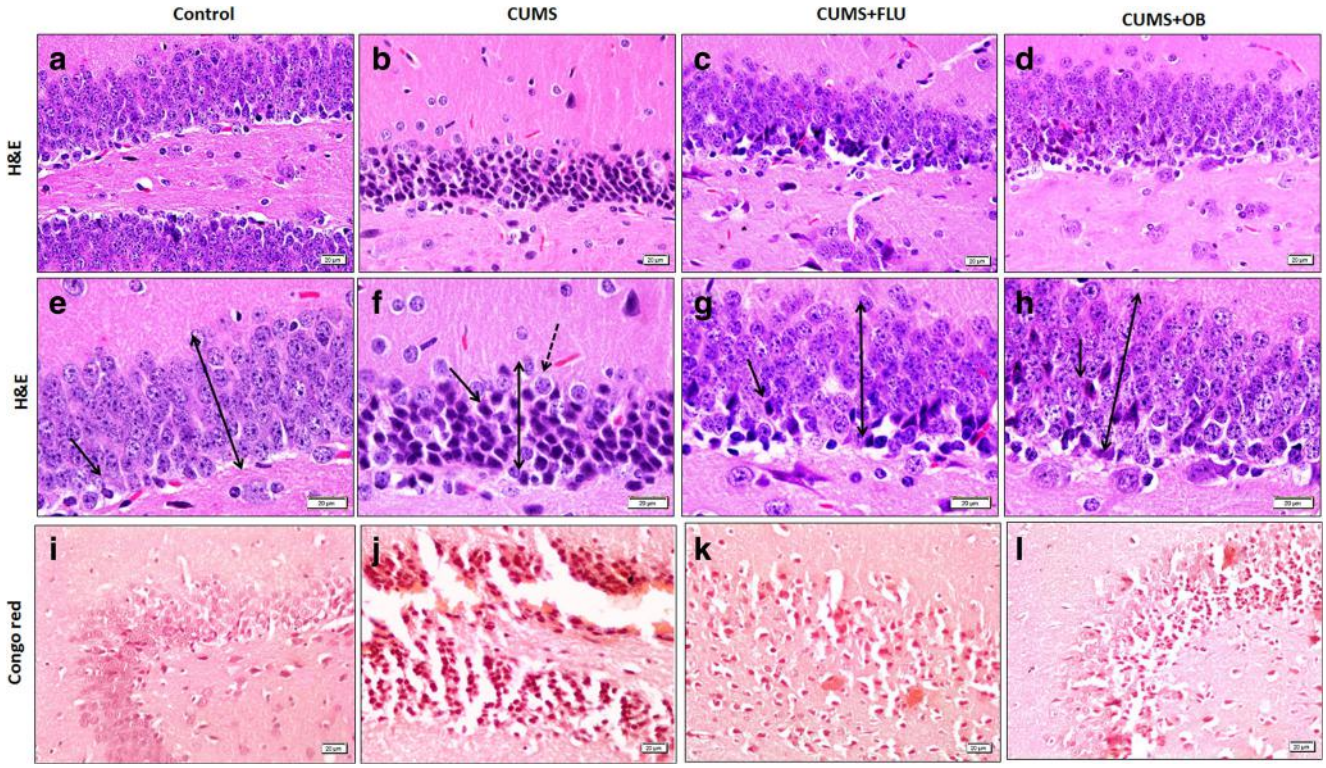

Fig. 6 The hippocampal dentate gyrus is formed of the pleomorphic layer (PLE), the granular cell layer (GL) and the molecular (MO). The thickness of the GL layer appears smaller in the chronic unpredictable mild stress (CUMS) compared to the control (bihead arrow). Some cells appear vacuolated cells (interrupted arrow) are observed. Note the increased number of the immature cell that have darkly stained nuclei (arrow). Immunoexpression of GFAP, Caspase-3, Ki67, BDNF and GR in the hippocampal dentate gyrus are shown (OB; *Ocimum basilicum)*

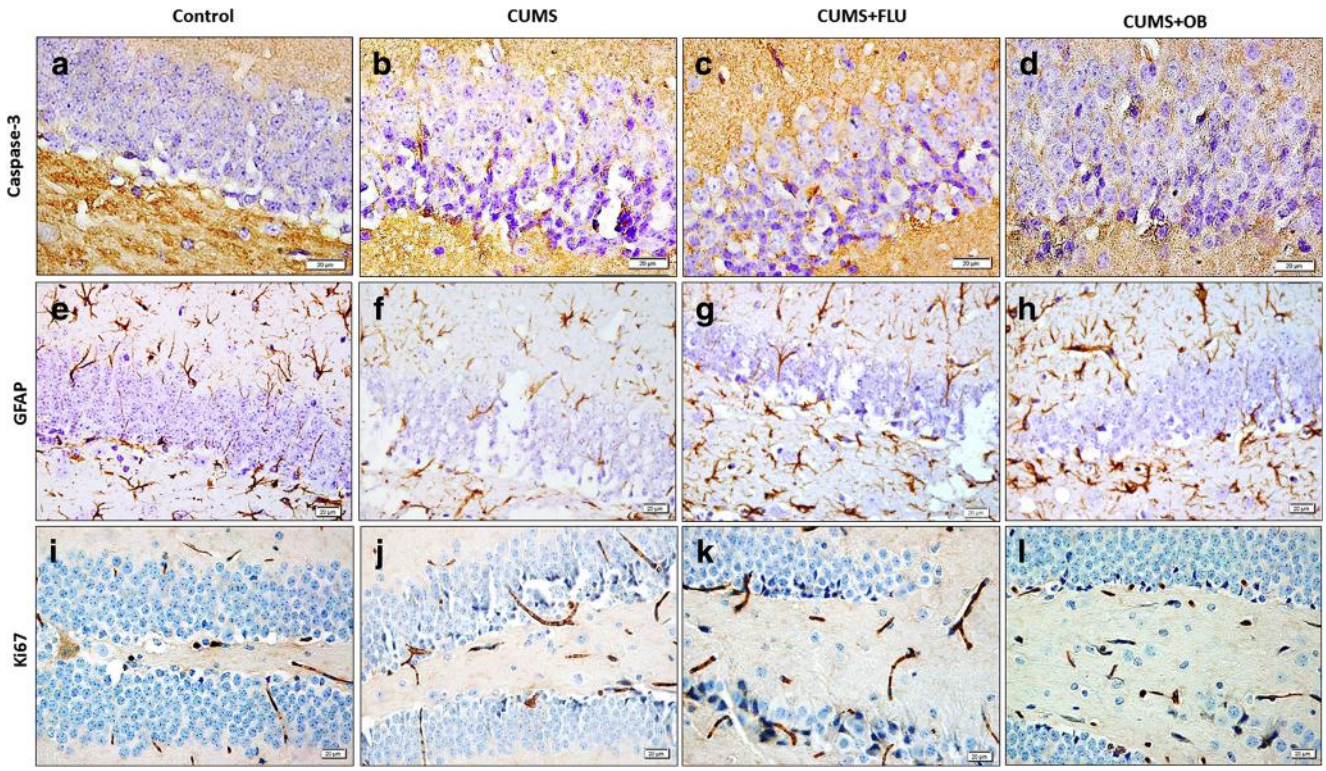

Fig. 7 Immunoexpression of GFAP, Caspase-3, Ki67, in the hippocampal dentate gyrus (A-D ×600 and E-L ×400). (CUMS: chronic unpredictable mild stress, FLU: fluoxetine and OB; *Ocimum basilicum)*

findings indicated that CUMS induced AD-like neurodegenerative changes in mice.

In the present study, the CUMS resulted in an increase in corticosterone level and this was in concordance with Gong et al. 2015. It was reported that stress activates the hypothalamic-pituitary-adrenal (HPA) axis resulting in glucocorticoids blood levels high enough to primarily activate type-II GR that translocate to the nucleus and trigger alterations in gene expression and cause negative consequences for hippocampal functions including cognition (Tsigos and Chrousos 2002; Duman and Monteggia 2006). This could explain the CUMS-induced neurodegenerative changes described above. Increased cerebrospinal fluid cortisol level in dementia of Alzheimer's type was also reported also by Popp et al. (2015). Another explanation of these changes is the reduction of the BDNF level which is confirmed biochemically in this study by assessing BDNF hippocampal protein level. Aleisa et al. 2006 reported that chronic stress significantly decrease BDNF levels in CA1 area of the hippocampus and therefore, interfere with the repair process and consequently exacerbating the effect of amyloid β (Aβ). Another possible mechanism of the stress impact is that stress may alter the processing and production of various AD-related proteins e.g. APP and drive its processing toward the amyloidogenic pathway which may account for the increased levels of Aβ (Srivareerat et al. 2011) and the increased amount of plaque formation that are also observed with stress (Lee et al. 2009).

Fluoxetine is reported by Dwivedi et al. 2006 to attenuate the behavioral changes induced by unpredictable stress and improve depression-associated changes in hippocampal CA1 region and corticosterone level. In addition, Marksteiner et al. 2003 reported an evidence that fluoxetine is a treatment option for patients with AD and highlights the importance of the serotoninergic system. These results were supportive to the findings of this study.

Chemical therapeutic for AD are often of no vast benefit. Aicardi (2013) reported that current pharmacological therapy of AD partially masks the symptoms while degenerative changes still progresses within the brain, therefore the need for a natural safe therapy for AD increased. In the present study, inhalation of OB alleviate the depressive status induced after exposure to CUMS. It also prevented the short memory and learning impairment in the mice as evidenced by shortening the TL and the time the mice spent in the closed arm during the EPM test. These findings are supported by Zahra et al. (2015) who reported that normal male albino mice exposed to OB exhibited better exploratory behavior and better mobility compared to the unexposed mice. Bora et al. (2011) attributed the ability of OB extract to prevent the impairment of short-term memory following global cerebral ischemia to its antioxidant properties. Some of the OB essential oils components, e.g., 1,8-cineole, linalool, caryophyllene, humulene and camphor, have been reported to exhibit anxiolytic and sedative effects (Satou et al. 2014), which is consistent with our findings. OB also reduced the corticosterone level as well as the pathological changes induced by stress on the hippocampal neuron in a pattern comparable to that induced by Fluoxetine and this might be attributed to its neuroprotective affect exerted by its phenolic, flavonoid and tannin contents, which are scavengers of reactive oxygen species (Bora et al. 2011).

In conclusion, essential oils of OB alleviated the memory impairment and hippocampal neurodegenerative changes induced by exposure to the chronic unpredictable stress indicating that it is the time to test its effectiveness on patients suffering from Alzheimer disease.

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Compliance with ethical standards

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