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Can *Ocimum basilicum* relieve chronic unpredictable mild stress-induced depression in mice?

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ABSTRACT

Background: Depression is one of the important world-wide health problems.
Objectives: This study aimed to assess the ameliorative effect of *Ocimum basilicum* (OB) essential oil on the behavioral, biochemical and histopathological changes resulted from exposure to chronic unpredictable mild stress (CUMS). It also aimed to investigate the underlying mechanism in an animal model of depression.
Materials and methods: Forty male Swiss albino mice were divided into four groups (n = 10): control, CUMS (exposed to CUMS for 4 weeks), CUMS plus fluoxetine, and CUMS plus OB. At the end of the experiment, behavioral changes, serum corticosterone level, protein and gene expressions of brain derived neurotropic factor (BDNF) and glucocorticoid receptors (GR) in the hippocampus was all assessed. Immunoexpression of surface makers of glial fibrillary acidic protein (GFAP), Ki67, Caspase-3, BDNF and GR in the hippocampus were estimated. Data were analyzed by using the statistical package for the social sciences (SPSS).
Results: OB alleviated both behavioral and biochemical changes recorded in mice after exposure to CUMS. It also reduced neuronal atrophy observed in the hippocampal region III cornu ammonis (CA3) and dentate gyrus and restored back astrocyte number. OB decreased apoptosis in both neurons and glial cells and increased neurogenesis in the dentate gyrus in a pattern comparable to that of fluoxetine. Increased BDNF and GR gene and protein expressions seems to be behind the antidepressant-like effect of OB.
Conclusion: Ocimum basilicum ameliorates the changes induced after exposure to the chronic stress. Assessing *Ocimum basilicum* efficacy on human as antidepressant is recommended in further studies.

1. Introduction

Depression is a worldwide health problem that affects both low and high income people in modern communities (Bromet et al., 2011). The conventional treatment of depression essentially includes both pharmacologic and psychological therapies (Schmidt et al., 2008). The complementary and alternative medicine (CAM) becomes widely utilized for treatment of many diseases (Ji et al., 2014). One of the CAM approaches, that is called “aromatherapy”, is the use of essential and aromatic oils of fruits and flowers to treat some medical problems. Although aromatherapy was not sufficiently investigated in terms of efficacy, it was reported to be relatively safe when compared to the conventional pharmacological agents. It is also suggested to have potential effects in alleviating some mental and psychologic problems (Schmidt et al., 2008).

Ocimum basilicum L. (Family Lamiaceae), which is an annual herb, is the scientific name of sweet basil or Reihan. It is widely cultivated in many Asian, African, European and American countries (Grayer et al., 1996). *Ocimum basilicum* (OB) is used as a fragrance in perfumes, soap, dental creams, and mouth washes as well as in Mediterranean foods such as soup, cream cheese and in pasta dishes (Khan and Abourashed, 2010). It has been widely used in traditional medicine as a treatment for anxiety, diabetes, cardiovascular disease and headache (Bora et al., 2011).

The chronic unpredictable mild stress (CUMS) model of depression was selected in this study because it represents a valid and reliable model in rodents and has proved etiological, face and predictive validity. CUMS elicits behavioral, biochemical and cellular consequences corresponding to those reported in patients with major depression and is being utilized to study the effect and mechanism of action of the antidepressants (Song and Leonard, 2005). Thus, this study aimed to evaluate the ameliorative effect of *Ocimum basilicum* on the CUMS-

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associated changes in an animal model of depression and to investigate the mechanism behind this effect.

2. Materials and methods

2.1. Animals

The experiment was performed at King Fahed Medical Research Center (KFMRC) at King Abdulaziz University (KAU), Jeddah, Saudi Arabia in collaboration with the research center at Mansoura Faculty of Medicine. It was conducted according to the guidelines of animals care set in at KFMRC, complied with the National Institute of Health Guide (NIH publication No. 80-23, revised 1996). Forty male Swiss albino mice weighting 30–40 g were purchased from the animal house at the KFMRC. They were housed in stainless steel cages and maintained in a 12-hour light-dark cycle, with a room temperature of 27 ± 1 °C under hygienic conditions with free access to water and the standard food.

2.2. Drug and chemicals

Ocimum basilicum, known as Reihan in Saudi Arabia, was obtained from the local garden at Jeddah. It was morphologically identified, as described by Simon et al. (1990), and confirmed by consulting a specialist in Botany from the Faculty of Science, KAU. Essential Oil of OB was extracted according to Ismail (2006). The constituents of OB were analyzed by using gas chromatography coupled to mass spectrometry (GC-MS; Agilent, Columbia, USA) with DB-5 ms column (30 m × 0.25 mm × 0.25 μm) and are shown in Table 1. As described by Chioca et al. (2013), OB essential oil was diluted by Propylene glycol (5%; Sigma, St. Louis, MO, USA) just prior to application.

Fluoxetine hydrochloride, the selective serotonin reuptake inhibitor (SSRI) antidepressant, was utilized as a positive control in this study for pharmacological validation according to Li et al. (2014) and Yu et al. (2014). It was obtained from Dar Al Dawa (DAD) Pharmaceuticals Co., Ltd. (Jordan). It was dissolved in 0.03% sodium carboxymethyl cellulose (CMC-Na) and was given once a day at a dose of 20 mg/kg through intragastric gavage according to Li et al. (2014). Amyl acetate (5%; Sigma, St. Louis, MO, USA) was administrated to the control group as it has no effect on anxiety as shown in the previous studies (Pavesi et al., 2011). Administration of OB and amyl acetate was through inhalation in odor-isolated chamber (32 cm × 24 cm × 32 cm) as described by Chioca et al. (2013). Inhalation was once a day and lasted for 15 min immediately after the CUMS procedure.

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

2.3. Experimental procedure

After 2-weeks of acclimatization, the mice were randomly divided into four groups (10 mice each) included, the control (not treated), CUMS (exposed to CUMS for 4 weeks plus amyl acetate during the last 2 weeks of the experiment), CUMS + fluoxetine (FLU) (exposed to the CUMS for 4 weeks plus FLU in the last 2 weeks) and CUMS + OB (exposed to the CUMS for 4 weeks plus OB in the last 2 weeks). The mice were subjected to CUMS procedure that was modified by Ducottet and Belzung (2004). They were exposed to different types of stressors at different time points during the day for 4 weeks. 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°, restraining the mice and water stress by placing mice in an empty cage with 1 cm of water at the bottom. After two weeks of exposure to CUMS, treatment of mice with FLU and OB is started daily and was continued throughout the following two weeks along with CUMS. At the end of the experiment the behavior tests were performed sequentially starting with the elevated plus maze test (EPM), open field test (OFT) and finally the forced swimming test (FST) on days 29, 30 and 31. Blood samples were collected then the mice were sacrificed by decapitation.

2.4. Assessment of behavior

2.4.1. Forced swim test (FST)

It was done according to Doron et al. (2014). The test procedure was previously described by Ayuob et al. (2016). The total time that the mouse spent immobile during the 6 min was measured and presented in seconds.

2.4.2. Elevated plus-maze (EPM)

It was carried out according to Carobrez and Bertoglio (2005) and previously described by Ayuob (2016). The numbers of closed arms entries in 5 min and time spent by each mouse inside the open arm were measured and expressed in seconds.

2.4.3. Open field test (OFT)

It was carried out according to Mineur et al. (2006) and described by Ayuob et al. (2016). The number of mouse rearing, counted by the observer in 25 min, was registered manually while the distance traveled in 25 min was quantified using the video tracking system (Columbus Instruments, OHIO 43204, USA).

2.5. Assessment of serum corticosterone level

After performing the behavior tests, blood samples were obtained in the morning from the retro-orbital venous plexus after anaesthetizing the mice using ether. Blood was collected in EDTA-coated tubes and was centrifuged for 10 min. The collected serum samples were kept at − 80 °C until the corticosterone levels were assessed using RIA (ELISA kits, ALPCO Diagnostics, Orangeburg, NY).

2.6. Assessment of hippocampal glucocorticoid receptors (GR) and brain derived neurotropic factor (BDNF) protein expression levels

Immediately after decapitation of the mouse, the brain was dissected out on an ice-plate and the whole hippocampus was isolated according to Paxinos and Watson (1998). Tissue punches from the left hippocampus were 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

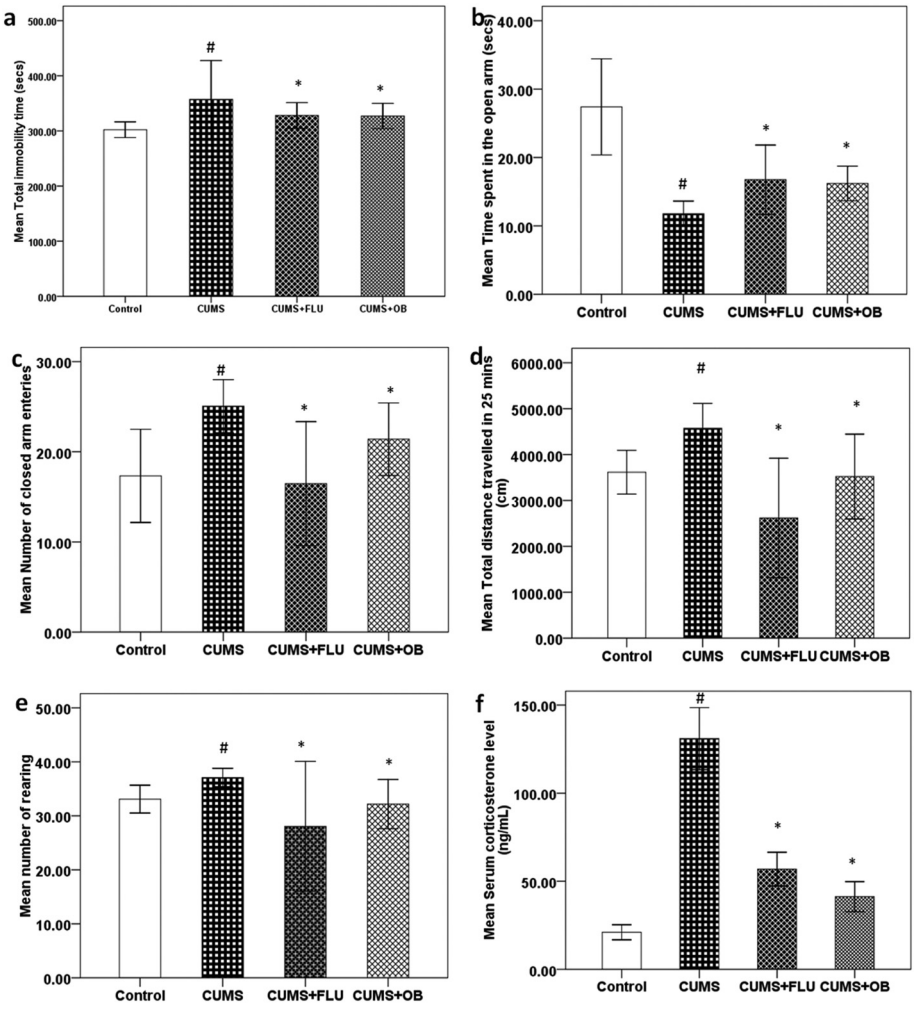

Fig. 1. Immobility time of the forced swimming test (a), elevated plus maze test (b, c), open field test (d, e) and serum level of corticosterone (f) of the control, chronic unpredictable mild stress (CUMS), fluoxetine-treated (FLU) and *Ocimum basilicum*-treated (OB) groups (n = 10 each). Data are shown as mean ± SD. # indicates significance compared to the control group, * indicates significance compared to the CUMS group.

leupeptin). Homogenates were acidified with 0.1 M HCl (pH 3.0), incubated at room temperature (22–24 °C) for 15 min, and neutralized (pH 7.6) with 0.1 M NaOH. Homogenates were then microfuged and BDNF and GR protein levels were assessed using sandwich enzyme-linked immunosorbent assay (ELISA) as described by Baker-Herman et al. (2004).

2.7. Assessment of hippocampal GR and BDNF gene expression levels

Total RNAs were isolated from 30 to 60 mg of hippocampus, using EZ RNA Clean up plus DNase Kit (EZ BioResearch, St Louis, MO, USA). RNA concentrations were measured using Nano Drop Spectrophotometer (Jenway, UK). Reverse transcriptions (RT) were performed using oligo-dT primers (BioneerInc, Daejeon, Republic of Korea) in a 20 μL reaction including 5 μL RNA. The cDNAs obtained were amplified by using PCR Master Mix (BioneerInc, Daejeon, Republic of Korea) with primers designed by metabion international AG, Semmelweisstr, Germany as follows: GR (forward 5′-AGCTCCCCCTGGTAGAGAC -3′; reverse 5′-GGTGAAGA CGCAGAAACCTT-3′), BDNF (forward 5′-TATTTCATACTTCGGTTGC-3′; reverse 5′-TGTCAGCCAGTGATGTCG-3′) and β-actin (forward 5′-TCTGGCACCACA CCTTCTA-3′; reverse 5′-AGGCATACAGGGACAGCAC-3′). PCR amplification was applied in a thermocycler (manufactured by Labnet International Inc.). The amplified fragments were analyzed by gel electrophoresis using a DNA ladder in order to assess the size of the amplicons products. The images were obtained using a gel documentation system (manufactured by Ultra-Violet Products Ltd.). The size of the amplicons was determined using a software available with the gel documentation system. The expression patterns of GR gene and BDNF gene in the hippocampus were performed through the real-time RT-PCR method using SYBR Green qPCR Master mix containing ROX as a reference dye (Biotool LLC, Houston, USA). All amplified fragments were achieved in three independent replicates; in addition, the results were normalized to β-actin as a reference gene using comparative Ct method.

2.8. Histopathological assessment

Animals were sacrificed by cervical decapitation to avoid any effect of anesthetic agent on brain histochemistry. The skull was opened and the brain was dissected out on iced plate then it was cut in the sagittal plane into 2 halves. The right one was fixed in 10% neutral buffered formalin overnight then processed to obtain paraffin blocks. Serial paraffin sections were cut into 3–4 μm thickness and stained with hematoxylin and eosin (H & E) for the histopathological examination (Bancroft and Gamble, 2008). Immunohistochemical studies were carried out using the peroxidase-labeled Streptavidin–Biotin Technique according to Makhlouf et al. (2014). The paraffin sections were deparaffinized then rehydrated. They were boiled in a microwave for 20 min in 0.01 M sodium citrate buffer (pH: 6) in order to retrieve antigen. 3% H_2O_2 in methanol was used for 5 min at room temperature to block endogenous peroxidase activity followed by washing twice in phosphate-buffered saline (PBS). In order to demonstrate astrocytes, slides were incubated overnight at 4 °C then they were incubated with 1:1000 diluted anti GFAP (Dako Cytomation-USA) for 1 h. In addition, 1:1000 diluted anti-Caspase-3 (Santa Cruz Biotechnology, USA) was

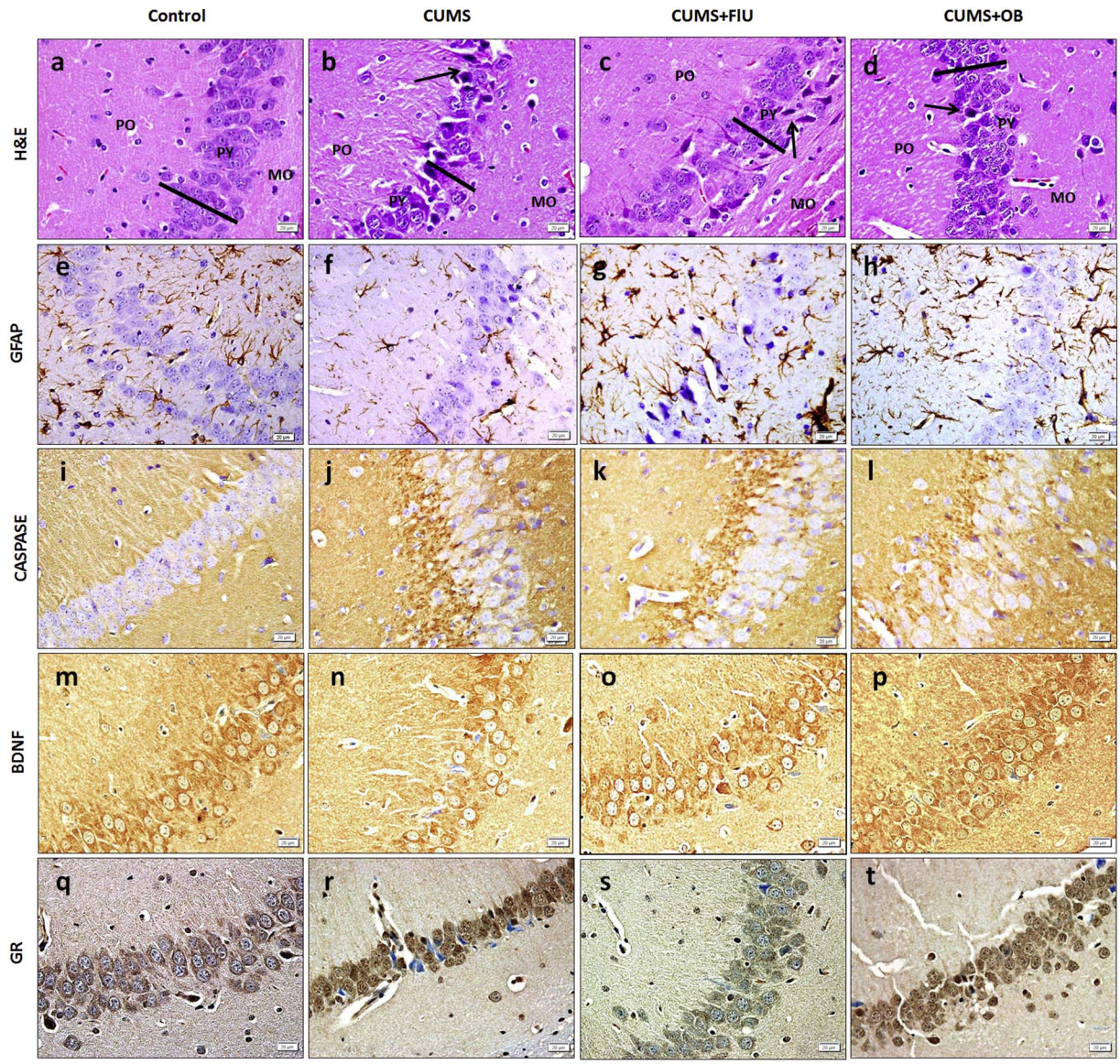

Fig. 2. The hippocampal CA3 is formed of the molecular layer (MO), the pyramidal layer (PY) and the polymorphic layer (PO). The thickness of the PY layer appears smaller in the chronic unpredictable mild stress (CUMS) compared to the control (black line). Some darkly stained cells (arrow) are observed (H & E a–d × 600, Scale bar = 20 μm). Immunoexpression of GFAP, Caspase, BDNF and GR in the hippocampal CA3 are shown. (e–t × 600, Scale bar = 20 μm) (OB; *Ocimum basilicum).*

utilized for 1 h to detect apoptosis. Anti-Ki-67 (rabbit polyclonal Ig G produced by Abcam, Cambridge, UK) was used at a dilution 1:100 to demonstrate proliferating cells. Rabbit anti-GR antibody and anti-BDNF (Santa Cruz Biotechnology, USA) were utilized with dilutions of 1:1000 and 1:400 respectively overnight at room temperature, then exposed to biotinylated goat anti-rabbit IgG and streptavidin peroxidase complex (1:200 dilution; Vector Laboratories) at room temperature. After slides washing, they were incubated with avidin-biotin-peroxidase complexes (Dako-USA) for 10 min, covered by DAB and incubated for 10 min then counterstained with hematoxylin.

2.9. Statistical and morphometric analyses

A light microscope (Olympus, BX-61, Los Anglos) connected to a digital camera was used for examining and photographing.

The CA3 of the C-shaped cornu ammonis (CA) of the hippocampus as well as the interlocking dentate gyrus (DG) were specifically examined in this study as they were the affected areas in depression (Gold et al., 2010). Both thickness and the surface area of the pyramidal cell layer in CA3 areas as well as the granular cell layer in the dentate gyrus (DG) areas were measured using Image Pro Plus Software media Cybernetics, Silver Spring, MD, USA (version 6.0). Assessment of 5 non-overlapping fields in each mouse and calculation of the mean of each mouse were done. In addition, the number of GFAP-positive cells in CA3 was counted in 5 high power field (× 400 magnification) in each mouse of the ten mice according to the method of Makhlouf et al. (2014). The number of Caspase-3 and Ki67-positive cells was assessed in mm^3 using the same software. The relative optical density (ROD) of BDNF and GR immunoexpression was assessed, as described by Chen et al. (2015).

The data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 16) software. Data were presented in the form of mean and standard deviation. For the parametric data, the different groups were compared using ANOVA (F-test) at degree of freedom (DF) between group = 3 and DF within groups = 6 and total DF = 9. ANOVA was followed by a Bonferroni post hoc test. Significance was considered at a p value < 0.05.

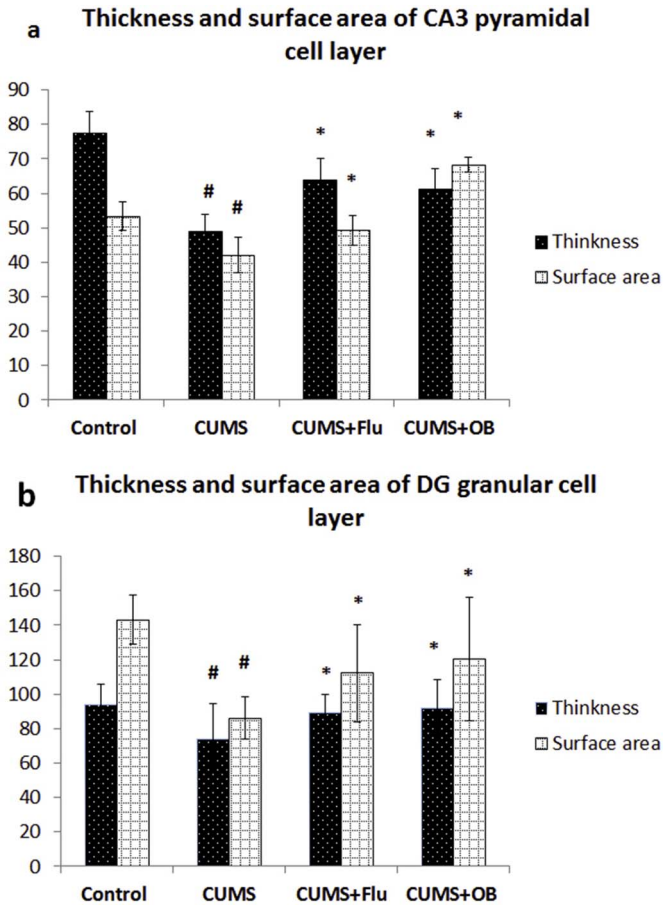

Fig. 3. Thickness and surface area of CA3 pyramidal cell layer and DG granular cell layer of the hippocampus of the studied groups. Data are shown as mean ± SD. # indicates significance compared to the control group, * indicates significance compared to the CUMS group.

3. Results

3.1. Effect on the behavior

A significant treatment effect on the immobility time of the FST, an indicator of the depressive-like behavior on using One way-ANOVA ($F = 2.6$, $p < 0.001$) was recorded. Post hoc test revealed a significant increase in this time after exposure to CUMS compared to the control group (control; 268.7 ± 41.2, CUMS; 305.3 ± 13.4, $p = 0.04$). Administration of either FLU or OB along with the CUMS significantly reduced the immobility time compared to the CUMS group (CUMS + FLU; 260.1 ± 47.8, $p = 0.01$, CUMS + OB; 263.4 ± 8.44, $p = 0.02$) (Fig. 1a).

The time spent in the open arms of the EPM, an indicator of the anxiety-like behavior, has been significantly changed after the treatment ($F = 52.7$, $p < 0.001$). It significantly decreased in mice exposed to CUMS compared to the control group (Control; 27.40 ± 3.51, CUMS; 11.77 ± 0.93, $p < 0.001$). Administration of either FLU or OB significantly increased this time compared to the CUMS group (CUMS + FLU; 16.76 ± 2.53, $p = 0.001$, CUMS + OB; 16.21 ± 1.3, $p = 0.003$) (Fig. 1b).

One way-ANOVA revealed a significant effect of treatment on the number of closed arm entries in the EPM ($F = 22.1$, $p < 0.001$). Post hoc test revealed a significant increase in this number in the CUMS group compared to the control mice (Control; 17.33 ± 2.6, CUMS; 25.7 ± 1.5, $p < 0.001$). Administration of either FLU or OB significantly reduced this number compared to the CUMS group (CUMS + FLU; 16.5 ± 3.4, $p < 0.001$, CUMS + OB; 21.4 ± 2.1, $p = 0.01$) (Fig. 1c).

The distance traveled by mice during the OFT, an indicator of the spontaneous locomotor activity, has been significantly changed after treatment ($F = 35.03$, $p < 0.001$). It was significantly longer in mice exposed to CUMS than in those of control mice (control; 3616.3 ± 238.2, CUMS; 4572.8 ± 270.3, $p < 0.001$). Exposure to either FLU or OB along with the CUMS significantly reduced this distance compared to the CUMS group (CUMS + FLU; 2617.8 ± 650.6, $p < 0.001$, CUMS + OB; 3521.4 ± 441.5, $p < 0.001$). Similarly, the number of mice rearing significantly changed after treatment ($F = 9.9$, $p < 0.001$). CUMS significantly increased it compared to the control group (control; 32.69 ± 1.5, CUMS; 37.1 ± 0.85, $p = 0.04$) while FLU or OB significantly reduced these movements compared to the CUMS group (CUMS + FLU; 28.05 ± 6.02, $p < 0.001$, CUMS + OB; 32.4 ± 4.3, $p = 0.01$) as revealed by post hoc (Fig. 1d, e).

3.2. Effect on serum corticosterone level

A significant effect of treatment on basal serum corticosterone using One way-ANOVA ($F = 458.9$, $p < 0.001$) was recorded. CUMS significantly ($p < 0.001$) increased it compared to the control; however, administration of either FLU or OB significantly ($p < 0.001$) reduced it compared to the CUMS group (Fig. 1f).

3.3. Effect on hippocampus histological structure

The CA of the hippocampus was formed of 3 areas; CA1, CA2 and CA3. The control CA3 was formed of three layers; polymorphic, pyramidal and the molecular cell layer. The pyramidal cell layer showed crowded pyramidal cells with large vesicular nuclei. Many of these pyramidal cells appeared smaller with dark cytoplasm and small condensed nuclei in mice exposed to CUMS. On the other hand, mice exposed to CUMS along with FLU or OB showed fewer numbers of these small dark cells while the majority of these cells appeared normal (Fig. 2). A significant increase in both thickness and surface area of the pyramidal cell layer was observed in groups exposed to FLU or OB compared to the CUMS group (Fig. 3a).

The control DG was formed of three layers: the molecular, granular and pleomorphic cell layers. The granular cell layer showed polyhedral cells with vesicular nuclei. Many of those cells appeared smaller with dark cytoplasm and small condensed nuclei (apoptotic) in mice exposed to CUMS. These dark cells were less frequently observed in mice exposed to CUMS along with FLU or OB (Fig. 4). A significant increase in both thickness and surface area of the granular cell layer is observed in groups exposed to FLU or OB compared to the CUMS group (Fig. 3b).

As for the immunohistochemical results, it was observed that immunoexpression of GFAP in both CA3 and DG of CUMS group significantly ($p = 0.004$, $p = 0.01$) decreased compared to the control group while it significantly increased in both areas compared to the CUMS group after administration of FLU ($p = 0.03$, $p = 0.04$) or OB ($p = 0.02$, $p = 0.04$) respectively (Figs. 2, 4, 5a).

Figs. 2, 4 and 5b illustrate the anti-apoptotic effect in the CUMS model. Administration of FLU ($p < 0.001$) or OB ($p < 0.001$) significantly decreased the number of Caspase-3-positive cells in both CA3 and DG compared to the CUMS group.

The effect on the DG neurogenesis was summarized in Figs. 2, 4 and 5c. Administration of FLU ($p = 0.003$) or OB ($p = 0.04$) significantly increased the number of proliferating Ki67-positive cells in the subgranular zone (SGZ) of DG compared to the CUMS group.

BDNF immunoexpression obviously decreased, in CA3 ($p = 0.001$) and DG ($p = 0.01$) of CUMS group compared to the control while it significantly increased in both areas after administration of FLU ($p < 0.001$) or OB ($p < 0.001$) (Figs. 2, 4, 6a).

A similar trend was observed in GR immunoexpression. It significantly decreased in CA3 ($p = 0.001$) and DG ($p = 0.02$) of CUMS group compared to the control while administration of FLU ($p < 0.001$) or OB ($p = 0.001$, $p = 0.004$) significantly increased it

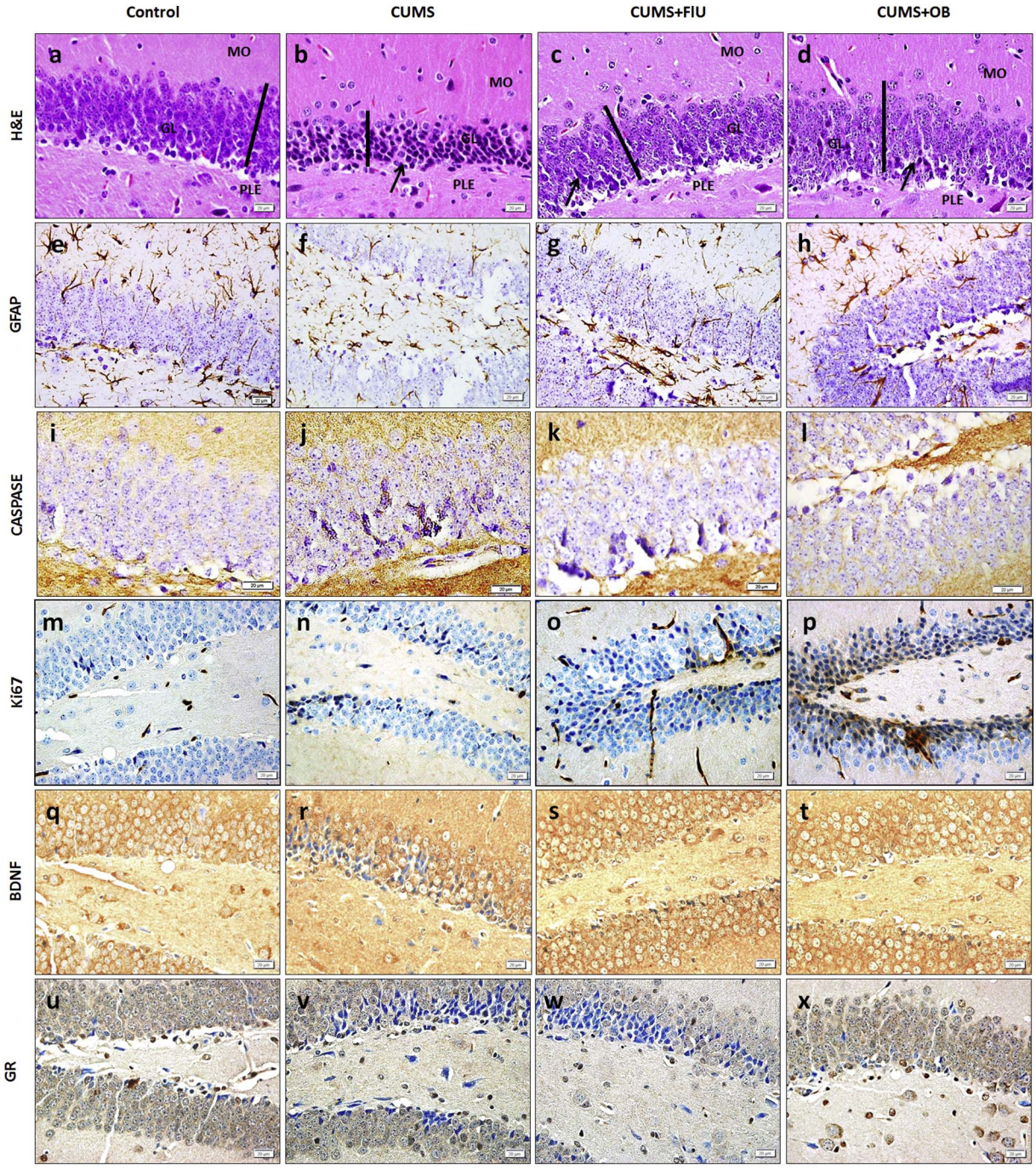

Fig. 4. The hippocampal dentate gyrus is formed of the pleomorphic layer (PLE), the granular cell layer (GL) and the molecular (MO). The thickness of the GL layer appears smaller in the chronic unpredictable mild stress (CUMS) compared to the control (black line). Some darkly stained cells (arrow) are observed (H & E a–d × 600, Scale bar = 20 μm). Immunoexpression of GFAP, Caspase-3, Ki67, BDNF and GR in the hippocampal dentate gyrus are shown. (e–x × 600, Scale bar = 20 μm) (OB; *Ocimum basilicum)*.

respectively compared to the CUMS group (Figs. 2, 4, 6d).

3.4. Effect on BDNF gene and protein expression levels

On assessing the BDNF mRNA expression level using the quantitative RT-PCR, it was found that it significantly diminished ($p = 0.03$) in the hippocampus of the CUMS group compared to the control one, while it was significantly increased compared to the CUMS group after administration of FLU or OB ($p < 0.001$, $p = 0.01$). Similar observations were noticed in BDNF protein expression assessed using ELISA as exposure to CUMS significantly ($p < 0.001$) down-regulated it compared to the control while administrating FLU or OB significantly ($p < 0.001$, $p = 0.01$) up-regulated it compared to the CUMS group (Fig. 6 b, c).

3.5. Effect on GR gene and protein expression levels

It was found that GR mRNA expression level was significantly ($p = 0.01$) reduced in the hippocampus of the CUMS group compared to the control one. However, FLU ($p < 0.001$) or OB ($p = 0.03$) administration significantly increased it compared to the CUMS group. A similar trend was observed in GR protein expression, since exposure to CUMS significantly ($p < 0.001$) down-regulated its level compared to the control. However, administrating FLU or OB significantly

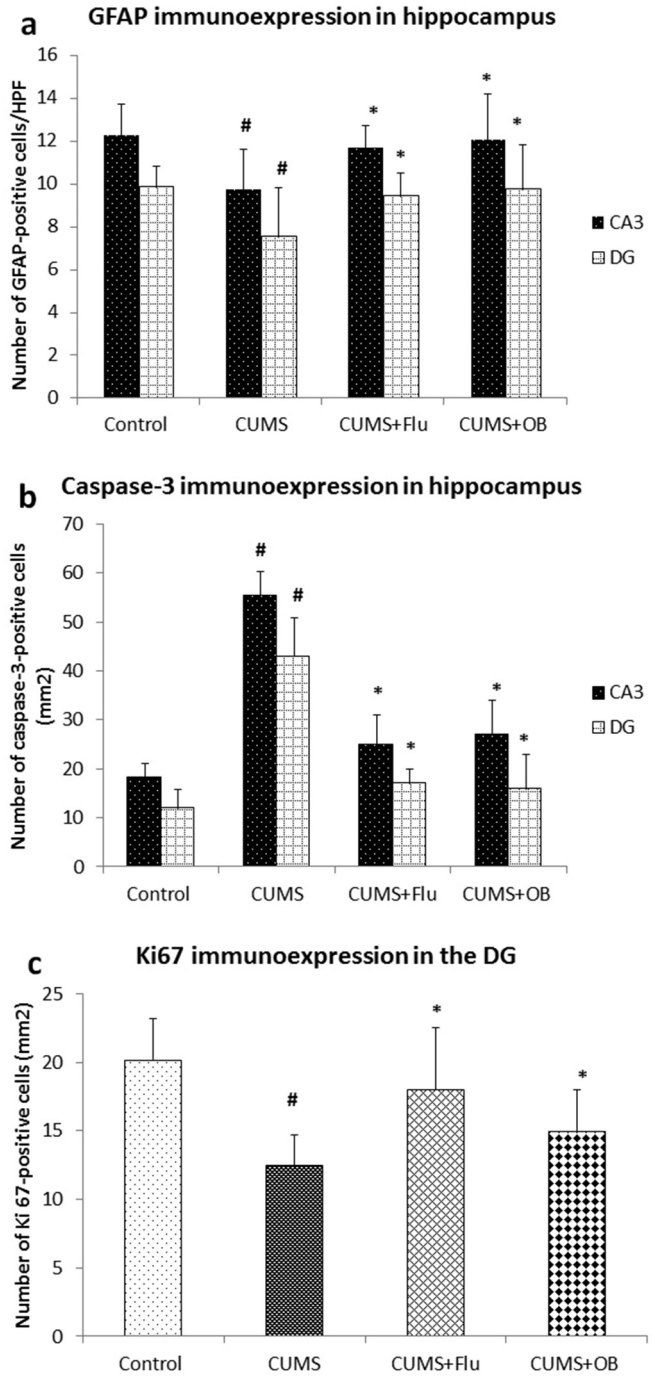

Fig. 5. GFAP, Caspase-3, Ki67 immunoexpression assessment in the hippocampus. Data are shown as mean ± SD. # indicates significance compared to the control group, * indicates significance compared to the CUMS group.

($p < 0.001$, $p = 0.04$) up-regulated this expression compared to the CUMS group (Fig. 6e, f).

4. Discussion

Many plants and herbs, in traditional medicine, have been used for the treatment of anxiety disorder and depression and found effective according to evidences (Rabbani et al., 2015). Accordingly, this study aimed to assess the effect of OB essential oil inhalation on alleviating CUMS-induced depression as well as understanding the involved mechanism eliciting that effect.

In this study, the results of GC/MS analysis of OB essential oil reveal that it contains about 23 compounds: Linalool (35.9%), Cineole (11.2%), Cadinol (10.4%), Farnesyl acetate (10.2%), Farnesene (4.9%), Ocimene (3.7%), Cadinene (3.1%) and Camphor (2.3%). These findings were in accordance with those of Rabbani et al. (2015) despite the difference in the component percentages which could be attributed to the collected plant or to the climatic factor that could affect plant growth.

In the current work, animals after CUMS exposure exhibited depressive status evidenced by lengthening of both immobility durations in the FST and period consumed in the open arms in the EPM, which was supported by increased spontaneous locomotor motion. The principal finding of the present work was the alleviation of these CUMS-induced behavioral changes by OB as well as the reduction of the increased serum corticosterone. OB could reduce the hippocampus nerve cell atrophy caused by CUMS and restored back the reduced number of the astrocytes. Moreover, OB diminished the apoptotic glial and nerve cells in the CUMS animals' hippocampus. OB diminished the CUMS-induced corticosterone level and cellular degeneration in a pattern comparable to that of fluoxetine. The antidepressant-like effect of OB was produced by up-regulation of gene and protein expression of BDNF and GR and this was the same for fluoxetine.

Depression symptoms were commonly observed to be accompanied with disturbed glucocorticoid secretion in patients with depression and in many animal models of depression. Therefore, dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis was investigated in the current work through assessing the serum corticosterone level (Zunszain et al., 2011). In this study, CUMS resulted in significant increase in the serum corticosterone which indicated hyperactivity in the HPA axis. Many previous studies reported similar findings (Mizuki et al., 2014). Fluoxetine and OB significantly reduced the corticosterone level in the present work. Liu et al. (2014) also observe a reduced corticosterone level after fluoxetine administration in stressed mice.

To estimate the hippocampal astrocyte number and integrity, a specific immune-marker GFAP was used (Webster et al., 2001). The number of the GFAP-positive astrocytes is markedly decreased in the CA3 area and DG of the CUMS mice. This finding is in accordance with that of Li et al. (2013) during their study on stressed rats and is supported by post-mortem studies of Webster et al. (2001) on patients with mood disorders and Bowley et al. (2002) on patients with depression. Banasr and Duman (2008) have postulated that reduced astrocyte GFAP expression is a contributing agent in depression symptoms evolution. Fluoxetine, as an antidepressant, was found to prevent both the decrease of GFAP expression and the glial atrophy (Liu et al., 2014) and this was manifest in the current work. OB has elicited a more or less similar effect as FLU. When it came to the chronic stress apoptotic effect, Liu et al. (2014) observed an increase in TUNEL-positive nerve cells in the hippocampus, an indicator of cell death. Furthermore, a more recent study of Yu et al. (2014) reported that CUMS induced an increase in bax and Caspase-3 as well as a decrease in the bcl-2 expression in the hippocampus and this is consistent with the present work regarding Caspase-3 expression. Administration of FLU along with CUMS has resulted in trivial reduction of Caspase-3 expression whereas administration of OB markedly reduced it. This anti-apoptotic effect of the antidepressants was previously described by many authors (Manji and Duman, 2001; Lucassen et al., 2004).

It was found, in this study, that exposure to CUMS was associated with decreased number of proliferating nerve cells in the SGZ of DG as detected by Ki67 immunostaining. This is in accordance with Alonso et al. (2004) and Zhang et al. (2015). Chronic, but not acute, antidepressant administration was found to increase SGZ proliferation and prevent the down-regulation of neurogenesis caused by chronic mild stress in mice (Alonso et al., 2004; Malberg et al., 2000). This new cell birth is necessary for the behavioral actions of these agents in selected rodent models (Banasr et al., 2011). This finding was supported, in this study, regarding fluoxetine as well as OB. Increased apoptosis of nerve

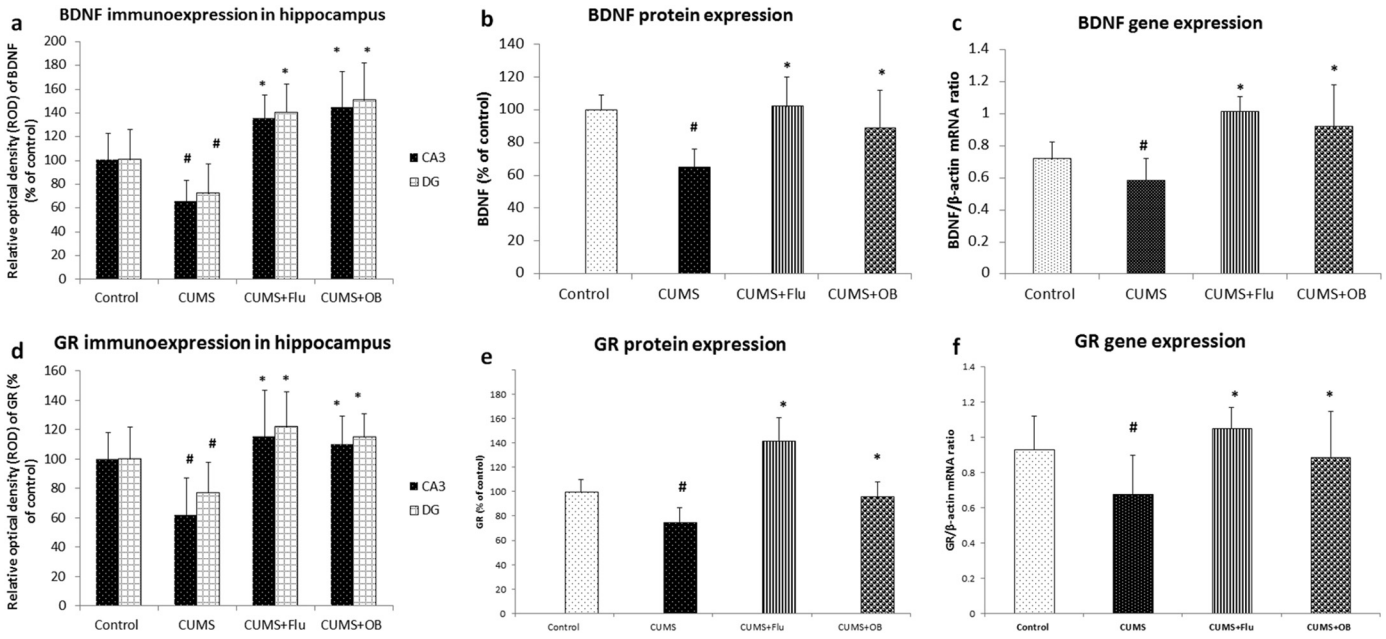

Fig. 6. Quantitative assessment of BDNF and GR immunoexpression, protein and gene expressions in the hippocampus. Data of immunoexpression are shown as mean ± SD and that of protein and gene are expressed as mean percent of control value ± SD. # indicates significance compared to the control group, * indicates significance compared to the CUMS group.

or glial cells and reduced neurogenesis in the SGZ of dentate gyrus seem to be behind the reduction in hippocampal volume reported by Sahay et al. (2007). It was also behind CA3 pyramidal and DG granular cell layers thickness diminution reported in the current work.

The BDNF is the brain neurotrophic/growth factor which is widely studied in stress and depression researches (Monteggia, 2007). Chronic stress and heterozygous deletion of BDNF in mouse resulted in atrophy of nerve cells in hippocampus and prefrontal cortex (Duman, 2004). When it came to GR, hippocampal nerve cells can be damaged by the high level of glucocorticoid because they are specifically rich in GR (Szyman´ ska et al., 2009). Repeated stress and glucocorticoid administration were reported to result in atrophy of CA3 pyramidal neurons (Warner-Schmidt and Duman, 2006). Because of that, both BDNF and GR were selected to be sensitive indicators of antidepressant effect of OB. Down-regulation of GR immuno- gene- and protein expressions following exposure to CUMS has been observed in this study. This was in accordance with the previous findings regarding the down-regulation in the number of GRs (Sapolsky et al., 1984), or of their mRNA expression (Mizoguchi et al., 2003) induced in hippocampus by exposure to different chronic stress protocols. This GR down-regulation has been ascribed to the sustained increase in corticosterone levels elicited by stress and resulted in a decreased responsiveness to glucocorticoids (Mizoguchi et al., 2003). In this study, both BDNF and GR immuno-gene- and protein expressions were up-regulated after administration of fluoxetine and OB. Increased level of circulating glucocorticoids, in response to chronic stress, seems to result primarily in activation of GR, which then translocate to the nucleus of the cell where they trigger changes in gene expression with subsequent long-lasting effects on the structure and functioning of the cells (Warner-Schmidt and Duman, 2006). Sustained stress with the subsequent release of pro-inflammatory cytokines lead to chronic neuroinflammation (Kim et al., 2016). They added that the elevated pro-inflammatory cytokine levels and hippocampal GRs functional resistance are among the most widely investigated factors in the pathophysiology of depression (Kim et al., 2016). OB was reported to have a neuroprotective effect, as it was reported to reduce the size of cerebral infarct and the lipid peroxidation in the brain (Bora et al., 2011). The authors attributed this to the reactive oxygen species scavenge effect of phenolic, flavonoids and tannin contents. Some of the OB essential oils components, detected in this study, have been reported in some previous studies to have anxiolytic and sedative effects such as: 1,8-Cineole, Linalool, Caryophyllene, Humulene and Camphor (Edewor-Kuponiyi, 2013; Satou et al., 2014), which is supportive to our findings.

In conclusion, this study indicated that inhalation of OB essential oils has antidepressant-like effect on CUMS induced depression in an animal model. The behavioral changes, elevated serum glucocorticoid level, neuronal and glial apoptosis, reduced neurogenesis in the dentate gyrus, down-regulated gene- and protein- expression levels of GR and BDNF in the hippocampus were all significantly improved after inhalation of OB during exposure to CUMS compared to the untreated group. Further investigation of the underlying mechanism as well as investigation of the antidepressant-like effect of OB on human are recommended.

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