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The antidepressant-like effect of Ocimum basilicum in an animal model of depression

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Abstract

We investigated the efficacy of *Ocimum basilicum* (OB) essential oils for treating depression related behavioral, biochemical and histopathological changes caused by exposure to chronic unpredictable mild stress (CUMS) in mice and to explore the mechanism underlying the pathology. Male albino mice were divided into four groups: controls; CUMS; CUMS plus fluoxetine, the antidepressant administered for pharmacological validation of OB; and CUMS plus OB. Behavioral tests included the forced swim test (FST), elevated plus-maze (EPM) and the open field test (OFT); these tests were performed at the end of the experiment. We assessed serum corticosterone level, protein, gene and immunoexpression of brain-derived neurotropic factor (BDNF) and glucocorticoid receptors (GRs) as well as immunoexpression of glial fibrillary acidic protein (GFAP), Ki67, caspase-3 in the hippocampus. CUMS caused depression in the mice as evidenced by prolonged immobility in the FST, prolonged time spent in the open arms during the EPM test and reduction of open field activity in the OFT. OB ameliorated the CUMS induced depressive status. OB significantly reduced the corticosterone level and up-regulated protein and gene expressions of BDNF and GR. OB reduced CUMS induced hippocampal neuron atrophy and apoptosis, and increased the number of the astrocytes and new nerve cells. OB significantly increased GFAP-positive cells as well as BDNF and GR immunoexpression in the hippocampus.

Key words: apoptosis, brain-derived neurotropic factor (BDNF), depression, glial fibrillary acidic protein (GFAP), glucocorticoid receptors (GRs), hippocampus, mice, neurogenesis, *Ocimum basilicum*

Depression is a mental disorder that is the second greatest disease load worldwide in 2020 (Brundtland 2001, Bromet et al. 2011). Although pharmacological therapies for depression are widely used (Rodgers et al. 2012), there remains a need for more and safer therapies.

Ocimum basilicum L. (Family Lamiaceae) (OB), commonly called sweet basil, is a widely cultivated annual herb (Grayer et al. 1996). OB also is used in perfumes and foods (Khan and Abourashed 2010), and as a traditional medicine owing to its anxiolytic, anti-diabetic, carminative and antispasmodic properties (Bora et al. 2011). A leaf extract of OB has been shown to improve neuromuscular coordination, exploratory behavior and short-term memory in healthy male albino mice (Zahra et al. 2015).

The brain is the central organ involved in perceiving and adapting to social and physical stressors owing to multiple interacting agents including excitatory amino acids and glucocorticoids

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together with a group of extra- and intracellular moderators such as brain-derived neurotrophic factor (BDNF) (McEwen et al. 2015).

Chronic unpredictable mild stress (CUMS) is a reliable animal model for depression in rodents. We used CUMS to assess the efficacy of OB for treating depression-related behavioral, biochemical and histopathological changes, and to explore the mechanisms underlying these effects.

Material and methods

Our study 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). Animals were treated according to the guidelines for animal care at the King Fahed Medical Research Center (KFMRC), KAU, Jeddah, SA. Forty 5-week-old 30–40 g male Swiss albino mice were purchased from the animal house at the KFMRC. Animals were housed in groups of 10 in 40 × 30 × 15 cm stainless steel cages with sawdust bedding that was renewed daily. Animals were maintained in a 12 h light:12 h dark cycle (lights on: 07:00 AM–7:00 PM) at 27 ± 1° C under hygienic conditions. Water and food were available *ad libitum*.

Chemicals and gas chromatography

OB, known as reihan in Saudi Arabia and Egypt, was obtained from the botanical gardens south of Jeddah in March 2015. It was identified morphologically according to Simon et al. (1990) and was confirmed by consultation with a specialist in botany from the Faculty of Science, KAU. The essential oils of OB were extracted according to Ismail (2006). The OB essential oils were analyzed by gas chromatography coupled to mass spectrometry (GC-MS; Agilent, Columbia, MD) using a 30 m × 0.25 mm × 0.25 µm DB-5 ms column. The OB essential oils were diluted with 5% propylene glycol (Sigma, St. Louis, MO) as described by Chioca et al. (2013) and administered by inhalation. Fluoxetine (FLU) hydrochloride was obtained from Dar Al Dawa (DAD) Pharmaceuticals Co., Ltd. (Amman, Jordan). FLU was dissolved in 0.03% sodium carboxymethyl cellulose (CMC-Na) and 20 mg/kg was administered by intragastric gavage (Li et al. 2014). Amyl acetate, 5% (Sigma), was administered by inhalation to the control group; it has been shown to have no effect on anxiety (Pavesi et al. 2011).

Experimental design

After 2 weeks for acclimatization, the mice were divided randomly into four groups of 10 and housed five mice/cage in the behavioral laboratory. The groups were: untreated control group, CUMS group exposed to CUMS for 4 weeks followed by amyl acetate for another 2 weeks, CUMS + FLU group exposed to CUMS for 4 weeks followed by FLU for another 2 weeks, and CUMS + OB group exposed to CUMS for 4 weeks followed by OB for another 2 weeks. We used the CUMS procedure described by Willner (1997) modified for mice by Ducottet and Belzung (2004). Mice were exposed to social stress by placing them in cages soiled by other mice, reversing the light/dark cycle, placing mice in cages with wet sawdust, tilting cages at 30°, restraining the mice and placing them in an empty cage with 1 cm of water on the bottom. Mice were exposed to one stressor/day at different times during the day for 4 weeks.

OB essential oil was diluted with propylene glycol immediately prior to the experiments. Inhalation of OB and amyl acetate was accomplished using a 32 × 24 × 32 cm odor-isolated acrylic box with two holes in its top wall. Two cotton balls soaked with 2.5 ml/unit of OB or amyl acetate were placed in these two holes for 15 min once/day immediately after the CUMS procedure. After each exposure session, the apparatus was cleaned with ethanol and the cotton wool containing the substance was replaced to maintain the concentration in the apparatus. At the end of the experiment, behavior tests were performed between 8:00 and 11:30 AM in a dimly lit room as described by Mineur et al. (2006) using the elevated plus maze test (EPM), open field test (OFT) and the forced swimming test (FST) on days 29, 30 and 31, respectively, with a 24 h break between tests. On day 32, approximately 1 ml blood samples were collected in the morning, then the mice were sacrificed by decapitation.

Behavioral tests

The FST was performed according to Doron et al. (2014). The total time the mouse spent not moving during 6 min was recorded in seconds.

For the EPM (Carobrez and Bertoglio 2005), the number of closed arm entries during 6 min and time spent by each mouse inside the open and closed arms were recorded in seconds.

The OFT was performed according to Mineur et al. (2006). The number of mouse rearings during 25 min was recorded and the distance traveled by

the mouse during this period was measured using a video tracking system (Columbus Instruments, Columbus, OH).

Biochemistry

Mice were anesthetized in the morning using ether and blood samples were obtained from the retro-orbital venous plexus and placed in EDTA-coated tubes. Samples then were centrifuged for 10 min at 7,000 × g at 37° C, then stored at −80° C until the corticosterone levels were assessed using radioimmunoassay (ELISA kits; ALPCO Diagnostics, Orangeburg, NY).

After blood sampling and while the mice were still anesthetized, they were sacrificed by cervical dislocation and the brain was immediately removed and placed on an ice plate. The entire hippocampus was isolated according to plate 11 of Paxinos and Watson's atlas (1998). Tissue punches were taken from the hippocampus of the left side, 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). Homogenates were acidified with 0.1 M HCl, pH 3.0, incubated at room temperature (22–24 ° C) for 15 min and adjusted to pH 7.6 with 0.1 M NaOH. The supernatants then were centrifuged at 7000 × g at 37° C for 10 min and processed using sandwich enzyme-linked immunosorbent assay (ELISA) as described by Baker-Herman et al. (2004) to assess the BDNF and GR.

GR and BDNF gene expression

The remaining tissues of the hippocampus of the left side were snap-frozen in liquid nitrogen, then processed for assessing both GR and BDNF gene expression as described by Ayuob et al. (2016). The primers used were designed by Metabion International (AG, Semmelweisstr, Germany) as follows: GR (forward 5′-AGCTCCCCCTGGTAGAGAC –3′; reverse 5′-GGTGAAGACGCAGAAACCTT-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 conducted in a thermocycler (Labnet International, Inc., Edison, NJ). The expression patterns of the GR and BDNF genes in the hippocampus were examined using RT-PCR SYBR Green qPCR Master mix that contained ROX as a reference dye (Biotool, Houston, TX).

Histopathology

The right cerebral hemisphere was fixed in 10% neutral buffered formalin overnight, dehydrated through an ascending series of alcohols, cleared in xylene and embedded in paraffin. Serial paraffin sections were cut at 3–4 µm and stained with hematoxylin and eosin (H & E) (Bancroft and Gamble 2008) for assessment of histopathology. Immunohistochemical studies were performed using the peroxidase-labeled streptavidin-biotin technique (Makhlouf et al. 2014). To demonstrate astrocytes, we used anti-glial fibrillary acidic protein (GFAP) antibody (Dako Cytomation, Minneapolis, MN) diluted 1:1,000 with PBS. To demonstrate apoptosis, we used anti-caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:1000 with PBS. We used anti-Ki-67 (rabbit polyclonal IgG; Abcam, Cambridge, UK) diluted 1:100 with PBS for indirect measurement of neurogenesis. We also used rabbit anti-GR antibody (Santa Cruz Biotechnology) and anti-BDNF (Santa Cruz Biotechnology) diluted 1:1,000 and 1:400 with PBS to detect expression of GR and BDNF, respectively, in the hippocampal tissue. A negative control section stained without the primary antibody was included for each antibody to verify primary antibody specificity. After washing, the slides were incubated with the avidin-biotin-peroxidase complex (Dako) for 10 min, covered with DAB, incubated for 10 min, then counterstained with hematoxylin, dehydrated and mounted.

We used a light microscope (Olympus, BX-61, Los Angeles, CA) connected to a digital camera for examination and photography. Both the thickness and surface area of the pyramidal cell layer in the CA3 area and the granular cell layer of the dentate gyrus (DG) were measured using Image ProPlus Software, (Media Cybernetics, Silver Spring, MD). We examined ten sections for each mouse. In each section, five non-overlapping fields were assessed and the mean for each of the ten mice was calculated. In addition, the number of GFAP-positive cells in CA3 was counted in five high power fields (x 400) in each mouse according to Makhlouf et al. (2014). The number of caspase-3 and Ki67-positive cells was counted in mm^2 using the same software. The relative optical density (ROD), an indicator of immunoexpression of BDNF and GR, was assessed using Image ProPlus Software (Chen et al. 2015).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 16) software. ANOVA (F-test), then Bonferroni *post hoc*

tests were used to compare parametric data of different groups. Values for $p \leq 0.05$ were considered significant.

Results

Gas chromatography-mass spectrometry

The components of the OB essential oil with their percentages determined by GC-MS are shown in Table 1.

Biochemistry

CUMS increased significantly the level of serum corticosterone compared to controls ($p < 0.001$). Administering either FLU ($p < 0.001$) or OB ($p < 0.001$) significantly reduced corticosterone levels compared to the CUMS group (Fig. 1a).

BDNF expression was decreased significantly compared to controls after exposure to CUMS ($p < 0.001$). Administering FLU or OB increased BDNF significantly compared to CUMS ($p < 0.001$ and 0.01, respectively) (Fig. 1b). BDNF mRNA expression assessed using quantitative RT-PCR was decreased significantly ($p = 0.03$) in the hippocampus of the CUMS group compared to controls; FLU or OB treatment increased these levels significantly compared to CUMS ($p < 0.001$ and 0.04, respectively) (Fig. 1c).

The GR protein expression level was decreased significantly after exposure to CUMS compared to controls ($p < 0.001$). Administering FLU or OB increased GR expression significantly compared to CUMS ($p < 0.001$ and 0.04, respectively) (Fig. 1d).

GR mRNA expression levels were reduced significantly in the hippocampus of the CUMS group compared to controls ($p = 0.01$), while FLU and OB treatment increased the level significantly compared to CUMS ($p < 0.001$ and 0.001, respectively) (Fig. 1e).

Table 1. Chemical composition of essential oil of OB

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
Unidentified compounds		4.177

Effect of OB on behavior

The FST immobility time was increased for the CUMS group compared to controls ($p < 0.001$). Treatment with FLU or OB significantly reduced the FST compared to the CUMS group ($p = 0.04$ and 0.01, respectively) (Fig. 2a).

We observed a significant decrease in the time spent in the open arms of the EPM test for the CUMS group compared to controls ($p < 0.001$). Administration of FLU or OB increased the time significantly compared to the CUMS group ($p = 0.001$ and < 0.001, respectively) (Fig. 2b). The number of closed arm entries during the EPM test was increased significantly for the CUMS group compared to controls ($p < 0.001$). FLU or OB reduced the number of entries significantly compared to the CUMS group ($p < 0.001$ and < 0.001, respectively) (Fig. 2c).

We observed an increase in the spontaneous locomotor activity of the CUMS group during the OFT compared to controls ($p < 0.001$). Treatment with either FLU or OB significantly reduced this activity compared to the CUMS group ($p < 0.001$ and $= 0.004$, respectively) (Fig. 2d, e).

Effect of OB on histology of the hippocampus

The CA3 area of the hippocampus contains polymorphic, pyramidal and molecular cell layers. In the control group, the pyramidal layer contained crowded pyramidal cells with large vesicular nuclei, while in the CUMS group, many of those cells appeared smaller with dark cytoplasm and small condensed nuclei. The groups treated with FLU or OB exhibited fewer affected cells (Fig. 3). Both thickness and surface area of the pyramidal cell layer were increased significantly in the groups

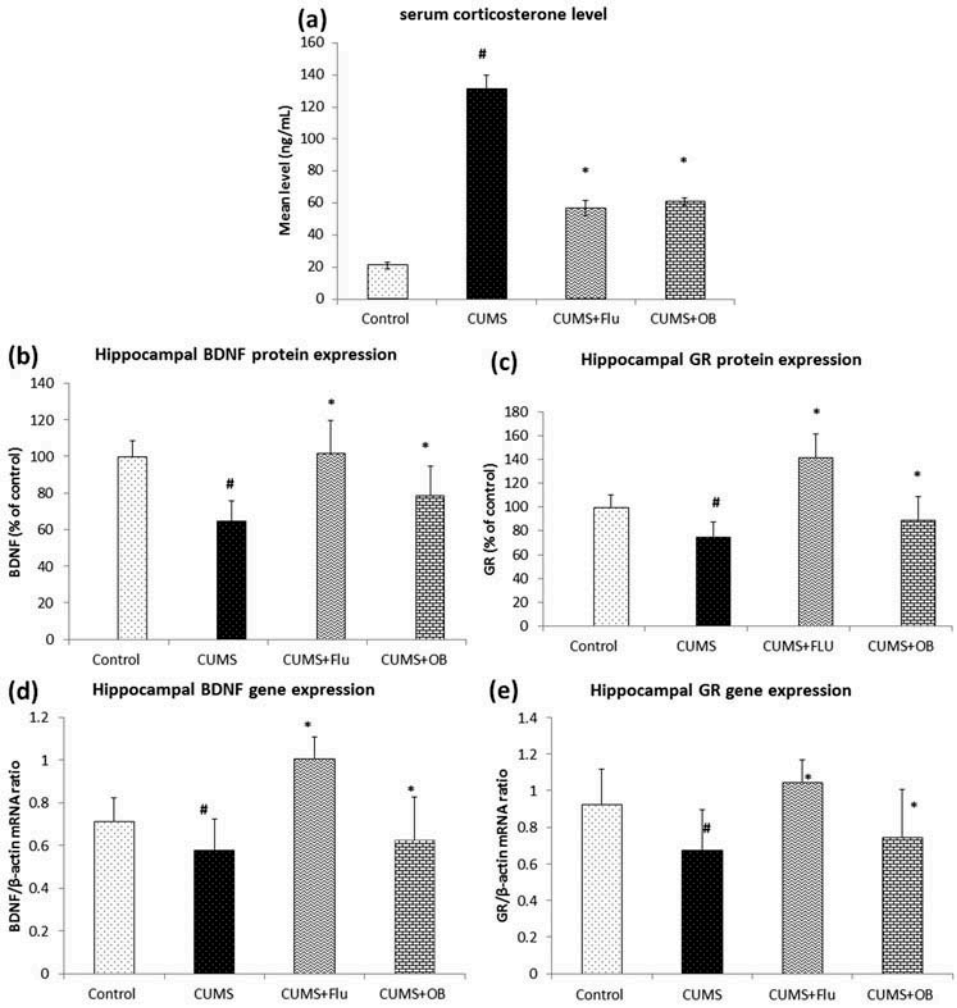

Fig. 1. Serum corticosterone level (a), and BDNF (b) and GR (c) expression levels in the hippocampus assessed by ELISA and expressed as percent of control value ± SD. BDNF mRNA (d) and GRm RNA (e) expression levels in the hippocampus assessed by quantitative RT-PCR (n = 10). #Significant vs. control, *significant vs. CUMS. CUMS, chronic unpredictable mild stress; FLU, fluoxtein; OB, *Ocimum basilicum*.

treated with FLU or OB compared to the CUMS group (Table 2).

The DG of the hippocampus also consists of molecular, granular cell and pleomorphic layers. The granular cell layer of the control group contained polyhedral cells with vesicular nuclei, while the CUMS group exhibited many smaller cells with dark cytoplasm and small condensed nuclei. The small cells were observed less frequently in the groups treated with FLU or OB (Fig. 4, Table 2). The thickness and surface area of the granular cell layer of groups treated with FLU or OB were increased significantly (Table 2).

Effect of OB on immunohistochemistry

Immunoexpression of GFAP, caspase, Ki67, BDNF and GR was assessed in the hippocampal CA3 and DG (Figs. 5, 6). The number of GFAP-positive cells was significantly decreased in the CA3 and DG of the CUMS group compared to the control (p = 0.003 and < 0.001, respectively). Treatment with FLU or OB increased GFAB significantly compared to CUMS (CA3: p = 0.04 and 0.02, respectively; DG: p = 0.04 and 0.01, respectively) (Fig. 7a).

Exposure to CUMS increased significantly the number of caspase-positive cells compared to controls in both the CA3 ($p < 0.001$) and DG ($p < 0.001$), while FLU and OB reduced significantly the number in both CA3 and DG compared to the CUMS group ($p < 0.001$ for both) (Fig. 7b).

CUMS exposure decreased significantly the number of Ki67-positive cells in the DG compared to the control group ($p < 0.001$), while FLU (p =

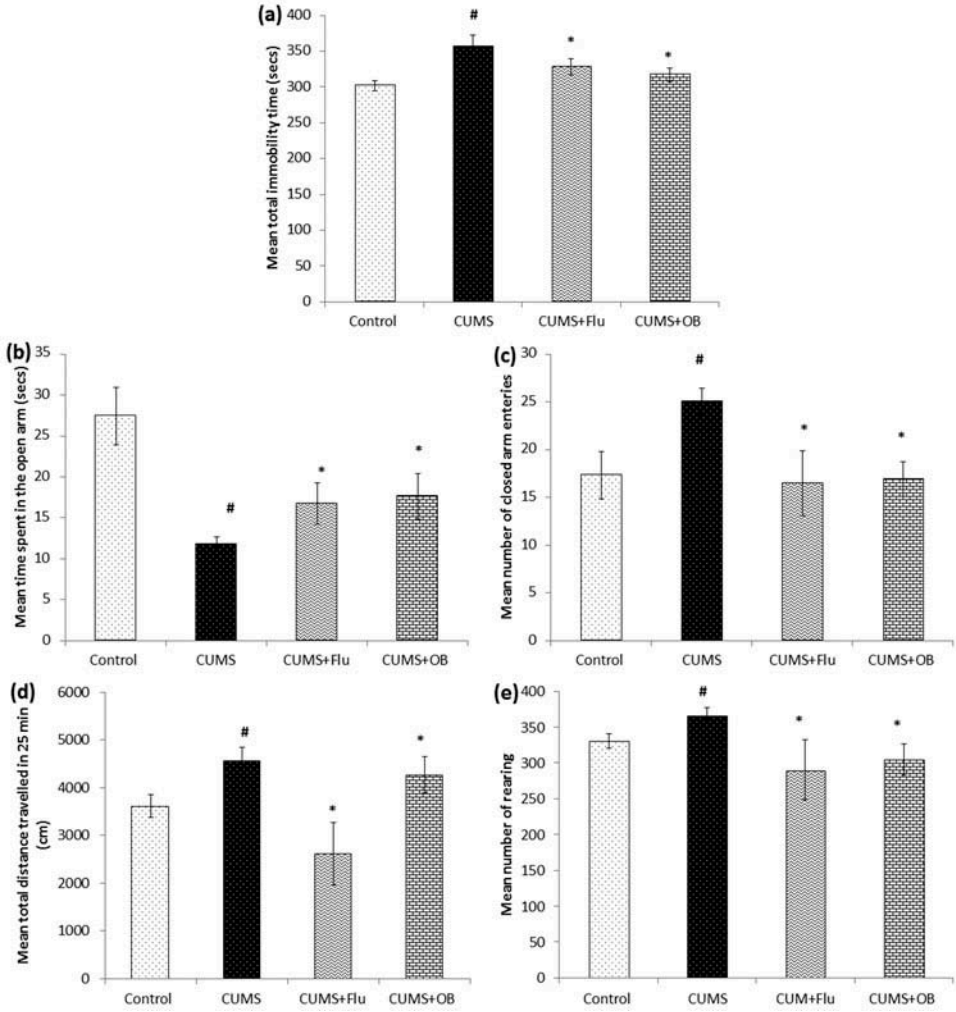

Fig. 2. Effect of treatment of CUMS with OB on the immobility time for the FST (a), time spent in the open arm (b), number of closed arm entries (c) of the EPM test, distance traveled in 25 min (d) and number of rearing behaviors (e). Data are means ± SD (n = 10). #Significant vs. control, *significant vs. CUMS. CUMS, chronic unpredictable mild stress; EPM, elevated plus maze; OFT, open field test; FlU, fluoxetine; OB, *Ocimum basilicum.*

0.003) and OB ($p = 0.01$) increased significantly the number of these cells compared to the CUMS group (Fig. 7c).

The ROD of the BDNF was decreased significantly in the CA3 ($p = 0.001$) and DG p = 0.01) of CUMS group compared to the control group. Treatment with FLU ($p < 0.001$) or OB ($p < 0.001$) increased significantly the ROD of the BDNF in both the CA3 and DG compared to the CUMS group (Fig. 7d). The ROD of the GR was decreased significantly in the CA3 and DG of the CUMS group compared to controls ($p = 0.001$ and 0.02, respectively), while FLU or OB treatment increased significantly ROD compared to CUMS (CA3: $p < 0.001$ and = 0.001, respectively; DG: $p < 0.001$ and = 0.003, respectively) (Fig. 7e).

Discussion

The side effects of currently available antidepressants often contribute to poor compliance by patients (Sadock et al. 2014); there is need for new and safer antidepressants with few or no side effects. We investigated the efficacy of inhalation of OB essential oil for treating CUMS-induced depression. GC/MS analysis of OB essential oil revealed that it contained about 23 compounds including linalool, cineole,

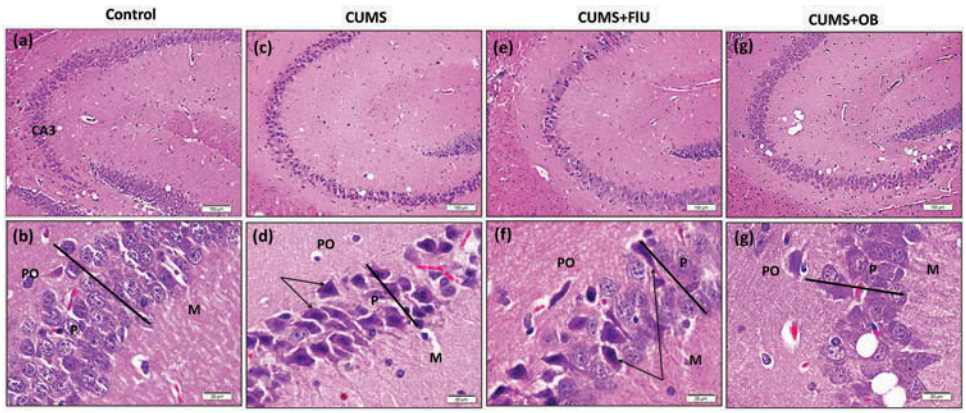

Fig. 3. CA3 region of hippocampus of control (a, b), CUMS (c, d), CUMS + FLU (e, f) and CUMS + OB (g, h) groups show polymorphic (PO), pyramidal (P) and molecular (M) layers. Note the reduction in thickness of pyramidal layer indicated by black line. H & E. (a, c, e, g) × 200; (b, d, f, h) × 1,000. CUMS, chronic unpredictable mild stress; FlU, fluoxetine; OB, *Ocimum basilicum.*

Table 2. Effect of treatment with OB following exposure to CUMS on morphometric measurement of the hippocampus

Parameter	Control	CUMS	CUMS + FLU	CUMS + OB
Thickness of CA3 pyramidal cell layer (μm)	77.7 ± 5.9	49.04 ± 4.7 $p < 0.001$	64.11 ± 6.1 $p^1 < 0.001$	52.92 ± 3.8 $p^1 = 0.06$
Surface area of CA3 pyramidal cell layer (× 10^3 μm^2)	53.37 ± 4.15	42.20 ± 5.14 $p = 0.002$	49.22 ± 4.27 $p^1 = 0.004$	46.81 ± 4.2 $p^1 = 0.04$
Thickness of DG granular cell layer (μm)	94.34 ± 11.2	74.22 ± 20 $p = 0.02$	89.54 ± 10 $p^1 = 0.04$	88.5 ± 9.5 $p^1 = 0.05$
Surface area of DG granular cell layer (× 103 μm^2)	143.2 ± 14.2	86.12 ± 12.33 $p < 0.001$	112.44 ± 28.2 $p^1 = 0.02$	102.3 ± 17.2 $p = 0.03$

Data are means ± SD; n = 10. p, significance vs. controls, p^1, significance vs. CUMS group. CUMS, chronic unpredictable mild stress; FlU, fluoxetine; OB, *Ocimum basilicum*.

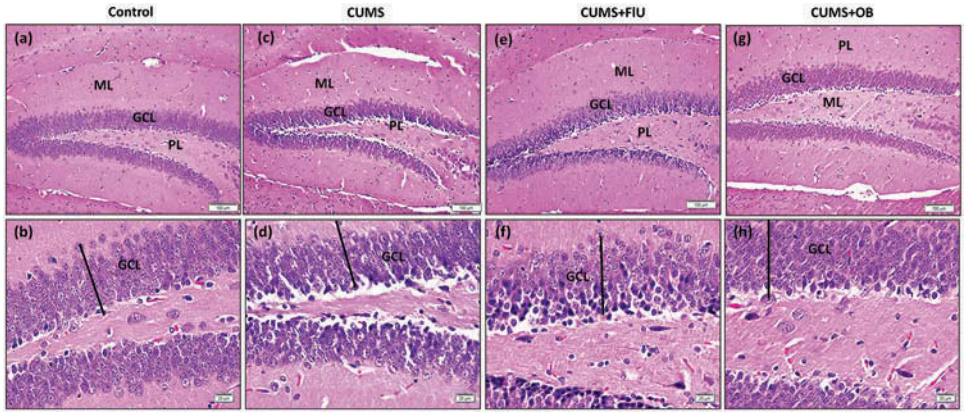

Fig. 4. DG of hippocampus of the control (a, b), CUMS (c, d), CUMS + FLU (e, f) and CUMS + OB (g, h) groups showing molecular (ML), granular cell (GCL) and pleomorphic layers (PL). Note the reduction in thickness of the granular cell layer indicated by the black line. H & E. (a, c, e, g) × 200; (b, d, f, h) × 600. CUMS, chronic unpredictable mild stress; FLU, fluoxetine; OB, *Ocimum basilicum.*

cadinol, farnesyl acetate, farnesene, ocimene, cadinene and camphor. Our findings were consistent with those reported by Rabbani et al. (2015) allowing for differences in the percentages of components that likely are due to individual plant differences or to climatic and soil factors.

We found that mice exposed to CUMS exhibited prolonged immobility during the FST,

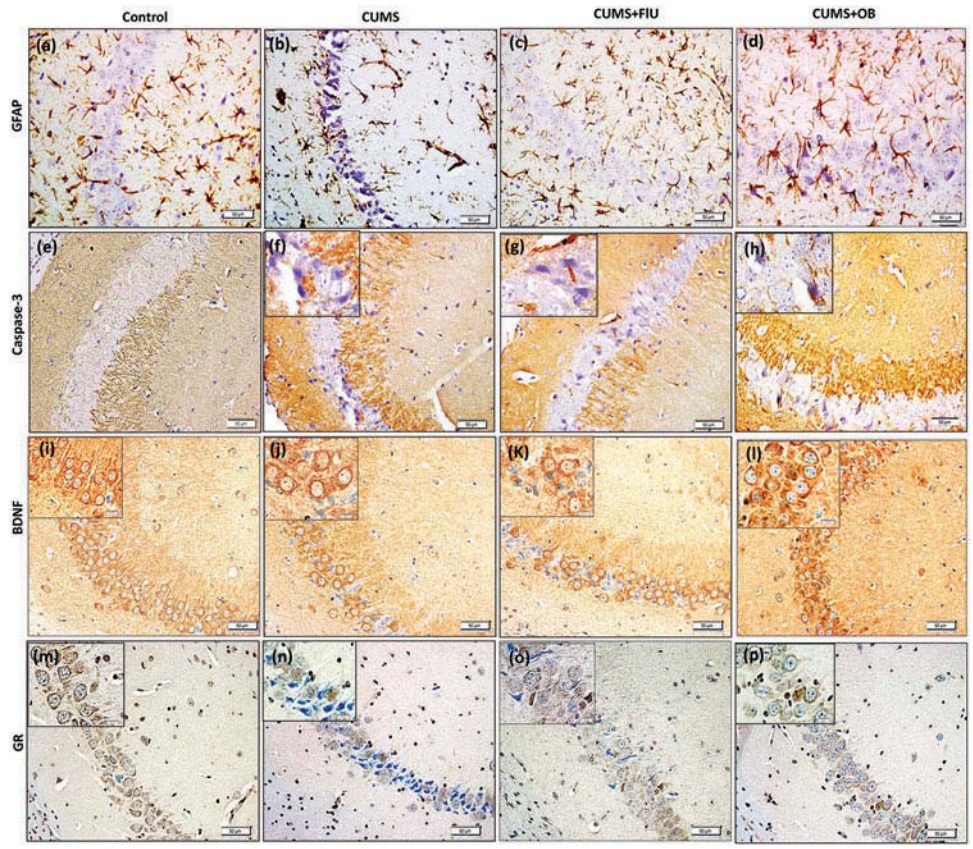

Fig. 5. Immunoexpression of GFAP (a–d), caspase (e–h), BDNF (i–l) and GR (m–p) in the CA3 region of the hippocampus. × 400; inset × 1000. CUMS, chronic unpredictable mild stress; FLU, fluoxetine; OB, *Ocimum basilicum.*

prolonged time spent in the open arms during the EPM test and reduction in open field activity, which indicated depression. Treating the mice exposed to CUMS with OB ameliorated these behavioral changes. Our findings are supported by Zahra et al. (2015) who reported that normal male albino mice exposed to OB exhibited improved exploratory behavior and improved 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 (Edewor-Kuponiyi 2013, Satou et al. 2014), which is consistent with our findings.

We found that CUMS caused a significant increase in serum corticosterone, which suggests stimulation of the hypothalamic-pituitary-adrenal (HPA) axis as suggested by others (Grippo et al. 2005, Mizuki et al. 2014). The hippocampus is rich in GR and MR. The GR, owing to its lower affinity for glucocorticoids, is activated primarily during periods of stress when circulating levels of glucocorticoids are relatively high. Upon activation, the glucocorticoid receptors translocate to the nucleus of the hippocampal cell where they trigger changes in gene expression that have long lasting effects on the structure and function of the cells (Warner-Schmidt and Duman 2006).

The reduced number of GFAP-positive astrocytes in the hippocampus that we observed has been reported previously in post mortem specimens from patients with depression (Bowley et al. 2002) and stressed rats (Li et al. 2014). Consistent with our observations, Liu et al. (2014) and Yu et al. (2014) reported that chronic stress caused apoptosis of hippocampal neurons. Reduced neurogenesis in the subgranular zone (SGZ) of the DG was among the sequelae of chronic stress (Malberg et al. 2000, Alonso et al. 2004). We used immunostaining with the primary antibody Ki67 and confirmed that CUMS resulted in reduced neurogenesis

Chronic stress has been reported to cause atrophy of neurons in the hippocampus and prefrontal cortex with subsequent reduction in hippocampal volume (Egan et al. 2003, Duman 2004, Sahay et al.

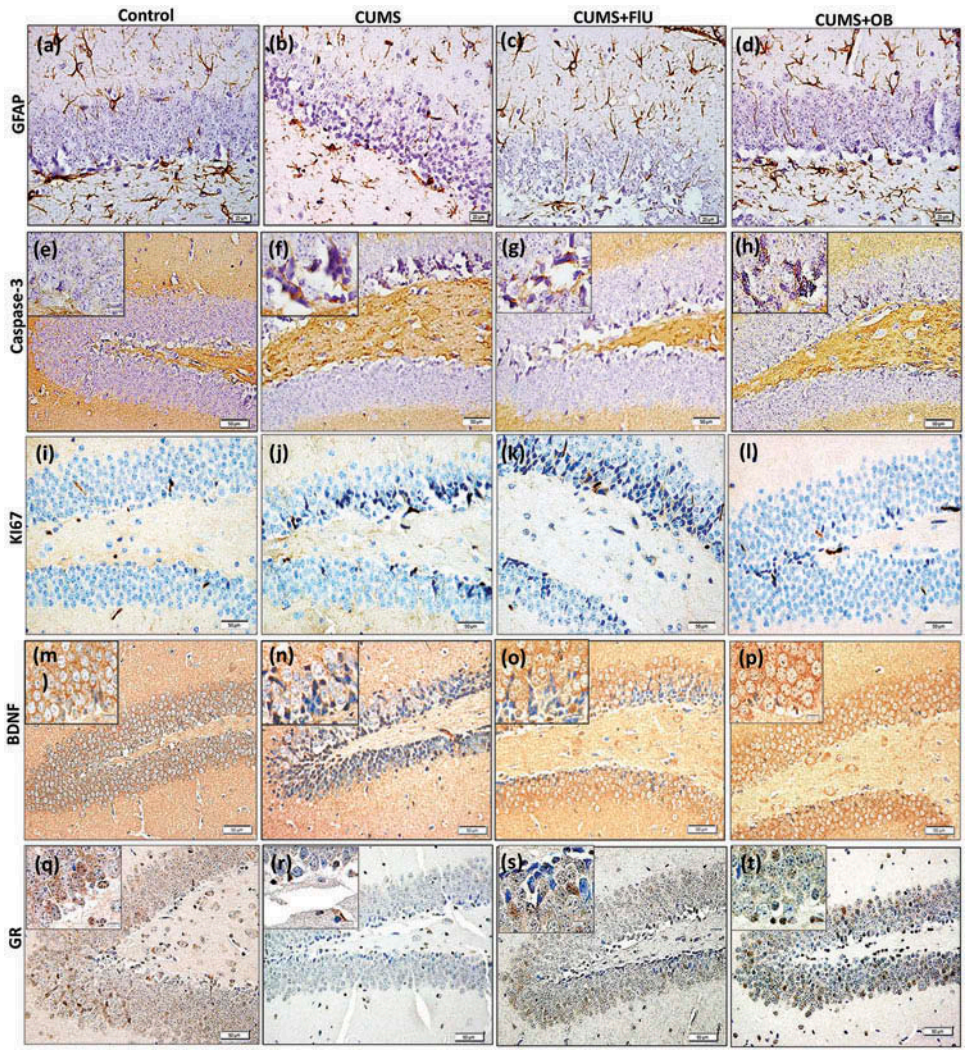

Fig. 6. Immunoexpression of GFAP (a–d), caspase-3 (e–h), Ki67 (i–l), BDNF (m–p) and GR (q–t) in DG of the hippocampus. a–l, × 600; m–t, × 400; inset × 1,000. CUMS, chronic unpredictable mild stress; FLU, fluoxetine; OB, *Ocimum basilicum*.

2007). We observed a reduction in the thickness of both the CA3 pyramidal cell layer and the DG granular cell layer in the stressed mice, which indicated atrophy of the hippocampus. Increased apoptosis of hippocampal neurons together with reduced SGZ neurogenesis appear to contribute to the reduction in hippocampus thickness.

Antidepressants increase neurogenesis and new cells are required for the behavioral actions of these agents in selected rodent models (Banasr et al. 2011). We found that both FLU and OB administration increased neurogenesis in the SGZ. BDNF plays an important role in dendritic remodeling and neural plasticity in the hippocampus. Therefore, BDNF participates in recovery from depression (McEwen et al. 2015, Duman 2004, Szymańska et al. 2009). We found that both BDNF and GR immune, gene and protein expressions were decreased after exposure to CUMS. Ridder et al. (2005) reported that compromised GR function caused BDNF dysregulation and predisposition to depressive behavior. These investigators reported decreased BDNF content in the hippocampus of GR-heterozygous mutant (GR+/-) mice. This is consistent with the neurotrophin hypothesis of depression postulated by Cryan and Mombereau (2004) and Lang et al. (2004).

FLU significantly reduced the corticosterone level, which is consistent with the report that it prevented reduction of GFAP expression (Liu et al. 2014). FLU also has been reported to exhibit

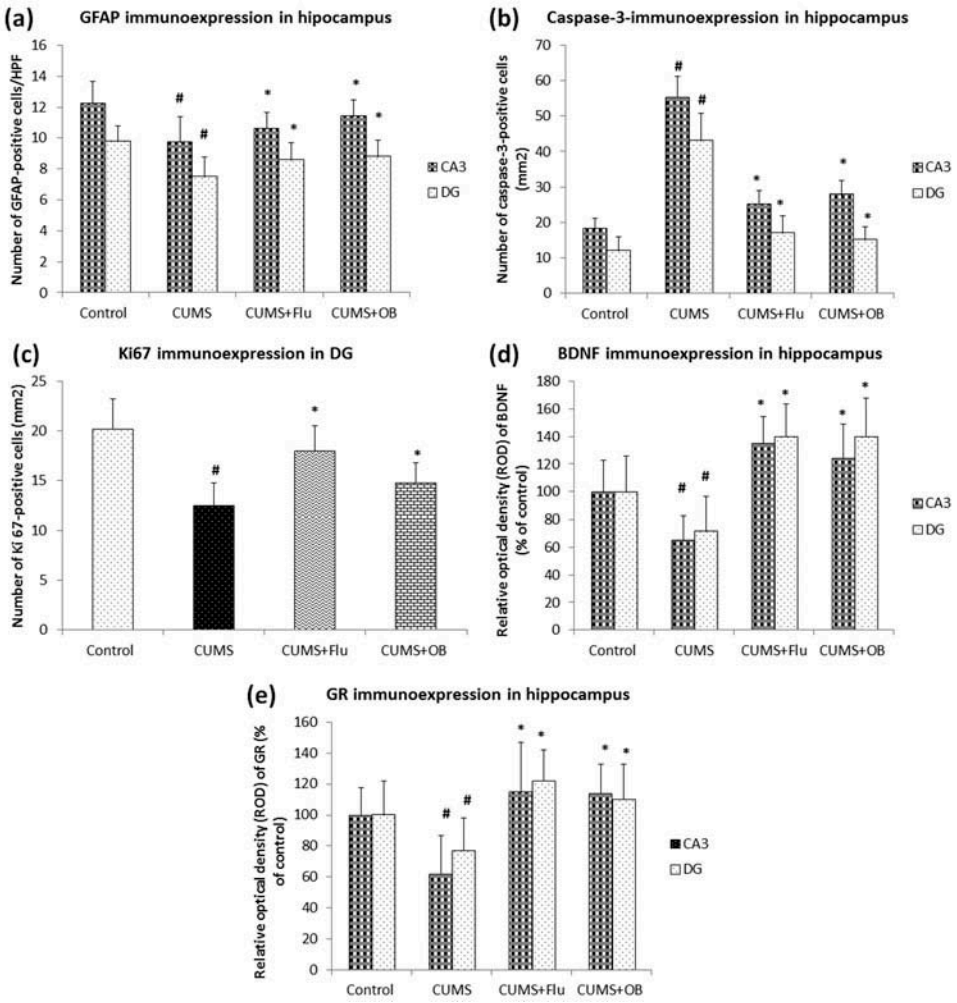

Fig. 7. Immunoexpression of GFAP (a), caspase-3 (b), Ki67 (c), BDNF (d) and GR (e) groups. Data are means or mean percent of control value in BDNF and GR ± SD. #Significant vs. control, *significant vs. CUMS. CUMS, chronic unpredictable mild stress; FLU, fluoxetine; OB, *Ocimum basilicum.*

an anti-apoptotic effect (Lucassen et al. 2004) in the psychosocially stressed tree shrew, an animal model of depression.

OB has been reported to exert a neuroprotective affect that was attributed to its phenolic, flavonoid and tannin contents, which are scavengers of reactive oxygen species (Bora et al. 2011). We found that OB reduced the elevated corticosterone level, and atrophy and apoptosis of hippocampal neurons, while it increased neurogenesis and the number of the astrocytes in a manner comparable to FLU.

Our study had some limitations. The connection between GR and BDNF expressions during stress procedures was not examined fully. Further studies are required to explore the detailed mechanism underlying the antidepressive effects of OB. Nevertheless, we found that inhalation of OB essential oils exhibited antidepressant-like effects on CUMS-induced depression in an animal model. Further investigation of the underlying mechanism of the antidepressant-like effect of OB on humans is recommended.

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