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ORIGINAL ARTICLE

Evaluation of the antidepressant-like effect of musk in an animal model of depression: how it works

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Abstract Depression has become a common public health problem that is showing increasing prevalence. Slow onset of action, low response rates and drug resistance are potential limitations of the current antidepressant drugs. Alternative therapy using natural substances, specifically aromatherapy, is currently tried to treat depression. This work aimed to assess the efficacy of musk in relieving the behavioral, biochemical and hippocampal histopathological changes induced by exposure to chronic mild stress in mice and explore the possible mechanism behind this antidepressant-like effect. Forty male albino mice were divided into four groups ($n = 10$): control, a group exposed to chronic unpredictable mild stress (CUMS) and two groups exposed to CUMS and then treated with fluoxetine or musk. Behavioral changes and serum corticosterone levels were assessed at the end of the experiment. Protein and gene expressions of brain-derived neurotropic factor (BDNF) and glucocorticoid receptors (GRs) in the hippocampus were assessed using ELISA and real-time RT-PCR, respectively. Histopathological examination of the hippocampus and immunohistochemical techniques using glial fibrillary acidic protein (GFAP), Ki67, caspase-3, BDNF and GR were performed. Inhalation of musk had an antidepressant-like effect in an animal model of depression. Musk alleviated the behavioral changes and elevated serum corticosterone levels induced by exposure to chronic stress. It reduced the hippocampal neuronal apoptosis and stimulated neurogenesis in the dentate gyrus. Musk's action may be related to the upregulation of hippocampal GR and BDNF expressions. Musk is considered a potential antidepressant so it is advisable to assess its efficacy in treating depressed patient.

Keywords Musk · Antidepressant · Neurogenesis · Apoptosis · BDNF · GR · Fluoxetine

Introduction

Depression has become a major public health problem, demonstrating a constant increase in prevalence (Milanović et al. 2015). It is a common illness worldwide, with an estimated 350 million people affected. Depression can cause the affected person to suffer greatly and function poorly at work, and at its worst, it can lead to suicide, which is the second leading cause of death in 15–29 year olds (World Health Organization; WHO 2015). Among the risk factors for depression are chronic medical illness, stress, being single, chronic pain and lack of social support (Douglas et al. 2012).

The brain is the central organ involved in perceiving and adapting to social and physical stressors via multiple interacting mediators including excitatory amino acids and glucocorticoids together with a group of extra- and intracellular mediators such as brain-derived neurotrophic factor (BDNF) (McEwen et al. 2015). It was reported that resilience, an active process that implies ongoing adaptive plasticity in the face of stress without external intervention, is a key aspect of a healthy brain (Russo et al. 2012). The key structures for the regulation of the stress response that are affected by social-environmental factors particularly include the amygdala, hippocampus and prefrontal regions

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(Tost et al. 2015). Chronic stress has harmful effects on the hippocampal structure and functions because it has plenty of glucocorticoid receptors (GRs) and is involved in cognition (McEwen and Magarinos 2001).

Chronic unpredictable mild stress (CUMS) is considered a valid and reliable animal model of depression in rodents as it has proved etiological, face and predictive validity, so many previous studies utilized it in investigating the antidepressant effect of drugs as well as their mechanism of action (Song and Leonard 2005). The pharmacological medications for depression include tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin (SSRIs) and others (Nemeroff 2007). These currently used anxiolytic and antidepressant drugs have shown potential limitations including slow onset of action, low rates of response and even development of drug resistance (Penn and Tracy 2012). Alternative therapy using natural substances from animal or plant sources has been tried to deal with many clinical therapeutic situations (Tillett and Ames 2010). Aromatherapy using essential oils has been tried to improve and treat a number of psychological disorders including stress (Bikmoradi et al. 2015).

Musk is a powerful odoriferous material obtained from the dried secretion of a gland of the male musk deer present under the abdomen near the pubis. It is used as a fragrance and fixative in perfumes. It has been used in Chinese medicine for thousands of years for treating stroke, coma, neurasthenia and convulsions (Khan and Abourashed 2010). Musk also was reported to decrease cortisol levels in males and therefore its odor was supposed to attenuate stress (Fukui et al. 2007). Therefore, its antidepressant effect was investigated in this study.

This study hypothesized that musk can treat depression induced by CUMS in mice, and its effect is mediated through upregulation of BDNF and GR. Therefore, the aim of this work was to assess the efficacy of musk in relieving the behavioral, biochemical and hippocampal histopathological changes induced by exposure to chronic mild stress in mice and explore the possible mechanism behind this antidepressant-like effect.

Materials and methods

Drugs

Fluoxetine hydrochloride 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 positive control group by inhalation as it has no effect on anxiety, as shown in previous studies (Pavesi et al. 2011).

Musk (*Moschus moschiferus*) was purchased from the local market in Jeddah, Saudi Arabia. The musk constituents were analyzed by gas chromatography coupled to mass spectrometry (GC-MS; Agilent, Columbia, MD, USA) with a DB-5ms column (30 m × 0.25 mm × 0.25 μm). The composition of musk is shown in Table 1. Musk was diluted in propylene glycol to obtain concentrations of 1.0 % (v/v) just prior to the experiments. Inhalation of musk and amyl acetate started after 4 weeks from exposure to CUMS and continued for another 4 weeks. Administration of musk and amyl acetate was through an inhalation apparatus consisting of an acrylic box (32 cm × 24 cm × 32 cm). Inhalation was once a day and lasted for 15 min immediately after the CUMS procedure as described by Chioca et al. (2013). Two cotton balls embedded with 2.5 ml per unit of musk were placed in the top wall holes of the apparatus. After each exposure session, the apparatus was cleaned and the cotton wool containing the substance was replaced to maintain the concentration in the apparatus.

Animals

The experiment was performed according to the guidelines of the animal care committee of the KFMRC, which comply with the National Institute of Health Guide (NIH publication no. 80–23, revised 1996). It was approved by the biomedical research ethics committee at the Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. Forty male Swiss albino mice weighting 30–40 g were obtained from the animal house of King Fahed Medical Research Center (KFMRC) at King Abdulaziz University (KAU), Jeddah, Saudi Arabia. After 2 weeks of acclimatization, the mice were randomly divided into four groups (10 mice each): control (left unexposed and not treated), CUMS (exposed to CUMS for 4 weeks and then amyl acetate for 2 weeks), CUMS and then FLU (exposed to CUMS for 4 weeks and then FLU for 2 weeks) and CUMS and then M (exposed to CUMS for 4 weeks and then musk for 2 weeks). The CUMS procedure used in this study was first used by Willner (1997) and modified to mice by Ducottet and Belzung (2004). During the CUMS procedure, mice 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, tilting cages to 30° and restraining the mice, and water stress by placing mice in an empty cage with 1 cm of water at the bottom. The whole experiment lasted 6 weeks (42 days) (Fig. 1).

Table 1 Chemical composition of musk obtained by GC-MS

Compound	Retention time (min)	Percentage
Steroids		13.801
Androstan-3-one semicarbazone	33.062	9.345
Spirostan-23-ol #; spirostan-23-ol	32.164	4.456
Essential oils		12.445
Alpha-cedrol	23.858	0.102
Other essential oils		2.343
Organic compound		41.515
5-Ethyl-5-[(E)-styryl] barbituric acid (sedative)	27.303	0.355
Other organic compounds		29.259
Alcohols and phenols		2.216
1,1′-Oxybis-2-propanol,	13.408	1.217
2-(2-Hydroxypropoxy)-1-propanol	13.804	0.799
3,3′-Oxybis-2-butanol,	14.235	0.158
Anise alcohol	17.599	0.028
2,4-Di-tert-butylphenol (antioxidants)	21.766	0.600
2-Fluoro-6-nitrophenol	24.447	0.008
Esters		9.423
Pyridine		8.233
Terpenes		0.091
Pyrans		5.126
Polcyclic musk		5.154
Others		1.996

At the end of the experiment, behavior tests were performed, and blood samples were collected from the retro-orbital vein. Then, the mice were killed by decapitation. The skull was opened, and the brain was dissected out on an ice plate. The whole hippocampus was isolated according to Paxinos and Watson's atlas (1998). The hippocampus of the left side was kept for assessing GR and BDNF protein and gene expression levels, while the right side was processed for histopathological examination.

Forced swim test (FST)

Each mouse was placed in a glass cylindrical container (height 20 cm, diameter 14 cm) with 15 cm depth of water at 25 ± 2 °C. The mouse was videotaped for 6 min using behavior software (Noldus Information Technology, EthoVision XT®), and the total time spent immobile during 6 min was measured and presented in seconds. Immobility was defined as the cessation of limb movements except the minor movement necessary to keep the mouse afloat (Doron et al. 2014).

Elevated plus-maze (EPM)

The maze was elevated 40 cm above the floor. Each mouse was placed in the center of the EPM, and its behavior was videotaped for 5 min using the behavior software (Noldus Information Technology, EthoVision XT®) and was presented in seconds. The numbers of closed arm entries and time spent inside the open arms were measured (Carobrez and Bertoglio 2005).

Open field test (OFT)

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 rearings (standing upright on the hind legs while the forepaws are free) was observed and registered manually. These vertical movement scores reflect exploratory behaviors. Locomotor activity (distance traveled in 25 min) was quantified using the video-tracking system (Columbus Instruments, Columbus, OH, USA) as described by Mineur et al. (2006).

Serum corticosterone level determination

At the end of the experiment, the mice were anesthetized using ether, and the blood sample was obtained in the morning from the retrorbital venous plexus into EDTA-coated tubes. They then were centrifuged for 10 min, and the collected serum samples were kept at −80 C until the corticosterone levels were assessed using RIA (ELISA kits, ALPCO Diagnostics, Orangeburg, NY).

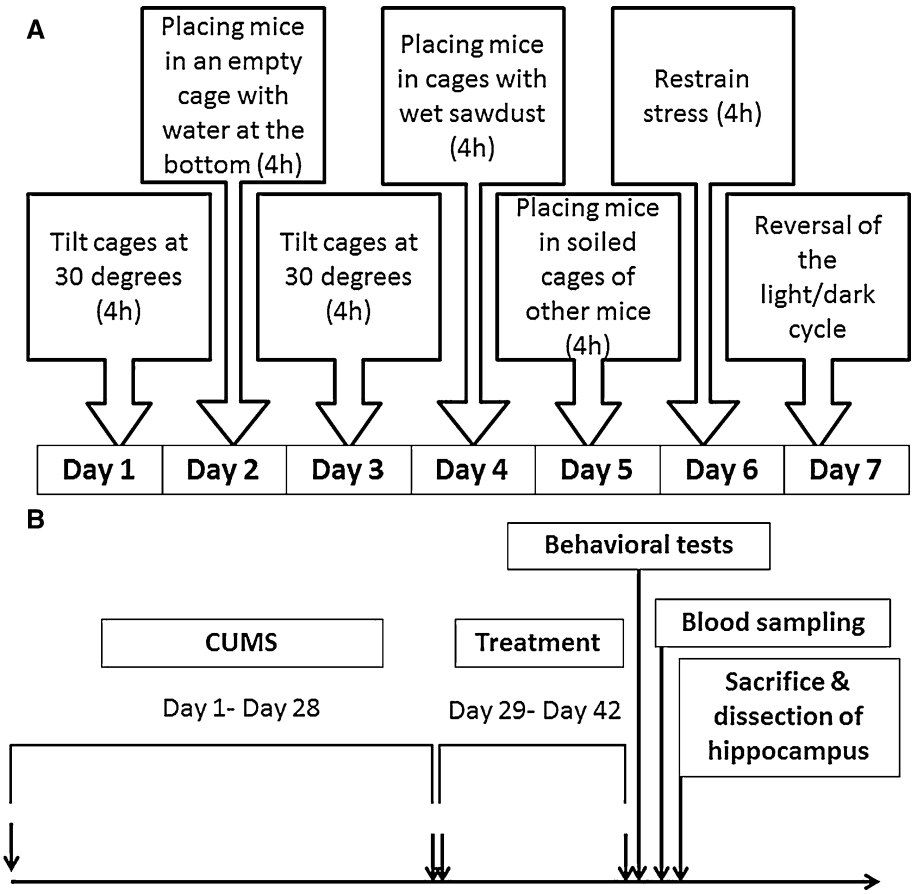

Fig. 1 **a** Stressor to which the mice were exposed during the CUMS procedure in 1 week. It was repeated for 4 weeks. To prevent habituation and to provide an unpredictable feature to the stressors, they were administered at different time points during the day (Doron et al. 2014). **b** Experimental design. In this experiment, mice ($n = 10$ in each group) were exposed to either chronic unpredictable mild stress (CUMS) daily from day 1 to day 28 or left unexposed to stress throughout the experiment. On days 29 to 42, the exposure of mice to CUMS was stopped, and daily exposure to the control treatment (amyl acetate), fluoxetine, or musk was started. On the days 43, 44 and 45, behavioral tests were sequentially done for all groups. On day 46, blood samples were obtained, and then the mice were killed and the brains dissected to obtain the hippocampus

Hippocampal GR and BDNF protein level determination

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's atlas (1998). Tissue punches 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 leupeptin). Homogenates were acidified with 0.1 MHCl (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 at 7000*g* for 10 min. BDNF and GR protein levels were assessed using the sandwich enzyme-linked immunosorbent assay (ELISA) according to Baker-Herman et al. (2004).

Assessment of hippocampal GR and BDNF mRNA level determination

Total RNAs were isolated from 30 to 60 mg of the hippocampus using the EZ RNA Clean Up Plus DNase Kit (EZ Bio Research, St Louis, MO, USA). RNA concentrations were measured using the Nano Drop Spectrophotometer (Jenway, UK). Reverse transcription (RT) was performed using oligo-dT primers (Bioneer Inc., Daejeon, Republic of Korea) in a 20-µl reaction including 5 µl RNA. The cDNAs obtained were amplified using PCR Master Mix (Bioneer Inc., Daejeon, Republic of Korea) with primers designed by Metabion International AG, Semmelweisstr, Germany, as follows: GR (forward 5′-AGCTCCCCCTGGTAGAGAC-3′; reverse 5′-GGTGAA-GACGCAGAAACCTT-3′), BDNF (forward 5′-TATTT-CATACTTCGGTTGC-3′; reverse 5′-TGTCAGCCAGTGATGTCG-3′) and β-actin (forward 5′-TCTGGCACCACA CCTTCTA-3′; reverse 5′-AGGCA-TACAGGGACAGCAC-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 amplicon products. The images were obtained using a gel documentation system (manufactured by Ultra-Violet Products, Ltd.). The size of the amplicons was determined using software available with the gel documentation system. The expression patterns of the GR gene and BDNF gene in the hippocampus were analyzed using the real-time RT-PCR method using SYBR Green

qPCR Master Mix containing ROX as a reference dye (Biotool LLC, Houston, TX, USA). All amplified fragments were achieved in three independent replicates; in addition, the results were normalized to β-actin as a reference gene using the comparative Ct method.

Hippocampal histopathological and immunohistochemical examination

The hippocampus of the right side was fixed in 10 % neutral buffered formalin overnight and then processed to obtain paraffin blocks. Serial paraffin sections were cut to 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 slides with the paraffin sections were deparaffinized and then rehydrated. They were boiled in a microwave for 20 min in 0.01 M sodium citrate buffer (pH: 6) to retrieve the 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). Incubation of slides overnight at 4 °C with antiglial fibrillary acidic protein (GFAP) (Dako Cytomation, USA) was done for 1 h with 1:1000 dilution for the demonstration of astrocytes. To demonstrate apoptosis, anti-caspase-3 (Santa Cruz Biotechnology, USA) was used at a dilution of 1:1000 for 1 h. Anti-Ki-67 (rabbit polyclonal Ig G produced by Abcam, Cambridge, UK) was used at a 1:100 dilution to demonstrate cell proliferation. Rabbit anti-GR antibody and anti-BDNF (Santa Cruz Biotechnology, USA) with 1:1000 and 1:400 dilution, respectively, overnight at room temperature were subsequently exposed to biotinylated goat anti-rabbit IgG and streptavidin peroxidase complex (1:200 dilution; Vector Laboratories) at room temperature. After washing, the slides were incubated with the avidin-biotin-peroxidase complexes (Dako-USA) for 10 min, covered by DAB and incubated for 10 min. They then were counterstained with hematoxylin, dehydrated, cleared and mounted.

Morphometric and statistical analysis

A digital camera connected to a light microscope (Olympus, BX-61, Los Angeles, CA) was used for photographing. Both the thickness and surface area of the pyramidal cell layer in the hippocampal CA3 area and the granular cell layer in the dentate gyrus (DG) areas were measured in five fields in each mouse using Image Pro Plus Software, (Media Cybernetics, Silver Spring, MD, USA, version 6.0). Assessment of the five non-overlapping fields in each mouse and calculation of the mean of each mouse of the ten mice were done. In addition, the number of GFAP-positive cells in CA3 was counted in five high-power fields (400× magnification) in each mouse of the ten mice according to the method of Makhlouf et al. (2014). The number of caspase-3, 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 as the mean and standard deviation. For the non-parametric data, Kruskal-Wallis analysis of variance (ANOVA) followed by a post hoc test (based on Dunnett's procedure) was used to analyze each pair of groups to avoid a multiple comparison effect. For the parametric data, the different groups were compared using ANOVA (F test) followed by a Bonferroni post hoc test. Significance was considered at a p value less than 0.05.

Results

Forced swimming test (FST)

Exposure to CUMS for 4 weeks resulted in depressive-like behavior as it significantly increased the immobility time of the FST (control; 62.2 ± 7.12, CUMS; 101 ± 15, $p < 0.001$). Treatment with FLU and musk after stopping exposure to CUMS significantly reduced the immobility time in comparison to the CUMS group (CUMS; 101 ± 15, CUMS then FLU; 85.11 ± 11.6, $p = 0.02$, CUMS then M; 77.4 ± 8.4, $p < 0.001$) (Fig. 2a).

Elevated plus-maze test (EPM)

Exposure to CUMS induced anxiety-like behavior as the time spent by the mice in this group in the open arms of the EPM was significantly decreased (control; 27.40 ± 3.51, CUMS; 11.77 ± 0.93, $p < 0.001$) compared with the control mice. Administration of FLU and musk significantly increased this time in comparison to the CUMS group (CUMS; 11.77 ± 0.93, CUMS then FLU; 16.76 ± 2.53, $p = 0.001$, CUMS then M; 19.29 ± 3.2, $p < 0.001$). On the other hand, the number of closed arm entries was significantly increased after CUMS exposure (control; 17.33 ± 2.59, CUMS; 25.06 ± 1.47, $p < 0.001$) compared with the control mice. Administration of FLU and musk significantly decreased it in comparison to the CUMS group (CUMS; 25.06 ± 1.47, CUMS then FLU; 16.47 ± 3.44, $p < 0.001$, CUMS then M; 18.5 ± 1.78, $p < 0.001$) (Fig. 2b, c).

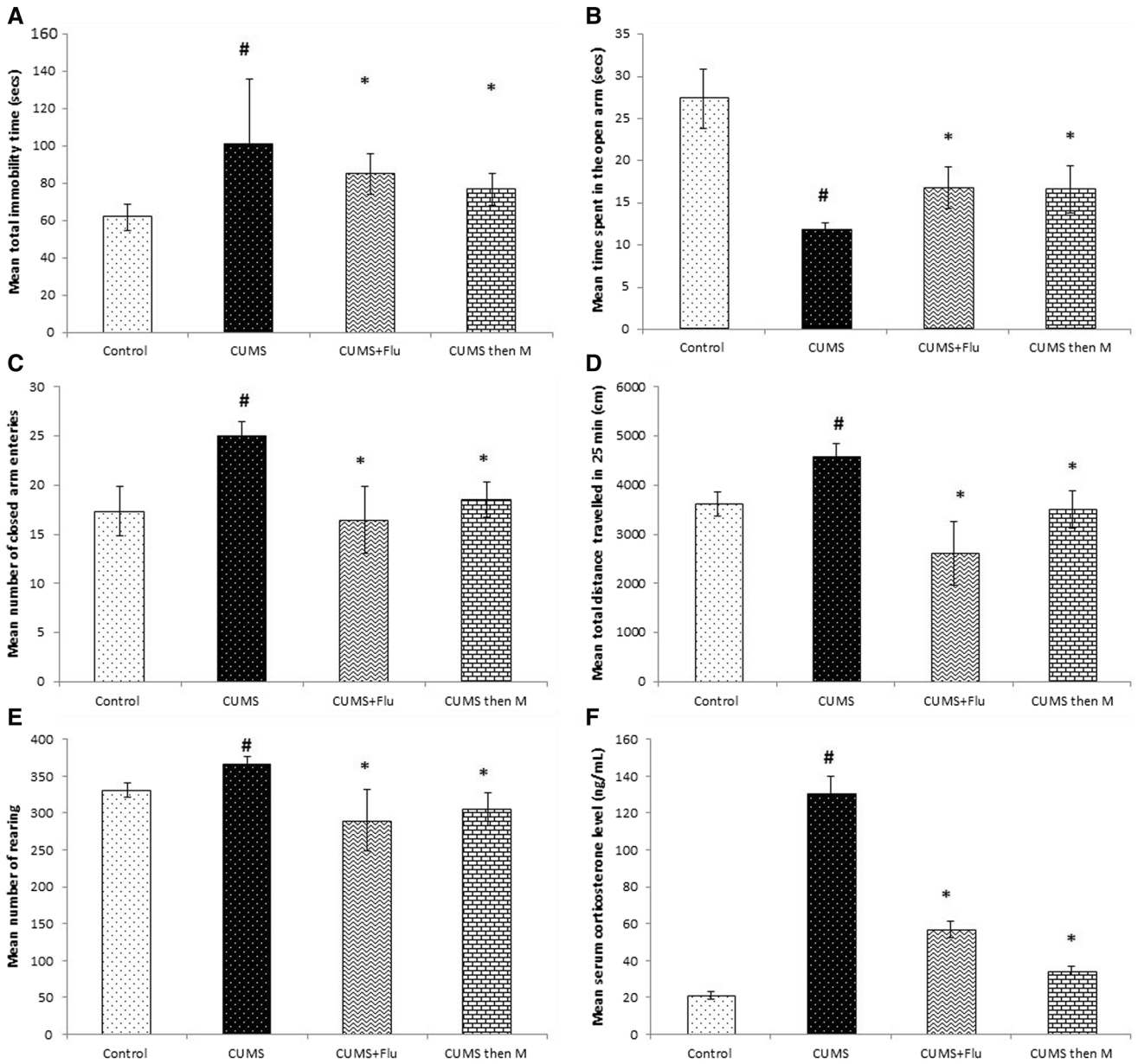

Fig. 2 Effect of musk inhalation on mice exposed to CUMS on the immobility time of the FST (**a**), the time spent in the open arm (**b**), number of closed arm entries (**c**) of the EPM test, distance traveled in 25 min (**d**) and number of rearings (**e**) of the OFT and the serum corticosterone level (**f**). Data were expressed as mean ± SD ($n = 10$). #Significance versus control, *significance versus CUMS. *EPM* elevated plus-maze, *OFT* open field test, *CUMS* chronic unpredictable mild stress, *FlU* fluoxetine, *M* musk

Open field test (OFT)

Mice exposed to CUMS traveled significantly more than the controls (control; 3616.3 ± 238.2, CUMS; 4572.8 ± 270.3, $p < 0.001$), which indicated increased spontaneous locomotor activity. Administration of FLU and musk significantly reduced this activity in comparison to the CUMS group (CUMS; 4572.8 ± 270.3, CUMS then FLU; 2617.8 ± 650.6, $p < 0.001$, CUMS then M; 3011.2 ± 102.9, $p < 0.001$) (Fig. 2d). CUMS also induced a significant increase in the number of rearings compared to the control (control; 331 ± 10.12, CUMS; 366.6 ± 10.53, $p < 0.001$), while FLU and musk significantly reduced rearings compared to the CUMS group (CUMS; 366.6 ± 10.53, CUMS then FLU; 290 ± 42.31, $p < 0.001$, CUMS then M; 305.4 ± 22.27, $p < 0.001$) (Fig. 2e).

Serum corticosterone level

The basal serum corticosterone level increased significantly ($p < 0.001$) after CUMS compared with the control, and the administration of FLU and musk reduced it significantly ($p < 0.001$) compared with the CUMS group (Fig. 2f).

Histopathological changes in the hippocampus

The control CA3, the hippocampal area that is reported to be affected in depression, was formed of polymorphic, pyramidal and molecular cell layers. The pyramidal layer had crowded pyramidal cells with large vesicular nuclei, while many of those cells in mice exposed to CUMS appeared smaller with dark cytoplasms and small condensed nuclei. On the other hand, mice treated with FLU or musk after being exposed to CUMS showed fewer numbers of these small dark cells, and both the thickness and surface area of this layer was significantly increased in comparison to the CUMS group (Fig. 3; Table 2).

The control DG, the other hippocampal area affected in depression, is formed of molecular cells, granular cells and pleomorphic layers. The granular cell layer has polyhedral cells with vesicular nuclei, while many of those cells appeared smaller with a dark cytoplasm and had small condensed nuclei in mice exposed to CUMS. These dark cells were less frequently observed in mice treated with FLU or musk, and both the thickness and surface area of the granular cell layer were significantly increased compared to the CUMS group (Fig. 3; Table 2).

Immunoexpression of GFAP

Figures 4 and 5 show the immunoexpression of GFAP in CA3 and DG. The number of GFAP-positive cells was significantly decreased (CA3; $p = 0.003$, DG; $p < 0.001$)

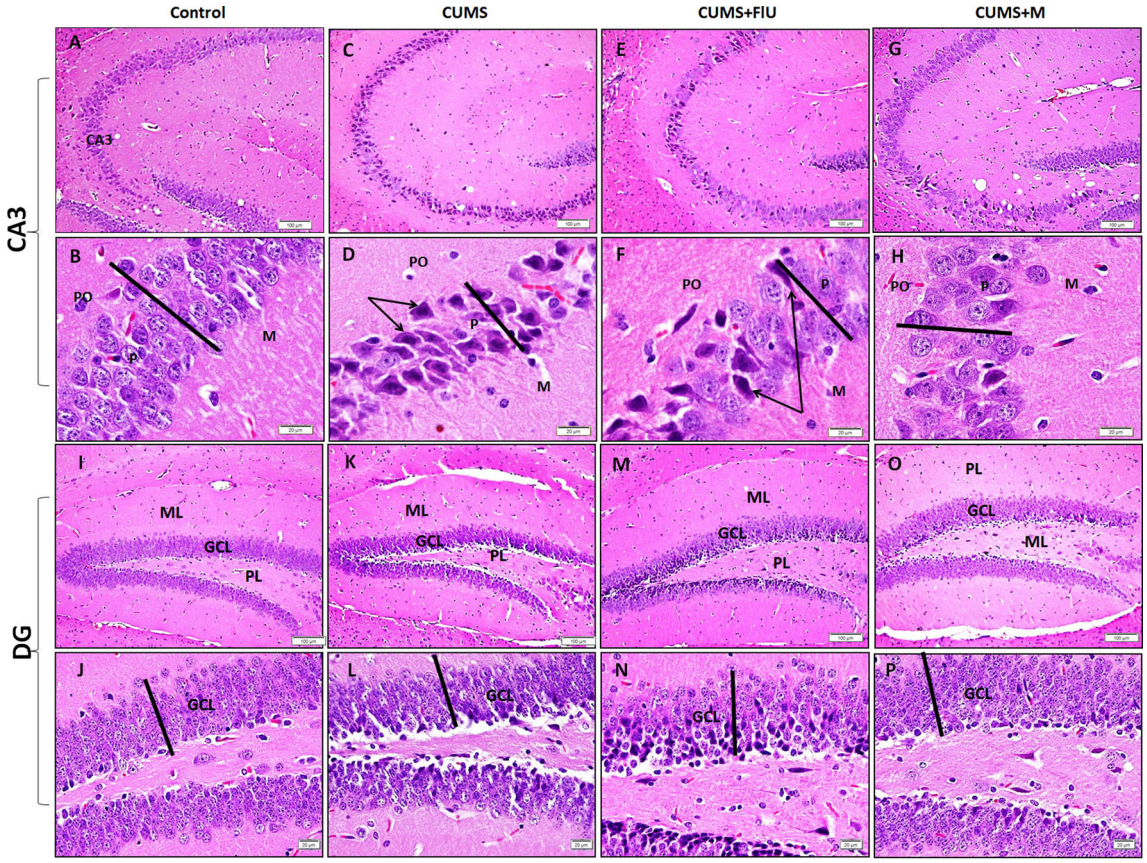

Fig. 3 The CA3 regions of the hippocampus of the control (**a**, **b**), CUMS (**c**, **d**), CUMS + FLU (**f**, **f**) and CUMS then M (**g**, **j**) groups show three layers: the polymorphic (PO), pyramidal (P) and molecular (M). Note the reduction in the pyramidal layer thickness indicated by the *black line* (H&E ×200 **a**, **c**, **e**, **g**; ×1000 **b**, **d**, **f**, **h**). The dentate gyrus of the hippocampus of the control (I, J), CUMS (K, L), CUMS + FLU (M, N) and CUMS then M (**o**, **p**) groups shows molecular (ML), granular cell (GCL) and pleomorphic layers (PL). Note the reduction in the granular cell layer thickness indicated by the *black line* (H&E ×200 **i**, **k**, **m**, **o**; ×600 **j**, **l**, **n**, **p**). *CUMS* chronic unpredictable mild stress, *FlU* fluoxetine, *M* Musk

Table 2 Effect of treatment with musk following exposure to CUMS on morphometric measurements of the hippocampus

Parameter	Control ($n = 10$)	CUMS ($n = 10$)	CUMS + Flu ($n = 10$)	CUMS then M ($n = 10$)
Thickness of CA3 pyramidal cell layer (μm)	77.7 ± 5.9	49.04 ± 4.7 $p < 0.001$	64.11 ± 6.1 $p1 < 0.001$	53.61 ± 4.5 $p1 = 0.03$
Surface area of CA3 pyramidal cell layer ($\times 10^3$ μm^2)	53.37 ± 4.15	42.20 ± 5.14 $p = 0.002$	49.22 ± 4.27 $p1 = 0.004$	47.30 ± 5.2 $p1 = 0.04$
Thickness of DG granular cell layer (μm)	94.34 ± 11.2	74.22 ± 20 $p = 0.02$	89.54 ± 10 $p1 = 0.04$	91.2 ± 10.2 $p1 = 0.02$
Surface area of DG granular cell layer ($\times 10^3$ μm^2)	143.2 ± 14.2	86.12 ± 12.33 $p < 0.001$	112.44 ± 28.2 $p1 = 0.02$	108.2 ± 21 $p1 = 0.01$

Data are expressed as mean ± SD

Significance is considered at $p < 0.05$

p significance versus control group, *p1* significance versus CUMS group

CUMS chronic unpredictable mild stress, *FlU* fluoxetine, *M* musk

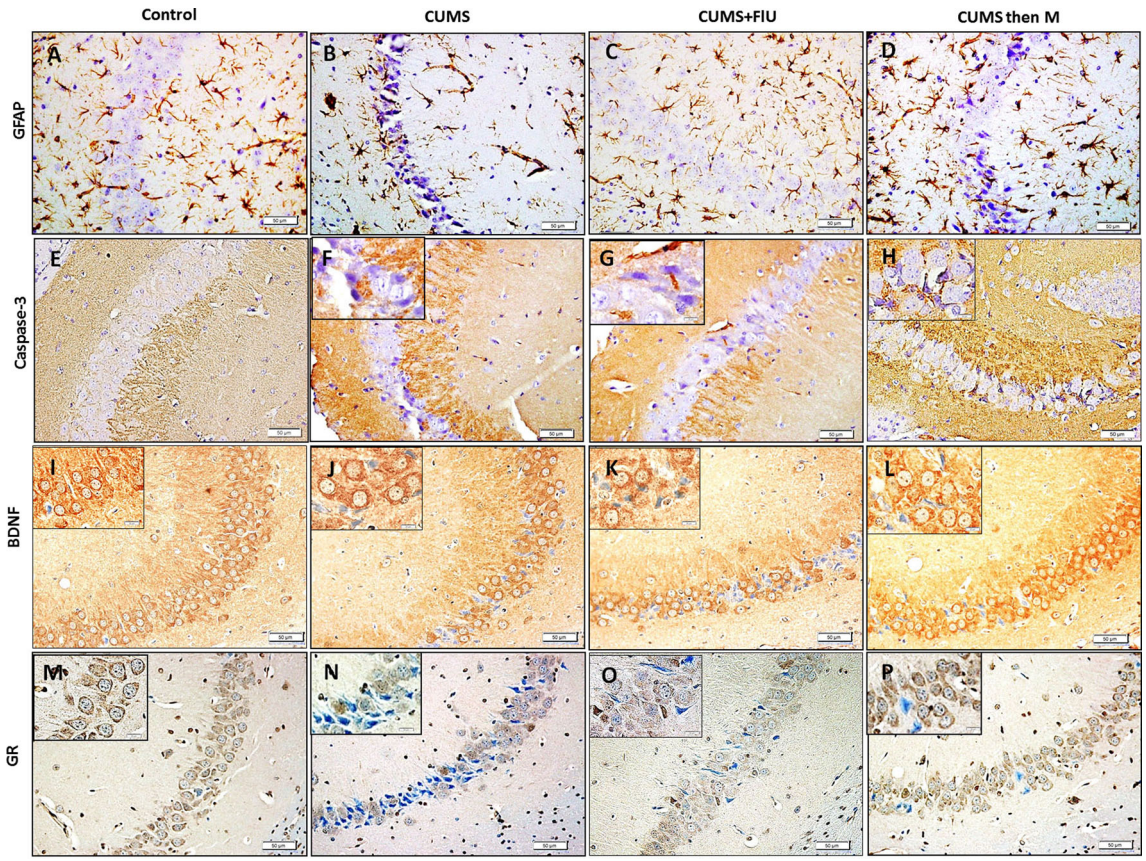

Fig. 4 Immunoexpression of GFAP (**a**–**d**), caspase (**e**, **f**), BDNF (**g**, **h**) and GR (**p**–**t**) in the CA3 region of the hippocampus in the studied group (×400, insert ×1000). *CUMS* chronic unpredictable mild stress, *FlU* fluoxetine, *M* musk

following exposure to CUMS compared to the control. FLU (CA3; $p = 0.04$, DG; $p = 0.05$) and musk (CA3; $p = 0.01$, DG; $p = 0.03$) administration significantly increased this number of GFAP-positive cells compared to the CUMS group (Fig. 6a).

Immunoexpression of caspase

Exposure to CUMS significantly ($p < 0.001$) increased the number of caspase-positive cells in both CA3 and DG compared to the control group, while FLU ($p < 0.001$) and

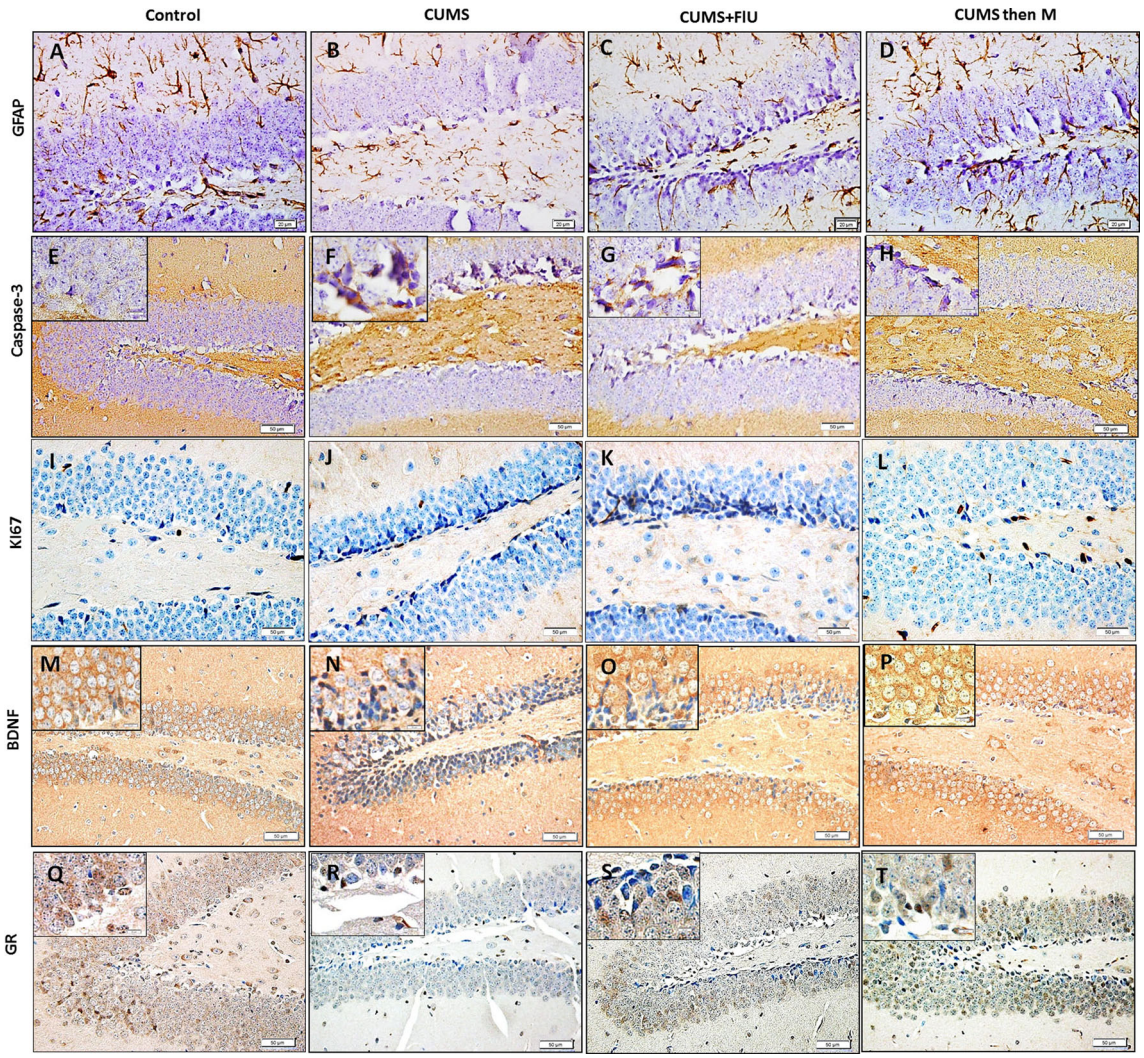

Fig. 5 Immunoexpression of GFAP (**a–d**), caspase-3 (**e**, **h**), Ki67 (**i–l**), BDNF (**m–p**) and GR (**q–t**) in the dentate gyrus of the hippocampus of the studied groups (**a–l** ×600, **m–t** ×400, insert ×1000). *CUMS* chronic unpredictable mild stress, *FlU* fluoxetine, *M* musk)

musk ($p < 0.001$) significantly decreased it in both CA3 and DG compared to the CUMS group (Fig. 6b).

Immunoexpression of Ki67

Neurogenesis in DG was significantly reduced ($p < 0.001$) after exposure to CUMS while treatment with FLU ($p = 0.003$) and musk ($p < 0.001$) significantly increased it back, as evidenced by the increased number of proliferating Ki-67-positive cells in the subgranular layer of the DG compared to the CUMS group (Fig. 6c).

Immunoexpression of BDNF

BDNF immune expression was obviously decreased in the CA3 and DG of the CUMS group compared to the control (CA3; $p = 0.001$, DG; $p = 0.01$). It was significantly increased in both areas following FLU ($p < 0.001$) and musk ($p < 0.001$) administration compared to the CUMS group (Fig. 6d).

Immunoexpression of GR

GR immune expression was significantly decreased ($p = 0.001$, $p = 0.02$) in the CA3 and DG of the CUMS group, respectively. Fluoxetine ($p < 0.001$) and musk ($p < 0.001$) administration could significantly increase it (Fig. 6e).

BDNF gene and protein expression level

The BDNF mRNA expression level was assessed using quantitative RT-PCR. It was significantly downregulated ($p = 0.03$) in the hippocampus of the CUMS group compared

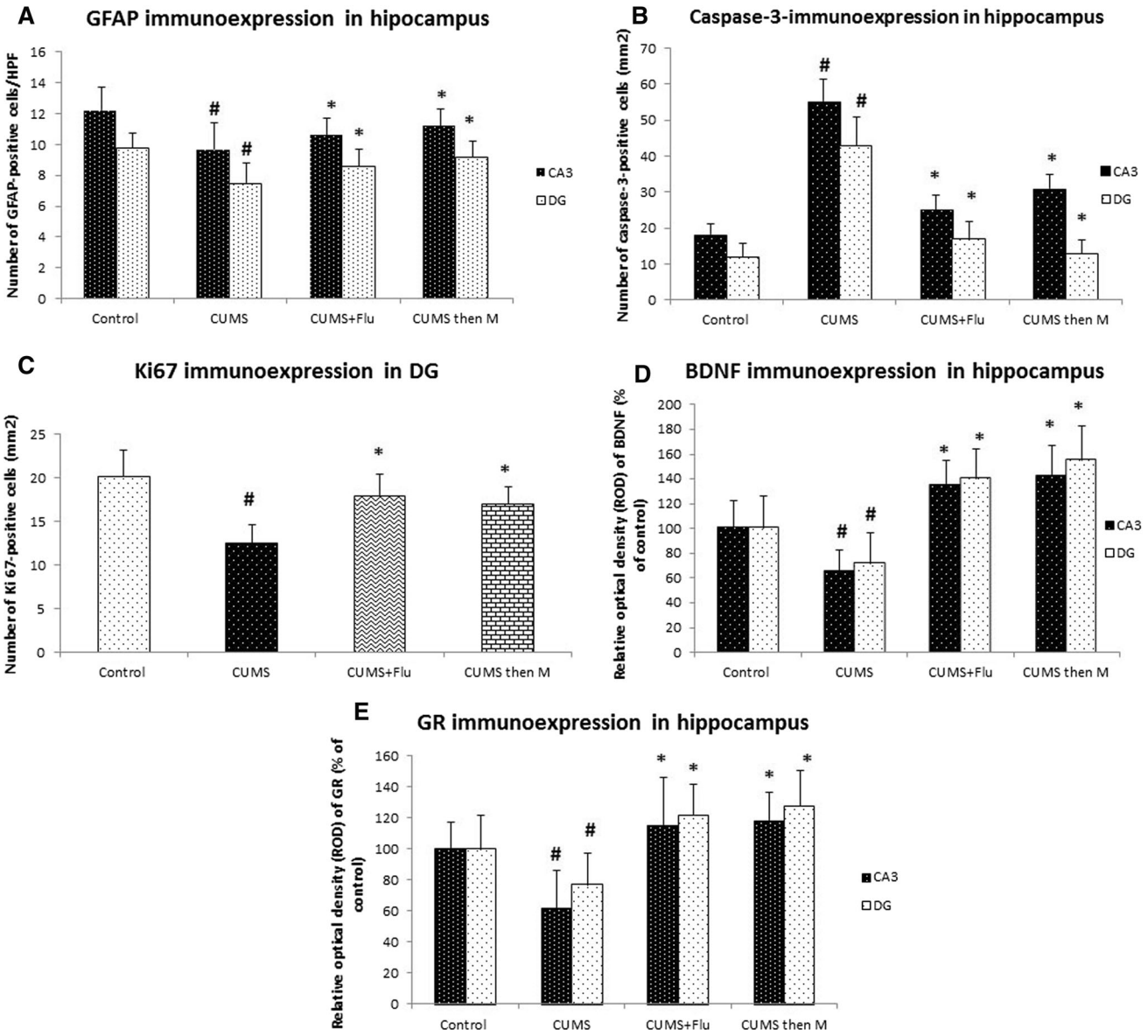

Fig. 6 Immunoexpression of GFAP (**a**), caspase-3 (**b**), Ki67 (**c**), BDNF (**d**) and GR (**e**) in the studied groups. Data are expressed as mean (or mean percent of the control value in BDNF and GR) ± SD. #Significance versus control, *significance versus CUMS. *CUMS* chronic unpredictable mild stress, *FLU* fluoxetine, *M* musk

to the control group, while treatment with FLU and musk significantly ($p < 0.001$ and $p = 0.01$) upregulated this expression, respectively. Similar observations were noticed in BDNF protein expression as CUMS significantly downregulated ($p < 0.001$) it compared with the control, while administrating FLU and musk significantly ($p < 0.001$, $p = 0.02$) upregulated it, respectively (Fig. 7a, b).

GR gene and protein expression level

GR mRNA expression levels were significantly downregulated ($p = 0.01$) in the hippocampus of the CUMS group compared to the control group, while FLU and musk treatment significantly ($p < 0.001$, $p = 0.03$) upregulated it. A similar trend was observed in GR protein expression as CUMS significantly downregulated ($p < 0.001$) its level compared to the control, while administrating FLU and musk significantly upregulated it ($p < 0.001$) (Fig. 7c, d).

Discussion

This work aimed to assess the antidepressant-like effect of musk, administered through inhalation, on mice exposed to CUMS and to explore the underlying mechanism of this effect. The hypothesis of this work was built on the

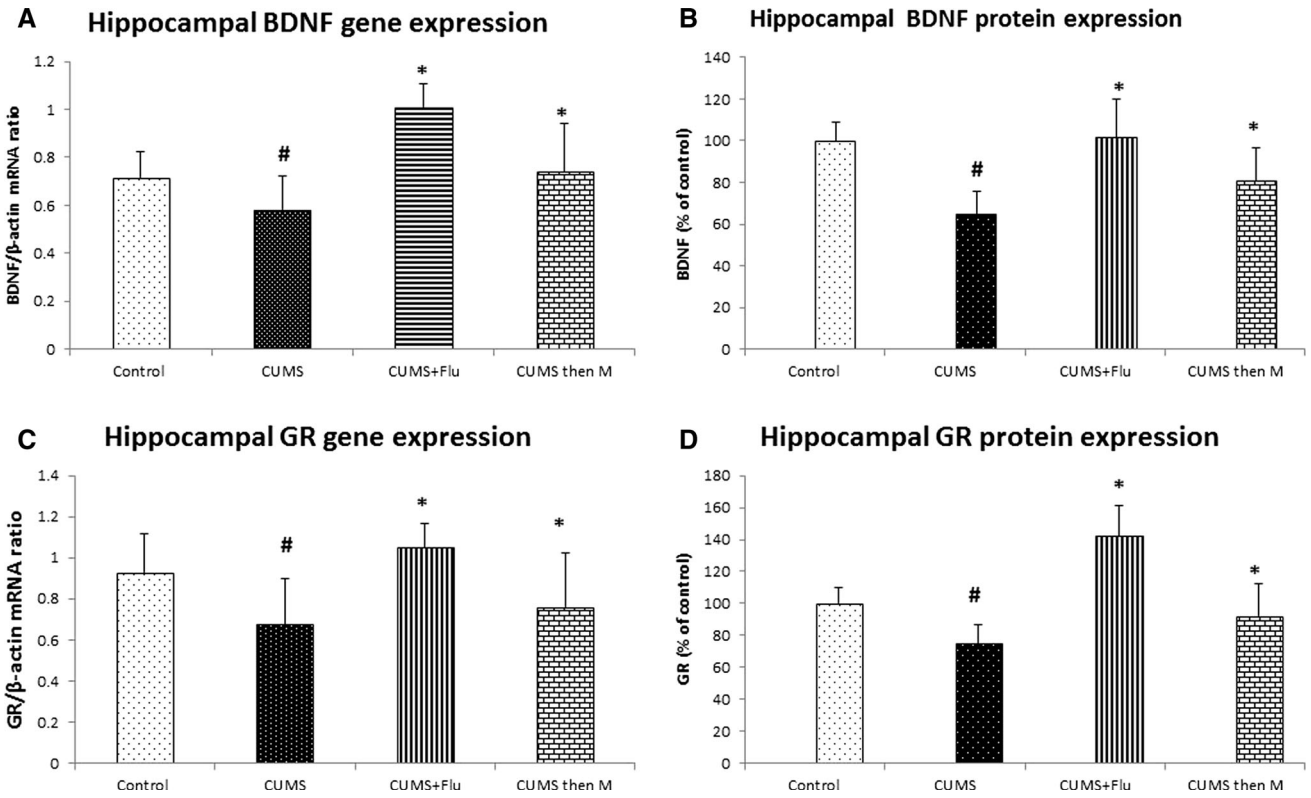

Fig. 7 BDNF mRNA (**a**) and GR mRNA (**c**) expression levels in the hippocampus of the studied groups assessed by quantitative RT-PCR. Data are expressed as mean ± SD ($n = 10$). BDNF protein (**b**) and GR protein (**d**) expression levels in the hippocampus assessed by ELISA and expressed as percent of control value ± SD. #Significance versus control, *significance versus CUMS. *CUMS* chronic unpredictable mild stress, *FLU*, fluoxetine, *M* musk

previous reports that aromatherapy has benefits in relieving some mental and psychologic disorders (Schmidt et al. 2008). In this study, exposure to CUMS induced a depressive status in mice as it resulted in increased immobility time in the FST. This depressive status was confirmed by the reduction of the time spent in the open arms of the EPM and the increased locomotor activity observed during the OFT. Inhalation of musk could ameliorate this depressive status. It also reduced the elevated corticosterone levels recorded in mice exposed to CUMS. The hippocampus, one of the most studied neural targets of stress and antidepressants (Lucassen et al. 2014; Wainwright and Galea 2013), was specifically examined and assessed in this study. It was found that musk alleviated the CUMS-induced histopathologic changes in the hippocampus, including neuronal atrophy and a reduced number of astrocytes and neuro- and glial apoptosis. Musk-induced changes were comparable to those with fluoxetine, the selective serotonin reuptake inhibitor (SSRI) antidepressant that was used in this study as a positive control for pharmacological validation.

Depression symptoms were frequently reported to be associated with disturbed glucocorticoid secretion in patients with depression as well as in many animal models of depression so dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis was investigated by assessing the serum corticosterone level (Zunszain et al. 2011). In this study, CUMS resulted in a significant increase in the serum corticosterone level, which indicated hyperactivity in the HPA axis. Many previous studies reported similar findings (Mizuki et al. 2014). Liu et al. (2014) reported a reduced corticosterone level after fluoxetine administration in stressed mice. Fukui et al. (2007) also reported that musk decreased cortisol levels in males, and they deduced that the musk odor can attenuate stress. Their study findings supported our findings. It seems that an elevated corticosterone level is one of the causes behind the hippocampal neuronal atrophy observed in this study, as Warner-Schmidt and Duman (2006) reported that repeated stress as well as glucocorticoid administration was the result of atrophy of the CA3 pyramidal neurons. Liston et al. (2013) also reported that excessive glucocorticoid exposure during chronic stress causes synapse loss. The BDNF, which is extensively studied in stress and depression research (Monteggia 2007), plays an important role in dendritic remodeling in the hippocampus (McEwen et al. 2015).

Atrophy of the hippocampal neurons was reported in mice exposed to chronic stress (Duman 2004) as well as in mice with heterozygous deletion of BDNF (Egan et al. 2003).

In this study, the BDNF immunohistochemical expression on hippocampal neurons was reduced, which could be attributed to its gene and hence its protein expression. Finally, reduced BDNF seems to be another cause of hippocampal neuronal atrophy observed in this study.

A selective immune marker, GFAP, was used in this study to assess the number and integrity of astrocytes in the hippocampus as described by Webster et al. (2001). The number of GFAP-positive astrocytes was found to be markedly reduced in both the CA3 and DG of the CUMS mice. This is consistent with what was described by Bowley et al. (2002) during their post-mortem studies of tissues from patients with depression as well as with the finding of the study done by Li et al. (2014) on stressed rats. Banasr and Duman (2008) have postulated that reduced GFAP expression in astrocytes is a contributing factor for developing depression symptoms. Fluoxetine, as an antidepressant, was reported to prevent the reduction of GFAP expression as well as glial atrophy (Liu et al. 2014). This was evident in this study, and musk induced a similar effect as FLU. The apoptotic effect of CUMS on hippocampal neurons was previously reported (Yu et al. 2014), and this effect was also confirmed in this study using the caspase-3 antibody. This anti-apoptotic effect of the antidepressants was previously reported in many studies (Lucassen et al. 2004). Administration of FLU and musk reduced the CUMS-induced apoptotic changes in the hippocampus.

A decreased number of proliferating neurons in the SGZ of the DG after exposure to CUMS was previously reported (Alonso et al. 2004). Lucassen et al. (2010) attributed stress-induced reductions in proliferation to apoptosis of progenitor cells or to cell cycle arrest. Reduced neurogenesis in the SGZ of the dentate gyrus as well as increased apoptosis of hippocampal neurons seems to be behind the reduction in hippocampal volume reported by Sahay et al. (2007) and behind the reduction in thickness of the CA3 pyramidal layer and DG granular cell layer observed in this study. Chronic antidepressant treatment was reported to prevent the downregulation of neurogenesis caused by chronic mild stress in mice (Alonso et al. 2004). New cell birth is necessary for the behavioral actions of these agents in selected rodent models (Banasr et al. 2011).

GR signaling may be a pivot of depression and the antidepressant effect. The acute glucocorticoid increase occurring in response to stress acted as a beneficial effect to keep the body away from harmful injury. Glucocorticoid acts via activation of GR and release of corticotrophin-releasing hormone (CRH), which in turn decreases the corticosterone secretion (Pariante and Miller 2001). In chronic stress, glucocorticoid hypersecretion decreases the GR levels and impairs HPA axis negative feedback; subsequently, damage occurs (Yi et al. 2012). The alteration of GR expression in the hippocampus was reported in human depression patients as well as depressive animal models (Anacker et al. 2011). Antidepressants could recover impaired feedback inhibition by regulating GR levels (mRNA and protein) in the brain, especially in the hippocampus (Wu et al. 2013). Notably, some reports showed that glucocorticoid/GR can influence BDNF expression and BDNF-mediated neuronal functions (Kumamaru et al. 2011). In this study, downregulation of GR mRNA and protein was recorded after exposure to CUMS for 4 weeks. FLU and musk could upregulated its level.

It was reported that psychosocial stressors are associated with activation of the sympathetic nervous system with subsequent secretion of catecholamines, which increase NF-kappa B activity in macrophages and induce the release of proinflammatory cytokines such as IL-6 and mitogen-activated protein kinase ERK-1/2 in the hippocampus and prefrontal cortex (Patki et al. 2013). Sustained stress and the subsequent release of proinflammatory cytokines lead to chronic neuroinflammation, which contributes to depression. On the other hand, neuroinflammation causes an imbalance between oxidative stress and the antioxidant system, which is also associated with depression (Kim et al. 2016). Elevated proinflammatory cytokine levels and hippocampal glucocorticoid receptor (GR) functional resistance are among the most widely investigated factors in the pathophysiology of depression (Kim et al. 2016).

Concerning the correlation between the action of FLU, the SSRI used in this study, and that of musk, it was reported that hippocampal neurogenesis is a necessary component of the FLU antidepressant response (Malberg and Duman 2003; David et al. 2009). Fluoxetine increases neurogenesis by augmenting proliferation of amplifying neural progenitor cells in the dentate gyrus (Encinas et al. 2006). In addition, chronic treatment with FLU upregulates BDNF (Schmidt and Duman 2006). In this study, musk increased hippocampal neurogenesis and unregulated BDNF in a comparable manner to FLU. In a more recent study on the unpredictable chronic mild stress (uCMS) rat model of depression, chronic stress was found to induce significant molecular changes that were generally reversed by fluoxetine. It primarily acted on neurons to reduce the expression of proinflammatory response genes and increased a set of genes involved in cell metabolism (Patrício et al. 2015). Musk was reported to have significant antiinflammatory activity long ago (Morishita et al. 1987), and Lin et al. (2004) reported that musk was used for treating inflammation in Chinese medicinal remedies. Wang et al. (2003) concluded that part of the mechanism underlying the antiinflammatory action of musk was

inhibiting the chemotaxis of polymorphonuclear leukocytes. Thus, the antiinflammatory action of musk could be one of the possible mechanisms explaining its antidepressant-like effect. Another suggested mechanism is the direct action of musk through GRs, which are reported to be abundant in the hippocampus (McEwen and Magarinos 2001). Natural musk was reported to contain steroids and androgen sex hormone derivatives (Sokolov et al. 1986; Oh et al. 2002), and this was consistent with what was observed in this study as steroids accounted for up to 14 % of musk's constituents.

One limitation of this study is that the antiinflammatory or antioxidant activities of musk could participate in its antidepressive effect, but these were not investigated in this study and will be targeted in a coming one.

In conclusion, this study showed that inhalation of musk had an antidepressant-like effect in an animal model of depression. Musk alleviated the behavioral changes and elevated serum corticosterone level induced by exposure to chronic stress. It reduced the hippocampal neuronal apoptosis and stimulated neurogenesis in the dentate gyrus. Therefore, musk might be considered a potential antidepressant, and its action may be related to the upregulation of hippocampal GR and BDNF expressions.

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Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

References

Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, Soubrie P (2004) Blockade of CRF1 or V1B receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. Mol Psychiatry 9:278–286

Anacker C, Zunszain PA, Carvalho LA, Pariante CM (2011) The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology 36(3):415–425

Baker-Herman TL, Fuller DD, Bavis RW, Zabka AG, Golder FJ, Doperalski NJ, Johnson RA, Watters JJ, Mitchell GS (2004) BDNF is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxia. Nat Neurosci 7(1):48–55

Banasr M, Duman RS (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry 64:863–870

Banasr M, Dwyer JM, Duman RS (2011) Cell atrophy and loss in depression: reversal by antidepressant treatment. Curr Opin Cell Biol 23(6):730–737

Bancroft JD, Gamble M (2008) Theory and practice of histological techniques, 6th edn. Churchill Livingstone, Philadelphia

Bikmoradi A, Seifi Z, Poorolajal J, Araghchian M, Safiaryan R, Oshvandi K (2015) Effect of inhalation aromatherapy with lavender essential oil on stress and vital signs in patients undergoing coronary artery bypass surgery: a single-blinded randomized clinical trial. Complement Ther Med. 23(3):331–338

Bowley MP, Drevets WC, Ongur D, Price JL (2002) Low glial numbers in the amygdala in major depressive disorder. Biol Psychiatry 52:404–412

Carobrez AP, Bertoglio LJ (2005) Ethological and temporal analyses of anxietylike behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev 29:1193–1205

Chen BH, Park JH, Cho JH, Kim IH, Shin BN, Ahn JH, Hwang SJ, Yan BC, Tae HJ, Lee JC, Bae EJ, Lee YL, Kim JD, Won MH, Kang IJ (2015) Ethanol extract of Oenanthe javanica increases cell proliferation and neuroblast differentiation in the adolescent rat dentate gyrus. Neural Regen Res. 10(2):271–276

Chioca LR, Ferro MM, Baretta IP, Oliveira SM, Ferreira J, Losso EM, Andreatini R, Silva CR (2013) Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not GABA neurotransmission. J Ethnopharmacol 147:412–418

David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R (2009) Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62(4):479–493. doi:10.1016/j.neuron.2009.04.017

Doron R, Lotan D, Versano Z, Benatav L, Franko M, Armoza S, Kately N, Rehavi M (2014) Escitalopram or novel herbal mixture treatments during or following exposure to stress reduce anxiety like behavior through corticosterone and BDNF modifications. PLoS One. 9(4):e91455. doi:10.1371/journal.pone.0091455

Douglas M, Maurer DM, Carl R (2012) Screening for depression. Am Fam Physician 85(2):139–144

Ducottet C, Belzung C (2004) Behaviour in the elevated plus-maze predicts coping after subchronic mild stress in mice. Physiol Behav 81:417–426

Duman R (2004) Role of neurotrophic factors in the etiology and treatment of mood disorders. NeuroMol Med 5:11–26

Egan M, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M et al (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257–269

Encinas JM, Vaahtokari A, Enikolopov G (2006) Fluoxetine targets early progenitor cells in the adult brain. Proc Natl Acad Sci USA 103:8233–8238

Fukui H, Komaki R, Okui M, Toyoshima K, Kuda K (2007) The effects of odor on cortisol and testosterone in healthy adults. Neuro Endocrinol Lett. 28(4):433–437

Khan IA, Abourashed EA (2010) Leung's encyclopedia of common natural ingredients. Wileyz, Hoboken, pp 455–465

Kim YK, Na KS, Myint AM, Leonard BE (2016) The role of proinflammatory cytokines in neuroinflammation, neurogenesis and the neuroendocrine system in major depression. Prog Neuropsychopharmacol Biol Psychiatry 4(64):277–284. doi:10.1016/j.pnpbp.2015.06.008 **Epub 2015 Jun 23**

Kumamaru E, Numakawa T, Adachi N, Kunugi H (2011) Glucocorticoid suppresses BDNF-stimulated MAPK/ERK pathway via inhibiting interaction of Shp2 with TrkB. FEBS Lett 585(20):3224–3228

Li M, Fu Q, Li Y, Li S, Xue J, Ma S (2014) Emodin opposes chronic unpredictable mild stress induced depressive-like behavior in mice by upregulating the levels of hippocampal glucocorticoid receptor and brain-derived neurotrophic factor. Fitoterapia 98:1–10

Lin DL, Chang HC, Huang SH (2004) Characterization of allegedly musk-containing medicinal products in Taiwan. J Forensic Sci 49(6):1187–1193

Liston C, Cichon JM, Jeanneteau F, Jia Z, Chao MV, Gan WB (2013) Circadian glucocorticoid oscillations promote learningdependent synapse formation and maintenance. Nat Neurosci 16(6):698–705. doi:10.1038/nn.3387

Liu D, Xie K, Yang X, Gu J, Ge L, Wang X, Wang Z (2014) Resveratrol reverses the effects of chronic unpredictable mild stress on behavior, serum corticosterone levels and BDNF expression in rats. Behav Brain Res 1(264):9–16. doi:10.1016/j.bbr.2014.01.039

Lucassen PJ, Fuchs E, Czéh B (2004) Antidepressant treatment with tianeptine reduces apoptosis in the hippocampal dentate gyrus and temporal cortex. Biol Psychiatry 8:789–796

Lucassen PJ, Meerlo P, Naylor AS, van Dam AM, Dayer AG, Fuchs E, Oomen CA, Czéh B (2010) Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. Eur Neuropsychopharmacol 20(1):1–17

Lucassen PJ, Pruessner J, Sousa N, Almeida OF, Van Dam AM, Rajkowska G et al (2014) Neuropathology of stress. Acta Neuropathol 127:109–135

Makhlouf NA, El-Beshbishy RA, Abousetta A (2014) Ginkgo modulates noise-induced hippocampal damage in male albino rats: a light and electron microscopic study. Egypt J Histol 37(1):159–174. doi:10.1097/01.EHX.0000444078.17248.ab

Malberg J, Duman RS (2003) Cell proliferation in adult hippocmpus is decreased by inescapable stress: reversal by fluoxetine treatment. Neuropsychopharmacology 28(9):1562–1571

McEwen BS, Magarinos AM (2001) Stress and hippocampal plasticity: implications for the pathophysiology of affective disorders. Hum Psychopharmacol 16:S7–S19

McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, Nasca C (2015) Mechanisms of stress in the brain. Nat Neurosci 18(10):1353–1363. doi:10.1038/nn.4086

Milanović SM, Erjavec K, Poljičanin T, Vrabec B, Brečić P (2015) Prevalence of depression symptoms and associated socio-demographic factors in primary health care patients. Psychiatria Danubina 27(1):31–37

Mineur YS, Belzung C, Crusio WE (2006) Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behav Brain Res 175:43–50

Mizuki D, Matsumoto K, Tanaka K, Le Thi X, Fujiwara H, Ishikawa T, Higuchi Y (2014) Antidepressant-like effect of Butea superba in mice exposed to chronic mild stress and its possible mechanism of action. J Ethnopharmacol 28(156):16–25. doi:10.1016/j.jep.2014.08.014 **(Epub 2014 Aug 23)**

Monteggia L (2007) Elucidating the role of brain-derived neurotrophic factor in the brain. Am J Psychiatry 164:1790

Morishita S, Mishima Y, Shoji M (1987) Pharmacological properties of musk. Gen Pharmacol 18(3):253–261

Nemeroff CB (2007) The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J Psychiatr Res 41:189–206

Oh SR, Lee JP, Chang SY, Shin DH, Ahn KS, Min BS, Lee HK (2002) Androstane alkaloids from musk of Moschus moschiferus. Chem Pharm Bull 50:663–664

Pariante CM, Miller AH (2001) Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry 49(5):391–404

Patki G, Solanki N, Atrooz F, Allam F, Salim S (2013) Depression, anxiety-ike behavior and memory impairment are associated with increased oxidative stress andinflammation in a rat model of social stress. Brain Res 20(1539):73–86. doi:10.1016/j.brainres.2013.09.033 **(Epub 2013 Oct 3)**

Patrício P, Mateus-Pinheiro A, Irmler M, Alves ND, Machado-Santos AR, Morais M, Correia JS, Korostynski M, Piechota M, Stoffel R, Beckers J, Bessa JM, Almeida OF, Sousa N, Pinto L (2015) Differential and converging molecular mechanisms of antidepressants' action in the hippocampal dentate gyrus. Neuropsychopharmacology. 40(2):338–349. doi:10.1038/npp.2014.176 **(Epub 2014 Jul 18)**

Pavesi E, Canteras NS, Carobrez AP (2011) Acquisition of Pavlovian fear conditioning using β-adrenoceptor activation of the dorsal premammillary nucleus as an unconditioned stimulus to mimic live predator-threat exposure. Neuropsychopharmacol 36:926–939

Paxinos G, Watson C (1998) The rat hippocampus in stereotaxic coordinates. Academic, San Diego

Penn E, Tracy DK (2012) The drugs don't work? Antidepressants and the current and future pharmacological management of depression. Ther Adv Psychopharmacol. 2(5):179–188. doi:10.1177/2045125312445469

Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ (2012) Neurobiology of resilience. Nat Neurosci 15(11):1475–1484. doi:10.1038/nn.3234 **(Epub 2012 Oct 14)**

Sahay A, Drew MR, Hen R (2007) Dentate gyrus neurogenesis and depression. Prog Brain Res 163:697–722

Schmidt HD, Duman RS (2006) The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Biol Psychiatry 59:1116e1127

Schmidt NB, Keough ME, Hunter LR, Funk AP (2008) Physical illness and treatment of anxiety disorders: a review. In: Zvolensky MJ, Smits J (eds) Series in anxiety and related disorders: anxiety in health behaviors and physical illness. Springer, New York, pp 341–366

Sokolov VE, Kagan MZ, Vasilieva VS, Prihodko VI, Zinkevich EP (1986) Musk deer (*Moschus moschiferus*): reinvestigation of main lipid components from preputial gland secretion. J Chem Ecol. 13(1):71–83

Song C, Leonard BE (2005) The olfactory bulbectomized rat as a model of depression. Neurosci Biobehav Rev 29:627–647

Tillett J, Ames D (2010) The uses of aromatherapy in women's health. J Perinat Neonatal Nurs. 24(3):238–245

Tost H, Champagne FA, Meyer-Lindenberg A (2015) Environmental influence in the brain, human welfare and mental health. Nat Neurosci 18(10):1421–1431. doi:10.1038/nn.4108 **Epub 2015 Sep 25**

Wainwright SR, Galea LA (2013) The neural plasticity theory of depression: assessing the roles of adult neurogenesis and PSANCAM within the hippocampus. Neural Plast 2013:1–14. doi:10.1155/2013/805497

Wang WJ, Zhong M, Guo Y, Zhou LE, Cheng GF, Zhu XY (2003) Effects of musk glucoprotein on chemotaxis of polymorphonuclear leukocytes in vivo and in vitro. Zhongguo Zhong Yao Za Zhi 28(1):59–62

Warner-Schmidt JL, Duman RS (2006) Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. Hippocampus. 16(3):239–249

Webster MJ, Knable MB, Johnston-Wilson N, Nagata K, Inagaki M, Yolken RH (2001) Immunohistochemical localization of phosphorylated glial fibrillary acidic protein in the prefrontal cortex and hippocampus from patients with schizophrenia, bipolar disorder, and depression. Brain Behav Immun 15:388–400

Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology 134(4):319–329

World health organization (WHO) (2015) Depression, fact sheet N°369 October 2015. http://www.who.int/mediacentre/factsheets/fs369/en/. 11 Nov 2015

Wu X, Wu J, Xia S, Li B, Dong J (2013) Icaritin opposes the development of social aversion after defeat stress via increases of GR mRNA and BDNF mRNA in mice. Behav Brain Res 256:602–608

Yi LT, Li J, Li HC, Su DX, Quan XB, He XC et al (2012) Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test. Prog Neuropsychopharmacol Biol Psychiatry 39(1):175–181

Yu HY, Yin ZJ, Yang SJ, Ma SP (2014) Baicalin reverse AMPA receptor expression and neuron apoptosis in chronic unpredictable mild stress rats. Biochem Biophys Res Commun 451(4):467–472. doi:10.1016/j.bbrc.2014.07.041

Zunszain PA, Anacker C, Cattaneo A, Carvalho LA, Pariante CM (2011) Glucocorticoids, cytokines and brain abnormalities in depression. Prog Neuropsychopharmacol Biol Psychiatry 35(3):722–729