NATURAL PRODUCTS AS SOURCES OF INNOVATIVE APPROACHES IN PSYCHIATRY

EDITED BY: Elaine Elisabetsky, Chun-Tao Che, Elizabeth Anne Olson and Zhang-Jin Zhang

PUBLISHED IN: Frontiers in Psychiatry and Frontiers in Pharmacology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88976-861-5
DOI 10.3389/978-2-88976-861-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

NATURAL PRODUCTS AS SOURCES OF INNOVATIVE APPROACHES IN PSYCHIATRY

Topic Editors:
Elaine Elisabetsky, Federal University of Rio Grande do Sul, Brazil
Chun-Tao Che, University of Illinois at Chicago, United States
Elizabeth Anne Olson, Southern Utah University, United States
Zhang-Jin Zhang, The University of Hong Kong Pokfulam, SAR China

Citation: Elisabetsky, E., Che, C.-T., Olson, E. A., Zhang, Z.-J., eds. (2022). Natural Products as Sources of Innovative Approaches in Psychiatry. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-861-5

Table of Contents

frontiers | Frontiers in Psychiatry

TYPE Editorial
PUBLISHED 26 July 2022
DOI 10.3389/fpsyt.2022.929139

OPEN ACCESS

EDITED BY
Zhang-Jin Zhang,
The University of Hong Kong,
Hong Kong SAR, China

REVIEWED BY
Yonghong Liu,
South China Sea Institute of Oceanology (CAS), China
Marcus Scotti,
Federal University of Paraíba, Brazil

*CORRESPONDENCE
Elaine Elisabetsky
elaine.elisabetsky@gmail.com

SPECIALTY SECTION
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

RECEIVED 26 April 2022
ACCEPTED 11 July 2022
PUBLISHED 26 July 2022

CITATION
Elisabetsky E and Che C-T (2022) Editorial: Natural products as sources of innovative approaches in psychiatry. *Front. Psychiatry* 13:929139. doi: 10.3389/fpsyt.2022.929139

Editorial: Natural products as sources of innovative approaches in psychiatry

Elaine Elisabetsky[1]* and Chun-Tao Che[2]

[1]Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [2]College of Pharmacy, University of Illinois at Chicago, Chicago, IL, United States

KEYWORDS

natural products, mental disorders, drug development, innovative antidepressants, medicinal plants revisited

Editorial on the Research Topic
Natural products as sources of innovative approaches in psychiatry

Between 1981 and 2010, more than thirty percent of the new medicines approved by the US FDA were based on natural products and/or their derivatives. Advantages of natural products in terms of drugability and bioactivity have been brilliantly reviewed and summarized by Harvey et al. (1) and Skirycz et al. (2), in part explaining why the vast majority of drugs have a close match with natural products (1). Today we also witness global interest in the use of plant-based therapies and botanical healthcare products. Such a trend has stimulated greater scientific awareness in exploring the pharmacologically active constituents of medicinal plants. This Special Issue is intended to be a compilation of scientific reports to demonstrate the potential of natural products for the management of psychiatric disorders. It is our hope that this Special Issue would serve those researchers who are interested in potentially useful molecules from natural sources for psychiatric applications.

The anti-depressant-like effects of natural products and herbal preparations were demonstrated by a series of studies.

Zhou et al. reported a Chinese herbal drug (Jie-Yu Pill) to be able to ameliorate mood disorder-like behavior and cognitive impairment using a mouse model induced by a combination of estrogen deprivation and chronic stress, implying the potential of Jie-Yu Pill in managing menopause-associated mood disorders.

Studies on depression-like behaviors and cognitive dysfunction was extended to microgravity and social isolation scenarios. Wang et al. reported that dammarane sapogenins (obtained from ginseng) could reverse the depressive-like behaviors and improve cognitive impairment in rats, and concluded that the protective effects might be driven in part by the modulation of cholinergic system in the hippocampus.

In a study by Ayuob et al., *Ocimum basilicum* essential oil was given to mice under chronic unpredictable mild stress (CUMS) in order to assess the effectiveness of the preparation on the main olfactory bulb (MOB). Their results revealed improvements in both biochemical and histopathological changes in the MOB. They suggested the effect might have been attained through an up-regulation of gene expression of GFAP and Ki67 and down-regulation of Caspase-3 in the MOB.

Jiang et al. reported the protective effect of a Chinese herbal drug (Shen Yuan, composed of *Panax ginseng* and *Polygala tenuifolia*) in the rat chronic unpredictable mild stress model. Shen Yuan reversed the depressive-like behaviors as well as biochemical depressive states correlates, including increased serum corticosterone and proinflammatory cytokines (IL-6, IL-1β, and TNF-α) levels, oxidative stress markers (SOD, CAT, and MDA), and diminished levels of hippocampal neurotransmitters (5-HT, DA, and NE) in rats exposed to CUMS. Furthermore, rats treated with Shen Yuan showed reduced hippocampus BDNF, p-TrkB, p-Akt, and p-mTOR proteins expression raised by CUMS exposure. The findings pointed to a preventive effect of Shen Yuan against hypothalamus-pituitary-adrenal axis dysfunction, decreased in neurotransmitters levels, reduced oxidative stress, neuroinflammation suppressio, and activation of the PI3K/Akt/mTOR-mediated BDNF/TrkB pathway. All of these effects were thought to be relevant to counteract depressive states.

Curcumin has been reported to exert beneficial effects on managing major depressive disorder. Highlights of the compound's clinical and non-clinical effects are presented in a review by Ramaholimihaso et al.

A significant amount of attention has recently been given to the potential therapeutic value of endogenous modulators.

Still on depression, Almeida et al. reported the antidepressant-like effects of chronic guanosine in the olfactory bulbectomy (OBX) mouse model. Guanosine reversed the OBX-induced recognition memory impairment and hyperlocomotion, increase in hippocampal BDNF and redox imbalance. Guanosine also mitigated the OBX-induced hippocampal neuroinflammation and increased metabolism.

Tonon et al. reviewed the evidences of melatonin for managing depressive states, noting that clinical evidence was inconsistent in regard to the benefits of melatonin or melatonin agonists in fighting depression, in contrast with the antidepressant-like effects observed in animal models. The authors argue that the understanding of melatonin in therapeutics must include melatonin specificities as an integrating molecule, associated with entrainment, metabolism, immunity, neurotransmission, and cell homeostasis.

Bitencourt et al. reviewed the path that led to the development of cannabidiol as an antiepileptic drug, highlighting early contributions by Brazilian scientists. Authors elaborated on the idea that CDB development model can be used to develop phytocannabinoids for other psychiatric conditions, including depression, anxiety, post-traumatic stress disorder (PTSD), addiction, neurodegenerative disorders and autism spectrum disorder (ASD).

Cognitive-enhancing effects of the Chinese medicinal plant, *Dendrobium nobile*, in sleep deprivation-induced amnesia in mice was reported by Jiang et al.. The results suggest amnesia was improved by the treatment in the novel object recognition and object location recognition tests. They also reported elevated levels of norepinephrine, dismutase and catalase activities, as well as a decrease in 5-HT and malondialdehyde, in the brain tissue obtained from the *Dendrobium* treated group. The authors conclude that *D. nobile* extract has beneficial effects in the prevention and improvement of cognitive impairment induced by sleep deprivation, effects possibly mediated through the regulation of neurotransmitters and alleviation of oxidative stress.

Bian et al. reviewed clinical and pre-clinical results of saffron (dried stigma of *Crocus sativus*) and its constituents (crocin, crocetin and safranal) on brain disorders. The review covers a range of pathologies such as depression, anxiety, Alzheimer's and Parkinson's diseases, post-traumatic stress disorder, schizophrenia, epilepsy, and stroke. Preclinical studies showed that saffron exerts its neuroprotective effects mostly via antioxidative stress, anti-neuroinflammation, and anti-apoptosis pathways. Clinical results supported that saffron could alleviate depressive and anxiety-like symptoms as well as improve cognition impairment. The authors suggested that these findings had provided a clear perspective that could aid the development of neuroprotective agents from saffron and its bioactive ingredients.

Zhao et al. suggest that bulleyaconitine A (BAA), a C19-diterpenoid alkaloid used in China for decades as nonnarcotic analgesic to treat chronic pain, is a candidate for treatment of opioids addiction. They showed that BAA attenuates morphine-induced withdrawal symptoms in mice, conditioned place preference and locomotor sensitization by stimulation of microglial (but not astrocytes or neurons) dynorphin A expression in nucleus accumbens and hippocampus.

Marx et al. reported a secondary analysis of a randomized placebo-controlled trial that investigated a 24-weeks intervention with mangosteen (*Garcinia mangostana* Linn.) pericarp extract supplementation in people diagnosed with schizophrenia. The secondary analysis investigated if the intervention was effective to improve cognition in this

population and revealed that mangosteen pericarp extract did not affect cognitive outcomes in people with schizophrenia.

In conclusion, the above studies illustrates the potential of natural products against various psychiatric conditions. As usual in drug development, to better characterize and understand their medicinal properties, additional preclinical and clinical investigations are warranted.

Author contributions

EE and C-TC contributed equally to the concept and execution of this editorial. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Discov*. (2015) 14:111–29. doi: 10.1038/nrd4510

2. Skirycz A, Kierszniowska S, Méret M, Willmitzer L, Tzotzos G. Medicinal bioprospecting of the Amazon Rainforest: a modern Eldorado? *Trends Biotechnol*. (2016) 34:781–90. doi: 10.1016/j.tibtech.2016.03.006

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 11 September 2020
doi: 10.3389/fpsyt.2020.569711

Ocimum basilicum (Basil) Modulates Apoptosis and Neurogenesis in Olfactory Pulp of Mice Exposed to Chronic Unpredictable Mild Stress

Nasra N. Ayuob[1,2,3*], *Maha J. Balgoon*[4], *Soad Ali*[3,5,6], *Ibrahim S. Alnoury*[7], *Hailah M. ALmohaimeed*[8] *and Amany A. AbdElfattah*[9]

[1] Department of Medical Histology and Cell Biology, Faculty of Medicine, Damietta University, Damietta, Egypt, [2] Department of Medical Histology, Faculty of Medicine, Delta University for Science and Technology, Mansoura, Egypt, [3] Yousef Abdullatif Jameel, Chair of Prophetic Medical Applications (YAJCPMA), Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, [4] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia, [5] Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, [6] Department of Histology, Faculty of Medicine, Assuit University, Assuit, Egypt, [7] Department of ENT, H&N Surgery, Faculty of Medicine, King Abdulaziz University Hospital, Jeddah, Saudi Arabia, [8] Department of Basic Science, Medical College, Princess Noruh bint Abdulrahman University (PNU), Riyadh, Saudi Arabia, [9] Department of Histology and Cell Biology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

OPEN ACCESS

Edited by:
Elaine Elisabetsky, Federal University of Rio Grande do Sul, Brazil

Reviewed by:
Kabirullah Lutfy, Western University of Health Sciences, United States
Kurt Leroy Hoffman, Autonomous University of Tlaxcala, Mexico

***Correspondence:**
Nasra N. Ayuob
nasraayuob@gmail.com;
nasra.ayuob@deltauniv.edu.eg

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *05 June 2020*
Accepted: *18 August 2020*
Published: *11 September 2020*

Citation:
Ayuob NN, Balgoon MJ, Ali S, Alnoury IS, ALmohaimeed HM and AbdElfattah AA (2020) Ocimum basilicum (Basil) Modulates Apoptosis and Neurogenesis in Olfactory Pulp of Mice Exposed to Chronic Unpredictable Mild Stress. Front. Psychiatry 11:569711. doi: 10.3389/fpsyt.2020.569711

Background: *Ocimum basilicum (O. basilicum*) was described to have antidepressant and anxiolytic activities. Although the relationship between the main olfactory bulb (MOB) and depression was recently reported, the chronic stress-induced dysfunction of the MOB is not clearly described.

Objectives: This study aimed to assess the efficacy of inhalation of *O. basilicum* essential oils in improving chronic unpredictable mild stress (CUMS)-induced changes in MOB of mice and understand the mechanism underlying such effect.

Materials and Methods: Adult male mice (n=40) were assigned into four groups included the control, CUMS-exposed, CUMS + fluoxetine (FLU), CUMS + *O. basilicum*. Behavioral changes, serum corticosterone level, and gene expression of GFAP, Ki 67, and caspase-3 were assessed using real-time PCR (RT-PCR). Histopathological and immunochemical examination of the MOB was performed.

Results: FLU and *O. basilicum* significantly down-regulated ($p = 0.002$, $p<0.001$) caspase-3 gene expression indicating reduced apoptosis and up-regulated ($p = 0.002$, $p < 0.001$) Ki67 gene expression indicating enhanced neurogenesis in MOB, respectively. FLU and *O. basilicum*-treated mice markedly improved MOB mitral cell layer distortion and shrinkage induced by CUMS.

Conclusion: *O. basilicum* relieved both biochemically and histopathological chronic stress-induced changes in the main olfactory bulb possibly through up-regulation of gene expression of GFAP and Ki67 and down-regulation of caspase-3 in the MOB.

Keywords: *Ocimum basilicum*, chronic stress, caspase-3, anti-glial fibrillary acidic protein, olfactory bulb, neurogenesis

INTRODUCTION

Chronic stress results in deterioration in mood, cognition, memory and may be a leading cause in the occurrence of many systemic diseases as Parkinson's disease, type 2 diabetes, gastric ulceration, and Alzheimer's disease (1).

Olfactory system is distinctive as it is considered the most proliferative CNS system entertaining differentiating progenitor cells, migrating from the subventricular zone to the olfactory bulb (OB), where they differentiate into tyrosine hydroxylase (THC) or GABA (C) interneurons (2). The main olfactory bulb (MOB) is an important part of the olfactory system that results in post developmental neurogenesis (3).

Unpredictable stress is associated with atrophy in cortical and limbic brain regions including the MOB. It was reported that olfactory deficits often accompany neurodegenerative diseases. This was noticed in idiopathic Parkinson's disease where advanced olfactory deficits were initial signs of the disease that occurs before the first motor dysfunctions (4). However, the mechanism of the dysfunction & the structural changes of MOB resulted from CUMS is still mostly unidentified (3). The model of (CUMS) is one of the common and verified models used to study depression. This model is characterized by the occurrence of long-lasting neurodegenerative changes including behavioral, neurochemical, and neuroendocrinal changes that mimic those observed in depressed patients (5).

Aromatherapy is the utilization of aromatic essences and pure essential oils, naturally-extracted from roots, leaves, and flowers of plants in promoting the health of body, mind as well as in treatment of some diseases. It was reported that it is potentially useful in the management of disruptive behaviors and the reduction of agitation in people with dementia (6, 7). *Ocimum basilicum* (*O. basilicum*), also called basil or Raihan, is an annual plant used as a spice for culinary purposes in salads and pasta sauces. It is extensively utilized as a flavoring agent and in production of perfumes and soap (8, 9). In addition, it is used for medicinal purposes due to its antidepressant, anxiolytic, sedative activities (10, 11). *O. basilicum* as a therapeutic supplement has been investigated in many animal models of cognitive deficits (12, 13). Recently, the therapeutic efficacy of *O. basilicum* against impairment of memory function in animal model of Multiple sclerosis was reported (14).

Although the relationship between MOB and depression was recently reported (15–17), the CUMS-induced dysfunction of MOB is not clearly described. Therefore, the current research was designed to evaluate the CUMS-induced alterations occurred in MOB in mice exposed to mild chronic depression and the efficacy of inhalation of *O. basilicum* essential oils in improving these changes as well as understand the possible mechanism underlying this effect.

MATERIAL AND STUDY DESIGN

Ethical approval and guidelines of animal care were obtained from "the biomedical research ethics committee" and King Fahad Medical Research Center (KFMRC), King Abdulaziz University (KAU), Jeddah, Saudi Arabia (SA).

Male Swiss albino mice (N=40), aged 5 weeks, and weighed (30–40 g), were obtained from the KFMRC and were maintained for 2 weeks before the experiment at 27 ± 1°C, were fed on a standard mice pellets and water *ad-libitum* in order to acclimatized and ensure normal growth and behavior.

Chemicals

Fluoxetine (FLU) is a selective serotonin reuptake inhibitor used as antidepressant to treat CUMS-induced depression in mice of the positive control group. It was purchased from Dar Al Dawa (DAD) Pharmaceuticals Co., Ltd. (Amman, Jordan). Sodium carboxymethyl cellulose (CMC-Na, 0.03%) was used to dissolve FLU and 20 mg/kg was given to the mice by intragastric gavage (18). *O. basilicum* was collected from the Jeddah gardens. A botanist from the Faculty of Science, KAU helped to morphologically identify *O. basilicum*. The essential oil of *O. basilicum* was prepared according to the method of (19). The constituents of *O. basilicum* essential oil were identified using "gas chromatography coupled to mass spectrometry" (GC-MS; Agilent, Columbia, MD). Essential oils of *O. basilicum* were diluted first with 5% propylene glycol before their use (Sigma, St. Louis, MO) as reported by (20), then given through inhalation. Amyl acetate, 5% (Sigma), was given to untreated CUMS mice by inhalation as it was reported by (21) that it has no effect on anxiety.

Experimental Design

After acclimatization period, the mice were assigned into four groups (n=10 each) at random. Each five mice were held together in a cage. The four groups included the control, CUMS, CUMS + FLU, CUMS + *O. basilicum*. The CUMS procedure used in this study was previously described. Mice were exposed to CUMS for continuous 4 weeks followed by 2 weeks treatment by amyl acetate, FLU, or *O. basilicum*.

Inhalation of *O. basilicum* and amyl acetate was performed according to Chioca, Ferro (20) using a "32 × 24 × 32 cm odor-isolated acrylic box".

Behavioral Assessment

Behavior tests were done after 6 weeks between 8:00 and 11:30 AM [Mineur, Belzung (22)] using the elevated plus maze test (EPM) and the forced swimming test (FST) spaced by a 24 h between tests.

The FST was carried as described by Doron, Lotan (23). The total time spent not moving by the mouse during 6 min was recorded in seconds as previously described by Ayuob, Firgany (24). Regarding the EPM, its procedure was described by Ali, Abd El Wahab (10). The number of times the mouse enter to the closed arm during 6 min and the time spent by each mouse inside the open and closed arms were registered in seconds using videotaped behavior software (Noldus Information Technology, EthoVision XT®).

Biochemical Assessment of Serum Corticosterone Level

Following finishing behavioral tests, blood samples from the retroorbital venous plexus were obtained in EDTA-coated

tubes from anesthetized mice, centrifuged for 10 min, and kept at −80°C for measuring the level of corticosterone by using radioimmunoassay (ELISA Kits; ALPCO Diagnostics, Orangeburg, NY).

Animal Dissection for Histological Study and Assessment of GFAP, Ki 67, and Caspase-3 Gene Expression Using Real-Time PCR

Immediately after taking the blood samples, the animals were sacrificed and the whole brain was carefully dissected with intact olfactory pulp, immersed in dried ice for farther dissection into left and right hemispheres, fixation in 10% neutral buffered formalin, and routinely processed for paraffin blocking in histopathology lab.

Paraffin processed samples were subjected to RNA extraction accord to methods adopted by from 100 mg of formalin-fixed paraffin-embedded (FFPE) sections obtained from the left brain hemisphere. They were deparaffinized in 1 ml of xylene, incubated at 56°C for 15 min, and centrifuged for 10 min at 13,000 g. The supernatant was discarded and the pellet washed twice with 1 ml 100% ethanol, centrifuged, the supernatant was discarded, and 1 ml Trizol was added to the pellet (25).

Extraction of total RNA using Trizol was done according to the supplier instruction (Invitrogen Life Technologies, Carlsbad, CA, USA). NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA) was used to measure the concentration of RNA. Reverse transcription was done using oligo-dT primers (Bioneer Inc., Daejeon, Republic of Korea) in a 20-ll reaction including 5 ll RNA. The resulted complementary DNAs (cDNAs) were amplified using PCR Master Mix (Bioneer Inc., Daejeon, Republic of Korea) with primers (Metabion International AG, Semmelweisstr, Germany). GFAP gene (forward 5′-CAAGCCAG ACCTCACAGCG-3′, reverse5′-GGTGTCCAGGCTGG-TTTCTC-3′, caspase-3 (forward 5′- *TGTATGCTTACTCTA CCGCACCCG*-3′, reverse5′-*GCGCAAAGTGACTGGATGAAC C*-3′), Ki67 (forward 5′-AAGAAGAGCCCACAGCACAGAG AA-3′, reverse5′-AAGAAGAGCCCACAGCACAGAGAA 3′), and β-actin (forward 5′-TCTGGCACCACA CCTTCTA-3′; reverse 5′-GGCATACAGGGACAGCAC-3′). PCR amplification was applied in a thermocycler (manufactured by Labnet International Inc.). The procedure was reported in a previous work of Ayuob, Firgany (24). Using comparative Ct method, normalization of current results to β-actin as a reference gene was done. Ct values were used to estimate the gene/β-actin ratio, with a value of 1.0 used as the control (calibrator). The normalized expression ratio was calculated using the 2−ΔΔCt (26). The mRNA level was expressed as a ratio or percent to that of corresponding β-actin

Histopathological Assessment

Five microns serial paraffin sections from brain hemispheres including olfactory lobe were, stained with hematoxylin and eosin (H & E) for general histological assessment (27). Immunohistochemical staining was performed using the peroxidase-labeled streptavidin-biotin technique (28). Anti-glial fibrillary acidic protein (GFAP) antibody (DakoCytomation, Minneapolis, MN) was used for demonstration of astrocytes and was diluted 1:1,000 with phosphate-buffered saline (PBS). Anti-caspase-3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used to demonstrate apoptosis, and diluted to 1:1,000 with PBS. Assessment of indirect neurogenesis was assessed using anti-Ki-67 antibody (rabbit polyclonal IgG; Abcam, Cambridge, UK). It was diluted to 1:100 with PBS. To verify the specificity of each primary antibody, a negative control section was done by omitting the primary antibody. Examination of slides was performed using light microscope connected to a digital camera (Olympus, BX-61, Los Angeles, CA) for photographing.

The percentage area of GFAP immune-expression, caspase-3-positive cells and Ki67-positive cells was counted in five non-overlapping high power fields (x400) of MOB in each mouse [Makhlouf, El-Beshbishy (28)] using ImageJ 1.52a (National Institutes of Health, USA). The thickness of the mitral cell layer of the MOB was measured in five non-overlapping high power fields (x 400) in each animal.

Statistical Analysis

Analysis of the data was carried out using the Statistical Package for the Social Sciences (SPSS, version 22) software. One-way ANOVA followed by LSD (least significant difference) *post hoc* were used to compare the parametric data of different groups. P values < 0.05 were considered significant.

RESULTS

Behavioral Results

Exposure of mice to CUMS for 4 weeks was found to result in significant increase in immobility time ($p < 0.001$). Administration of FLU or *O. basilicum* for 2 weeks after induction of depression significantly decreased immobility time compared to untreated mice ($p = 0.02$, $p < 0.001$) respectively (**Figure 1**).

CUMS also exhibited significantly decreased ($p < 0.001$) time spent in the open arms with a significant increase time spent ($p < 0.001$) in the closed arm during the EPM compared to control mice. On the other hand, both FLU and *O. basilicum* could significantly increase ($p=0.001$, $p < 0.001$) the duration the mice spent in the open arm during the EPM and significantly reduce ($p < 0.001$) entries to closed arms compared to untreated animals, respectively. This indicating the ability of FLU and *O. basilicum* to reduce the anxiety-like behavior in CUMS-exposed mice (**Figure 1**).

Biochemical Results

Serum Corticosterone Level

Exposure of mice to CUMS for 4 weeks produced a significant increase ($p < 0.001$) in corticosterone serum level compared to the control. A significant reduction in corticosterone serum level was observed following treatment with either FLU ($p < 0.001$) or *O. basilicum* ($p < 0.001$) in comparison with untreated group (**Figure 1**).

Gene Expression of GFAP, Caspase-3, Ki 67

Real-time PCR (RT-PCR) revealed significantly lowered ($p < 0.001$) GFAP gene expression in MOB of CUMS group in

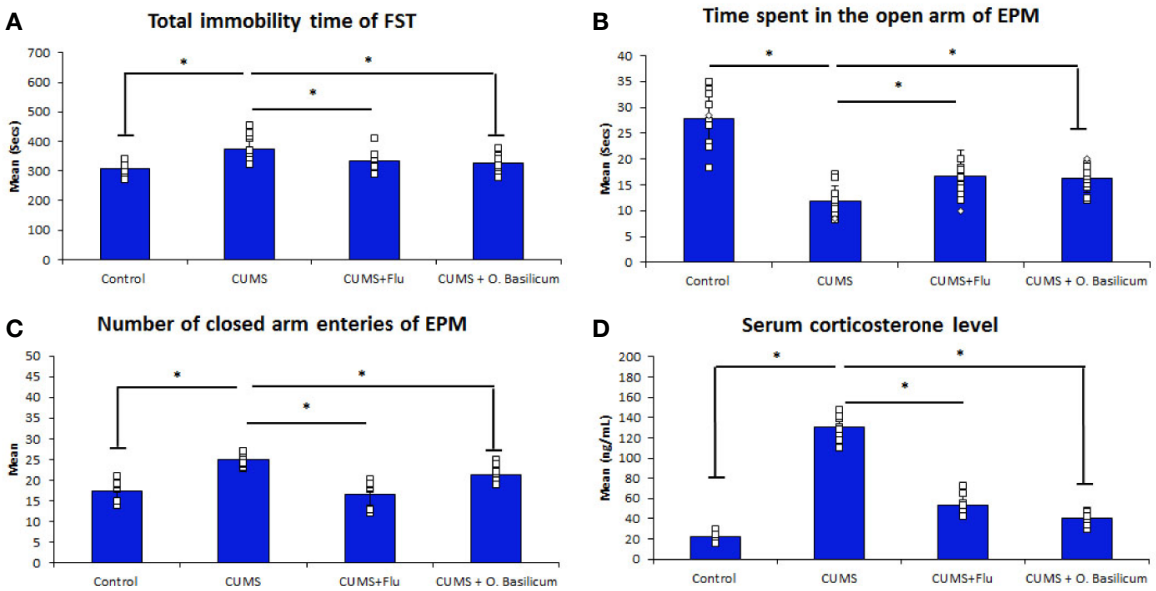

FIGURE 1 | Effect of *Ocimum basilicum* on the immobility time of the forced swimming test (FST) **(A)**, the time spent in the open arm **(B)**, the number of closed arm entries **(C)** of the elevated plus maise (EPM) test, and serum corticosterone level **(D)**. Data are expressed as mean ± SD (n = 10). * significance with $p < 0.05$. CUMS, chronic unpredictable mild stress; FLU fluoxetine; *O. basilicum*, *Ocimum basilicum*.

comparison with control while FLU- or *O. basilicum*-treated groups showed significant higher levels ($p = 0.003$, $p<0.001$) in comparison with untreated CUMS exposed mice, respectively (**Figure 2**).

On the other hand, caspase-3 gene expression was significantly increased ($p < 0.001$) in the MOB of CUMS group, while it was reduced significantly ($p = 0.002$, $p<0.001$) in both FLU- or *O. basilicum*-treated groups compared to untreated CUMS group, respectively (**Figure 2**).

Ki67 gene expression, was reduced significantly ($p < 0.001$) in MOB of CUMS group, in comparison to significant higher level in the groups received FLU- or *O. basilicum* ($p = 0.002$, $p < 0.001$) in comparison with untreated CUMS group respectively (**Figure 2**).

Histopathological Results

Examination of MOB of control group (H&E stain) showed that it was consisted of six distinct layers include from outside inward; olfactory nerve of multipolar nerve cells called mitral cells, glomerular, external plexiform, mitral cell, internal plexiform, with the most inner granular cell layer. The mitral cells have large vesicular lightly stained nuclei with well-defined nucleoli and abundant amount of basophilic granular cytoplasm. Smaller nerve cells from granular cell layer, with dark nuclei and little amount of cytoplasm, appeared scattered between the mitral cells.

Examination of the MOB of the CUMS group revealed that the mitral cell layer was affected where nerve cells, in this layer, appeared smaller and distorted in shape and had deeply stained cytoplasm, dark pyknotic nuclei, and wide pericellular spaces (**Figure 3**). The thickness of this layer was significantly reduced in this group ($p < 0.001$) in comparison to control. Groups treated with FLU or *O. basilicum* showed restoration of the normal appearance of mitral nerve cells while few mitral cells appeared distorted with significant increase in thickness ($p < 0.001$) when compared to untreated CUMS mice. Insignificant difference could be recognized between the two animal groups ($p=0.035$) (**Figures 3** and **4**).

Immunoexpression of Caspase-3, Ki 67, and GFAP in the Main Olfactory Bulb

It was observed that caspase-3 immunoexpression was increased in the MOB of CUMS group, while it was reduced back in both FLU- and *O. basilicum*-treated groups (**Figure 4**). A significant increase ($p < 0.001$) in caspase-3 positive cells in the MOB of CUMS group was observed upon statistical analysis of area density in comparison to control mice. In FLU- and *O. basilicum*-treated groups, expression was significantly lower ($p < 0.001$) compared to the untreated mice. There was insignificant difference ($p = 0.39$) in caspase-3 positive cells between FLU- and *O. basilicum*-treated groups (**Figure 4**).

Regarding GFAP immunoexpression, the astrocytes processes in various layers of the control MOB showed moderate positive immunoexpression. A significant reduction ($p = 0.001$) in percentage area of GFAP immune expression in the MOB of CUMS group was observed upon statistical analysis compared to control animals.

On contrast, a significant rise ($p < 0.001$) in GFAP immunoexpression was recorded in FLU- and O. *basilicum*-treated groups compared to the untreated mice. Insignificant

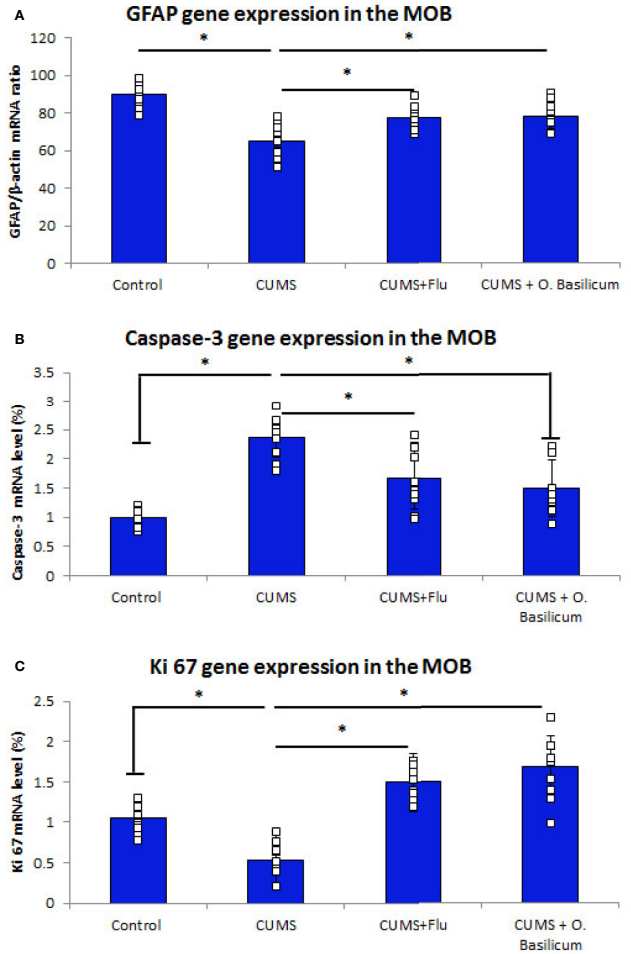

FIGURE 2 | Effect of *Ocimum basilicum* on GFAP **(A)**, caspase-3 **(B)**, Ki 67 **(C)** mRNA levels detected by real-time PCR (RT-PCR) in the main olfactory bulb (MOB) after treatment of chronic unpredictable mild stress (CUMS) in rats with fluoxetine (FLU) and *O. basilicum*. The messenger RNA (mRNA) level was expressed as a ratio or percent to that of corresponding β-actin. Data were presented as mean ± SD (n=10 in each group). * significance with $p<0.05$.

difference ($p = 0.81$) was observed between FLU- and *O. basilicum*-treated groups (**Figure 4**).

Many nerve cells in the MOB exhibited immune positive expression for Ki 67. Statistical analysis revealed a significant ($p < 0.001$) reduction in Ki67-positive cells in the MOB in comparison to its expression in control mice while a significant increase was found in FLU- and O. *basilicum*-treated groups ($p < 0.001$) in comparison with untreated CUMS group with no significant difference ($p = 0.30$) between the two groups (**Figure 4**).

DISCUSSION

Olfactory function plays an important role in health and behavior. The laminar organization of olfactory bulb combines a unique neuronal morphology and complex synaptic connections which play a basic role in smell perception and modify the response of the output neurons for sharp tuning of a given odor (29). Olfactory dysfunction is considered a premotor sign of neurodegeneration that appears early in the degenerative process as in Alzheimer disease and Parkinson disease (30, 31). Moreover, the olfactory bulbectomy of rats has been validated as a model of depression over the past 30 years, suggesting a close relationship between MOB and depression (15–17). Reviewing literature showed that the, pathophysiology of MOB induced by CUMS is still unclear. Therefore, the present study was designed to describe the pathological alteration occurred in MOB of mice after exposure to CUMS and to investigate the efficacy of inhalation of *O. basilicum* essential oils in improving these changes as well as to understand the possible mechanism underlying this effect.

Mice exposed to the CUMS, in this study, showed depressive-like behavior verified by increased time of immobility during the FST and confirmed by decreasing the time spent in the open arms of the EPM as well as increasing of serum level of corticosterone. Similar results were reported by (32).

In this study, FLU induced an improvement in the behavioral, biochemical, and structural changes occurred in MOB of mice exposed to CUMS. In consistent with these findings, some previous studies revealed that FLU alleviated the behavioral changes induced by CUMS and improve the CUMS-induced structural changes in the hippocampus (33).

It was stated that stress enhanced lipid peroxidation and decreased oxidative stress defense in depressed patients that endorses undesirable effects on many cellular functions as showed by decreased plasma antioxidant defenses (34). It was reported that oxidative stress has a crucial role in the pathophysiology of depression in rodents and humans (35) and (36). Methanol, ethanol, or water extracts of *O. basilicum* seeds were described to have good antioxidants activity (9). Therefore, *O. basilicum* was used, in this study, to relieve the CUMS-induced changes on the MOB. Inhalation of *O. basilicum* alleviated this depressive status, evident by the behavioral tests, and reduced elevated corticosterone levels documented in animals subjected to CUMS. It was proposed that *O. basilicum* antidepressant capacity might be related to the increased brain level of enzymatic and non-enzymatic antioxidants endorsed by *O. basilicum* extracts (37).

This work demonstrated that exposure to CUMS caused structural alteration in the olfactory bulb of mice represented by distortion and shrinkage of mitral cells. This might be responsible for the atrophy reported in this study as well as some previous studies. Exposure to CUMS was reported to result in cortical and limbic brain regions atrophy that including also hippocampus and MOB (38) elevation of inflammatory mediators in hippocampal regions (39), and disturbances in hypothalamic-pituitary-adrenal (HPA) axis that might explain the increased corticosterone level in CUMS mice (40). *O. basilicum* was found to ameliorate CUMS-induced neuronal changes in MOB especially mitral cells. This effect might be attributed to the bioactive compounds present in *O. basilicum*

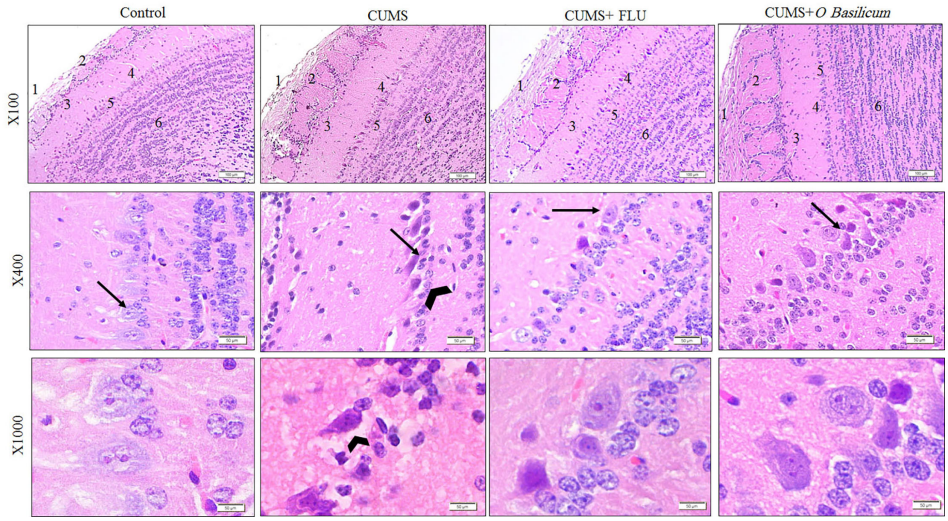

FIGURE 3 | The histological structure of the main olfactory bulb (MOB) of the studied groups show nerve fiber layer (1), glomerular layer (2), external plexiform layer (3), mitral layer (4), internal plexiform layer (5), and granular layers (6). Note the affected mitral layer in the CUMS group appear with higher magnification and show distorted and small Mitral cells (arrow) compared with other group together with widening of pericellular space around them (arrow head). H&E staining. CUMS, chronic unpredictable mild stress; FLU, fluoxetine; *O. basilicum*, *Ocimum basilicum*.

essential oils like linalool, eugenol, cineole, and many other compounds that exert free radical scavenging activity (8, 24). These essential oils inhibit liposomal peroxidation and scavenge hydroxyl radicals, NO, and superoxide anion.

GFAP immunoreaction was used as a selective marker for estimation of astrocytes integrity in MOB as reported by (41). In the present study, administration of FLU and *O. basilicum* reversed the CUMS-induced reduction in GFAP expression. This was for a certain extent is in agreement with (32) who reported that fluoxetine could prevent GFAP reduction and glial atrophy and restore the integrity of astrocytes in CUMS-induced animal model of depression. *O. basilicum*, in this study, could be considered to have a similar effect as fluoxetine. In consistent with this study (14), recently reported that *O. basilicum* (at the dose of 100 and 200 µl/kg) induced neuroprotective effect against ethidium bromide-induced cognitive deficit through amelioration of neuroinflammation, mitochondrial dysfunction, and astrogliosis in the prefrontal cortex of the animals.

The olfactory bulb together with hippocampal dentate gyrus subventricular and the sub-granular zones, represent the three regions in the brain that undergo adult neurogenesis; the process that maintains continuous turnover of bulbar interneurons. Granular and periglomerular neurons of OB are well known to be differentiating and from cell precursors located in the subventricular zone and migrating to OB where it can respond to odor stimulations (42–44). In this study, Ki67 positive cells, which represent the newly proliferating nerve cells, was observed in the control MOB. This was previously described by (45). Exposure to CUMS resulted in a significant decrease in proliferating cells number in MOB of CUMS-exposed mice and this finding was consistent with that of Ki67 gene expression that revealed a considerable down-regulation in CUMS animals when compared to control. Similar results were recorded by (46) and (47) in the MOB of chronic stressed rats using the cell proliferation markers; PSA-NCAM, DCX, and BrdU. In addition, previous studies reported a significant suppression of neurogenesis in hippocampal regions in mice model of depression (12, 48). This effect might be attributed to increased plasma levels of corticosterone in CUMS-exposed rats, recorded in this study as well as many other studies which concluded that chronic exposure to corticosterone, or stressors that increase its secretion, has powerful suppressor effect on proliferation process (10, 49, 50).

Administration of FLU and *O. basilicum* significantly improved the neurogenesis and evidenced by upregulated Ki67 gene expression. This was supported by other studies conducted on MOB of mice (47), hippocampus of mice (51), and hippocampus of human (52). Brain derived neurotropic factor and other neurotrophins expressions were reported to be increased by antidepressant drugs and thus stimulate neurogenesis and repairing of damaged neurons (24, 53).

In this study, increased neuronal apoptosis, evidenced by increase number of caspase-3 positive cells as well as upregulation of caspase-3 gene expression was detected in MOB of mice exposed to CUMS. In agreement with this (54), and Meyer, Glaser (4) reported marked increase of apoptosis in rat and mice olfactory bulbs in models of experimentally induced neurodegeneration as well as in the hippocampus of CUMS-exposed mice (48).

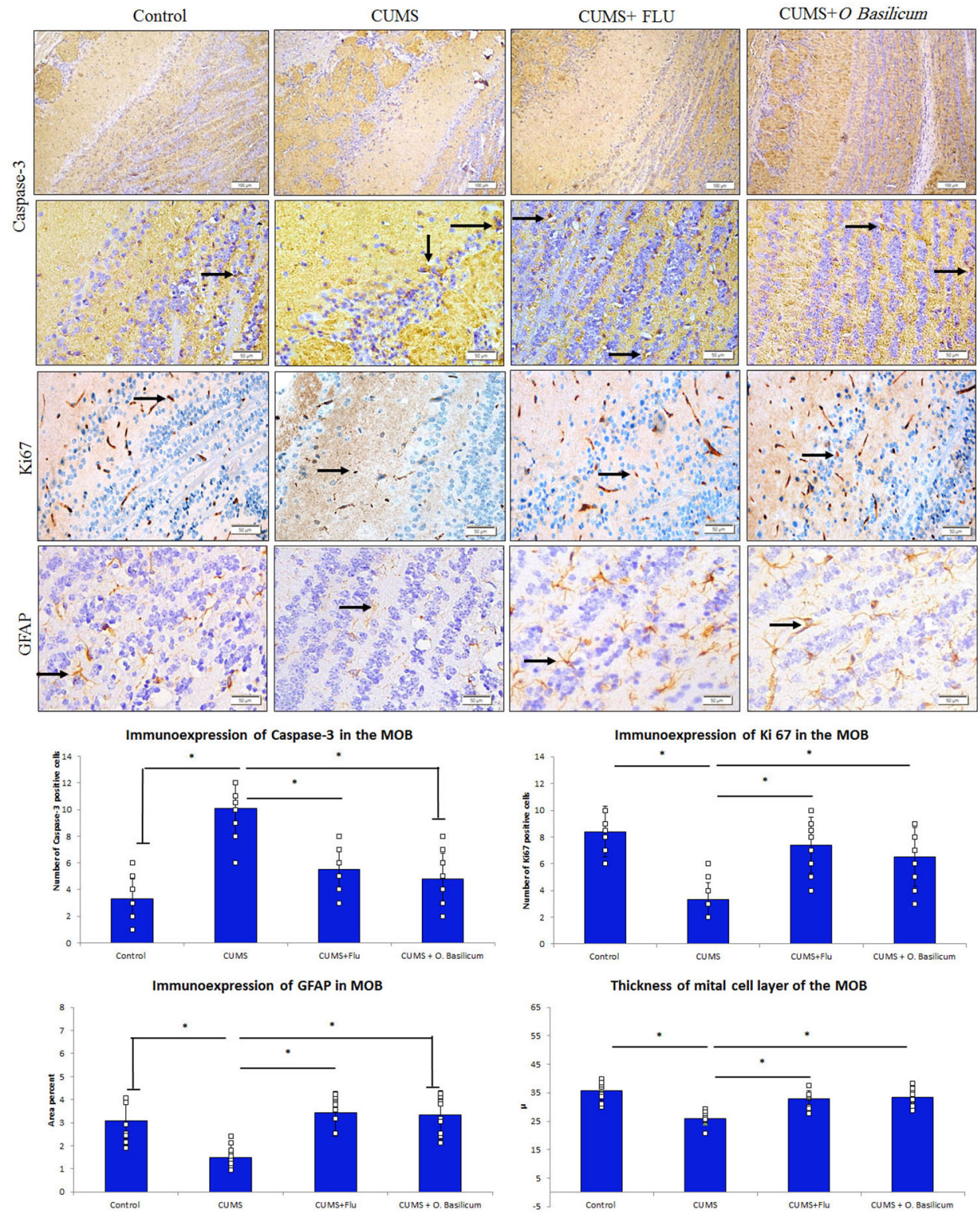

FIGURE 4 | Effect of *Ocimum basilicum* on the immunoexpression of caspase-3, Ki67, and GFAP in the MOB of the studied groups. Main olfactory bulb (MOB), chronic unpredictable mild stress (CUMS), fluoxetine (FLU), *O. basilicum (Ocimum basilicum).* * significance with $p < 0.05$.

In the present study, *O. basilicum* reduced caspase-3 positive cells population *via* downregulated caspase-3 gene expression. In consistent with such finding neuroprotective effect of O. *basilicum* with reduced size of cerebral infarct and lipid peroxidation were described in the brain by (55). It reduced the corticosterone level, apoptosis of hippocampal neurons, and increased both newly formed nerve cells and astrocytes numbers in a manner comparable to FLU (10). This effect was attributed to effect of phenolic, flavonoids, and tannin contents of *O. basilicum* essential oils which were previously reported to act as reactive oxygen species scavengers [Garabadu and Singh (14)] recently described that *O. basilicum* attenuated significantly mitochondria-dependent apoptosis induced by ethidium bromide in rat prefrontal cortex.

Among the limitations of this study was the absence of the in-depth analysis of the detailed mechanism of the *O. basilicum* neuroprotective effect which needs further future study.

CONCLUSION

The present paper showed that *O. basilicum* relieved depressive-like behavioral alterations induced in mice following exposure to chronic mild stress. O. *basilicum* also was found to ameliorate stress-induced changes in the main olfactory bulb as evident both biochemically and histopathologically. These effects might be mediated down-regulation of caspase-3 in addition to up-regulation of GFAP and Ki67 gene expression in the MOB.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: https://figshare.com/s/d5a87f5869ea80e2598f.

ETHICS STATEMENT

The animal study was reviewed and approved by Biomedical research ethics committee, Faculty of medicine, Kind Abdulaziz University, Jeddah, Saudi Arabia.

AUTHOR CONTRIBUTIONS

NA and SA designed the study, conducted the analyses and wrote the initial version of this manuscript. MB, IA, HA, and AA collected the samples, data, performed the literature review and interpret the results. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Saudi Arabia under grant No. (D-701-247-1441). The authors, therefore, gratefully acknowledge DSR technical and financial support.

REFERENCES

1. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: Meta-analysis of model reliability. *Neurosci Biobehav Rev* (2019) 99:101–16. doi: 10.1016/j.neubiorev.2018.12.002
2. Schwob JE. Neural regeneration and the peripheral olfactory system. *Anat Rec* (2002) 269(1):33–49. doi: 10.1002/ar.10047
3. Cheng K, Li J, Yang D, Yang Y, Rao C, Zhang S, et al. 2D-gel based proteomics unravels neurogenesis and energetic metabolism dysfunction of the olfactory bulb in CUMS rat model. *Behav Brain Res* (2016) 313:302–9. doi: 10.1016/j.bbr.2016.05.026
4. Meyer A, Glaser A, Brauer AU, Wree A, Strotmann J, Rolfs A, et al. Olfactory Performance as an Indicator for Protective Treatment Effects in an Animal Model of Neurodegeneration. *Front Integr Neurosci* (2018) 12:35. doi: 10.3389/fnint.2018.00035
5. Czeh B, Fuchs E, Wiborg O, Simon M. Animal models of major depression and their clinical implications. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 64:293–310. doi: 10.1016/j.pnpbp.2015.04.004
6. Fu CY, Moyle W, Cooke M. A randomised controlled trial of the use of aromatherapy and hand massage to reduce disruptive behaviour in people with dementia. *BMC Complementary Altern Med* (2013) 13:165. doi: 10.1186/1472-6882-13-165
7. Reis D, Jones T. Aromatherapy: Using Essential Oils as a Supportive Therapy. *Clin J Oncol Nurs* (2017) 21(1):16–9. doi: 10.1188/17.CJON.16-19
8. Dhawan SS, Shukla P, Gupta P. Lal RK. A cold-tolerant evergreen interspecific hybrid of Ocimum kilimandscharicum and Ocimum basilicum: analyzing trichomes and molecular variations. *Protoplasma* (2016) 253(3):845–55. doi: 10.1007/s00709-015-0847-9
9. Sestili P, Ismail T, Calcabrini C, Guescini M, Catanzaro E, Turrini E, et al. The potential effects of Ocimum basilicum on health: a review of pharmacological and toxicological studies. *Expert Opin Drug Metab Toxicol* (2018) 14(7):679–92. doi: 10.1080/17425255.2018.1484450
10. Ali SS, Abd El Wahab MG, Ayuob NN, Suliaman M. The antidepressant-like effect of Ocimum basilicum in an animal model of depression. *Biotechnic Histochem Off Publ Biol Stain Commission* (2017) 92(6):390–401. doi: 10.1080/10520295.2017.1323276
11. Rabbani M, Sajjadi SE, Vaezi A. Evaluation of anxiolytic and sedative effect of essential oil and hydroalcoholic extract of Ocimum basilicum L. and chemical composition of its essential oil. *Res Pharm Sci* (2015) 10(6):535–43.
12. Ayuob NN, El Wahab MGA, Ali SS, Abdel-Tawab HS. Ocimum basilicum improve chronic stress-induced neurodegenerative changes in mice hippocampus. *Metab Brain Dis* (2018) 33(3):795–804. doi: 10.1007/s11011-017-0173-3
13. Singh V, Krishan P, Shri R. Improvement of memory and neurological deficit with Ocimum basilicum L. extract after ischemia reperfusion induced cerebral injury in mice. *Metab Brain Dis* (2018) 33(4):1111–20. doi: 10.1007/s11011-018-0215-5
14. Garabadu D, Singh D. Ocimum basilicum attenuates ethidium bromide-induced cognitive deficits and pre-frontal cortical neuroinflammation, astrogliosis and mitochondrial dysfunction in rats. *Metab Brain Dis* (2020) 35(3):483–95. doi: 10.1007/s11011-020-00536-z
15. Song C, Leonard BE. The olfactory bulbectomised rat as a model of depression. *Neurosci Biobehav Rev* (2005) 29(4-5):627–47. doi: 10.1016/j.neubiorev.2005.03.010
16. Nakagawasai O, Yamada K, Odaira T, Takahashi K, Nemoto W, Sakuma W, et al. Liver hydrolysate improves depressive-like behavior in olfactory bulbectomized mice: Involvement of hippocampal neurogenesis through the

AMPK/BDNF/CREB pathway. *J Pharmacol Sci* (2020) 143(1):52–5. doi: 10.1016/j.bbr.2020.112640
17. Zhou Y, Tao X, Wang Z, Feng L, Wang L, Liu X, et al. Hippocampus Metabolic Disturbance and Autophagy Deficiency in Olfactory Bulbectomized Rats and the Modulatory Effect of Fluoxetine. *Int J Mol Sci* (2019) 20(17):4282. doi: 10.3390/ijms20174282
18. Li M, Fu Q, Li Y, Li S, Xue J, Ma S. 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* (2014) 98:1–10. doi: 10.1016/j.fitote.2014.06.007
19. Ismail M. Central properties and chemical composition of Ocimum basilicum. essential oil. *Pharm Biol* (2006) 44(8):619–26. doi: 10.1080/13880200600897544
20. Chioca LR, Ferro MM, Baretta IP, Oliveira SM, Silva CR, Ferreira J, et al. Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not GABAA/benzodiazepine neurotransmission. *J Ethnopharmacol* (2013) 147(2):412–8. doi: 10.1016/j.jep.2013.03.028
21. Pavesi E, Canteras NS, Carobrez AP. Acquisition of Pavlovian fear conditioning using β-adrenoceptor activation of the dorsal premammillary nucleus as an unconditioned stimulus to mimic live predator-threat exposure. *Neuropsychopharmacology* (2011) 36(5):926–39. doi: 10.1038/npp.2010.231
22. Mineur YS, Belzung C, Crusio WE. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. *Behav Brain Res* (2006) 175(1):43–50. doi: 10.1016/j.bbr.2006.07.029
23. Doron R, Lotan D, Einat N, Yaffe R, Winer A, Marom I, et al. A novel herbal treatment reduces depressive-like behaviors and increases BDNF levels in the brain of stressed mice. *Life Sci* (2014) 94(2):151–7. doi: 10.1016/j.lfs.2013.10.025
24. Ayuob NN, Firgany AEL, El-Mansy AA, Ali S. Can Ocimum basilicum relieve chronic unpredictable mild stress-induced depression in mice? *Exp Mol Pathol* (2017) 103(2):153–61. doi: 10.1016/j.yexmp.2017.08.007
25. Pikor LA, Enfield KS, Cameron H, Lam WL. DNA extraction from paraffin embedded material for genetic and epigenetic analyses. *J Vis Exp* (2011) 26 (49), 2763. doi: 10.3791/2763
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2– ΔΔCT method. *Methods* (2001) 25(4):402–8. doi: 10.1006/meth.2001.1262
27. Suvarna K, Layton C, Bancroft J. *Bancroft"s Theory and Practice of Histological Techniques. 7th ed*. Churchill Livingstone: Elsevier (2013).
28. Makhlouf NA, El-Beshbishy RA, Abousetta A. Ginkgo modulates noise-induced hippocampal damage in male albino rats: a light and electron microscopic study. *Egy J Histol* (2014) 37(1):159–74. doi: 10.1097/01.EHX.0000444078.17248.ab
29. Bahaa N, Raafat M. Effect of intranasally-administered adipose derived stem cells on age-related changes of rat olfactory bulb: A histological and immunohistochemical study. *Egyptian J Histol* (2017) 40(4):459–75. doi: 10.21608/ejh.2017.5687
30. Zhang ZH, Chen C, Wu QY, Zheng R, Chen Y, Liu Q, et al. Selenomethionine Ameliorates Neuropathology in the Olfactory Bulb of a Triple Transgenic Mouse Model of Alzheimer's Disease. *Int J Mol Sci* (2016) 17(10):1595. doi: 10.3390/ijms17101595
31. Zheng R, Zhang ZH, Zhao YX, Chen C, Jia SZ, Cao XC, et al. Transcriptomic Insights into the Response of the Olfactory Bulb to Selenium Treatment in a Mouse Model of Alzheimer's Disease. *Int J Mol Sci* (2019) 20(12):2998. doi: 10.3390/ijms20122998
32. Liu D, Xie K, Yang X, Gu J, Ge L, Wang X, et al. Resveratrol reverses the effects of chronic unpredictable mild stress on behavior, serum corticosterone levels and BDNF expression in rats. *Behav Brain Res* (2014) 264:9–16. doi: 10.1016/j.bbr.2014.01.039
33. Abd El Wahab MG, Ali SS, Ayuob NN. The Role of Musk in Relieving the Neurodegenerative Changes Induced After Exposure to Chronic Stress. *Am J Alzheimers Dis Other Demen* (2018) 33(4):221–31. doi: 10.1177/1533317518755993
34. Filho CB, Jesse CR, Donato F, Giacomeli R, Del Fabbro L, da Silva Antunes M, et al. Chronic unpredictable mild stress decreases BDNF and NGF levels and Na (+), K (+)-ATPase activity in the hippocampus and prefrontal cortex of mice: antidepressant effect of chrysin. *Neuroscience* (2015) 289:367–80. doi: 10.1016/j.neuroscience.2014.12.048
35. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry* (2011) 35(3):676–92. doi: 10.1016/j.pnpbp.2010.05.004
36. Freitas AE, Bettio LE, Neis VB, Santos DB, Ribeiro CM, Rosa PB, et al. Agmatine abolishes restraint stress-induced depressive-like behavior and hippocampal antioxidant imbalance in mice. *Prog Neuropsychopharmacol Biol Psychiatry* (2014) 50:143–50. doi: 10.1016/j.pnpbp.2013.12.012
37. Zahra K, Khan MA, Iqbal F. Oral supplementation of Ocimum basilicum has the potential to improves the locomotory, exploratory, anxiolytic behavior and learning in adult male albino mice. *Neurol Sci* (2015) 36(1):73–8. doi: 10.1007/s10072-014-1913-3
38. Marsden WN. Synaptic plasticity in depression: molecular, cellular and functional correlates. *Prog Neuropsychopharmacol Biol Psychiatry* (2013) 43:168–84. doi: 10.1016/j.pnpbp.2012.12.012
39. Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav Immun* (2011) 25(2):181–213. doi: 10.1016/j.bbi.2010.10.015
40. Menard C, Pfau ML, Hodes GE, Russo SJ. Immune and Neuroendocrine Mechanisms of Stress Vulnerability and Resilience. *Neuropsychopharmacology* (2017) 42(1):62–80. doi: 10.1038/npp.2016.90
41. Webster M, Knable M, Johnston-Wilson N, Nagata K, Inagaki M, Yolken RH. 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* (2001) 15(4):388–400. doi: 10.1006/brbi.2001.0646
42. Fares J, Bou Diab Z, Nabha S, Fares Y. Neurogenesis in the adult hippocampus: history, regulation, and prospective roles. *Int J Neurosci* (2019) 129(6):598–611. doi: 10.1080/00207454.2018.1545771
43. Lledo PM, Valley M. Adult Olfactory Bulb Neurogenesis. *Cold Spring Harb Perspect Biol* (2016) 8(8):a018945. doi: 10.1101/cshperspect.a018945
44. Zhu Y, Qiu Y, Chen M, Zhang Y, Cao L, Su Z, et al. Hemopexin is required for adult neurogenesis in the subventricular zone/olfactory bulb pathway. *Cell Death Dis* (2018) 9(3):268. doi: 10.1038/s41419-018-0328-0
45. Olude MA, Ogunbunmi TK, Olopade JO, Ihunwo AO. The olfactory bulb structure of African giant rat (Cricetomys gambianus, Waterhouse 1840) I: cytoarchitecture. *Anat Sci Int* (2014) 89(4):224–31. doi: 10.1007/s12565-014-0227-0
46. Yang D, Li Q, Fang L, Cheng K, Zhang R, Zheng P, et al. Reduced neurogenesis and pre-synaptic dysfunction in the olfactory bulb of a rat model of depression. *Neuroscience* (2011) 192:609–18. doi: 10.1016/j.neuroscience.2011.06.043
47. Siopi E, Denizet M, Gabellec M-M, De Chaumont F, Olivo-Marin J-C, Guilloux J-P, et al. Anxiety-and depression-like states lead to pronounced olfactory deficits and impaired adult neurogenesis in mice. *J Neurosci* (2016) 36(2):518–31. doi: 10.1523/JNEUROSCI.2817-15.2016
48. Qi X, Xu H, Wang L, Zhang Z. Comparison of Therapeutic Effects of TREK1 Blockers and Fluoxetine on Chronic Unpredicted Mild Stress Sensitive Rats. *ACS Chem Neurosci* (2018) 9(11):2824–31. doi: 10.1021/acschemneuro.8b00225
49. Willner P. The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiol Stress* (2017) 6:78–93. doi: 10.1016/j.ynstr.2016.08.002
50. Tong J, Zhou Z, Qi W, Jiang S, Yang B, Zhong Z, et al. Antidepressant effect of helicid in chronic unpredictable mild stress model in rats. *Int Immunopharmacol* (2019) 67:13–21. doi: 10.1016/j.intimp.2018.11.052
51. Guilloux JP, Mendez-David I, Pehrson A, Guiard BP, Reperant C, Orvoen S, et al. Antidepressant and anxiolytic potential of the multimodal antidepressant vortioxetine (Lu AA21004) assessed by behavioural and neurogenesis outcomes in mice. *Neuropharmacology* (2013) 73:147–59. doi: 10.1016/j.neuropharm.2013.05.014
52. Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, et al. Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology* (2009) 34(11):2376–89. doi: 10.1038/npp.2009.75

53. Willner P, Scheel-Kruger J, Belzung C. Resistance to antidepressant drugs: the case for a more predisposition-based and less hippocampocentric research paradigm. *Behav Pharmacol* (2014) 25(5-6):352–71. doi: 10.1097/FBP.0000000000000066
54. Yang D, Liu X, Zhang R, Cheng K, Mu J, Fang L, et al. Increased apoptosis and different regulation of pro-apoptosis protein bax and anti-apoptosis protein bcl-2 in the olfactory bulb of a rat model of depression. *Neurosci Lett* (2011) 504(1):18–22. doi: 10.1016/j.neulet.2011.08.046
55. Bora KS, Arora S, Shri R. Role of Ocimum basilicum L. in prevention of ischemia and reperfusion-induced cerebral damage, and motor dysfunctions in mice brain. *J Ethnopharmacol* (2011) 137(3):1360–5. doi: 10.1016/j.jep.2011.07.066

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 06 October 2020
doi: 10.3389/fphar.2020.579052

Neuroprotective Potency of Saffron Against Neuropsychiatric Diseases, Neurodegenerative Diseases, and Other Brain Disorders: From Bench to Bedside

Yaqi Bian, Chen Zhao and Simon Ming-Yuen Lee *

State Key Laboratory of Quality Research in Chinese Medicine and Institute of Chinese Medical Sciences, University of Macau, Macau, China

OPEN ACCESS

Edited by:
Elizabeth Anne Olson, Southern Utah University, United States

Reviewed by:
Andy Wai Kan Yeung, The University of Hong Kong, Hong Kong
Wenda Xue, Nanjing University of Chinese Medicine, China

***Correspondence:**
Simon Ming-Yuen Lee
simonlee@umac.mo

Specialty section:
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

Received: *01 July 2020*
Accepted: *15 September 2020*
Published: *06 October 2020*

Citation:
Bian Y, Zhao C and Lee SM-Y (2020) Neuroprotective Potency of Saffron Against Neuropsychiatric Diseases, Neurodegenerative Diseases, and Other Brain Disorders: From Bench to Bedside. Front. Pharmacol. 11:579052. doi: 10.3389/fphar.2020.579052

The increasing morbidity rates of brain disorders and conditions such as anxiety, depression, Alzheimer's disease, and Parkinson's disease have become a severe problem in recent years. Although researchers have spent considerable time studying these diseases and reported many positive outcomes, there still are limited drugs available for their treatment. As a common traditional Chinese medicine (TCM), saffron was employed to treat depression and some other inflammatory diseases in ancient China due to its antioxidant, anti-inflammatory, and antidepressant properties. In modern times, saffron and its constituents have been utilized, alone and in TCM formulas, to treat neuropsychiatric and neurodegenerative diseases. In this review, we mainly focus on recent clinical and preclinical trials of brain disorders in which saffron was applied, and summarize the neuroprotective properties of saffron and its constituents from chemical, pharmacokinetic, and pharmacological perspectives. We discuss the properties of saffron and its constituents, as well as their applications for treating brain disorders; we hope that this review will serve as a comprehensive reference for studies aimed at developing therapeutic drugs based on saffron.

Keywords: saffron, saffron constituents, anti-depressant effect, anxiolytic effect, neuroprotective effects, traditional Chinese medicine

INTRODUCTION

Brain disorders, i.e. neuropsychiatric and neurodegenerative diseases, have emerged as a majorproblem in recent years. Anxiety and depression are neuropsychiatric disorders that mainly result from intense interpersonal relationships, certain medications, and major stressful life events (divorce or death of a loved one, etc.). Patients suffering from mental disorders usually have symptoms like a decrease or increase in appetite, hypersomnia or insomnia, psychomotor agitation or retardation, and chronic fatigue (Breen et al., 2011). Genetic factors also contribute to the development of depression and anxiety. Chromosome 3p25-26 has been found in more than 800 families with recurrent depression (Pitsikas, 2015). In addition, one of the more common

comorbidities is that of anxiety and depression (Bui and Fava, 2017). Accumulating evidence suggest that the underlying pathogenesis of anxiety and depression involve numerous common mechanisms such as control of hormones secretion, functional disturbance of GABAergic system (γ-aminobutyric acid, GABA) (Kalueff and Nutt, 2007) and dysfunction of glutamate-related nervous system (Howells and Russell, 2008; Jia et al., 2020). Furthermore, several signaling pathways involved in the regulations of oxidative stress, neuroinflammation, neurotransmitter dysfunction, and neurotrophic factors (e.g. brain derived neurotrophic factor, BDNF) also contribute to the pathogenesis of anxiety and depression (Kalueff et al., 2006; Ehsanifar et al., 2019). Therefore, treatments targeting these common mechanisms may achieve a more effective therapeutic effect. However, symptoms of depression and anxiety patients sometimes are not exactly the same. For instances, the patients with a diagnosis of major depression are more likely to show a depressed or a sad mood while the patients with a diagnosis of major anxiety mainly display an anxious or a panic mood (Clark et al., 1994). In these cases, the selection of suitable therapeutic strategy becomes more challenging and difficult (Gallagher-Michaels, 2013).

Neurodegenerative diseases are the most prevalent senile diseases in aging populations (especially those aged over 70 years), and include Parkinson's disease (PD) and Alzheimer's disease (AD). Cognitive decline, slow and involuntary movements, progressive dementia, and changes of personality are the common symptoms of these two diseases; however, the psychological disorder associated with PD and AD should not be overlooked. Anxiety and depression are secondary changes seen not only in neurodegenerative diseases, but also in other brain disorders. This indicates that the overlap among brain disorders is complex. Since AD and PD are multifactorial disorders without effective cures, nearly all of the drugs on the market aim mainly to alleviate the symptoms (Finley and Gao, 2017). Natural products contain multiple chemical constituents, which are more effective than single chemicals in addressing the pathogenesis of multifactorial disorders through their effects on multiple targets. This explains why drugs developed from natural products with preventive activities against brain disorders are particularly desirable. For example, sodium oligomannate (GV-971®) is a marine algae-derived oral oligosaccharide conditionally approved in China for the treatment of mild-to-moderate AD (to improve cognitive function) in November 2019. Unlike most previous anti-PD and anti-AD drugs on the markets, by acting directly on specific target in neuronal cells, GV-971 constitutes a novel agent that therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit AD progression (Wang et al., 2019).

Saffron—the dry red stigma of *Crocus sativus L*—is one of the most expensive herbs on the market today. The flower of *Crocus sativus L* has been widely used as a natural additive in cooking to enhance flavor, color, and aroma. The origin of *Crocus sativus* can be traced back to the Late Bronze Age in Crete; since then, saffron has been cultivated all over the world, but especially in Mediterranean Europe, India, and south-western Asia. Cultivation of saffron requires fertile clay soil and direct sunlight under natural environmental conditions, or greenhouse conditions (which can improve yield) (Galigani and Garbati, 1999; Gresta et al., 2008; Cavusoglu et al., 2009). Saffron is referred to as "red gold," due to the high market price attributable to hand-harvesting and low production volumes. According to an analysis of food ingredient fraud based on 677 references, saffron is one of the most commonly adulterated products (Moore et al., 2012). Thus, quality control of saffron is important for authentication. For this purpose, various chromatographic and spectrometric methods, such as UV, HPLC, GC, NIR combined with MS, and PTR-TOF-MS have been established and optimized to analyze the components of saffron (Tarantilis et al., 1995; Masi et al., 2016; Grinan-Ferre et al., 2018).

Several active ingredients are present in saffron, including carotenoids (crocin, crocetin), monoterpene aldehydes (picrocrocin, safranal), monoterpenoids (crocusatines), isophorones, and flavonoids (Rameshrad et al., 2018). The contents of these active compounds varies from region to region. Saffron has been used in traditional medicine for its hypolipidemic, anti-cancer, antioxidant, anti-inflammatory, and antidepressant properties (Rios et al., 1996). As saffron has pharmacological effects on nervous system, it has also been tested in clinical trials of depression, anxiety, AD, and other brain disorders (Moshiri et al., 2015; Hosseini et al., 2018; Samarghandian and Farkhondeh, 2020). In this review, we summarized preclinical and clinical studies of the use of saffron and its constituents for treating neuropsychiatric diseases, neurodegenerative diseases, and other brain disorders (**Figure 1**).

CONSTITUENTS OF SAFFRON

Saffron is composed of water, nitrogenous matter, sugars, soluble extract, volatile oil, and fibers, in varying amounts. Among all the components, soluble extract accounts for the highest proportion (41–44%), followed by water (14–16%), sugar (12–15%), and nitrogenous matter (11–13%) (Christodoulou et al., 2015). Saffron contains two vitamins essential to the human body: riboflavin (vitamin B2) and thiamine (vitamin B1). The riboflavin content of saffron ranges from 56 to 138 μg/g, which is the highest amount among all foods (Bhat and Broker, 1953). Apart from these two essential vitamins, small quantities of β-carotene, essential fatty acids, linoleic and linolenic are also found in saffron. Sterols including campesterol, stigmasterol, and β-sitosterol have been identified, as well as oleanolic, ursolic, palmitoleic, palmitic, and oleic acids. Most of the volatile compounds are terpenes, terpene alcohols, and their esters. Non-volatile compounds include picrocrocin safranal, crocetin, crocins, and flavonoids (quercetin and kaempferol), among which safranal is the major component (Pitsikas, 2015). About 150 volatile and non-volatile compounds, and nearly 50 constituents, have been identified in saffron (Boskabady and Farkhondeh, 2016). Particularly, the water-soluble carotenoid,

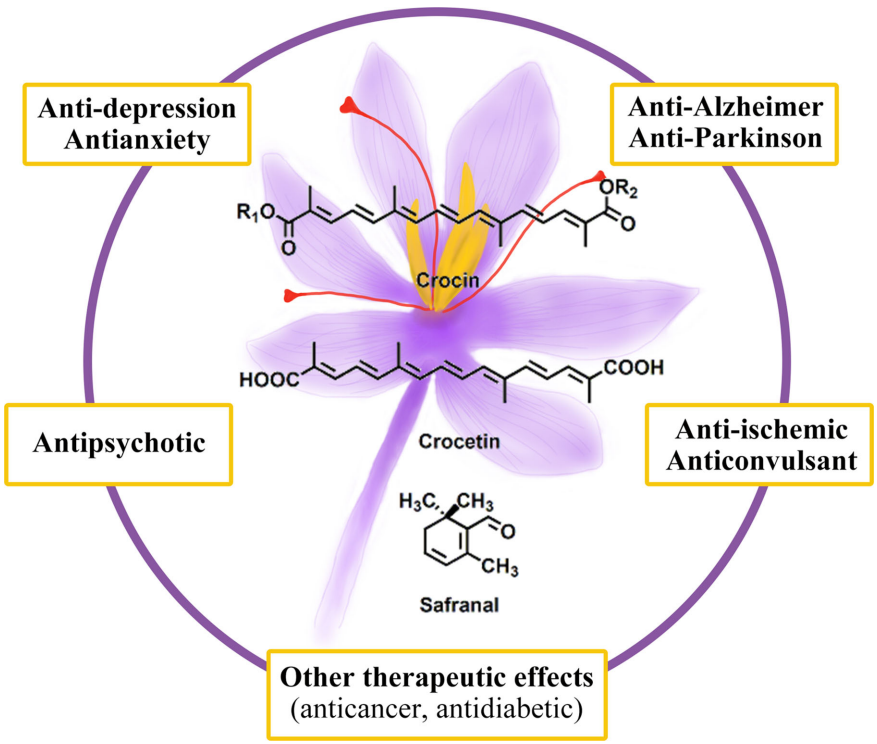

FIGURE 1 | The therapeutic effects of saffron.

crocin, determines saffron's color. Picrocrocin, the glycoside of safranal, is responsible for its bitterness, while safranal provides the characteristic aroma of saffron. Saffron contains four main bioactive compounds: crocin, crocetin, picrocrocin, and safranal. These four compounds contribute to saffron's high value and versatility in food and pharmaceuticals. We describe the chemical constituents, neuropharmacological activities, and safety profile of saffron in the following sections (**Figure 2**).

Crocin

Crocin (8,8'-diapo-8,8'-carotenedioicdioic acid with different glycosides), with a molecular weight of 976.96, is a hydrophilic carotenoid responsible for saffron's red color. A variety of crocin analogues can be produced *via* the substitution of different glycosyl esters, such as glucose, gentiobiose, and triglucose, into the R1 and/or R2 positions of the side chain (**Figure 2**). As the most abundant crocins in saffron, crocin 1 (or α-crocin) is formed by disaccharide gentiobiose and the dicarboxylic acid crocetin (Samarghandian and Borji, 2014). Qualitative and quantitative analysis of different glycosyl moieties and cis-/trans-isomeric forms of crocins can be aid in the authentication, quality control, standardization, and process traceability of saffron products (Rocchi et al., 2018).

Picrocrocin

Picrocrocin ($C_{16}H_{26}O_7$), a crystalline terpene-glucoside of saffron with a molecular weight of 330.37, is the de-glycosylated precursor of saffron's aromatic components and contributes to its bitter taste (Lage and Cantrell, 2009). Picrocrocin releases hydroxy-safranal (aglycone4–hydroxy–2, 6, 6–trimethyl–1–cyclohexene–1–carboxaldehyde) through the action of β-glucosidase, by dehydration *via* heating and enzymatic reactions occurring in storage. Under natural conditions, safranal is yielded by dehydration during drying (**Figure 3**) (Samarghandian and Borji, 2014).

Safranal

As the main component of essential volatile oil, safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) is responsible for the characteristic aroma of saffron. During the dehydration that occurs in post-harvest processing, picrocrocin can produce safranal after being de-glycosylated; thus, the concentration of safranal in saffron is determined by storage time and conditions. As an essential volatile oil, a high safranal content can only maintained for one year after harvesting (Maggi et al., 2010).

PHARMACOKINETICS AND SAFETY EVALUATION

Crocin and crocetin exhibit very different pharmacokinetic profiles. Crocin can be hydrolyzed to crocetin before (in the gastrointestinal lumen) or during (in the intestinal mucosa) intestinal absorption (Asai et al., 2005). Although crocetin acts

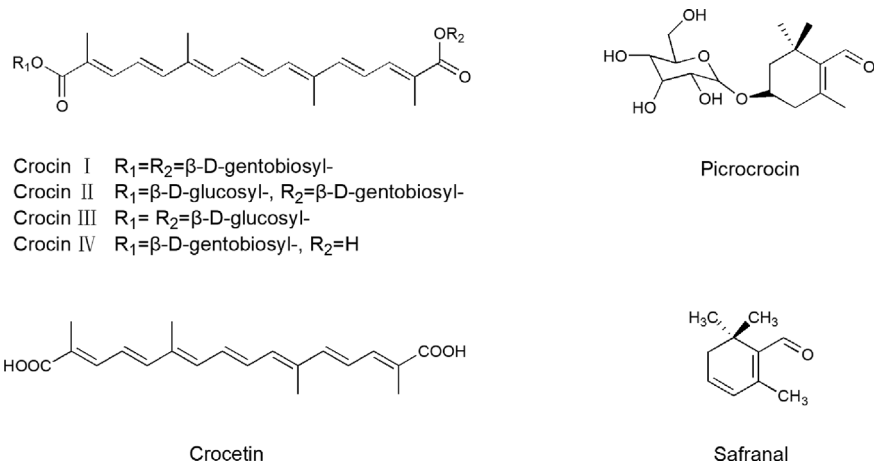

FIGURE 2 | The structural formula of saffron.

as the bioactive compound in rat plasma, oral administration of crocin is preferable to that of crocetin, due to the poor dissolution of the latter substance in intestinal fluid (Zhang et al., 2017). After hydrolysis, crocetin is partly metabolized into mono- and diglucuronide conjugates in the intestinal mucosa (during absorption), liver (after absorption), or both (Asai et al., 2005; Zhang et al., 2017). A clinical pharmacokinetic trial of healthy adult human volunteers showed that, after a single oral administration, crocetin reached a maximum concentration 4 to 4.8 h after administration, and was eliminated with a corresponding half-life of 6.1 to 7.5 h. The results also showed that crocetin exhibited no serious adverse reactions, even up to 22.5 mg. Due to its small molecules and hydrophilic nature, crocetin shows more rapid absorption in the portal vein than in the lymphatics when transported into the bloodstream (Umigai et al., 2011).

After intravenous injection, crocin is converted into crocetin in the gastrointestinal tract. Crocetin has a widespread distribution in tissue and low plasma concentration because of the weak crocetin-albumin interaction. Also, crocetin has therapeutic effects on neurodegenerative diseases due to its ability to penetrate blood-brain barrier (BBB) (Hosseini et al., 2017). To investigate the underlying permeation mechanisms, Lautenschlager et al. established models based on Caco-2 monolayer cells, porcine brain capillary endothelial cells (BCEC), and blood cerebrospinal fluid barrier (BCSFB). Crocin-1 could not penetrate Caco-2 monolayers even at a high concentration of 1,000 μM, which indicates its poor penetration of the intestinal barrier. In contrast, trans-crocetin not only penetrates the intestinal barrier in a dose-dependent manner, but can also gradually permeate the BBB. Instead of the paracellular rout, trans-crocetin is mostly absorbed *via* passive transcellular diffusion (Lautenschlager et al., 2015).

In both experimental and clinical investigations, saffron shows no significant toxicity in therapeutic doses. In acute, sub-acute, sub-chronic, and chronic toxicity tests, no marked changes have been reported in biochemical parameters, hematological parameters, or body organs, among other factors. However, as the key determinant of acute toxicity, the LD_{50} of safranal is lower than that of saffron and crocin, which indicates greater toxicity (Bostan et al., 2017). In a sub-acute toxicity study, although safranal reduced the levels of total cholesterol, triglyceride, and alkaline phosphatase (ALP), it also increased those of lactic acid dehydrogenase (LDH) and serum urea nitrogen (BUN). Moreover, histological results indicate safranal exhibits toxicity in kidney and lung (Hosseinzadeh et al., 2013). In a short-term, double-blinded placebo-controlled clinical trial, saffron tablets were given to patients orally at a dose of 200 or 400 mg for 7 days, and showed an excellent safety (Modaghegh et al., 2008). Crocin tablets were also relatively safe in healthy volunteers at a dose of 20 mg dose for 1 month (Mohamadpour et al., 2013). In studies comparing the efficacy of saffron and placebo in patients with neuropsychiatric diseases, no serious side effects were observed (Mousavi et al., 2015; Mazidi et al., 2016; Lopresti and Drummond, 2017). As the bioactive compounds of saffron can interact with CYP enzymes, drugs with the same function will likely increase the risk of low pharmacological efficacy when co-administrated with saffron (Dovrtělová et al., 2015).

PHARMACOLOGICAL ACTIONS AND POTENTIAL THERAPEUTIC USES OF SAFFRON

Pharmacological Effects of Saffron on the Central Nervous System (CNS) and Psychological Disorders

Depression and Anxiety

Depression

Depression is the most prevalent psychiatric disease worldwide, and is associated with high economic costs and a large social burden. Depression is projected to affect up to 21% of the world's

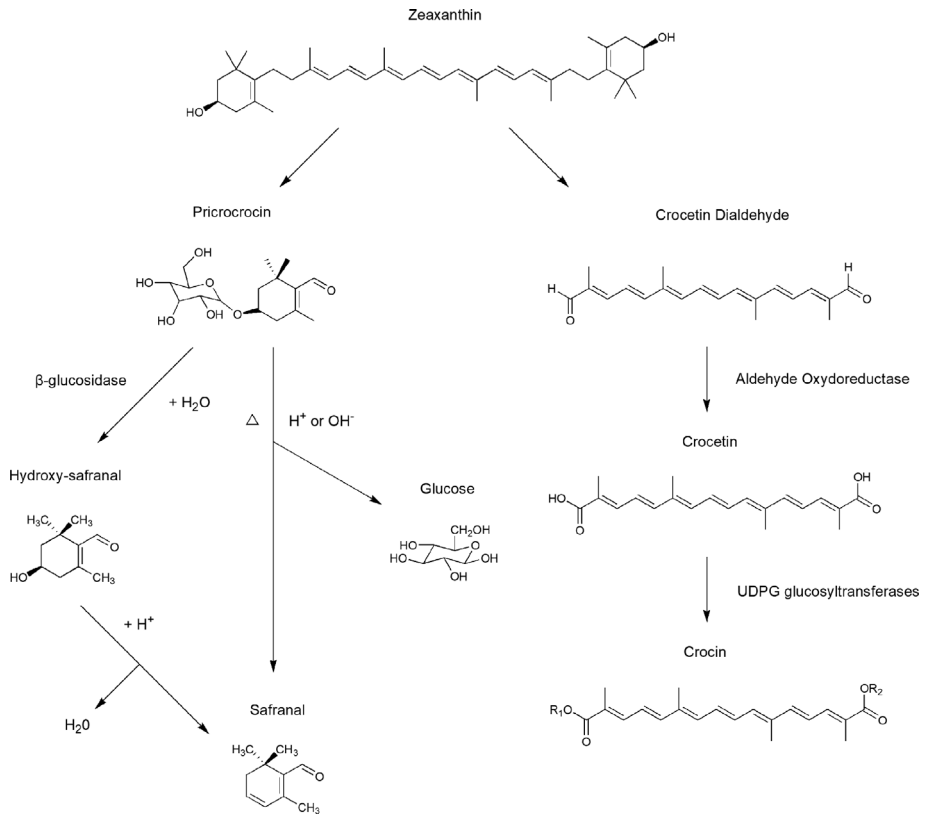

FIGURE 3 | Biosynthesis pathways of crocin, crocetin, safranal, and other important compounds in saffron stigmas.

population by the end of 2020 (Murray and Lopez, 1997). Tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and selective serotonin noradrenaline reuptake inhibitors (SSNRIs) are the most commonly used antidepressants. Antidepressants mainly work by increasing the availability of serotonin and certain other neurotransmitters, thereby reducing depressive symptoms (Nelson et al., 2008). Unfortunately, given the lack of precision in targeting symptoms, it is not surprising that the outcomes of nearly all drugs on the market are less than satisfactory. Hence, combination treatments (using serotonergic, noradrenergic, and serotonergic and noradrenergic [and dopaminergic] drugs) involving drugs with two or more mechanisms of action are used to obtain a synergistic effect, or to improve tolerability. Psychotherapy and electroconvulsive therapy are also utilized as adjuvant therapeutic measures to improve the efficacy of drug treatment (Moret, 2005). Typical side effects include insomnia, somnolence, dry mouth, constipation, and tachycardia. Low rates of full remission, prolonged delays in symptom resolution, substantial residual symptoms after treatment, and high relapse rates are the major problems associated with currently available antidepressants (Si and Yu, 2016). Promisingly, some natural products have antidepressant effects, such as saffron, resveratrol, green tea catechins, cocoa, omega-2, anthocyanins, and B vitamins. Therefore, new drugs developed from extracts of natural products, especially those that have been shown to have low side effects in the treatment of depression, are becoming increasingly desirable (Siddiqui et al., 2018).

Preclinical Studies. Extracts of saffron (aqueous and ethanolic) were demonstrated to have antidepressant effects in a rodent depression model. Hosseinzadeh et al. confirmed the antidepressant effect of saffron in a forced swimming test completed by mice. The results showed that safranal (0.15–0.5 mg/kg), crocin (50–600 mg/kg), and the extracts of saffron stigma (0.2–0.8 g/kg) reduced the immobility time compared to the saline group. Swimming time was increased by both the extracts and safranal, in a manner comparable to fluoxetine. This indicated that the underlying mechanism may involve the activation of dopaminergic, noradrenergic, and serotonergic systems (Hosseinzadeh et al., 2004). In a similar study, also conducted by Hosseinzadeh, another constituent of saffron, kaempferol, had positive effects in both mice and rats depression models. In another preclinical study, Wang et al. confirmed therapeutic effects of saffron on depression. In this study, the aqueous ethanol extract of saffron was fractionated, based on the polarity at which the petroleum ether fraction and dichloromethane fraction showed dose-dependent antidepressant effects in a behavioral model of depression (Wang et al., 2010). Amin et al. discovered that crocetin has a stronger antidepressant effect than crocin, since a

higher dose of the latter was needed in acute and sub-acute administration regimens (Amin et al., 2015). Several other studies found that aqueous extracts of saffron showed antidepressant effects in various experimental depression models that involved modulation of the BDNF, CREB, and VGF pathways (Dorri et al., 2015; Ghasemi et al., 2015; Razavi et al., 2017; Asrari et al., 2018). Moreover, crocin has exhibited anti-inflammatory effects by suppressing the expression of NF-kB and NLRP3 signaling pathway activity in an LPS-induced mouse model; neuroinflammation has been suggested to be a potential mechanism (Zhang et al., 2018).

Clinical Studies. Due to the efficacy of saffron in the treatment of depression demonstrated in many preclinical studies, several clinical trials on saffron have been performed over the last few decades. Moshiri et al. and Akhondzadeh et al. obtained the same result, i.e., that that patients who received saffron 30 mg/day (*b.i.d.*) for 6 weeks showed better outcomes according to the Hamilton Depression Rating Scale than patients who received placebo (*b.i.d.*). Both studies included 40 outpatients who met the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV) criteria for depression and received either saffron or placebo treatment (Akhondzadeh et al., 2005; Moshiri et al., 2006). Another clinical trial showed that saffron can reduce depression severity. That 4-week study involved 40 patients suffering from major depression according to the DSM-IV criteria; the patients were randomly assigned to a fluoxetine and saffron group or fluoxetine and placebo group. Even though depression severity was reduced in both groups, at the end of the study there were no significant group differences, indicating no additional benefit of saffron taken in conjunction with fluoxetine. Further investigation of longer-term treatment outcomes is merited (Sahraian et al., 2016). In another randomized double-blind study, Lopresti and colleagues observed 123 patients for 12 weeks. They concluded that various doses of curcumin and combined curcumin/saffron treatment can reduce depressive and anxiolytic symptoms in patients with major depressive disorder. Similarly, Mazidi et al. found that, compared with the placebo group, patients taking 50 mg saffron (*b.i.d.*) for 12 weeks showed an improvement in anxiolytic and depressant symptoms (Mazidi et al., 2016; Lopresti and Drummond, 2017). Postpartum depression is a subtype of depression that can affect new mothers after childbirth. A randomized, double-blind placebo-controlled trial was conducted on 60 women suffering from postpartum depression. At the final assessment, 96% patients in the saffron group were in remission compared to 43% in placebo group. The complete response rate of the saffron group reached 60%, which was higher than that of the placebo group (Tabeshpour et al., 2017). In addition, several clinical studies have been carried out to evaluate the antidepressant effects of the active ingredient in saffron, crocin. Talaei et al. reported that crocin combined with one SSRI (fluoxetine, sertraline or citalopram) had a greater antidepressant effect than SSRI combined with placebo (Talaei et al., 2015).

As well as clinical studies comparing the antidepressant effects of saffron and placebo, several studies compared saffron with clinical antidepressants. Shahmansouri et al. and Noorbala et al. both compared saffron and fluoxetine, in terms of therapeutic effects on depression, in a 6-week study. In both studies, saffron had a comparable therapeutic effect to that of fluoxetine on mild-to-moderate depression. In a pilot double-blind randomized trial, Akhondzadeh et al. reported a similar result to the two studies mentioned above, with no side effects (Noorbala et al., 2005; Akhondzadeh Basti et al., 2007; Shahmansouri et al., 2014). A double-blind, randomized clinical trial found that, after receiving saffron (15 mg capsule, *b.i.d.*) or fluoxetine (20 mg capsule, *b.i.d.*), nearly 50% of the patients in both groups experienced an improvement of depressant symptoms and a reduction in depression scores. There were no significant differences between the groups (Kashani et al., 2017). Two other studies compared the antidepressant effects of saffron with either imipramine or citalopram. Despite the similar results to those reported above, saffron exerted fewer adverse effects compared with imipramine (Akhondzadeh et al., 2004; Ghajar et al., 2017). Thus, substantial clinical evidence indicates that saffron is an effective alternative solution to antidepressant drugs for the management of depression.

Anxiety

Anxiety is a serious psychiatric condition that can manifest as panic disorder, phobias such as agoraphobia or claustrophobia, social anxiety disorder etc.; anxiety affects more than 6% of the world's population. SSRIs, serotonin, and noradrenaline reuptake inhibitors (SNRIs), and pregabalin are still the first-line drugs recommended by international guidelines. However, side effects, delayed action, and worsening of anxious symptoms at the beginning of treatment make it hard to achieve an ideal therapeutic outcome and tend to preclude continuous treatment. Meanwhile, natural products such as bacopa monniera, centella asiatica, galphimia glauca and matricaria recutita etc. have demonstrated anti-anxiety effects (Sarris, 2018). New drugs synthesized from natural products may have a bright future owning to fewer side effects and a shorter onset time (Maron and Nutt, 2017).

Preclinical Studies. Hosseinzadeh et al. compared the anxiolytic and hypnotic effects of saffron extract, crocin, and safranal using an elevated plus maze test in a mouse model of anxiety. The results showed that saffron aqueous extract and safranal, but not crocins, had anxiolytic and hypnotic effects (Hosseinzadeh and Noraei, 2009). Another study obtained similar results in an animal model of anxiety using a light/dark test. However, there were differences from the experiments conducted by Hosseinzadeh et al., in that both crocin and diazepam could increase the "darkness entering latency of rats" in a light/dark test, suggesting that crocin had anxiolytic-like effects (Pitsikas et al., 2008). Ghalandari-Shamami et al. exposed rats to stress during adolescence to evaluate the anxiolytic effects of crocin and physical activity (voluntary wheel running exercise). Crocin, physical activity, and the combined intervention all alleviated the behavioral and morphological deficits induced by adolescent stress (Ghalandari-Shamami et al., 2019). Other studies investigated the therapeutic effects of crocin on obsessive-compulsive disorder and stress-induced anorexia. The results showed that both crocin and aqueous extracts of saffron had the ability to attenuate symptoms, albeit to differing extents (Halataei et al., 2011; Georgiadou et al., 2012).

Clinical Studies. Several randomized double-blind clinical trials have been performed to evaluate the efficacy of saffron or saffron extracts on anxiety. Another 6-week study, involving 66 patients who suffered from major depression accompanied with anxiety, compared the anxiolytic effects of saffron (30 mg/day) and citalopram (40 mg/day). Obvious improvement of anxiety symptoms was observed and no severe side effects were seen in either group (Ghajar et al., 2017). Two clinical trials were designed to investigate the anxiolytic effect of affron® (a novel saffron extract) on both adults and youths. In the first study, Kell et al. found that affron® (28 mg/day for 4 weeks) notably improved anxiety-like symptoms in healthy adults (Kell et al., 2017). The second trial was focused on youths (aged 12–16 years) with mild-to-moderate anxiety or depression symptoms. The results showed that administration of affron® (14 mg, *b.i.d.*) for 8 weeks improved anxiety and depressive symptoms in the youths (Lopresti et al., 2018). Furthermore, Milajerdi et al. investigated whether saffron has a therapeutic effect on mild-to-moderate depression-anxiety in type 2 diabetes mellitus (DM) patients. Anxiety and sleep disturbance in the DM patients were relieved after 8 weeks of treatment with saffron (Milajerdi et al., 2018). However, a 12-week, double-blind randomized placebo-controlled clinical trial obtained different results. Men and women with on-pump coronary artery bypass grafting (CABG) were included in the study and received either saffron capsules (15 mg/twice daily) or placebo. The results did not support the hypothesis of a therapeutic effect of saffron in post-CABG patients with symptoms of depression and anxiety. The limitations of the study included a small sample size, short study duration, and non-comprehensive study design (Moazen-Zadeh et al., 2018).

Alzheimer's Disease and Parkinson's Disease

Alzheimer's Disease

AD is a slowly progressing neurodegenerative disease associated with progressive loss of learning and memory function. Pathological changes, such as the formation of neurofibrillary tangles (NFT) and amyloid plaques, can cause a range of biological dysfunctions in the brain, ultimately leading to memory and learning ability loss. AD is one of the most common causes of dementia, especially in elderly populations. There will be approximately 50 million people with dementia associated with AD by 2040 (Finley and Gao, 2017). Thus, new drugs are urgently needed for the treatment of AD. Natural products are currently the "hot topic" in neurodegenerative diseases. Regarding its therapeutic effects on brain disorders, saffron has been proven to alleviate the symptoms of AD.

Depression and anxiety are two frequent and challenging comorbidities of AD. When accompanied by personality changes, depression and anxiety are often neglected and negatively impact quality of life. Studies have shown that patients with severe AD were more likely to have depression. Moreover, depression and anxiety could accelerate the progression, and increase the mortality, of AD patients. Patients are likely to benefit from saffron because of its antidepressant and anti-anxious effects (Van der Mussele et al., 2013; Chi et al., 2015; Gracia-Garcia et al., 2015).

Preclinical Studies. Saffron and its constituents have neuroprotective effects against chemically induced cognitive impairment in experimental animal models (Dashti et al., 2012; Naghibi et al., 2012; Naghizadeh et al., 2013; Asadi et al., 2015; Ghaffari et al., 2015). Moreover, amyloid-β (Aβ) peptide, phosphorylated tau proteins, and their associated signaling pathways are potentially crucial therapeutic targets for AD intervention. The neuroprotective effects of crocin and crocetin were demonstrated by several *in vitro* studies. The results showed that both crocin and crocetin could provide neuroprotection by reducing Aβ aggregation, phosphorylated tau formation, and synaptic loss. AD is an intractable neurodegenerative disease and complex mechanisms regulate its progress. Several studies have proved that saffron and its constituents, especially crocin and crocetin, achieve a neuroprotective effect *via* attenuating oxidant stress, endoplasmic reticulum stress, neuroinflammation, damage of the BBB, and neuronal cell apoptosis (Papandreou et al., 2006; Ahn et al., 2011; Deslauriers et al., 2011; Ghahghaei et al., 2012; Ghahghaei et al., 2013; Kong et al., 2014; Karakani et al., 2015; Rashedinia et al., 2015). A recent study showed that the neuroprotective effects of saffron may involve the MAPK and PI3K pathways (Rafieipour et al., 2019).

Clinical Studies. Since there are no effective drugs for AD, natural products are being emphasized in the development of therapeutics. Few clinical studies have compared the effects of saffron with either first-line drugs or placebo. Tsolaki et al. reported that saffron could be a good choice for management of mild cognitive impairment, where it improved Mini-Mental State Examination scores in patients (Tsolaki et al., 2016). To compare the effects of saffron with the first-line drugs used by AD patients, Akhondzadeh et al. conducted a clinical trial. The results showed that saffron (30 mg/day) was as effective as donepezil (10 mg/day) for mild-to-moderate AD patients. Another double-blind, randomized study compared saffron with memantine in terms of their ability to alleviate cognitive impartment. In that study, saffron was comparable to memantine in terms of reducing cognitive decline in AD patients. It has also been reported that saffron exerts synergistic effects with other nutraceuticals (Bacopa monnieri, l-theanine, copper, folate, and vitamins of B) to influence cognitive function (Akhondzadeh et al., 2010a; Akhondzadeh et al., 2010b; Farokhnia et al., 2014; Cicero et al., 2017).

Parkinson's Disease

PD is a common neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta. The symptoms of PD include tremor, bradykinesia, rigid muscles, impaired balance, and loss of automatic movements. Preclinical results indicate that saffron may be a promising target for curative drugs for PD (Pan et al., 2016).

PD in the early stages may manifest as non-motor symptoms such as sleep disorder, depression, and anxiety. In some cases, non-motor symptoms may even be the first symptoms. Studies have shown that the morbidity of depression in patients suffering from PD ranges from 2.7 to 90% due to differences in diagnostic criteria and study populations. According to epidemiological

data, nearly 97% of PD patients have two or more non-motor symptoms, including anxiety. It is clear that the severity of PD is positively correlated with the development of non-motor symptoms. Saffron deserves more attention as a potential therapeutic for PD given its antidepressant and anxiolytic effects (Arabia et al., 2007; Pontone et al., 2009; Schrag and Taddei, 2017; Ryan et al., 2019).

Preclinical Studies. Saffron has been shown to exert multiple neuroprotective effects in different disease models. Abdullah et al. found that crocetin, one of the constituents of saffron, exerted neuroprotective effects in a 6-OHDA-induced rat PD model by attenuating oxidative stress (Ahmad et al., 2005). In another study, saffron exerted a neuroprotective effect on nigral and retinal dopaminergic cells in MPTP-treated mice (Purushothuman et al., 2013). Guo-Feng Zhang et al. showed that another constituent of saffron, crocin, protected pheochromocytoma (PC-12) cells against MPP^+-induced injury through inhibiting mitochondrial dysfunction and ER stress (Zhang et al., 2015). Crocin was found to improve motor deficits and reduce inflammatory cytokines in a malathion-induced rat model (Mohammadzadeh et al., 2018). The antioxidative and antiapoptotic effects of safranal were investigated in an *in vitro* model of rotenone-induced PD. Safranal protected primary dopaminergic cells against oxidative stress and apoptosis *via* the Keap1/Nrf2 signaling pathway (Pan et al., 2016). Saffron and its constituents crocin and crocetin were also shown to exert neuroprotective effects by inhibiting the aggregation and accumulation of α-synuclein (Inoue et al., 2018). In a study conducted by Tamegart et al., saffron reversed dopaminergic and noradrenergic damage induced by lead (Tamegart et al., 2019). As well as in animal and cell models, neuroprotective effects of saffron and crocin were also confirmed by Rao et al. in a drosophila model of parkinsonism (Rao et al., 2016).

Other Brain Disorders

Post-Traumatic Stress Disorder (PTSD)

Post-traumatic stress disorder (PTSD) is a mental disorder, which is caused by experiencing or witnessing a catastrophic incident such as natural disaster, war, serious accident, assault, rape, and abuse. Flashback of trauma, avoidance of certain places, feeling tense, insomnia, and nightmares are the most common symptoms occurred in PTSD patients (Auxemery, 2018). Hormones changes, such as the increase of adrenaline, vasopressin, and corticotropin-releasing hormone (CHR), are generally considered to be responsible for the cause of the psychological disorders including different types of anxiety and depression (Newport and Nemeroff, 2000; Asalgoo et al., 2015). The recommended standard treatments for PTSD patients are psychological therapies (e.g. cognitive behavioral treatment, and talk therapy), medications (e.g. antidepressants, and cannabinoids), or combination of different methods (Watson, 2019).

Some preclinical studies on testing the effects of saffron and its constituents on PTSD animals have obtained encouraging results. Iranian scientists found out that both saffron extract and crocin could significantly reduce the plasma corticosterone level as well as the anorexic time in a PTSD rat model (Sahraei et al., 2012). Asalgoo et al. and his colleague reported that saffron extract and crocin could enhance the ability of spatial learning and attenuate the freezing behavior also in a PTSD rat model (Asalgoo et al., 2018). Regarding management of anxiety behavior, another study showed that combination oral intake of saffron and deep brain stimulation (DBS) exhibited a more efficient therapeutic effect than monotherapy of DBS (Hashtjini et al., 2018). All these promising pre-clinical results shall be further validated in clinical study in PTSD patients.

Schizophrenia

Schizophrenia is a severe mental disorder characterized by abnormal behavior, strange speech, a decreased ability to understand reality, and social, occupational, and individual dysfunction. The aetiology and pathophysiology of schizophrenia remain unknown. The complexity of schizophrenia is reflected in the different types of enduring and persistent psychotic symptoms (positive symptoms, negative symptoms, and cognitive disturbances). Because of the complexity of schizophrenia, current antipsychotic drugs have shown efficacy only for positive symptoms, such as hallucinations, delusions, catatonic behavior. There are no effective drugs for the negative symptoms (social withdrawal, anhedonia, avolition) or cognitive disturbances (deficits in attention and memory) (Pitsikas, 2016).

Few studies have investigated the effects of saffron in schizophrenia-like models. Georgia Georgiadou et al. found that crocin (50 mg/kg, *i.p.*) could attenuate the hypermotility, stereotypies, and ataxia induced by ketamine. Moreover, crocin (50 mg/kg, *i.p.*) counteracted ketamine-induced social isolation in the social interaction test (Georgiadou et al., 2014). Using a novel object recognition task (NORT), another study showed that crocin (15 and 30 mg/kg) reversed recognition memory deficits induced by apomorphine in rat schizophrenia-like models (Pitsikas and Tarantilis, 2017). Two clinical studies used saffron in schizophrenia patients but only investigated its effects on non-schizophrenia symptoms. Fadai et al. reported that saffron extract could alleviate metabolic syndrome, while Mousavi et al. found that saffron aqueous extract and crocin (15 mg *b.i.d.*) had no side effects in patients suffering from schizophrenia (Fadai et al., 2014; Mousavi et al., 2015).

Epilepsy

Epilepsy is characterized by abnormal hypersynchrony of neuronal activity due to an imbalance between glutamatergic signaling pathway-mediated excitatory neurotransmission and the GABAergic signaling pathway. Epilepsy constitutes a highly significant health concern and financial burden that affects about 50–65 million people worldwide. The main symptom of epilepsy is recurrent seizures (Eyo et al., 2017).

It was shown that safranal could reduce seizure duration and delay the onset of tonic convulsions (Hosseinzadeh and Talebzadeh, 2005; Hosseinzadeh and Sadeghnia, 2007). In addition, a study found that safranal exerted anticonvulsant activity through the $GABA_A$-benzodiazepine receptor complex and might have interact with opioid receptors (Hosseinzadeh and Sadeghnia, 2007). Results obtained by Iranian researchers showed that administration of crocin (100 μg) had a comparable

anticonvulsant effect to diazepam (10 μg) in a penicillin-induced epilepsy rat model, indicating involvement of the GABAA-benzodiazepine receptor (Tamaddonfard et al., 2012). In another study, crocin (5, 10, and 20 mg/kg *p.o.*) improved cognitive impairment in male Swiss albino mice by suppressing ROS generation and NF-κB pathway signaling (Mazumder et al., 2017). Additionally, hydroethanolic saffron extract (CSE) (10–200 μg/ml) inhibited evoked postsynaptic potentials (PSPs) and decrease glutamate-induced membrane depolarization (Berger et al., 2011). Other researches have shown that aqueous and ethanolic extracts of *Crocus sativus L.* stigma may benefit both absence and tonic clonic seizures (Hosseinzadeh and Khosravan, 2002).

Stroke

Stroke is one of the major causes of morbidity and mortality in developed and developing countries. Increasing evidence indicates that oxidative stress, inflammation, mitochondrial dysfunction, and excitotoxicity in ischemic areas account for the pathogenic progression of stroke (Luo et al., 2019). Oxidative stress is particularly implicated in stroke, and is one of the causes of dysfunction and death of neuronal cells (Barnham et al., 2004). Therefore, drugs that target oxidative stress may be useful in the treatment of stroke.

As a potent antioxidant, crocin has the ability to prevent the death of PC-12 cells by suppressing the generation of ROS (Ochiai et al., 2004; Ochiai et al., 2007). By the same token, saffron extract (Saleem et al., 2006), crocin (Ochiai et al., 2007; Zheng et al., 2007; Vakili et al., 2014), crocetin(Higashino et al., 2014), and safranal (Hosseinzadeh and Sadeghnia, 2005; Sadeghnia et al., 2017) exerted protective effects against ischemic injury by ameliorating excessive oxidation and increasing antioxidant activities in rat and mouse models. Neuroprotective effects of crocin were attributed to the regulation of MDA, SOD, GPx, and the c-jun kinase (JNK) pathway in the ischemic cortex (Ochiai et al., 2007; Vakili et al., 2014). In a recent randomized clinical trial, patients with acute ischemia stroke were randomly divided into two groups and subjected to either routine stroke care or routine stroke care with saffron capsule treatment (200 mg/day), for a 3-month follow-up observation. Based on the Institute of Health Stoke Scale (NIHSS), the severity of stroke was significantly alleviated in saffron-treated group during the first 4 days. Decreased serum neuron specific enolase, s100 and increased BDNF were also observed in saffron-treated group. At the end of this trial, patients in saffron-treated group showed a higher mean Barthel index, which measures functional independence and mobility in patients with chronic and disabling conditions, than patients in control group (Asadollahi et al., 2019).

CONCLUSION

As one of the most expensive spices in the world, saffron and its constituents, such as crocin, crocetin, and safranal, have shown various biochemical and pharmacological functions. In this comprehensive review, we aimed to summarize the chemical profiles, pharmacological activities, and therapeutic applications of saffron and its constituents in diseases of the central nervous system. Both preclinical and clinical trials suggested that saffron was effective and safe without serious side effects. According to current scientific evidence, saffron and its bioactive compounds have multiple therapeutic effects in many conditions, including psychological disorders, neurodegenerative diseases, cancer, diabetes, and cardiovascular diseases. Preclinical studies proved that saffron exerts its neuroprotective effects mostly *via* antioxidative stress, anti-neuroinflammation, anti-apoptosis and certain other related pathways. Clinical trials also confirmed that saffron could alleviate depressive and anxiety-like symptoms in both depression and anxiety patients. Improvement of cognition impairment was observed in clinical studies using saffron for treating neurodegenerative diseases such as AD and PD. Taken together, the findings provide a fresh perspective that could aid the development of novel neuroprotective drugs from saffron and its bioactive compounds. More investigation of saffron is needed, including preclinical and clinical studies, in terms of its potential to treat neuropsychiatric diseases.

AUTHOR CONTRIBUTIONS

YB and CZ contributed equally to this work. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by The Science and Technology Development Fund, Macau SAR (File no. 0058/2019/A1 and 0016/2019/AKP), and University of Macau (File no. MYRG2019-00105-ICMS).

REFERENCES

Ahmad, A. S., Ansari, M. A., Ahmad, M., Saleem, S., Yousuf, S., Hoda, M. N., et al. (2005). Neuroprotection by crocetin in a hemi-parkinsonian rat model. *Pharmacol. Biochem. Behav.* 81 (4), 805–813. doi: 10.1016/j.pbb.2005.06.007

Ahn, J. H., Hu, Y., Hernandez, M., and Kim, J. R. (2011). Crocetin inhibits beta-amyloid fibrillization and stabilizes beta-amyloid oligomers. *Biochem. Biophys. Res. Commun.* 414 (1), 79–83. doi: 10.1016/j.bbrc.2011.09.025

Akhondzadeh Basti, A., Moshiri, E., Noorbala, A. A., Jamshidi, A. H., Abbasi, S. H., and Akhondzadeh, S. (2007). Comparison of petal of Crocus sativus L. and fluoxetine in the treatment of depressed outpatients: a pilot double-blind randomized trial. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31 (2), 439–442. doi: 10.1016/j.pnpbp.2006.11.010

Akhondzadeh, S., Fallah-Pour, H., Afkham, K., Jamshidi, A. H., and Khalighi-Cigaroudi, F. (2004). Comparison of Crocus sativus L. and imipramine in the treatment of mild to moderate depression: a pilot double-blind randomized trial [ISRCTN45683816]. *BMC Complement. Altern. Med.* 4, 12. doi: 10.1186/1472-6882-4-12

Akhondzadeh, S., Tahmacebi-Pour, N., Noorbala, A. A., Amini, H., Fallah-Pour, H., Jamshidi, A. H., et al. (2005). Crocus sativus L. in the treatment of mild to moderate depression: a double-blind, randomized and placebo-controlled trial. *Phytother. Res.* 19 (2), 148–151. doi: 10.1002/ptr.1647

Akhondzadeh, S., Sabet, M. S., Harirchian, M. H., Togha, M., Cheraghmakani, H., Razeghi, S., et al. (2010a). A 22-week, multicenter, randomized, double-blind controlled trial of Crocus sativus in the treatment of mild-to-moderate Alzheimer's disease. *Psychopharmacology* 207 (4), 637–643. doi: 10.1007/s00213-009-1706-1

Akhondzadeh, S., Shafiee-Sabet, M., Harirchian, M. H., Togha, M., Cheraghmakani, H., Razeghi, S., et al. (2010b). Saffron in the treatment of patients with mild to moderate Alzheimer's disease: a 16-week, randomized and placebo-controlled trial. *J. Clin. Pharm. Ther.* 35 (5), 581–588. doi: 10.1111/j.1365-2710.2009.01133.x

Amin, B., Nakhsaz, A., and Hosseinzadeh, H. (2015). Evaluation of the antidepressant-like effects of acute and sub-acute administration of crocin and crocetin in mice. *Avicenna J. Phytomed.* 5 (5), 458–468.

Arabia, G., Grossardt, B. R., Geda, Y. E., Carlin, J. M., Bower, J. H., Ahlskog, J. E., et al. (2007). Increased risk of depressive and anxiety disorders in relatives of patients with Parkinson disease. *Arch. Gen. Psychiatry* 64 (12), 1385–1392. doi: 10.1001/archpsyc.64.12.1385

Asadi, F., Jamshidi, A. H., Khodagholi, F., Yans, A., Azimi, L., Faizi, M., et al. (2015). Reversal effects of crocin on amyloid beta-induced memory deficit: Modification of autophagy or apoptosis markers. *Pharmacol. Biochem. Behav.* 139, 47–58. doi: 10.1016/j.pbb.2015.10.011

Asadollahi, M., Nikdokht, P., Hatef, B., Sadr, S. S., Sahraei, H., Assarzadegan, F., et al. (2019). Protective properties of the aqueous extract of saffron (Crocus sativus L.) in ischemic stroke, randomized clinical trial. *J. Ethnopharmacol.* 238:111833. doi: 10.1016/j.jep.2019.111833

Asai, A., Nakano, T., Takahashi, M., and Nagao, A. (2005). Orally Administered Crocetin and Crocins Are Absorbed into Blood Plasma as Crocetin and Its Glucuronide Conjugates in Mice. *J. Agric. Food Chem.* 53, 7302–7306. doi: 10.1021/jf0509355

Asalgoo, S., Jahromi, G. P., Meftahi, G. H., and Sahraei, H. (2015). Posttraumatic Stress Disorder (PTSD): Mechanisms and Possible Treatments. *Neurophysiology* 47 (6), 482–489. doi: 10.1007/s11062-016-9559-9

Asalgoo, S., Jahromi, G. P., Hatef, B., and Sahraei, H. (2018). The Effect of Saffron Aqueous Extract and Crocin on PTSD Rat Models: The Focus on Learning and Spatial Memory. *J. Adv. Med. BioMed. Res.* 26 (119), 34–42.

Asrari, N., Yazdian-Robati, R., Abnous, K., Razavi, B. M., Rashednia, M., Hasani, F. V., et al. (2018). Antidepressant effects of aqueous extract of saffron and its effects on CREB, P-CREB, BDNF, and VGF proteins in rat cerebellum. *J. Pharmacopuncture* 21 (1), 35–40. doi: 10.3831/KPI.2018.21.005

Auxemery, Y. (2018). Post-traumatic psychiatric disorders: PTSD is not the only diagnosis. *Presse. Med.* 47 (5), 423–430. doi: 10.1016/j.lpm.2017.12.006

Barnham, K. J., Masters, C. L., and Bush, A. I. (2004). Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discov.* 3 (3), 205–214. doi: 10.1038/nrd1330

Berger, F., Hensel, A., and Nieber, K. (2011). Saffron extract and trans-crocetin inhibit glutamatergic synaptic transmission in rat cortical brain slices. *Neuroscience* 180, 238–247. doi: 10.1016/j.neuroscience.2011.02.037

Bhat, J. V., and Broker, R. (1953). Riboflavine and thiamine contents of saffron, Crocus sativus linn. *Nature* 172 (4377), 544. doi: 10.1038/172544a0

Boskabady, M. H., and Farkhondeh, T. (2016). Antiinflammatory, Antioxidant, and Immunomodulatory Effects of Crocus sativus L. and its Main Constituents. *Phytother. Res.* 30 (7), 1072–1094. doi: 10.1002/ptr.5622

Bostan, H. B., Mehri, S., and Hosseinzadeh, H. (2017). Toxicology effects of saffron and its constituents: a review. *Iran J. Basic Med. Sci.* 20 (2), 110–121. doi: 10.22038/ijbms.2017.8230

Breen, G., Webb, B. T., Butler, A. W., van den Oord, E. J., Tozzi, F., Craddock, N., et al. (2011). A genome-wide significant linkage for severe depression on chromosome 3: the depression network study. *Am. J. Psychiatry* 168 (8), 840–847. doi: 10.1176/appi.ajp.2011.10091342

Bui, E., and Fava, M. (2017). From depression to anxiety, and back. *Acta Psychiatr. Scand.* 136 (4), 341–342. doi: 10.1111/acps.12801

Cavusoglu, A., Erkel, E. I., and Sulusoglu, M. (2009). Saffron (Crocus sativus L.) studies with two mother corm dimensions on yield and harvest period under greenhouse condition. *Am.-Eurasian J. Sustain. Agric.* 3 (2), 126–129.

Chi, S., Wang, C., Jiang, T., Zhu, X. C., Yu, J. T., and Tan, L. (2015). The prevalence of depression in Alzheimer's disease: a systematic review and meta-analysis. *Curr. Alzheimer Res.* 12 (2), 189–198. doi: 10.2174/1567205012666150204124310

Christodoulou, E., Kadoglou, N. P., Kostomitsopoulos, N., and Valsami, G. (2015). Saffron: a natural product with potential pharmaceutical applications. *J. Pharm. Pharmacol.* 67 (12), 1634–1649. doi: 10.1111/jphp.12456

Cicero, A. F., Bove, M., Colletti, A., Rizzo, M., Fogacci, F., Giovannini, M., et al. (2017). Short-Term Impact of a Combined Nutraceutical on Cognitive Function, Perceived Stress and Depression in Young Elderly with Cognitive Impairment: A Pilot, Double-Blind, Randomized Clinical Trial. *Jpad-J. Prev. Alzheimers Dis.* 4 (1), 12–15. doi: 10.14283/jpad.2016.110

Clark, D. A., Beck, A. T., and Beck, J. S. (1994). Symptom differences in major depression, dysthymia, panic disorder, and generalized anxiety disorder. *Am. J. Psychiatry* 151 (2), 205–209. doi: 10.1176/ajp.151.2.205

Dashti, R. M., Zeinali, F., Anvari, M., and Hosseini, S. M. (2012). Saffron (Crocus sativus L.) extract prevents and improves D-galactose and NaNO2 induced memory impairment in mice. *EXCLI J.* 11, 328–337.

Deslauriers, A. M., Afkhami-Goli, A., Paul, A. M., Bhat, R. K., Acharjee, S., Ellestad, K. K., et al. (2011). Neuroinflammation and endoplasmic reticulum stress are coregulated by crocin to prevent demyelination and neurodegeneration. *J. Immunol.* 187 (9), 4788–4799. doi: 10.4049/jimmunol.1004111

Dorri, S. A., Hosseinzadeh, H., Abnous, K., Hasani, F. V., Robati, R. Y., and Razavi, B. M. (2015). Involvement of brain-derived neurotrophic factor (BDNF) on malathion induced depressive-like behavior in subacute exposure and protective effects of crocin. *Iran J. Basic Med. Sci.* 18 (10), 958–966.

Dovrtělová, G., Nosková, K., Juřica, J., Turjap, M., and Zendulka, O. (2015). Can bioactive compounds of Crocus sativus L. influence the metabolic activity of selected CYP enzymes in the rat? *Physiol. Res.* 644), S453–S458. doi: 10.33549/physiolres.933203

Ehsanifar, M., Tameh, A. A., Farzadkia, M., Kalantari, R. R., Zavareh, M. S., Nikzaad, H., et al. (2019). Exposure to nanoscale diesel exhaust particles: Oxidative stress, neuroinflammation, anxiety and depression on adult male mice. *Ecotoxicol. Environ. Saf.* 168, 338–347. doi: 10.1016/j.ecoenv.2018.10.090

Eyo, U. B., Murugan, M., and Wu, L. J. (2017). Microglia-Neuron Communication in Epilepsy. *Glia* 65 (1), 5–18. doi: 10.1002/glia.23006

Fadai, F., Mousavi, B., Ashtari, Z., Ali beigi, N., Farhang, S., Hashempour, S., et al. (2014). Saffron aqueous extract prevents metabolic syndrome in patients with schizophrenia on olanzapine treatment: a randomized triple blind placebo controlled study. *Pharmacopsychiatry* 47 (4-5), 156–161. doi: 10.1055/s-0034-1382001

Farokhnia, M., Sabet, M. S., Iranpour, N., Gougol, A., Yekehtaz, H., Alimardani, R., et al. (2014). Comparing the efficacy and safety of Crocus sativus L. with memantine in patients with moderate to severe Alzheimer's disease: a double-blind randomized clinical trial. *Hum. Psychopharmacol.-Clin. Exp.* 29 (4), 351–359. doi: 10.1002/hup.2412

Finley, J. W., and Gao, S. (2017). A Perspective on Crocus sativus L. (Saffron) Constituent Crocin: A Potent Water-Soluble Antioxidant and Potential Therapy for Alzheimer's Disease. *J. Agric. Food Chem.* 65 (5), 1005–1020. doi: 10.1021/acs.jafc.6b04398

Galigani, P. F., and Garbati, P. F. (1999). "Mechanized saffron cultivation including harvesting," in *Saffron*. Ed. M. Negbi (Australia: Crocus sativus L. Harwood Academic Publishers), 115–126.

Gallagher-Michaels, J. (2013). Treatment Plans and Interventions for Depression and Anxiety Disorders, 2nd edition. *Behav. Cogn. Psychother.* 41 (1), 123–124. doi: 10.1017/S1352465812000938

Georgiadou, G., Tarantilis, P. A., and Pitsikas, N. (2012). Effects of the active constituents of Crocus Sativus L., crocins, in an animal model of obsessive-compulsive disorder. *Neurosci. Lett.* 528 (1), 27–30. doi: 10.1016/j.neulet.2012.08.081

Georgiadou, G., Grivas, V., Tarantilis, P. A., and Pitsikas, N. (2014). Crocins, the active constituents of Crocus Sativus L., counteracted ketamine-induced behavioural deficits in rats. *Psychopharmacology* 231 (4), 717–726. doi: 10.1007/s00213-013-3293-4

Ghaffari, S., Hatami, H., and Dehghan, G. (2015). Saffron ethanolic extract attenuates oxidative stress, spatial learning, and memory impairments induced by local injection of ethidium bromide. *Res. Pharm. Sci.* 10 (3), 222–232.

Ghahghaei, A., Bathaie, S. Z., and Bahraminejad, E. (2012). Mechanisms of the Effects of Crocin on Aggregation and Deposition of A beta 1-40 Fibrils in

Alzheimer's Disease. *Int. J. Pept. Res. Ther.* 18 (4), 347–351. doi: 10.1007/s10989-012-9308-x

Ghahghaei, A., Bathaie, S. Z., Kheirkhah, H., and Bahraminejad, E. (2013). The protective effect of crocin on the amyloid fibril formation of a beta 42 peptide in vitro. *Cell. Mol. Biol. Lett.* 18 (3), 328–339. doi: 10.2478/s11658-013-0092-1

Ghajar, A., Neishabouri, S. M., Velayati, N., Jahangard, L., Matinnia, N., Haghighi, M., et al. (2017). Crocus sativus L. versus Citalopram in the Treatment of Major Depressive Disorder with Anxious Distress: A Double-Blind, Controlled Clinical Trial. *Pharmacopsychiatry* 50 (4), 152–160. doi: 10.1055/s-0042-116159

Ghalandari-Shamami, M., Nourizade, S., Yousefi, B., Vafaei, A. A., Pakdel, R., and Rashidy-Pour, A. (2019). Beneficial Effects of Physical Activity and Crocin Against Adolescent Stress Induced Anxiety or Depressive-Like Symptoms and Dendritic Morphology Remodeling in Prefrontal Cortex in Adult Male Rats. *Neurochem. Res.* 44 (4), 917–929. doi: 10.1007/s11064-019-02727-2

Ghasemi, T., Abnous, K., Vahdati, F., Mehri, S., Razavi, B. M., and Hosseinzadeh, H. (2015). Antidepressant Effect of Crocus sativus Aqueous Extract and its Effect on CREB, BDNF, and VGF Transcript and Protein Levels in Rat Hippocampus. *Drug Res. (Stuttg)* 65 (7), 337–343. doi: 10.1055/s-0034-1371876

Gracia-Garcia, P., de-la-Camara, C., Santabarbara, J., Lopez-Anton, R., Quintanilla, M. A., Ventura, T., et al. (2015). Depression and incident Alzheimer disease: the impact of disease severity. *Am. J. Geriatr. Psychiatry* 23 (2), 119–129. doi: 10.1016/j.jagp.2013.02.011

Gresta, F., Lombardo, G. M., Siracusa, L., and Ruberto, G. (2008). Saffron, an alternative crop for sustainable agricultural systems. A review. *Agron. Sustain. Dev.* 28 (1), 95–112. doi: 10.1051/agro:2007030

Grinan-Ferre, C., Corpas, R., Puigoriol-Illamola, D., Palomera-Avalos, V., Sanfeliu, C., and Pallas, M. (2018). Understanding Epigenetics in the Neurodegeneration of Alzheimer's Disease: SAMP8 Mouse Model. *J. Alzheimers Dis.* 62 (3), 943–963. doi: 10.3233/JAD-170664

Halataei, B. A. S., Khosravi, M., Arbabian, S., Sahraei, H., Golmanesh, L., Zardooz, H., et al. (2011). Saffron (Crocus sativus) Aqueous Extract and its Constituent Crocin Reduces Stress-induced Anorexia in Mice. *Phytother. Res.* 25 (12), 1833–1838. doi: 10.1002/ptr.3495

Hashtjini, M. M., Jahromi, G. P., Meftahi, G. H., Esmaeili, D., and Javidnazar, D. (2018). Aqueous extract of saffron administration along with amygdala deep brain stimulation promoted alleviation of symptoms in post-traumatic stress disorder (PTSD) in rats. *Avicenna J. Phytomed.* 8 (4), 358–369.

Higashino, S., Sasaki, Y., Giddings, J. C., Hyodo, K., Sakata, S. F., Matsuda, K., et al. (2014). Crocetin, a carotenoid from Gardenia jasminoides Ellis, protects against hypertension and cerebral thrombogenesis in stroke-prone spontaneously hypertensive rats. *Phytother. Res.* 28 (9), 1315–1319. doi: 10.1002/ptr.5130

Hosseini, A., Razavi, B. M., and Hosseinzadeh, H. (2017). Pharmacokinetic Properties of Saffron and its Active Components. *Eur. J. Drug Metab. Pharmacokinet.* 43 (4), 383–390. doi: 10.1007/s13318-017-0449-3

Hosseini, A., Razavi, B. M., and Hosseinzadeh, H. (2018). Pharmacokinetic Properties of Saffron and its Active Components. *Eur. J. Drug Metab. Pharmacokinet.* 43 (4), 383–390. doi: 10.1007/s13318-017-0449-3

Hosseinzadeh, H., and Khosravan, V. (2002). Anticonvulsant effects of aqueous and ethanolic extracts of Crocus sativus L. stigmas in mice. *Arch. Irn. Med.* 5 (1), 44–47.

Hosseinzadeh, H., and Noraei, N. B. (2009). Anxiolytic and hypnotic effect of Crocus sativus aqueous extract and its constituents, crocin and safranal, in mice. *Phytother. Res.* 23 (6), 768–774. doi: 10.1002/ptr.2597

Hosseinzadeh, H., and Sadeghnia, H. R. (2005). Safranal, a constituent of Crocus sativus (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus. *J. Pharm. Pharm. Sci.* 8, 394–399.

Hosseinzadeh, H., and Sadeghnia, H. R. (2007). Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. *Phytomedicine* 14 (4), 256–262. doi: 10.1016/j.phymed.2006.03.007

Hosseinzadeh, H., and Talebzadeh, F. (2005). Anticonvulsant evaluation of safranal and crocin from Crocus sativus in mice. *Fitoterapia* 76 (7-8), 722–724. doi: 10.1016/j.fitote.2005.07.008

Hosseinzadeh, H., Karimi, G., and Niapoor, M. (2004). Antidepressant effect of Crocus sativus L. stigma extracts and their constituents, crocin and safranal, in mice. *Proc. 1st Int. Symp. Saffron Biol. Biotechnol.* 650), 435–445. doi: 10.17660/ActaHortic.2004.650.54

Hosseinzadeh, H., Shakib, S. S., Sameni, A. K., and Taghiabadi, E. (2013). Acute and Subacute Toxicity of Safranal, a Constituent of Saffron, in Mice and Rats. *Iran J. Pharm. Res.* 12 (1), 93–99.

Howells, F. M., and Russell, V. A. (2008). Glutamate-stimulated release of norepinephrine in hippocampal slices of animal models of attention-deficit/hyperactivity disorder (spontaneously hypertensive rat) and depression/anxiety-like behaviours (Wistar-Kyoto rat). *Brain Res.* 1200, 107–115. doi: 10.1016/j.brainres.2008.01.033

Inoue, E., Shimizu, Y., Masui, R., Hayakawa, T., Tsubonoya, T., Hori, S., et al. (2018). Effects of saffron and its constituents, crocin-1, crocin-2, and crocetin on alpha-synuclein fibrils. *J. Nat. Med.* 72 (1), 274–279. doi: 10.1007/s11418-017-1150-1

Jia, Y. F., Wininger, K., Ho, A. M., Peyton, L., Baker, M., and Choi, D. S. (2020). Astrocytic Glutamate Transporter 1 (GLT1) Deficiency Reduces Anxiety- and Depression-Like Behaviors in Mice. *Front. Behav. Neurosci.* 14, 57. doi: 10.3389/fnbeh.2020.00057

Kalueff, A. V., and Nutt, D. J. (2007). Role of GABA in anxiety and depression. *Depress Anxiety* 24 (7), 495–517. doi: 10.1002/da.20262

Kalueff, A. V., Avgustinovich, D. F., Kudryavtseva, N. N., and Murphy, D. L. (2006). BDNF in anxiety and depression. *Science* 312 (5780), 1598–1598. doi: 10.1126/science.312.5780.1598

Karakani, A. M., Riazi, G., Mahmood Ghaffari, S., Ahmadian, S., Mokhtari, F., Jalili Firuzi, M., et al. (2015). Inhibitory effect of corcin on aggregation of 1N/4R human tau protein in vitro. *Iran J. Basic Med. Sci.* 18 (5), 485–492.

Kashani, L., Eslatmanesh, S., Saedi, N., Niroomand, N., Ebrahimi, M., Hosseinian, M., et al. (2017). Comparison of Saffron versus Fluoxetine in Treatment of Mild to Moderate Postpartum Depression: A Double-Blind, Randomized Clinical Trial. *Pharmacopsychiatry* 50 (2), 64–68. doi: 10.1055/s-0042-115306

Kell, G., Rao, A., Beccaria, G., Clayton, P., Inarejos-Garcia, A. M., and Prodanov, M. (2017). affron (R) a novel saffron extract (Crocus sativus L.) improves mood in healthy adults over 4 weeks in a double-blind, parallel, randomized, placebo controlled clinical trial. *Complement. Ther. Med.* 33, 58–64. doi: 10.1016/j.ctim.2017.06.001

Kong, Y., Kong, L. P., Luo, T., Li, G. W., Jiang, W., Li, S., et al. (2014). The Protective Effects of Crocetin on A beta(1-42)-Induced Toxicity in Ht22 Cells. *CNS Neuro. Disord.-Drug Targets* 13 (9), 1627–1632. doi: 10.2174/1871527313666140806125410

Lage, M., and Cantrell, C. L. (2009). Quantification of saffron (Crocus sativus L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. *Sci. Hortic.* 121 (3), 366–373. doi: 10.1016/j.scienta.2009.02.017

Lautenschlager, M., Sendker, J., Huwel, S., Galla, H. J., Brandt, S., Dufer, M., et al. (2015). Intestinal formation of trans-crocetin from saffron extract (Crocus sativus L.) and in vitro permeation through intestinal and blood brain barrier. *Phytomedicine* 22 (1), 36–44. doi: 10.1016/j.phymed.2014.10.009

Lopresti, A. L., and Drummond, P. D. (2017). Efficacy of curcumin, and a saffron/curcumin combination for the treatment of major depression: A randomised, double-blind, placebo-controlled study. *J. Affect. Disord.* 207, 188–196. doi: 10.1016/j.jad.2016.09.047

Lopresti, A. L., Drummond, P. D., Inarejos-Garcia, A. M., and Prodanov, M. (2018). affron (R), a standardised extract from saffron (Crocus sativus L.) for the treatment of youth anxiety and depressive symptoms: A randomised, double-blind, placebo-controlled study. *J. Affect. Disord.* 232, 349–357. doi: 10.1016/j.jad.2018.02.070

Luo, S., Li, H., Mo, Z., Lei, J., Zhu, L., Huang, Y., et al. (2019). Connectivity map identifies luteolin as a treatment option of ischemic stroke by inhibiting MMP9 and activation of the PI3K/Akt signaling pathway. *Exp. Mol. Med.* 51 (3), 37. doi: 10.1038/s12276-019-0229-z

Maggi, L., Carmona, M., Zalacain, A., Kanakis, C. D., Anastasaki, E., Tarantilis, P. A., et al. (2010). Changes in saffron volatile profile according to its storage time. *Food Res. Int.* 43 (5), 1329–1334. doi: 10.1016/j.foodres.2010.03.025

Maron, E., and Nutt, D. (2017). Biological markers of generalized anxiety disorder. *Dialogues Clin. Neurosci.* 19 (2), 147–158. doi: 10.1176/appi.focus.16205

Masi, E., Taiti, C., Heimler, D., Vignolini, P., Romani, A., and Mancuso, S. (2016). PTR-TOF-MS and HPLC analysis in the characterization of saffron (Crocus sativus L.) from Italy and Iran. *Food Chem.* 192, 75–81. doi: 10.1016/j.foodchem.2015.06.090

Mazidi, M., Shemshian, M., Mousavi, S. H., Norouzy, A., Kermani, T., Moghiman, T., et al. (2016). A double-blind, randomized and placebo-controlled trial of Saffron (Crocus sativus L.) in the treatment of anxiety and depression. *J. Complement. Integr. Med.* 13 (2), 195–199. doi: 10.1515/jcim-2015-0043

Mazumder, A. G., Sharma, P., Patial, V., and Singh, D. (2017). Crocin Attenuates Kindling Development and Associated Cognitive Impairments in Mice via Inhibiting Reactive Oxygen Species-Mediated NF-κB Activation. *Basic Clin. Pharmacol. Toxicol.* 120, 426–433. doi: 10.1111/bcpt.12694

Milajerdi, A., Jazayeri, S., Shirzadi, E., Hashemzadeh, N., Azizgol, A., Djazayery, A., et al. (2018). The effects of alcoholic extract of saffron (Crocus satious L.) on mild to moderate comorbid depression-anxiety, sleep quality, and life satisfaction in type 2 diabetes mellitus: A double-blind, randomized and placebo-controlled clinical trial. *Complement. Ther. Med.* 41, 196–202. doi: 10.1016/j.ctim.2018.09.023

Moazen-Zadeh, E., Abbasi, S. H., Safi-Aghdam, H., Shahmansouri, N., Arjmandi-Beglar, A., Hajhosseinn Talasaz, A., et al. (2018). Effects of Saffron on Cognition, Anxiety, and Depression in Patients Undergoing Coronary Artery Bypass Grafting: A Randomized Double-Blind Placebo-Controlled Trial. *J. Altern. Complement. Med.* 24 (4), 361–368. doi: 10.1089/acm.2017.0173

Modaghegh, M. H., Shahabian, M., Esmaeili, H. A., Rajbai, O., and Hosseinzadeh, H. (2008). Safety evaluation of saffron (Crocus sativus) tablets in healthy volunteers. *Phytomedicine* 15 (12), 1032–1037. doi: 10.1016/j.phymed.2008.06.003

Mohamadpour, A. H., Ayati, Z., Parizadeh, M. R., Rajbai, O., and Hosseinzadeh, H. (2013). Safety Evaluation of Crocin (a constituent of saffron) Tablets in Healthy Volunteers. *Iran J. Basic Med. Sci.* 16 (1), 39–46.

Mohammadzadeh, L., Hosseinzadeh, H., Abnous, K., and Razavi, B. M. (2018). Neuroprotective potential of crocin against malathion-induced motor deficit and neurochemical alterations in rats. *Environ. Sci. Pollut. Res. Int.* 25 (5), 4904–4914. doi: 10.1007/s11356-017-0842-0

Moore, J. C., Spink, J., and Lipp, M. (2012). Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. *J. Food Sci.* 77 (4), R118–R126. doi: 10.1111/j.1750-3841.2012.02657.x

Moret, C. (2005). Combination/augmentation strategies for improving the treatment of depression. *Neuropsychiatr. Dis. Treat* 1 (4), 301–309.

Moshiri, E., Basti, A. A., Noorbala, A. A., Jamshidi, A. H., Hesameddin Abbasi, S., and Akhondzadeh, S. (2006). Crocus sativus L. (petal) in the treatment of mild-to-moderate depression: a double-blind, randomized and placebo-controlled trial. *Phytomedicine* 13 (9-10), 607–611. doi: 10.1016/j.phymed.2006.08.006

Moshiri, M., Vahabzadeh, M., and Hosseinzadeh, H. (2015). Clinical Applications of Saffron (Crocus sativus) and its Constituents: A Review. *Drug Res. (Stuttg)* 65 (6), 287–295. doi: 10.1055/s-0034-1375681

Mousavi, B., Bathaie, S. Z., Fadai, F., Ashtari, Z., Ali Beigi, N., Farhang, S., et al. (2015). Safety evaluation of saffron stigma (Crocus sativus L.) aqueous extract and crocin in patients with schizophrenia. *Avicenna J. Phytomed.* 5 (5), 413–419.

Murray, C. J., and Lopez, A. D. (1997). Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 349 (9064), 1498–1504. doi: 10.1016/S0140-6736(96)07492-2

Naghibi, S. M., Hosseini, M., Khani, F., Rahimi, M., Vafaee, F., Rakhshandeh, H., et al. (2012). Effect of Aqueous Extract of Crocus sativus L. @ on Morphine-Induced Memory Impairment. *Adv. Pharmacol. Sci.* 2012:494367. doi: 10.1155/2012/494367

Naghizadeh, B., Mansouri, M. T., Ghorbanzadeh, B., Farbood, Y., and Sarkaki, A. (2013). Protective effects of oral crocin against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats. *Phytomedicine* 20 (6), 537–542. doi: 10.1016/j.phymed.2012.12.019

Nelson, J. C., Thase, M. E., Khan, A., and Nelson, J. C. (2008). Are antidepressants effective? What's a clinician to think? *J. Clin. Psychiatry* 69 (6), 1014–1015. doi: 10.4088/jcp.v69n0619

Newport, D. J., and Nemeroff, C. B. (2000). Neurobiology of posttraumatic stress disorder. *Curr. Opin. Neurobiol.* 10 (2), 211–218. doi: 10.1016/S0959-4388(00)00080-5

Noorbala, A. A., Akhondzadeh, S., Tahmacebi-Pour, N., and Jamshidi, A. H. (2005). Hydro-alcoholic extract of Crocus sativus L. versus fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial. *J. Ethnopharmacol.* 97 (2), 281–284. doi: 10.1016/j.jep.2004.11.004

Ochiai, T., Ohno, S., Soeda, S., Tanaka, H., Shoyama, Y., and Shimeno, H. (2004). Crocin prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effects stronger than those of alpha-tocopherol. *Neurosci. Lett.* 362 (1), 61–64. doi: 10.1016/j.neulet.2004.02.067

Ochiai, T., Shimeno, H., Mishima, K., Iwasaki, K., Fujiwara, M., Tanaka, H., et al. (2007). Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. *Biochim. Biophys. Acta* 1770 (4), 578–584. doi: 10.1016/j.bbagen.2006.11.012

Pan, P. K., Qiao, L. Y., and Wen, X. N. (2016). Safranal prevents rotenone-induced oxidative stress and apoptosis in an in vitro model of Parkinson's disease through regulating Keap1/Nrf2 signaling pathway. *Cell. Mol. Biol.* 62 (14), 11–17. doi: 10.14715/cmb/2016.62.14.2

Papandreou, M. A., Kanakis, C. D., Polissiou, M. G., Efthimiopoulos, S., Cordopatis, P., Margarity, M., et al. (2006). Inhibitory activity on amyloid-beta aggregation and antioxidant properties of Crocus sativus stigmas extract and its crocin constituents. *J. Agric. Food Chem.* 54 (23), 8762–8768. doi: 10.1021/jf061932a

Pitsikas, N., and Tarantilis, P. A. (2017). Crocins, the active constituents of Crocus sativus L., counteracted apomorphine-induced performance deficits in the novel object recognition task, but not novel object location task, in rats. *Neurosci. Lett.* 644, 37–42. doi: 10.1016/j.neulet.2017.02.042

Pitsikas, N., Boultadakis, A., Georgiadou, G., Tarantilis, P. A., and Sakellaridis, N. (2008). Effects of the active constituents of Crocus sativus L., crocins, in an animal model of anxiety. *Phytomedicine* 15 (12), 1135–1139. doi: 10.1016/j.phymed.2008.06.005

Pitsikas, N. (2015). The Effect of Crocus sativus L. and Its Constituents on Memory: Basic Studies and Clinical Applications. *Evid. Based Complement. Alternat. Med.* 2015:926284. doi: 10.1155/2015/926284

Pitsikas, N. (2016). Constituents of Saffron (Crocus sativus L.) as Potential Candidates for the Treatment of Anxiety Disorders and Schizophrenia. *Molecules* 21 (3):303. doi: 10.3390/molecules21030303

Pontone, G. M., Williams, J. R., Anderson, K. E., Chase, G., Goldstein, S. A., Grill, S., et al. (2009). Prevalence of anxiety disorders and anxiety subtypes in patients with Parkinson's disease. *Mov. Disord.* 24 (9), 1333–1338. doi: 10.1002/mds.22611

Purushothuman, S., Nandasena, C., Peoples, C. L., El Massri, N., Johnstone, D. M., Mitrofanis, J., et al. (2013). Saffron pre-treatment offers neuroprotection to Nigral and retinal dopaminergic cells of MPTP-Treated mice. *J. Parkinsons Dis.* 3 (1), 77–83. doi: 10.3233/JPD-130173

Rafieipour, F., Hadipour, E., Emami, S. A., Asili, J., and Tayarani-Najaran, Z. (2019). Safranal protects against beta-amyloid peptide-induced cell toxicity in PC12 cells via MAPK and PI3 K pathways. *Metab. Brain Dis.* 34 (1), 165–172. doi: 10.1007/s11011-018-0329-9

Rameshrad, M., Razavi, B. M., and Hosseinzadeh, H. (2018). Saffron and its derivatives, crocin, crocetin and safranal: a patent review. *Expert Opin. Ther. Pat.* 28 (2), 147–165. doi: 10.1080/13543776.2017.1355909

Rao, S. V., Muralidhara,, Yenisetti, S. C., and Rajini, P. S. (2016). Evidence of neuroprotective effects of saffron and crocin in a Drosophila model of parkinsonism. *Neurotoxicology* 52, 230–242. doi: 10.1016/j.neuro.2015.12.010

Rashedinia, M., Lari, P., Abnous, K., and Hosseinzadeh, H. (2015). Protective effect of crocin on acrolein-induced tau phosphorylation in the rat brain. *Acta Neurobiol. Exp. (Wars)* 75 (2), 208–219.

Razavi, B. M., Sadeghi, M., Abnous, K., Vahdati Hasani, F., and Hosseinzadeh, H. (2017). Study of the Role of CREB, BDNF, and VGF Neuropeptide in Long Term Antidepressant Activity of Crocin in the Rat Cerebellum. *Iran J. Pharm. Res.* 16 (4), 1452–1462.

Rios, J. L., Recio, M., Giner, R., and Manez, S. (1996). An Update Review of Saffron and its Active Constituents. *Phytother. Res.* 10 (3), 189–193. doi: 10.1002/(SICI)1099-1573(199605)10:3<189::AID-PTR754>3.0.CO;2-C

Rocchi, R., Mascini, M., Sergi, M., Compagnone, D., Mastrocola, D., and Pittia, P. (2018). Crocins pattern in saffron detected by UHPLC-MS/MS as marker of quality, process and traceability. *Food Chem.* 264, 241–249. doi: 10.1016/j.foodchem.2018.04.111

Ryan, M., Eatmon, C. V., and Slevin, J. T. (2019). Drug treatment strategies for depression in Parkinson disease. *Expert Opin. Pharmacother.* 20 (11), 1351–1363. doi: 10.1080/14656566.2019.1612877

Sadeghnia, H. R., Shaterzadeh, H., Forouzanfar, F., and Hosseinzadeh, H. (2017). Neuroprotective effect of safranal, an active ingredient of Crocus sativus , in a

rat model of transient cerebral ischemia. *Folia Neuropathol.* 55 (3), 206–213. doi: 10.5114/fn.2017.70485

Sahraei, H., Fatahi, Z., Eidi, A., Haeri-Rohani, A., Hooshmandi, Z., Shekarforoush, S., et al. (2012). Inhibiting Post Traumatic Stress Disorder (PTSD) induced by electric shock using ethanol extract of saffron in rats. *J. Biol. Res.-Thessaloniki* 18, 320–327.

Sahraian, A., Jelodar, S., Javid, Z., Mowla, A., and Ahmadzadeh, L. (2016). Study the effects of saffron on depression and lipid profiles: A double blind comparative study. *Asian J. Psychiatr.* 22, 174–176. doi: 10.1016/j.ajp.2015.10.012

Saleem, S., Ahmad, M., Ahmad, A. S., Yousuf, S., Ansari, M. A., Khan, M. B., et al. (2006). Effect of saffron (Crocus sativus) on neurobehavioral and neurochemical changes in cerebral ischemia in rats. *J. Med. Food* 9, 246–254. doi: 10.1089/jmf.2006.9.246

Samarghandian, S., and Borji, A. (2014). Anticarcinogenic effect of saffron (Crocus sativus L.) and its ingredients. *Pharmacogn. Res.* 6 (2), 99–107. doi: 10.4103/0974-8490.128963

Samarghandian, S., and Farkhondeh, T. (2020). "Saffron and Neurological Disorders," in *Saffron* (Elsevier), 103–116.

Sarris, J. (2018). Herbal medicines in the treatment of psychiatric disorders: 10-year updated review. *Phytother. Res.* 32 (7), 1147–1162. doi: 10.1002/ptr.6055

Schrag, A., and Taddei, R. N. (2017). Depression and Anxiety in Parkinson's Disease. *Int. Rev. Neurobiol.* 133, 623–655. doi: 10.1016/bs.irn.2017.05.024

Shahmansouri, N., Farokhnia, M., Abbasi, S. H., Kassaian, S. E., Noorbala Tafti, A. A., Gougol, A., et al. (2014). A randomized, double-blind, clinical trial comparing the efficacy and safety of Crocus sativus L. with fluoxetine for improving mild to moderate depression in post percutaneous coronary intervention patients. *J. Affect. Disord.* 155, 216–222. doi: 10.1016/j.jad.2013.11.003

Si, T., and Yu, X. (2016). Current Problems in the Research and Development of more Effective Antidepressants. *Shanghai Arch. Psychiatry* 28 (3), 160–165. doi: 10.11919/j.issn.1002-0829.216017

Siddiqui, M. J., Saleh, M. S. M., Basharuddin, S., Zamri, S. H. B., Mohd Najib, M. H. B., Che Ibrahim, M. Z. B., et al. (2018). Saffron (Crocus sativus L.): As an Antidepressant. *J. Pharm. Bioallied Sci.* 10 (4), 173–180. doi: 10.4103/JPBS.JPBS_83_18

Tabeshpour, J., Sobhani, F., Sadjadi, S. A., Hosseinzadeh, H., Mohajeri, S. A., Rajabi, O., et al. (2017). A double-blind, randomized, placebo-controlled trial of saffron stigma (Crocus sativus L.) in mothers suffering from mild-to-moderate postpartum depression. *Phytomedicine* 36, 145–152. doi: 10.1016/j.phymed.2017.10.005

Talaei, A., Moghadam, M. H., Tabassi, S. A. S., and Mohajeri, S. A. (2015). Crocin, the main active saffron constituent, as an adjunctive treatment in major depressive disorder: A randomized, double-blind, placebo-controlled, pilot clinical trial. *J. Affect. Disord.* 174, 51–56. doi: 10.1016/j.jad.2014.11.035

Tamaddonfard, E., Gooshchi, N. H., and Seiednejad-Yamchi, S. (2012). Central effect of crocin on penicillin-induced epileptiform activity in rats. *Pharmacol. Rep.* 64 (1), 94–101. doi: 10.1016/s1734-1140(12)70735-1

Tamegart, L., Abbaoui, A., Makbal, R., Zroudi, M., Bouizgarne, B., Bouyatas, M. M., et al. (2019). Crocus sativus restores dopaminergic and noradrenergic damages induced by lead in Meriones shawi: A possible link with Parkinson's disease. *Acta Histochem.* 121 (2), 171–181. doi: 10.1016/j.acthis.2018.12.003

Tarantilis, P. A., Tsoupras, G., and Polissiou, M. (1995). Determination of saffron (Crocus sativus L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. *J. Chromatogr. A.* 699 (1), 107–118. doi: 10.1016/0021-9673(95)00044-N

Tsolaki, M., Karathanasi, E., Lazarou, I., Dovas, K., Verykouki, E., Karacostas, A., et al. (2016). Efficacy and Safety of Crocus sativus L. @ in Patients with Mild Cognitive Impairment: One Year Single-Blind Randomized, with Parallel Groups, Clinical Trial. *J. Alzheimers Dis.* 54 (1), 129–133. doi: 10.3233/JAD-160304

Umigai, N., Murakami, K., Ulit, M. V., Antonio, L. S., Shirotori, M., Morikawa, H., et al. (2011). The pharmacokinetic profile of crocetin in healthy adult human volunteers after a single oral administration. *Phytomedicine* 18 (7), 575–578. doi: 10.1016/j.phymed.2010.10.019

Vakili, A., Einali, M. R., and Bandegi, A. R. (2014). Protective effect of crocin against cerebral ischemia in a dose-dependent manner in a rat model of ischemic stroke. *J. Stroke Cerebrovasc. Dis.* 23 (1), 106–113. doi: 10.1016/j.jstrokecerebrovasdis.2012.10.008

Van der Mussele, S., Bekelaar, K., Le Bastard, N., Vermeiren, Y., Saerens, J., Somers, N., et al. (2013). Prevalence and associated behavioral symptoms of depression in mild cognitive impairment and dementia due to Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 28 (9), 947–958. doi: 10.1002/gps.3909

Wang, Y., Han, T., Zhu, Y., Zheng, C. J., Ming, Q. L., Rahman, K., et al. (2010). Antidepressant properties of bioactive fractions from the extract of Crocus sativus L. *J. Nat. Med.* 64 (1), 24–30. doi: 10.1007/s11418-009-0360-6

Wang, X., Sun, G., Feng, T., Zhang, J., Huang, X., Wang, T., et al. (2019). Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. *Cell Res.* 29 (10), 787–803. doi: 10.1038/s41422-019-0216-x

Watson, P. (2019). PTSD as a Public Mental Health Priority. *Curr. Psychiatry Rep.* 21 (7), 1–12. doi: 10.1007/s11920-019-1032-1

Zhang, G. F., Zhang, Y., and Zhao, G. (2015). Crocin protects PC12 cells against MPP+-induced injury through inhibition of mitochondrial dysfunction and ER stress. *Neurochem. Int.* 89, 101–110. doi: 10.1016/j.neuint.2015.07.011

Zhang, Y., Fei, F., Zhen, L., Zhu, X., Wang, J., Li, S., et al. (2017). Sensitive analysis and simultaneous assessment of pharmacokinetic properties of crocin and crocetin after oral administration in rats. *J. Chromatogr. B. Analyt. Technol. BioMed. Life Sci.* 1044–1045, 1–7. doi: 10.1016/j.jchromb.2016.12.003

Zhang, L., Previn, R., Lu, L., Liao, R. F., Jin, Y., and Wang, R. K. (2018). Crocin, a natural product attenuates lipopolysaccharide-induced anxiety and depressive-like behaviors through suppressing NF-kB and NLRP3 signaling pathway. *Brain Res. Bull.* 142, 352–359. doi: 10.1016/j.brainresbull.2018.08.021

Zheng, Y. Q., Liu, J. X., Wang, J. N., and Xu, L. (2007). Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. *Brain Res.* 1138, 86–94. doi: 10.1016/j.brainres.2006.12.064

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 29 October 2020
doi: 10.3389/fpsyt.2020.579995

Jie-Yu Pill, A Proprietary Herbal Medicine, Ameliorates Mood Disorder-Like Behavior and Cognitive Impairment in Estrogen-Deprived Mice Exposed to Chronic Unpredictable Mild Stress: Implication for a Potential Therapy of Menopause Syndrome

Xi-Dan Zhou[1], *Xin-Jing Yang*[1], *Yu Zheng*[1], *Zong-Shi Qin*[1], *Wei Sha*[2], *Gang Chen*[3] *and Zhang-Jin Zhang*[1,4]*

[1] *Li Ka Shing (LKS) Faculty of Medicine, School of Chinese Medicine, The University of Hong Kong, Hong Kong, China,* [2] *Department of Research and Development, Henan Taifeng Biological Technology Corporation Limited, Kaifeng, China,* [3] *Interdisciplinary Institute for Personalized Medicine in Brain Disorders, Jinan University, Guangzhou, China,* [4] *Department of Chinese Medicine, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China*

OPEN ACCESS

Edited by:
Nazzareno Cannella, University of Camerino, Italy

Reviewed by:
Kabirullah Lutfy, Western University of Health Sciences, United States
Piotr Podlasz, University of Warmia and Mazury, Poland

***Correspondence:**
Zhang-Jin Zhang
zhangzj@hku.hk

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *04 July 2020*
Accepted: *29 September 2020*
Published: *29 October 2020*

Citation:
Zhou X-D, Yang X-J, Zheng Y, Qin Z-S, Sha W, Chen G and Zhang Z-J (2020) Jie-Yu Pill, A Proprietary Herbal Medicine, Ameliorates Mood Disorder-Like Behavior and Cognitive Impairment in Estrogen-Deprived Mice Exposed to Chronic Unpredictable Mild Stress: Implication for a Potential Therapy of Menopause Syndrome. Front. Psychiatry 11:579995. doi: 10.3389/fpsyt.2020.579995

Jie-Yu Pill (JYP) is a proprietary herbal medicine initially developed to treat menstrual mood disorders. This study sought to determine whether JYP could alleviate menopausal psychiatric symptoms in ovariectomized (OVX) mice, an animal model of estrogen deprivation, exposed to chronic unpredictable mild stress (CUMS) and the underlying mechanisms in comparison with estrogen therapy. The OVX+CUMS mice were treated with 0.3 mg/kg estradiol (E_2), 2.5 g/kg or 5 g/kg JYP for 36 days, and tested in multiple behavioral paradigms. Serum, uterus, and brain tissues were collected for the measurement of hypothalamus-pituitary-ovarian axis (HPO) and hypothalamus-pituitary-adrenal (HPA) axis hormones, γ-aminobutyric acid (GABA), glutamate, neurotrophins, and estrogen receptors. JYP and E_2 had comparable efficacy in reducing anxiety- and depression-like behavior and cognitive impairment of the OVX+CUMS mice. E_2 strikingly increased ratio of uterus to body weight of the OVX+CUMS mice, but JYP did not. Both agents suppressed HPO-axis upstream hormones, inhibited HPA-axis hyperactivity by reinstating hypothalamic GABA, restored hippocampal and prefrontal glutamate contents and its receptor expression in the OVX+CUMS mice. While JYP and E_2 protected against decreases in hippocampal and prefrontal neurotrophins and estrogen receptors of the OVX+CUMS mice, unlike E_2, JYP had no significant effects on these biomarkers in the uterus. These results suggest that JYP has comparable efficacy in ameliorating mood disorder-like behavior and cognitive impairment induced by a combination of estrogen deprivation and chronic stress in association with certain differential

uterus-brain mechanisms compared to estrogen therapy. JYP may be a potential therapy for menopause-associated psychiatric disorders.

Keywords: Jie-Yu Pill, herbal medicine, menopause syndrome, psychiatric disorders, estrogen deprivation, ovariectomized mice

INTRODUCTION

Menopause is an important and physiological process in a woman's life, manifesting as the permanent cessation of menstruation due to the failure of ovarian follicular activity (1). Multiple psychiatric symptoms, including anxiety, depression, and cognitive decline, are frequent complaints of menopausal women and have become a significant obstacle to the quality of their life (1, 2). Although estrogen therapy is the mainstay of the management of menopausal syndrome, its efficacy in improving psychiatric symptoms is limited and even controversial; long-term estrogen therapy even increases the risk of breast and ovarian cancer, stroke, and cardiovascular disease (3). Therefore, the development of novel treatment strategies for menopausal women is greatly desired.

Jie-Yu Pill (JYP), consisting of 10 herbal materials (**Table 1**), is a proprietary agent which was initially developed from classic Chinese medicine formulae to treat menstrual cycle related mood symptoms (4, 5). Clinical studies have shown the effectiveness of JYP in the treatment of depressive and anxiety disorders, including climacteric depression, postpartum depression, generalized anxiety disorder, and psychosomatic disorders (4, 6). A series of previous studies have confirmed anxiolytic and antidepressant effects of JYP in normal rodents and its modulatory effects on the hypothalamic-pituitary-adrenal (HPA) axis and monoamine neurotransmitters in the prefrontal cortex, hippocampus, and hypothalamus, the three brain regions that play key roles in the development of menopausal psychiatric disorders (7–12).

On the other hand, one herbal medicine formula derived from JYP's two herbal materials, paeoniae radix and glycyrrhizae radix, could normalize dopamine hyperactivity induced female sex hormone dysfunction (13). Several constituents contained in JYP, such as paeoniflorin (14), saikosaponin A (15), and polysaccharides of lily bulb (*Lilium lancifolium* Thunb.) (16), could reduce estrogen deprivation induced anxiety, depression, and cognitive impairment in animal models. Total polysaccharides of lily bulb and estrogen therapy exerted differential modulatory effects on different neurotrophins and estrogen receptor subtypes in the uterus and brain regions (16, 17). In addition, brain γ-aminobutyric acid (GABA) and glutamatergic neuronal system are directly involved in the pathophysiology of psychiatric disorders occurred during menopausal transition (18–20). Brain neurotrophins modulate estrogen-associated learning, memory, and the underlying neuroplasticity (21, 22).

We therefore hypothesized that JYP could produce comparable efficacy in alleviating menopause-related psychiatric disorders compared to estrogen therapy in association with differential mechanisms at peripheral reproductive organs and the brain. Ovariectomized (OVX) animals are often used as a model of estrogen deprivation as it is well-validated to represent most estrogen deprivation-associated clinical features in the adult human (23). We have modified this model by exposing the OVX mice to chronic unpredictable mild stress (CUMS) to facilitate the development of mood disorder-like behavior and cognitive impairment (16, 17). In this study, the psychotropic effects of JYP were evaluated in behavioral paradigms of anxiety, depression, and cognition in OVX mice exposed to CUMS. We also examined the effects of JYP on serum hormones of the hypothalamus-pituitary-ovary (HPO) and HPA axes, the expression of GABA, glutamate, neurotrophins, estrogen receptor α (ERα) and β (ERβ) in the prefrontal cortex, hippocampus, and hypothalamus.

TABLE 1 | Individual Medicinal materials of the JYP formula[a].

Material name (in Chinese)	%	Major bioactive constituents
Paeoniae Radix Alba (Bai-Shao)	16.395	Paeoniflorin
Bupleuri Radix (Chai-Hu)	12.298	Saikosaponins A
Angelicae Sinensis Radix (Dang-Gui)	8.195	Ferulic Acid
Curcumae Radix (Yu-Jin)	8.195	Essential oils, curcuminoids
Poria (Fu-Ling)	9.835	Polysaccharides
Lily bulb (Bai-He)	9.835	Polysaccharides
Silktree Albizia Bark (He-Huan-Pi)	9.835	Albitocin
Wheat (Xiao-Mai)	12.298	
Licorice (Gao-Cao)	4.919	Liquiritin and glycyrrhizic acid
Chinese Date (Da-Zao)	8.195	Ziziphussaponins, jujuboside B

[a] *The detailed information can be seen at: https://patentimages.storage.googleapis.com/68/b2/47/ed1207e1332697/CN1404864A.pdf.*

MATERIALS AND METHODS

Animals and Experimental Time Course

All experimental procedures were approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong (CULATR 3812-15). A total of 50 C57BL/6N female mice weighing 18–22 g at 8 weeks of age were purchased from Charles River Laboratory (Wilmington, MA, USA). Mice were housed in 3–4 per cage at a constant temperature (23 ± 2°C) and maintained on a 12 h/12 h light/dark cycle (lights on 7:00–19:00) with *ad libitum* access to food and water.

The experimental time course is illustrated in **Figure 1**. After 1 week of acclimatization, animals received OVX or sham surgery and were allowed for 2 weeks of recovery. Our preliminary study found that OVX itself could not consistently evoke remarkable

psychiatric disorder-like behavior. Chronic unpredictable mild stress (CUMS) procedure was then added to accelerate the development of mood disorder-like behavior and cognitive impairment. Multiple behavioral tests were carried out at 4 weeks post-OVX. Treatment started at 1 day after OVX and throughout 36 days.

Ovariectomy (OVX) and CUMS

For OVX surgery, mice were anesthetized with a mixture of ketamine (10 mg/kg) and xylazine (10 mg/kg) *via* intraperitoneal injection (i.p.). Following small bilateral dorsal flank incisions, bilateral ovaries were removed immediately. An additional group of mice who received sham surgery with similar incisions but without ovary removal served as control.

At 2 weeks after surgery, OVX mice received CUMS for 14 days with various types of mild stressors, including tail clamping for 1 min, water deprivation for 15 h, food deprivation for 15 h, restraint in a plastic tube for 4 h, cage tilting at 45 degree for 15 h, empty cage without nesting for 15 h, illumination in dark phase, and wet bedding (50 g sawdust/200 ml water) for 15 h. The animals received only one stressor per day and the same stressor was applied for no more than 2 consecutive days such that animals could not predict the occurrence of future stressor events. Our previous studies have confirmed that the addition of CUMS could better mimic distress experienced during menopause transition (16, 17).

Drug Preparation and Treatment

There were five treatment groups. They were OVX+CUMS mice who received a treatment with vehicle, 0.3 mg/kg 17β-estradiol (E_2), 2.5 g/kg JYP, or 5 g/kg JYP. The control group with sham surgery received vehicle treatment. All agents were delivered *via* oral gavage on a daily basis for 36 days.

For estrogen treatment, E_2 (Sigma-Aldrich, St. Louis, MO) was prepared in stock ethanol solution (20 mg/ml) and then diluted with distilled water to a concentration of 0.03 mg/ml, at which the ethanol concentration of 0.15% yielded served as vehicle. OVX+CUMS mice were treated with 0.3 mg/kg E_2 in a volume of roughly 0.2 ml vehicle. The dose of 0.3 mg/kg E2 was an optimal dose that can well mimic biochemical effects of estrogen therapy based on previous dose-dependent studies (24, 25).

JYP has been approved for marketing in China in 2002. JYP used in this study was generously provided by Henan Taifeng Biotechnology Co., Ltd (Zhengzhou, China) (manufacturer batch number: 20170901). The detailed manufacturing procedure of JYP was carried out in strict compliance with Good Manufacturing Practice (GMP) (https://patentimages.storage.googleapis.com/68/b2/47/ed1207e1332697/CN1404864A.pdf). The quality control of JYP adhered to the specifications and test procedures according to the internal standard. For animal treatment, JYP was dissolved in the vehicle and administered at 30 min before the CUMS stressors. The two doses of JYP (2.5 g/kg and 5 g/kg) used in mice were based on a clinically recommended dose of 12 gram per day for adults weighing 60 kg.

Behavioral Tests

Anxiety-like behavior was examined in open-field test (OFT) and elevated plus maze (EPM). Depression-like behavior was examined using sucrose preference test (SPT), forced-swimming test (FST), and tail suspension test (TST). Cognitive performance was measured using Morris water maze.

Open-Field Test (OFT)

The test was conducted in a white square box (40 × 40 × 40 cm), in which the white floor is divided into the central zone (15 × 15 cm) and the surrounding zone. Each mouse was placed on the surrounding zone and allowed to explore freely for 5 min under white fluorescent light from above. Its movement trajectory in the box was recorded with video tracking software (Noldus Information Technology, Leesburg, VA, USA). The total distance moved was analyzed to evaluate locomotor activity. The time spent in and number of entries into the central zone were obtained to evaluate the extent of anxiety. The box was cleaned with 70% ethanol between tests.

Elevated Plus Maze (EPM)

EPM consists of two open arms (35 × 5 cm) and two closed arms (35 × 5 × 15 cm), arranged such that the two arms of each type are opposite to each other. The maze is elevated to a height of 70 cm from the floor. The mouse was placed in the center area of the maze with its head directed toward an open arm and was then allowed to explore the maze freely for 5 min. The movement trajectory in the maze was recorded with video tracking software (Noldus Information Technology, Leesburg, VA, USA). Any subsequent visit to one of the four arms was counted when all four paws of a mouse entered. The time spent in and number of entries into the open arms were obtained to evaluate the extent of anxiety. The maze was cleaned with 70% ethanol between tests.

Sucrose Preference Test (SPT)

To acclimatize sucrose preference, mice were exposed to two bottles containing 1% sucrose solution (w/v) with *ad libitum* access for 24 h in groups of three to five per cage. On day 2, one bottle containing 1% sucrose solution and another containing tap water were accessible for 24 h. On day 3, the position of the two bottles was switched for 24 h. At the end of the adaptation period, mice were deprived from food and water for 22 h. After that, SPT was conducted in an individual mouse housed in a cage with free access to two respective bottles containing 1% sucrose solution and tap water for 2 h. To prevent side preference in drinking behavior, the position of the two bottles was switched in the middle of testing. Water and sucrose consumption were measured as changes in weight of fluid consumed. The sucrose preference was calculated from the following formula: sucrose preference (%) = sucrose intake (g)/[sucrose intake (g) + water intake (g)] × 100%.

Forced-Swimming Test (FST)

A polycarbonate cylinder (30 cm in height and 20 cm in diameter) was used for FST. The cylinder was filled with water to a depth of 15 cm at 23 to 25°C. A mouse was placed in the

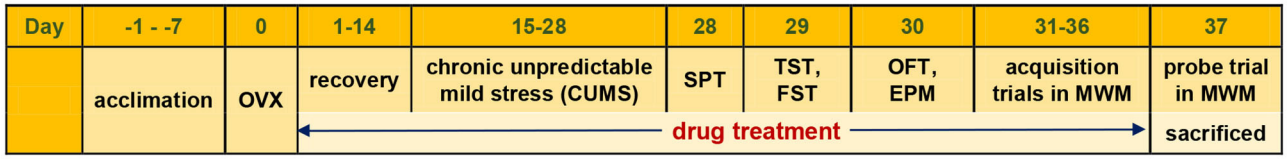

FIGURE 1 | Experimental time course. OVX, ovariectomized; SPT, sucrose preference test; TST, tail suspension test; FST, forced-swimming test; OFT, open-field test; EPM, elevated plus maze; MWM, Morris water maze.

cylinder for 6 min and its movement was recorded on videotape. Immobility time, defined as the absence of all movements except for motions required to maintain the head above the water, was obtained from the last 4 min of the trial with EthoVision XT7 software.

Tail Suspension Test (TST)

The test was conducted in a specially manufactured tail suspension box. Each mouse was suspended 50 cm above the floor of the box by fixing its tail tip (1 cm in length) with adhesive tape. Immobility time, defined as the absence of any movements of limbs and trunk except for whisker movement and respiration, was recorded on videotape over 6 min of testing and analyzed with EthoVision XT7 software.

Morris Water Maze (MWM) Test

The apparatus for MWM consists of an open circular pool (90 cm in diameter and 45 cm in height) filled with water (22 ± 2°C). The pool was conceptually divided into four quadrants, and a hidden platform (6 cm in diameter and 39 cm in height) was placed in one of the quadrants and submerged 1 cm beneath the water surface. The test was divided into the two phases: 6 days of acquisition trials and 1 day of probe trial.

For acquisition trials, at Day 1 the mice were allowed to freely swim in clear water with the visual cues on the hidden platform. During the subsequent 5 days, the mice were given four training trials per day in the milky water by placing titanium dioxide into the water. Different starting points were used on each of the five daily trials and the order of starting point was random. If a mouse located the platform, it was allowed to remain on the platform for 10 s. If a mouse failed to locate the platform within 60 s, it was placed on the platform for 10 s. The mice were towel-dried and returned to their home cage after each trial. The latency to the hidden platform was recorded from each training trial and averaged across the four training trials each day for data analysis. One mouse of the 5 JYP group died at Day 2 during the acquisition trials.

The probe trial was conducted at Day 7. The platform was removed from the pool and a mouse was placed onto the pool and allowed to search the platform for 60 s. The movement trajectory in the maze was recorded with video tracking software (Noldus Information Technology, Leesburg, VA, USA). The total swimming distance was calculated to evaluate the locomotor activity. The latency to the target zone, the duration spent in the target zone, and the crossing number into the target zone were obtained to measure the spatial memory.

Tissue Preparation and Measurement of Serum HPO- and HPA-Related Hormones

Following the completion of behavioral tests, animals were anesthetized with ketamine/xylazine (120/18 mg/kg). A volume of 0.5–0.6 ml blood was collected *via* cardiac puncture. The uterus and whole brains were removed. The prefrontal cortex, hippocampus, and hypothalamus were dissected from the brains for Western blot analysis (see below). Sera were immediately separated by centrifuging at 3,500 rpm for 15 min at 4°C and stored at −80°C until assay. Adrenocorticotropic hormone (ACTH) (Cat No.: RK02566), Follicle Stimulating Hormone (FSH) (Cat No.: RK02819) and Luteinizing Hormone (LH) (Cat No.: RK02986) levels were measured using their corresponding commercial enzyme-linked immunosorbent assay (ELISA) kits (ABclonal Technology, Wuhan, China) according to the manufacturer's instructions. Estradiol (E_2) (Cat No.: KGE014) and Corticosterone (CORT) (Cat No.: KGE009) were also tested with ELISA kits (R&D systems Inc., Minneapolis, USA).

Determination of Brain Regional GABA and Glutamate Levels

High performance liquid chromatography (HPLC) with diode-array detector (DAD) was used to measure the contents of regional brain GABA and glutamate (Glu) as described previously (26). Briefly, the hypothalamus, hippocampus, and prefrontal cortex were dissected from the brain and homogenized in 100–200 μl of acetonitrile. The homogenate was centrifuged at 13,000 rpm at 4°C for 20 min; the supernatant was collected and evaporated under a gentle stream of nitrogen. The dried residue was reconstituted in 50–100 μl of Na_2CO_3-$NaHCO_3$ buffer (pH 9.5). The 25–50 μl of dansyl chloride (10 mmol/L) was added and vortex mixed for derivatization procedure, and the mixture was incubated in dark at 65°C for 25 min and cooled to room temperature. The solution was then centrifuged at 13,000 rpm for 20 min, and 10 μl of the supernatant was directly injected into a Thermo 3000 series UPLC equipped with an ACE Excel 2 C18 column (100 mm × 2.1 mm × 1.7 μm) and DAD. The mobile phase consisted of acetonitrile (A) and 0.6% acetic acid in water/0.008% trithylamine (B). The gradient program was developed with 70–55% B for 0–20 min. The flow rate was kept at 0.4 mL/min and the detective wavelength was selected at 254 nm.

Western Blot Analysis

Western blot analysis was used to detect the effects of JYP on the expression levels of N-methyl-D-aspartate receptor subunit 1 (NMDAR1), the three neurotrophins, brain-derived

neurotrophic factor (BDNF), nerve growth factor (NGF), and glial cell-derived neurotrophic factor (GDNF), and the two estrogen receptors, ERα and ERβ, in the brain regions and the uterus. Brain and uterine tissues were homogenized in radio-immunoprecipitation assay buffer (RIPA Buffer, Sigma Aldrich, USA) containing 2% phenylmethanesulfonyl fluoride (PMSF, Sigma-Aldrich, USA) at 4°C for 30 min. All tissues were centrifuged at 13,000 rpm for 20 min. The supernatant was collected and their protein concentration were measured with the Bradford method using Coomassie brilliant blue G-250 (Bio-Rad Laboratories Inc.). Proteins were separated by electrophoresis on 10% SDS-PAGE gels and subsequently transferred onto polyvinylidene difluoride membranes (PVDF, 0.22 μM, Bio-Rad Laboratories, Inc.). After being blocked with 5% BSA in TBST, the membranes were then blotted with the primary rabbit antibodies against NMDAR1 (1:2000, Santa Cruz Biotechnology, USA, Cat No.: sc-1468), BDNF (1:1000, Santa Cruz Biotechnology, USA, Cat No.: sc-546), NGF (1:1000, Abcam, Cambridge, USA, Cat No.: ab52918), GDNF (1:1000, Abcam, Cambridge, USA, Cat No.: ab176564), ERα (1:1000, invitrogen, USA, Cat No.: PA5-16440), ERβ (1:1000, invitrogen, USA, Cat No.: PA1-310B), and mouse anti-GAPDH (1:5000, Immunoway, USA, Cat No.:YM3029) at 4°C overnight. After rinsing with TBST, the membranes were incubated with suitable secondary antibodies (1:2000, Santa Cruz Biotechnology, USA) at 4°C for 4 h. Chemiluminescence was detected using an enhanced chemiluminescence detection kit (GE Healthcare, UK). The intensity of the bands was quantified by scanning densitometry using Image Lab 5.1 software (Bio-Rad, Laboratories, Inc.). The mean value of the intensity was obtained from at least three independent experiments.

Statistical Analysis

Based on our previous studies (16, 17), $n = 10$ per group should be sufficient to detect statistical differences in behavioral variables among treatment groups at a power of 0.8 and a significance level of 0.05. Two-way repeated measure analysis of variance (ANOVA) was used to detect the effects of JYP on the latency to the hidden platform over acquisition trials. One-way ANOVA was used to examine other variables. Between-group differences were further analyzed using Student–Newman–Keuls test. All data were expressed as mean ± standard error of the mean (SEM). Statistical significance was defined as <0.05 of *P*-value. All statistical analysis was conducted with SAS (version 9.4; SAS Institute, Cary, NC).

RESULTS

Effects of JYP and E_2 on Body Weight and Uterine Weight

Significant effects of group were observed on body weight [$F_{(4, 44)} = 6.088$, $P < 0.001$] and ratio of uterus to body weight [$F_{(4, 44)} = 27.6$, $P < 0.001$] (**Figure 2**). OVX+CUMS caused a striking increase in body weight ($P = 0.004$) (**Figure 2A**) and a dramatic decrease in ratio of uterus to body weight ($P < 0.001$) compared to sham surgery (**Figure 2B**). E_2 treatment partially reversed OVX+CUMS-induced changes in the two variables ($P \leq 0.002$).

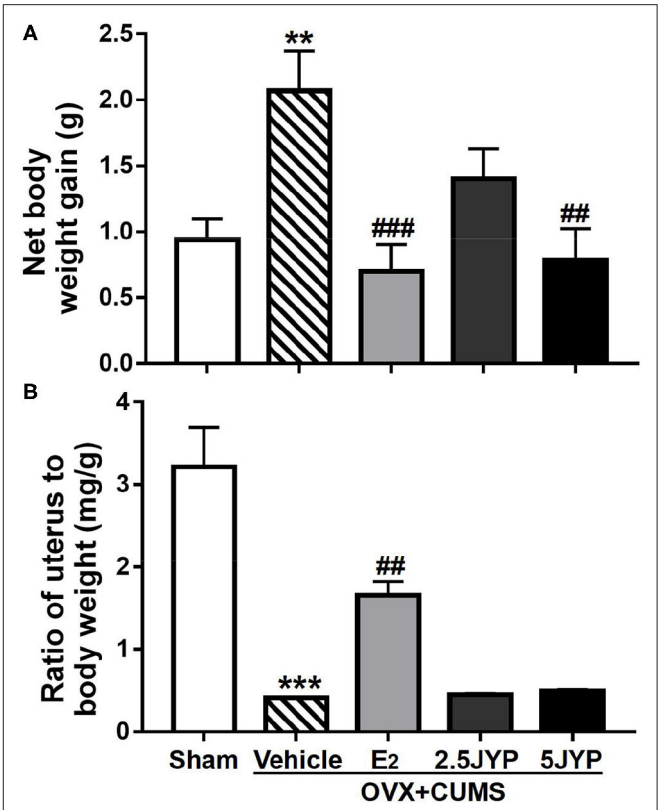

FIGURE 2 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on net changes in body weight **(A)** and ratio of uterus to body weight **(B)** of OVX+CUMS mice. Data are expressed as mean ± SEM (n = 9–10) and examined with one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. vehicle group.

JYP (5 g/day) significantly suppressed the OVX+CUMS-induced weight gain ($P = 0.001$), but either dose of JYP had no effects on the ratio of uterus to body weight compared to vehicle ($P \geq 0.997$).

Effects of JYP and E_2 on Anxiety-Like Behavior

There were significant differences among the four groups in time spent in [$F_{(4, 44)} = 2.754$, $P = 0.037$] and number of entries into the central zone of OFT [$F_{(4, 44)} = 4.388$, $P = 0.005$], and time spent in [$F_{(4, 44)} = 3.35$, $P = 0.018$] and number of entries into open arms of EPM [$F_{(4, 44)} = 4.828$, $P = 0.003$] (**Figure 3**). In OFT, OVX+CUMS mice treated with E_2 spent markedly more time in and had more entries into the central zone ($P \leq 0.027$) compared to those treated with vehicle (**Figures 3A,B**). Both doses of JYP significantly increased the entry number to the central zone ($P \leq 0.031$). In EPM, OVX+CUMS mice treated with E_2 and 5 g/kg JYP spent significantly more time in and had more entries into open arms than those treated with vehicle ($P \leq 0.020$) (**Figures 3C,D**). OVX+CUMS mice receiving 2.5 g/kg JYP also showed significantly more entries into open arms than those vehicle-treated OVX+CUMS mice ($P = 0.008$).

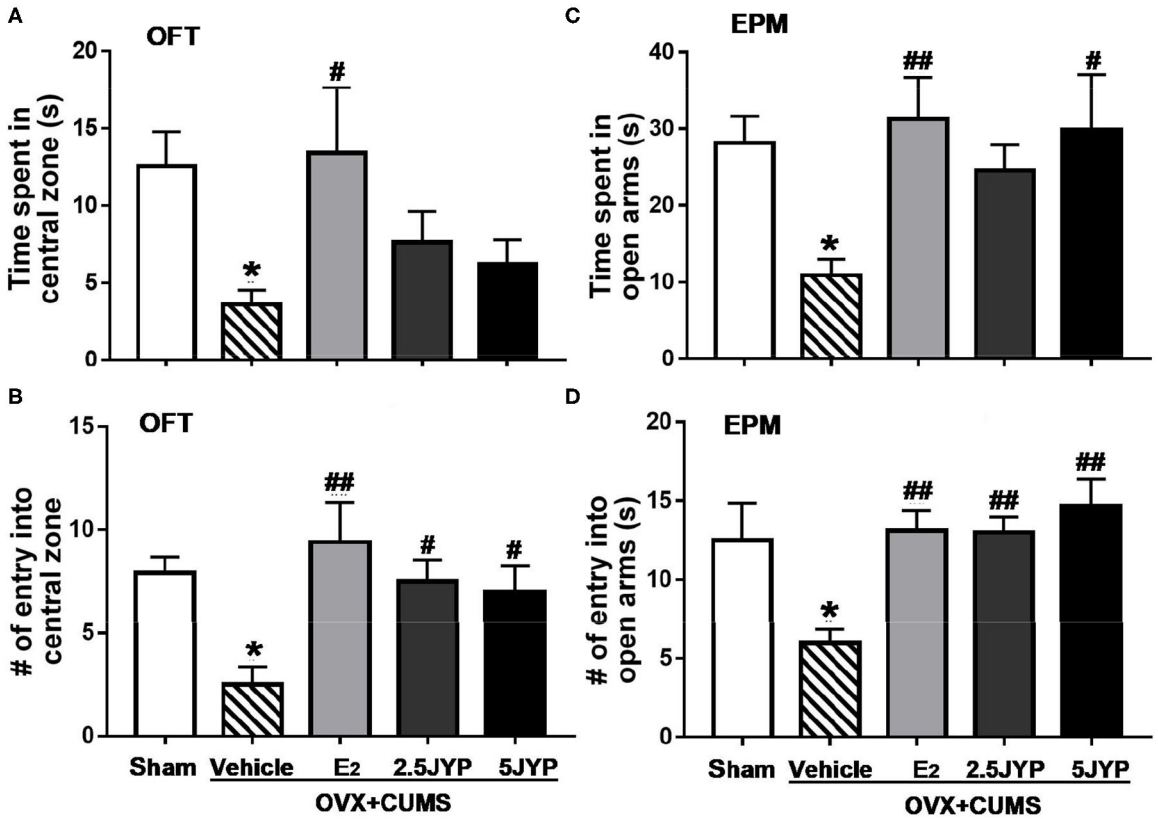

FIGURE 3 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on OVX+CUMS-induced anxiety-like behavior: **(A)** Duration in the central zone in the open field test (OFT); **(B)** Number of entries into the central zone in the OFT; **(C)** Duration in the open arms in the elevated plus maze (EPM) test; and **(D)** Number of entries into the open arms in EPM. Data are expressed as mean ± SEM (n = 9–10) and examined with one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{*}P < 0.05$ vs. sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. vehicle group.

Effects of JYP and E_2 on Depression-Like Behavior

Significant effects of group were present on sucrose consumption in SPT [$F_{(4, 44)} = 6.221$, $P < 0.001$] (**Figure 4A**), immobility time in TST [$F_{(4, 44)} = 4.01$, $P = 0.007$] (**Figure 4B**), and FST [$F_{(4, 44)} = 3.138$, $P = 0.024$] (**Figure 4C**). OVX+CUMS resulted in a marked reduction of sucrose consumption in SPT and a significant increase in immobility time spent in TST and FST compared to sham surgery ($P \leq 0.026$). E_2 completely reversed OVX+CUMS-induced changes in the three variables ($P \leq 0.045$). Both doses of JYP significantly increased sucrose consumption ($P \leq 0.023$) and 5 g/kg JYP additionally reduced immobility time spent in FST ($P < 0.039$) as compared to vehicle.

Effects of JYP and E_2 on Cognitive Performance

Two-way ANOVA showed no significant interaction between group and time on the escape latency to the platform [$F_{(20, 220)} = 1.300$, $P = 0.203$], but significant main effects were observed on group [$F_{(4, 44)} = 11.10$, $P < 0.001$] and time [$F_{(5, 220)} = 41.61$, $P < 0.001$] on the latency in acquisition trials (**Figure 5A**). Vehicle-treated OVX+CUMS mice spent much longer latency to find the platform than mice with sham surgery at Day 2 through Day 6 ($P \leq 0.033$). The latency of OVX+CUMS mice treated with E_2 was markedly less than that of OVX+CUMS mice treated with vehicle at Day 2 through Day 6 ($P \leq 0.040$). OVX+CUMS mice treated with 2.5 g/kg JYP spent less time locating the platform at Day 5 and Day 6 ($P \leq 0.009$). OVX+CUMS mice treated with 5 g/kg JYP showed a shorter latency to find the platform at Day 3 through Day 6 ($P \leq 0.016$).

In the probe trial, representative individual swim paths from each group are shown in **Figures 5B–F**. Treatment had no effects on swimming speed [$F_{(4, 44)} = 1.755$, $P = 0.155$] (**Figure 5G**), but significantly changed time spent in [$F_{(4, 44)} = 3.278$, $P = 0.020$] (**Figure 5H**), number of entries into [$F_{(4, 44)} = 5.142$, $P = 0.002$] (**Figure 5I**), and latency to the target zone [$F_{(4, 44)} = 3.000$, $P = 0.028$] (**Figure 5J**). OVX+CUMS markedly reduced time stayed in ($P = 0.016$) and number of entries into the target zone ($P < 0.001$), and increased the latency to the target zone ($P = 0.044$) compared to sham surgery. OVX+CUMS mice treated with E_2 and 5 g/kg JYP remarkably increased time spent in ($P \leq 0.022$), frequency crossed ($P \leq 0.035$), and shorter latency to the target zone ($P \leq 0.034$) compared to those treated with vehicle.

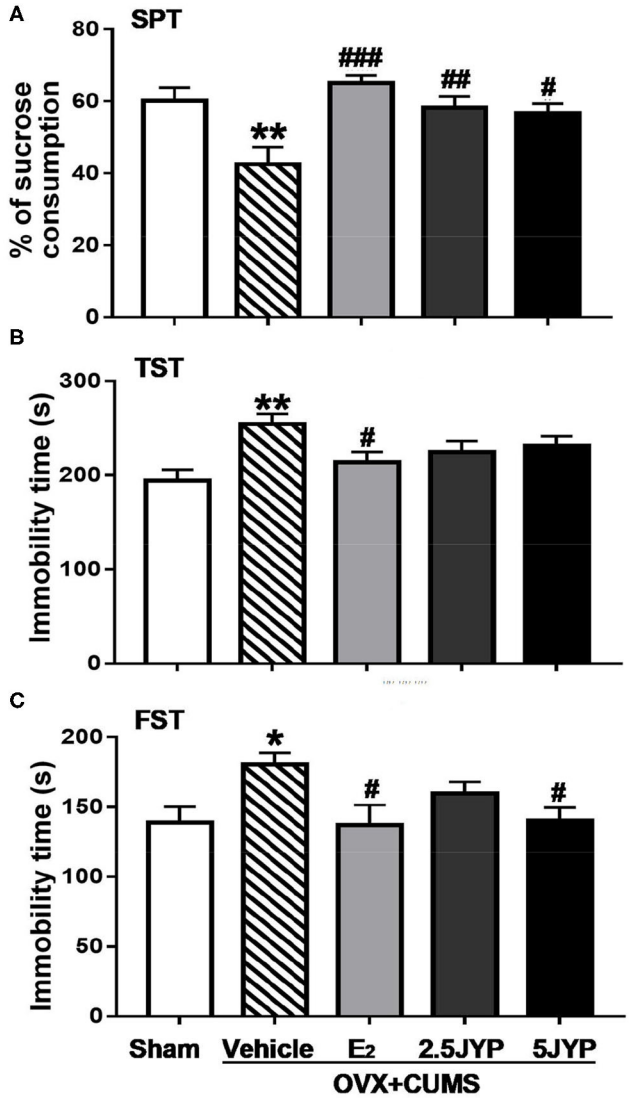

FIGURE 4 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on OVX+CUMS-induced depression-like behavior: **(A)** percentage of sucrose consumption in sucrose preference test (SPT); **(B)** immobility time of tail suspension test (TST); and **(C)** immobility time of forced swimming test (FST). Data are expressed as mean ± SEM ($n = 9$–10) and examined with one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{*}P < 0.05$, $^{**}P < 0.01$ vs. sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. vehicle group.

Effects of JYP and E_2 on HPO-and HPA-Related Hormones

Significant main effects of groups were observed on serum E_2 (**Figure 6A**), FSH (**Figure 6B**), and LH [$F_{(4, 15)} \geq 5.543$, $P \leq 0.006$] (**Figure 6C**). OVX+CUMS strikingly decreased serum level of E_2 and increased FSH and LH levels compared with sham surgery ($P \leq 0.033$). E_2 treatment completely reversed OVX-induced changes in levels of the three hormones. Both doses of JYP significantly suppressed the elevated levels of FSH ($P \leq 0.045$); 5 g/kg JYP additionally suppressed the OVX+CUMS-induced elevation of LH level ($P = 0.017$).

Significant differences were observed in serum level of CORT (**Figure 6D**) and ACTH (**Figure 6E**) across the five groups [$F_{(4, 15)} \geq 4.059$, $P \leq 0.020$]. OVX+CUMS resulted in a significant elevation of the two hormones ($P \leq 0.028$), which were completely reversed by E_2 and 5 g/kg JYP. JYP at 2.5 g/kg also completely reversed OVX+CUMS-induced elevation of ACTH level ($P \leq 0.038$).

Effects of JYP and E_2 on Brain Regional Contents of GABA and Glutamate

There were significant differences in the contents of GABA and glutamate in the hypothalamus (**Figure 7A**), hippocampus (**Figures 7B,D**), and prefrontal cortex (**Figures 7C,E**), among groups [$F_{(4, 24)} \geq 3.544$, $P \leq 0.021$]. OVX+CUMS mice showed marked decreases of GABA in the three brain regions ($P \leq 0.010$), which were significantly restored by E_2 treatment ($P \leq 0.046$). The high dose of JYP also restored the decrease of hypothalamic GABA ($P \leq 0.023$). The contents of hippocampal and prefrontal glutamate of OVX+CUMS mice were much higher than those with sham surgery ($P \leq 0.013$). OVX+CUMS mice exposed to stress and treated with E_2 and the high dose of JYP completely reversed the treatment ($P \leq 0.038$). The contents of glutamate in the hypothalamus failed to be detected.

Effects of JYP and E_2 on Regional Brain NMDAR1

Significant group effects were detected on the expression of NMDAR1 in the hippocampus (**Figure 8A**) and prefrontal cortex (**Figure 8B**) [$F_{(4, 10)} \geq 12.51$, $P \leq 0.001$]. The expression levels of NMDAR1 in both brain regions of OVX+CUMS mice were ∼2-fold higher than those with sham surgery ($P \leq 0.002$), but completely reversed by treatment with E_2 and the high dose JYP ($P \leq 0.016$). The low dose JYP also strikingly suppressed the OVX+CUMS-induced increase of prefrontal NMDAR1 expression ($P < 0.001$).

Effects of JYP and E_2 on Brain Regional and Uterine Neurotrophins

GDNF, BDNF, and NGF expression levels significantly differed among groups in the uterus (**Figure 9A**), hippocampus (**Figure 9B**), and prefrontal cortex (**Figure 9C**) [$F_{(4, 10)} \geq 4.691$, $P \leq 0.022$] OVX+CUMS dramatically suppressed the expression of the three neurotrophins in the three tissues ($P \leq 0.046$) except for hippocampal and prefrontal NGF compared to sham surgery. E_2 completely restored all the three neurotrophin expression in the three tissues examined ($P \leq 0.046$) compared to vehicle treatment. Both doses of JYP also completely reversed the OVX+CUMS-induced decreases of hippocampal GDNF and BDNF and prefrontal NGF ($P \leq 0.024$), but had no significant effects on uterine neurotrophins. JYP-treated OVX+CUMS mice displayed strikingly higher expression levels of hippocampal NGF and prefrontal GDNF with the high dose ($P \leq 0.006$) and prefrontal NGF ($P = 0.012$) with the low dose than those treated with vehicle.

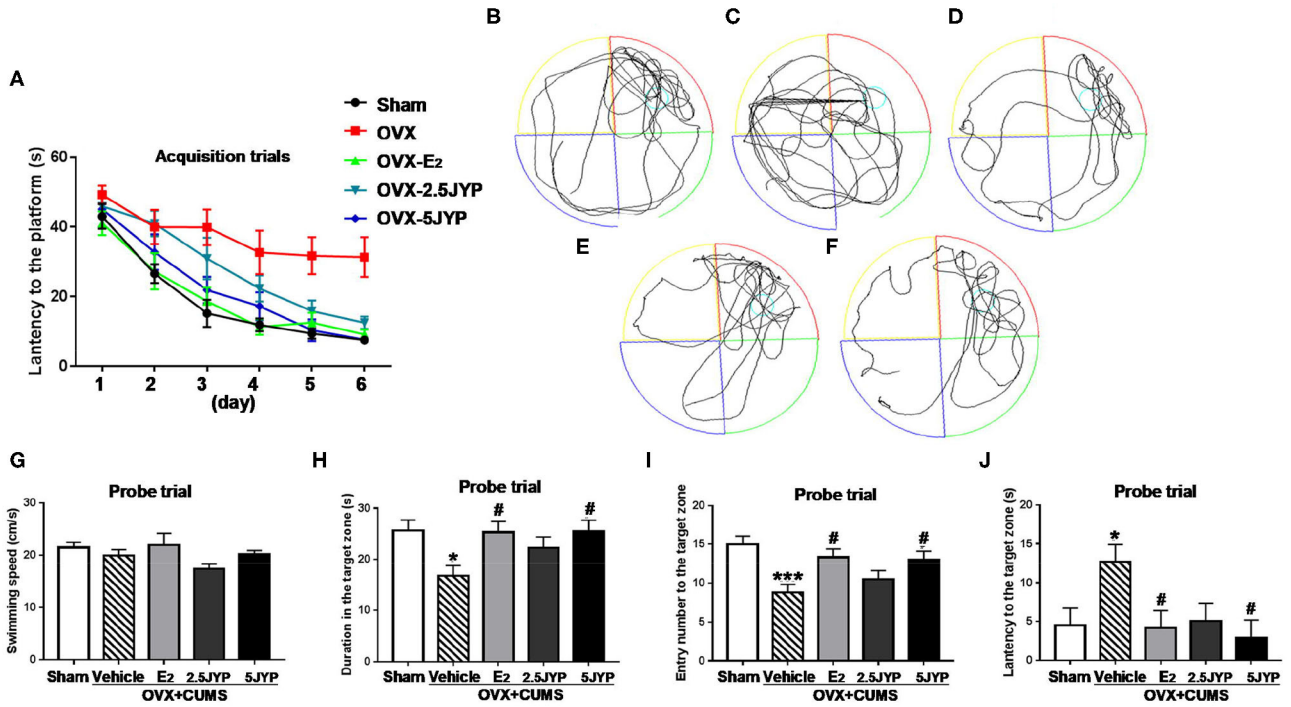

FIGURE 5 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) in Morris water maze test of OVX+CUMS mice: escape latency in the acquisition trials **(A)**; representative individual swim paths in the probe trial of sham group **(B)**, vehicle group **(C)**, E_2 group **(D)**, 2.5 JYP group **(E)**, and 5 JYP group **(F)**; swimming speed **(G)**, duration in the target zone **(H,I)** entry number into the target zone **(I)**, and latency to the target zone **(J)** in the probe trial. Data are expressed as mean ± SEM ($n = 9$–10) and examined with two-way or one-way analysis of variance (ANOVA) and one-way ANOVA, followed by followed by *post hoc* between-group comparisons: $^{*}P < 0.05$, $^{***}P < 0.001$ vs. sham group; $^{\#}P < 0.05$ vs. vehicle group.

Effects of JYP and E_2 on Brain Regional and Uterine Estrogen Receptors

There were marked differences in the expression level of ERα and ERβ in the uterus (**Figure 10A**), hypothalamus (**Figure 10B**), hippocampus (**Figure 10C**), and prefrontal cortex (**Figure 10D**) among groups [$F_{(4, 10)} \geq 3.626$, $P \leq 0.045$]. OVX+CUMS markedly suppressed the expression of both receptor types in all the four tissues examined compared to sham surgery ($P \leq 0.031$). E_2 partially or completely restored the expression of the two receptor types in the four tissues compared to vehicle ($P \leq 0.030$). Both doses of JYP significantly increased the expression level of hypothalamic ERβ ($P \leq 0.010$), but had no effects on both receptor types in the uterus. The high dose JYP partially or completely restored the expression of hippocampal and hypothalamic ERα ($P \leq 0.013$), and the low dose almost completely restored the expression level of hippocampal and prefrontal ERβ ($P \leq 0.021$) compared to vehicle.

DISCUSSION

The main purpose of this study was to evaluate the therapeutic effects of JYP as a novel therapy in improving mood and cognitive symptoms associated with menopause. In previous studies, we found that OVX alone could not constantly evoke anxiety- and mood disorder-like behavior; the addition of CUMS exposure however augmented aberrant behaviors (16, 17). This combination model also has been well-validated in several recent studies (28–30). Indeed, in this study, OVX mice exposed to CUMS showed remarkable anxiety-like behavior, manifesting as marked decrease in time spent and number of entry in central zone of the open field and in open arms of EPM compared to the control group. OVX combined with CUMS also evoked depression-like behavior, with significant decrease in sucrose consumption and increase in immobility time in TST and FST. The combination of OVX and CUMS further impaired spatial learning and memory ability, evidenced by strikingly longer latency in finding the platform in training trials and in reaching the target zone in the probe trial, and less duration and number of entry in the target zone than the control group in water maze test. These results, once again, confirm that OVX mice with stress exposure is a valid model in mimicking estrogen deprivation-induced psychiatric disorders.

Treatment with JYP in particularly higher dose, however, suppressed anxiety- and depression-like behavior and prevent spatial learning and memory to a similar degree as did E_2, suggesting comparable efficacy of JYP in the treatment of estrogen deprivation induced psychiatric disorders. This is also highly consistent with anxiolytic and antidepressant effects of JYP observed in male rodents with (10, 11) and without exposure

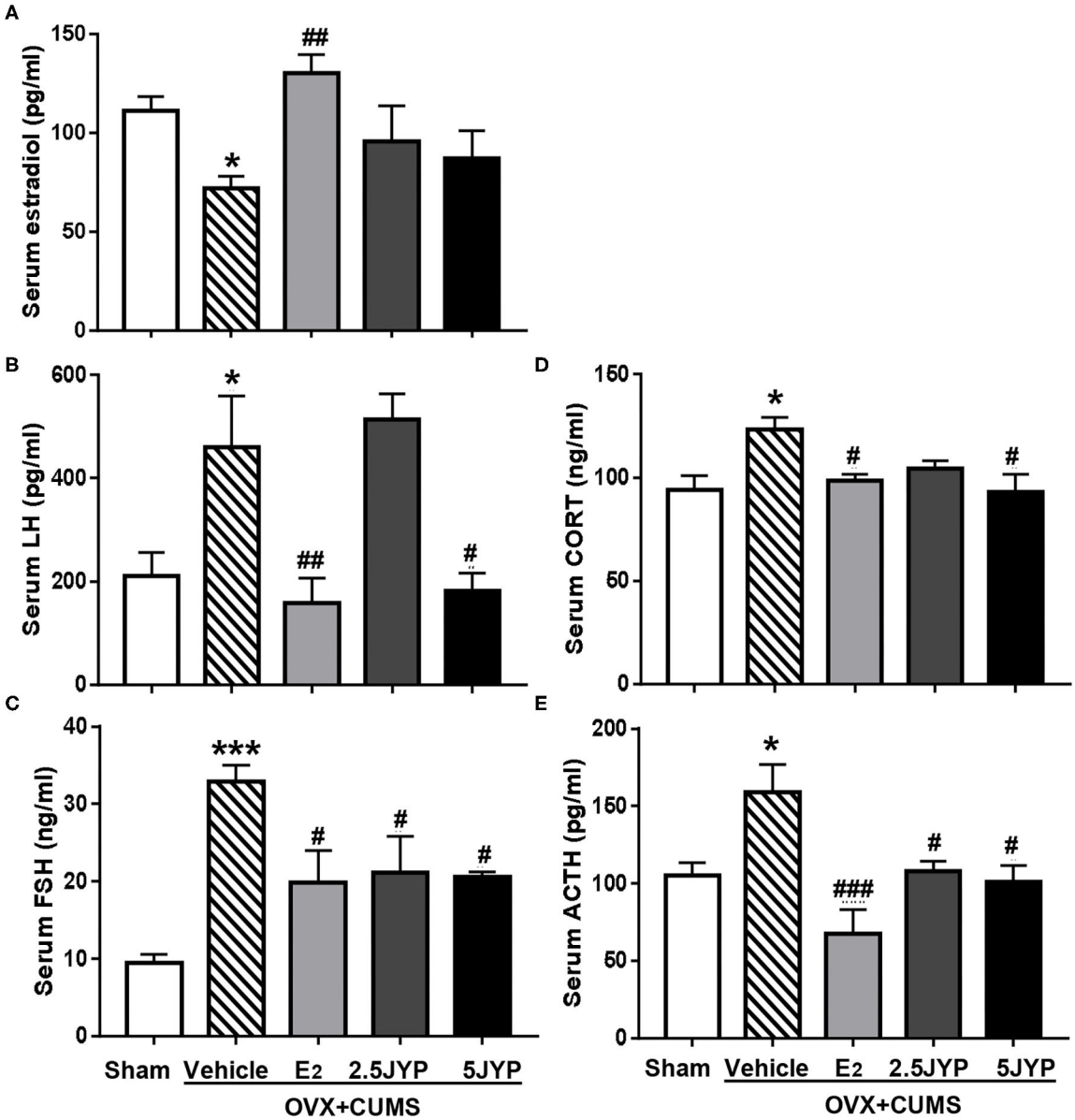

FIGURE 6 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on serum hypothalamic–pituitary–adrenal (HPA) and hypothalamic-pituitary-ovarian (HPO)-axis hormones with E_2 **(A)**, luteinizing hormone (LH) **(B)**, follicle stimulating hormone (FSH) **(C)**, corticosterone (CORT) **(D)**, and adrenocorticotropic hormone (ACTH) **(E)**. Data are expressed as mean ± SEM ($n = 4$) and examined with one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{*}P < 0.05$, $^{***}P < 0.001$ vs. sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. vehicle group.

to CUMS (7, 8). It therefore appears that JYP not only could reduce "generalized" anxiety and "endogenous" depression, but is also effective in alleviating stress-associated mood disorders.

This study further revealed that the OVX mice exposed to stress displayed large weight gain and uterine shrinkage. Weight gain and shrinkage of the urogenital organs are the two major physical changes occurring during menopausal transition (31, 32). While long-term repeated E_2 treatment suppressed OVX+CUMS-induced weight gain, it also caused uterine hyperplasia that may be indicative of adenomyosis, uterine fibroids, ovarian cysts, and even endometrial cancer (33). Such side effect has been widely observed in hormone replacement therapy in menopause women (34). However, unlike E_2, JYP, in either low or high dose, did not influence changes in either body or uterine weight induced by OVX combined with CUMS. It seems that JYP has minor or even no effects on the uterus. It appears that, while JYP had comparable efficacy in improving estrogen deprivation-induced psychiatric symptoms, JYP may possess a better safety profile than estrogen therapy. Such therapeutic advantages also have been observed in

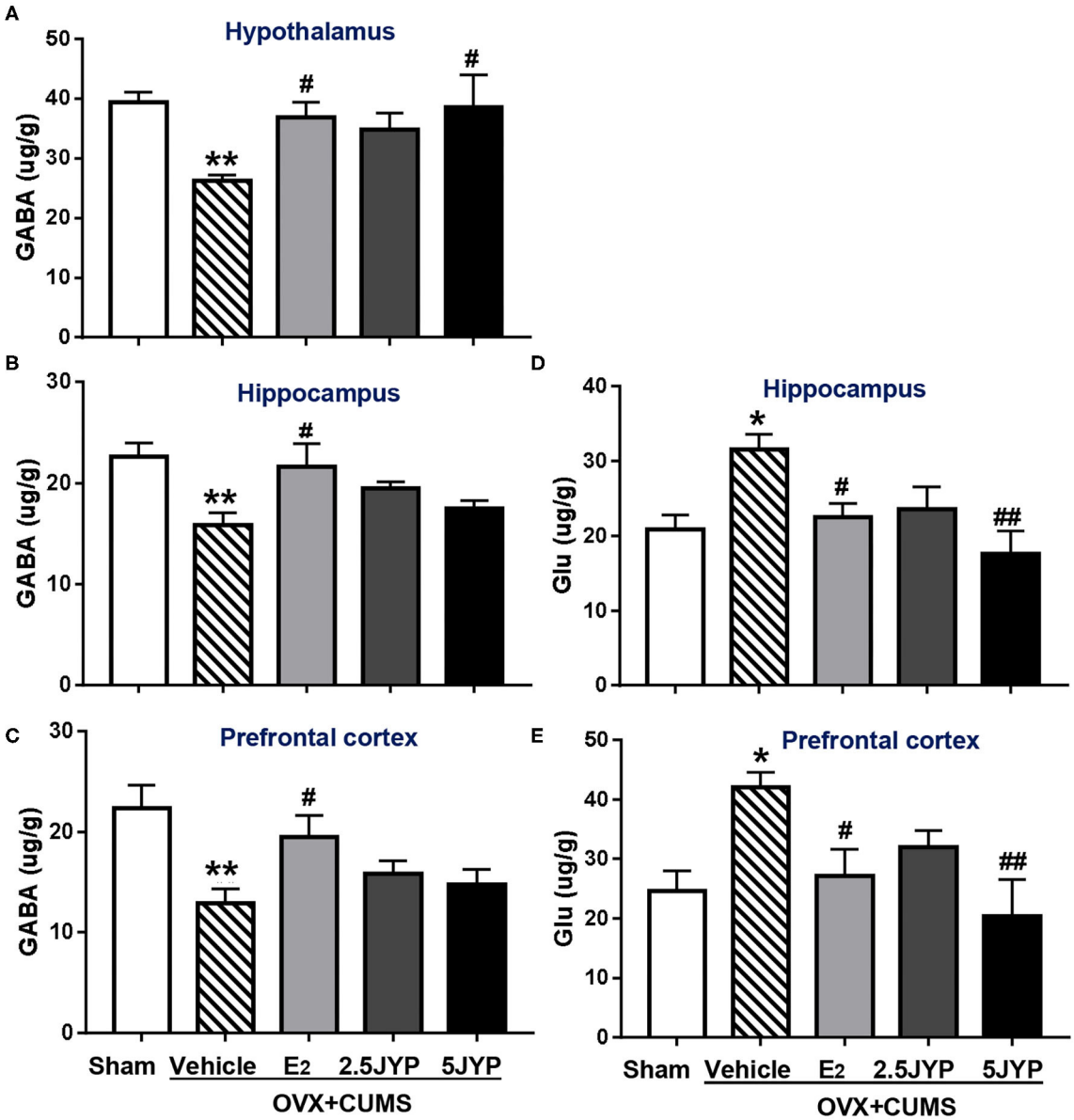

FIGURE 7 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on brain regional γ-Aminobutyric acid (GABA) and glutamate (Glu) contents. **(A)** GABA in hypothalamus; **(B)** GABA in hippocampus; **(C)** GABA in prefrontal cortex; **(D)** Glu in hippocampus; **(E)** Glu in prefrontal cortex. Data are expressed as mean ± SEM ($n = 5$–6) and examined with one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{*}P < 0.05$, $^{**}P < 0.01$ vs. sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. vehicle group.

several herbal medicines that contain JYP individual materials in OVX+CUMS mice (16, 17) and patients with menopause syndrome and hyperprolactinemia (35, 36).

Ovarian hormones regulate the response of the HPA axis to menstrual cycle-associated stress *via* brain GABAergic neuronal system (19). In this study, we found that ovarian hormone deprivation evoked strikingly elevated serum levels of FSH and LH, which are indicative of ovarian failure, but exhausted blood estradiol level and hypothalamic GABA contents. OVX mice with stress exposure also exhibited markedly elevated serum levels of corticosterone (CORT) and ACTH, the two key stress hormones that play the crucial roles in the pathogenesis of menopausal anxiety and mood symptoms (19, 37). These results support the notion that estrogen deprivation caused anxiety and mood disorders may be derived from fluctuations in female sex hormones that result in altered GABAergic regulation of the HPA axis (19, 37, 38). Chronic E_2 and JYP in particular the high dose almost completely reversed OVX+CUMS-induced elevated FSH, LH, CORT and ACTH to control levels, and restored the

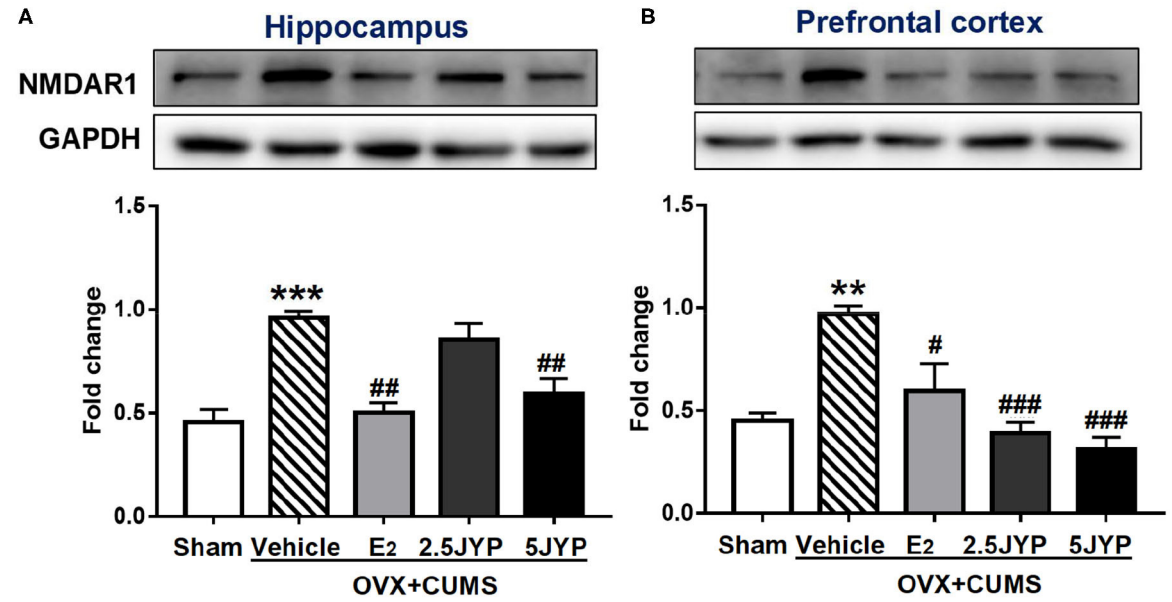

FIGURE 8 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on the expression of N-methyl-D-aspartate (NMDA) receptor unit 1 (NMDAR1) in the hippocampus **(A)** and prefrontal cortex **(B)**. Data are expressed as mean ± SEM ($n = 3$) and examined with one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. vehicle group.

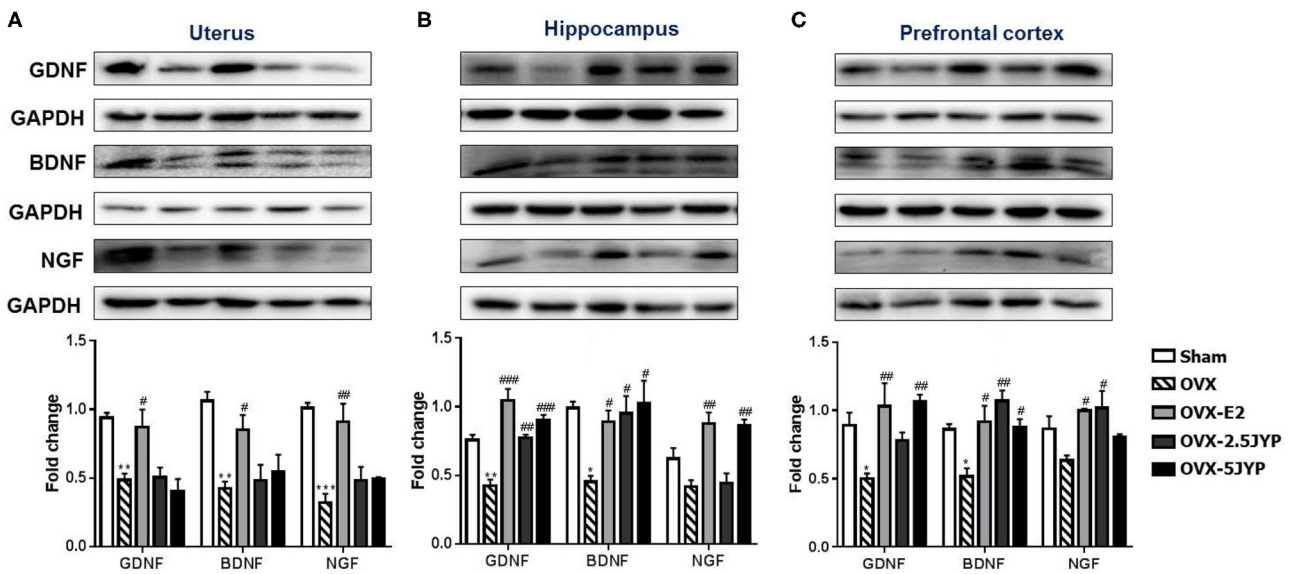

FIGURE 9 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on the expression of glial cell-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) in the uterus **(A)**, hippocampus **(B)**, and prefrontal cortex **(C)**. Data are expressed as mean ± SEM ($n = 3$) and examined using one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{*}P < 0.05$, $^{**}P < 0.01$, vs. sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. vehicle group.

hypothalamic GABA contents. However, unlike E2, JYP did not affect blood estradiol level. JYP appears to mainly modulate the upstream factors of the HPO axis and inhibit the HPA-axis hyperactivity by reinstating the hypothalamic GABAergic neuronal function.

GABA and glutamate are two key neurotransmitters that work together to maintain a balance between excitatory and inhibitory transmission in the brain (39). Dramatic fluctuations in the levels of ovarian hormones during menopause transition profoundly disturbs this functional balance, causing

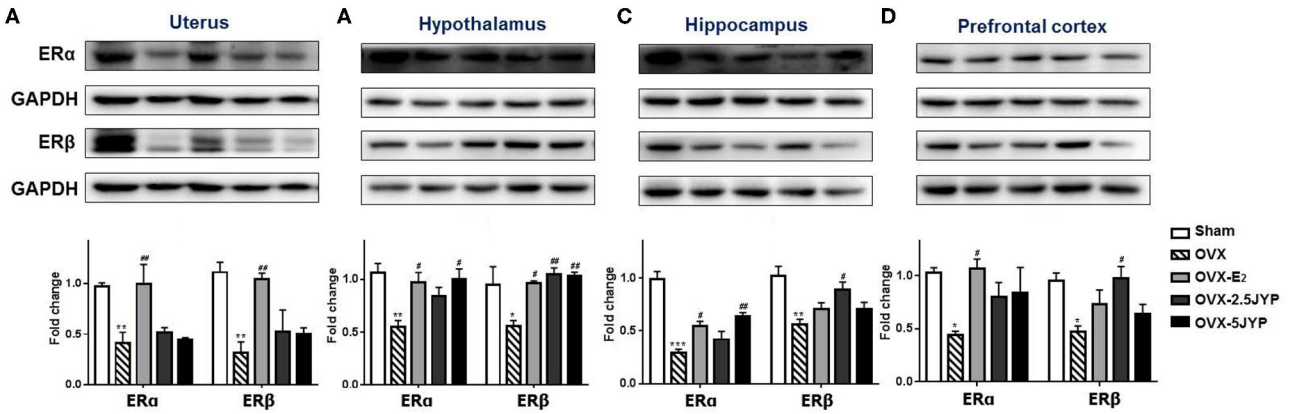

FIGURE 10 | Effects of 2.5 g/kg JYP (2.5 JYP), 5.0 g/kg JYP (5 JYP), and 0.3 mg/kg estradiol (E2) on the expression of ERα and ERβ in the uterus **(A)**, hypothalamus **(B)**, hippocampus **(C)**, and prefrontal cortex **(D)**. Data are expressed as mean ± SEM ($n = 3$) and examined with one-way analysis of variance (ANOVA), followed by *post hoc* between-group comparisons: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, vs. vehicle group.

pathological anxiety, mood, and cognitive disorders (40). This study revealed that, in addition to the hypothalamic GABA, ovarian hormone deprivation plus stress exposure also exhausted GABA contents in the hippocampus and prefrontal cortex, but largely restored the glutamate contents and expression of its receptor NMDR1 in the two brain regions, probably causing an imbalance of opposite effects of the two amino acid transmitters. Both E_2 and the high dose JYP entirely suppressed OVX+CUMS-induced elevation of glutamate contents and NMDR1 expression; however, unlike E_2, JYP had no significant effects on GABA contents in the two brain regions examined. It seems that JYP may dominantly modulate glutamatergic neuronal functions in the brain regions associated with learning and memory.

Neurotrophins play the crucial roles in the survival, maintenance, and regeneration of specific neurons as well as uterine growth and proliferation (41–43). NGF, GDNF, and BDNF are the three most abundant neurotrophins which widely exist in the adult brain and are highly expressed in the female reproductive system (44, 45). High level of peripheral NGF and BDNF has been proven to be associated with ovarian and breast cancer, polycystic ovarian syndrome (PCOS), and endometriosis (27, 46, 47). Similar to our previous studies (16, 17), this study confirmed that OVX+CUMS profoundly suppressed the expression of the three neurotrophins in the uterus, hippocampus, and prefrontal cortex, but not NGF in the two brain tissues, suggesting that ovarian hormone deprivation may evoke differential effects on brain neurotrophic systems. While the OVX+CUMS-induced decreases of the three neurotrophins in the two brain regions examined were entirely reversed by E_2 and either or both doses of JYP, E_2 additionally overturned and even enhanced the expression of the three neurotrophins in the uterus, but JYP did not. It therefore seems that increased risk of breast and endometrial cancer often occurred in estrogen therapy may be related to its enhancement effects on peripheral neurotrophins (48, 49), and the potential better safety profile of JYP observed in this study is, at least in part, derived from it tissue-specific effects on neurotrophins, particularly without effects on uterine neurotrophins.

The estrogen receptors, ERα and ERβ, are closely associated with the pathophysiology of menopause-related metabolic, neurological, and psychiatric disorders (50–52). The two subtypes have distinct anatomical distribution patterns, different physiological processes in the brain and peripheral organs, and even counteract each other (53, 54). There have been contradictory studies on the effects of OVX on the expression of estrogen receptors in different brain regions (54–56). In this study, we revealed that the removal of the ovaries caused a widespread suppression of the expression of the two receptor subtypes across the brain regions examined and the uterus. Chronic E_2 reversed the OVX+CUMS-induced suppression of the two subtype expression in the uterus, ERα in all the three brain regions, and ERβ in the hypothalamus, but failed to reverse hippocampal and prefrontal ERβ expression. The high dose JYP reversed the OVX+CUMS-induced decreases of hypothalamic and hippocampal ERα. Either or both doses of JYP also prevented ERβ expression in the three brain regions from a combination of OVX and CUMS. However, either dose of JYP had no significant effects on OVX+CUMS-induced changes in uterine ERα and ERβ. These results demonstrated that, like the tissue-specific effects of JYP on neurotrophins, the effects of JYP on the estrogen receptors also seem to be tissue-specific, i.e., JYP may have brain-predominant effects in modulating estrogen receptors, with minor effects or even without effects on estrogen receptors of female peripheral reproductive organs. The aberrant expression of estrogen receptors has been suggested to be associated with ovarian cancer, breast cancer, and other human cancers (57–59). Therefore, the minor and even no effects of JYP on uterine estrogen receptors may be an additional factor contributing to the potential better safety profile of JYP observed in this study.

LIMITATIONS

Several limitations of this study should be considered. First, JYP as a whole preparation was evaluated in this study. We were unable to determine which individual materials or constituents play the principal roles in the psychotropic effects of JYP observed in this study. Further characterization of bioactive constituents of JYP could help better understand a phytochemical profile of this herbal agent, probably resulting in the discovery of novel constituents for treating estrogen deprivation-association disorders in particular psychiatric symptoms. Second, this study did not directly examine the beneficial effects of JYP in improving other estrogen deprivation-related symptoms, such as hot flush and night sweats. Previous studies have revealed the benefits of several herbal preparations in reducing hot flush in menopausal women (5). Finally, we did not consider the effects of the ovarian cycle in the sham mice as control group. Nevertheless, one recent study has shown that there were only differences in the severity of anxiety- and depression-like behavior in female C57BL mice across the estrous cycle (60). It means that we took "average" levels of anxiety and depression across the estrous cycle to serve as control.

Collectively, JYP has comparable efficacy in reducing psychiatric disorders observed in OVX mice exposed to stress with a better safe profile. The therapeutic advantages of JYP may be associated with certain uterus-brain mechanisms distinct from estrogen therapy. It deserves a clinical assessment as an alternative therapy in menopausal women with apparent psychiatric symptoms.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong (CULATR 3812-15).

AUTHOR CONTRIBUTIONS

X-DZ and Z-JZ were involved in the conception and design of the study, data analysis, and preparation of the manuscript. X-DZ, X-JY, YZ, and Z-SQ developed and conducted experiments. WS provided consultants and technical support. GC provided critical consultants on experiments and critical comments on the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by Henan Taifeng Biological Technology Corporation Limited (TF20190301, Z-JZ), the National Key R&D Program of China (2018YFC1705801, Z-JZ), and General Research Fund (GRF) of Research Grant Council of Hong Kong (17115017, Z-JZ). The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

REFERENCES

1. Monteleone P, Mascagni G, Giannini A, Genazzani AR, Simoncini T. Symptoms of menopause - global prevalence, physiology and implications. *Nat Rev Endocrinol*. (2018) 14:199–215. doi: 10.1038/nrendo.2017.180
2. Soares CN. Depression and menopause: an update on current knowledge and clinical management for this critical window. *Med Clin North Am*. (2019) 103:651–67. doi: 10.1016/j.mcna.2019.03.001
3. Fernandez E, Gallus S, Bosetti C, Franceschi S, Negri E, La Vecchia C. Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. *Int J Cancer*. (2003) 105:408–12. doi: 10.1002/ijc.11083
4. Ji Y, Wang Z, Chen W, Lu L, Xie W, Deng J. Research progress of Jieyu pill in treating depressive disorder. *Chin J Drug Depend*. (2019) 28:329–34. doi: 10.13936/j.cnki.cjdd1992.2019.05.002
5. Zhu X, Liew Y, Liu ZL. Chinese herbal medicine for menopausal symptoms. *Cochrane Database Syst Rev*. (2016) 3:CD009023. doi: 10.1002/14651858.CD009023.pub2
6. Wang L, Huang S, Pan J, Huang C, Yang X. Systematic review of randomized controlled trials of Jieyu Pill in the treatment of depression. *Med Recapitul*. (2017) 23:2046–51. doi: 10.3936/j.issn.1006-2084.2017.10.036
7. Ma R, Yao HY, Ku BS, Qian RQ, Zang XL, Guo JG. Difference of the antidepressant effects between Jieyu wan and its components in mouse models of depression. *Chin J Clin Rehabil*. (2005) 9:115–6.
8. Ma R, Yao HY, Ku BS, Qian RQ. The experimental research on anxiolytic and hypnogenetic effects of Jieyuwan. *Chin J Exp Trad Med Formul*. (2006) 12:50–3.
9. Ma R, Qian RQ, Yao HY, Ku BS. Preliminary research on mechanism of antidepressant effect of Jieyuwan. *Chin J Exp Trad Med Formul*. (2010) 16:168–72.
10. Shi GL, Ku BS, Yao HY, Xu Y. Effects of jieyuwan on behavior and monoamine oxidase activity in different brain regions of chronic stress rats. *Chin J Behav Med Sci*. (2007) 16:393–5.
11. Shi GL, Ku BS, Yao HY. Effects of jieyuwan on HPA axis and immune system in chronic stress models in rats. *Zhongguo Zhong Yao Za Zhi*. (2007) 32:1551–4.
12. Pruessner JC, Dedovic K, Pruessner M, Lord C, Buss C, Collins L, et al. Stress regulation in the central nervous system: evidence from structural and functional neuroimaging studies in human populations – 2008 Curt Richter Award Winner. *Psychoneuroendocrinology*. (2010) 35:179–91. doi: 10.1016/j.psyneuen.2009.02.016
13. Wang D, Wong HK, Zhang L, McAlonan GM, Wang XM, Sze SC, et al. Not only dopamine D2 receptors involved in Peony-Glycyrrhiza Decoction, an herbal preparation against antipsychotic-associated hyperprolactinemia. *Prog Neuropsychopharmacol Biol Psychiatry*. (2012) 39:332–8. doi: 10.1016/j.pnpbp.2012.07.005
14. Huang H, Zhao J, Jiang L, Xie Y, Xia Y, Lv R, et al. Paeoniflorin improves menopause depression in ovariectomized rats under chronic unpredictable mild stress. *Int J Clin Exp Med*. (2015) 8:5103–11.
15. Chen XQ, Chen SJ, Liang WN, Wang M, Li CF, Wang SS, et al. Saikosaponin A attenuates perimenopausal depression-like symptoms by chronic unpredictable mild stress. *Neurosci Lett*. (2018) 662:283–9. doi: 10.1016/j.neulet.2017.09.046
16. Zhou XD, Zheng Y, Sharma R, Zhang ZJ. Total polysaccharides of lily bulb ameliorate menopause-like behavior in ovariectomized mice: multiple

mechanisms distinct from estrogen therapy. *Oxid Med Cell Longev*. (2019) 2019:6869350. doi: 10.1155/2019/6869350
17. Zhou XD, Shi DD, Wang HN, Tan QR, Zhang ZJ. Aqueous extract of lily bulb ameliorates menopause-like behavior in ovariectomized mice with novel brain-uterus mechanisms distinct from estrogen therapy. *Biomed Pharmacother*. (2019) 117:109114. doi: 10.1016/j.biopha.2019.109114
18. Gava G, Orsili I, Alvisi S, Mancini I, Seracchioli R, Meriggiola MC. Cognition, mood and sleep in menopausal transition: the role of menopause hormone Therapy. *Medicina*. (2019) 55:668. doi: 10.3390/medicina55100668
19. Gordon JL, Girdler SS, Meltzer-Brody SE, Stika CS, Thurston RC, Clark CT, et al. Ovarian hormone fluctuation, neurosteroids, and HPA axis dysregulation in perimenopausal depression: a novel heuristic model. *Am J Psychiatry*. (2015) 172:227–36. doi: 10.1176/appi.ajp.2014.14070918
20. Smith CC, Vedder LC, McMahon LL. Estradiol and the relationship between dendritic spines, NR2B containing NMDA receptors, and the magnitude of long-term potentiation at hippocampal CA3-CA1 synapses. *Psychoneuroendocrinology*. (2009) 34:S130–42. doi: 10.1016/j.psyneuen.2009.06.003
21. Engler-Chiurazzi E, Tsang C, Nonnenmacher S, Liang WS, Corneveaux JJ, Prokai L, et al. Tonic Premarin dose-dependently enhances memory, affects neurotrophin protein levels and alters gene expression in middle-aged rats. *Neurobiol Aging*. (2011) 32:680–97. doi: 10.1016/j.neurobiolaging.2009.09.005
22. Van Kempen TA, Gorecka J, Gonzalez AD, Soeda F, Milner TA, Waters EM. Characterization of neural estrogen signaling and neurotrophic changes in the accelerated ovarian failure mouse model of menopause. *Endocrinology*. (2014) 155:3610–23. doi: 10.1210/en.2014-1190
23. Souza VR, Mendes E, Casaro M, Antiorio ATFB, Oliveira FA, Ferreira CM. Description of ovariectomy protocol in mice. *Methods Mol Biol*. (2019) 1916:303–9. doi: 10.1007/978-1-4939-8994-2_29
24. Matsumoto YK, Kasai M, Tomihara K. The enhancement effect of estradiol on contextual fear conditioning in female mice. *PLoS ONE*. (2018) 13:e0197441. doi: 10.1371/journal.pone.0197441
25. Wang Y, Shoemaker R, Thatcher SE, Batifoulier-Yiannikouris F, English VL, Cassis LA. Administration of 17β-estradiol to ovariectomized obese female mice reverses obesity-hypertension through an ACE2-dependent mechanism. *Am J Physiol Endocrinol Metab*. (2015) 308:E1066–75. doi: 10.1152/ajpendo.00030.2015
26. Huo T, Chang B, Zhang Y, Chen Z, Li W, Jiang H. Alteration of amino acid neurotransmitters in brain tissues of immature rats treated with realgar. *J Pharm Biomed Anal*. (2012) 57:120–4. doi: 10.1016/j.jpba.2011.08.032
27. Streiter S, Fisch B, Sabbah B, Ao A, Abir R. The importance of neuronal growth factors in the ovary. *Mol Hum Reprod*. (2016) 22:3–17. doi: 10.1093/molehr/gav057
28. Jing Q, Ren L, Deng X, Zhang N, Fu M, Wang G, et al. Electroacupuncture promotes neural proliferation in hippocampus of perimenopausal depression rats *via* Wnt/β-catenin signaling pathway. *J Acupunct Meridian Stud*. (2020) 13:94–103. doi: 10.1016/j.jams.2020.03.065
29. Koshkina A, Dudnichenko T, Baranenko D, Fedotova J, Drago F. Effects of vitamin D3 in long-term ovariectomized rats subjected to chronic unpredictable mild stress: BDNF, NT-3, and NT-4 implications. *Nutrients*. (2019) 11:1726. doi: 10.3390/nu11081726
30. Zhang K, Wang Z, Pan X, Yang J, Wu C. Antidepressant-like effects of Xiaochaihutang in perimenopausal mice. *J Ethnopharmacol*. (2020) 248:112318. doi: 10.1016/j.jep.2019.112318
31. Calleja-Agius J, Brincat MP. The urogenital system and the menopause. *Climacteric*. (2015) 18:18–22. doi: 10.3109/13697137.2015.1078206
32. Kapoor E, Collazo-Clavell ML, Faubion SS. Weight gain in women at midlife: a concise review of the pathophysiology and strategies for management. *Mayo Clin Proc*. (2017) 92:1552–8. doi: 10.1016/j.mayocp.2017.08.004
33. Genc M, Genc B, Sahin N, Celik E, Turan GA, Gur EB, et al. Endometrial pathology in postmenopausal women with no bleeding. *Climacteric*. (2015) 18:241–5. doi: 10.3109/13697137.2014.944152
34. Edey KA, Rundle S, Hickey M. Hormone replacement therapy for women previously treated for endometrial cancer. *Cochrane Database Syst Rev*. (2018) 5:CD008830. doi: 10.1002/14651858.CD008830.pub3
35. Man SC, Li XB, Wang HH, Yuan HN, Wang HN, Zhang RG, et al. Peony-Glycyrrhiza decoction for antipsychotic-related hyperprolactinemia in women with schizophrenia: a randomized controlled trial. *J Clin Psychopharmacol*. (2016) 36:572–9. doi: 10.1097/JCP.0000000000000607
36. Yuan HN, Wang CY, Sze CW, Tong Y, Tan QR, Feng XJ, et al. A randomized, crossover comparison of herbal medicine and bromocriptine against risperidone-induced hyperprolactinemia in patients with schizophrenia. *J Clin Psychopharmacol*. (2008) 28:264–370. doi: 10.1097/JCP.0b013e318172473c
37. Weiser MJ, Handa RJ. Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic-pituitary-adrenal axis *via* estrogen receptor alpha within the hypothalamus. *Neuroscience*. (2009) 159:883–95. doi: 10.1016/j.neuroscience.2008.12.058
38. Barbaccia ML, Lello S, Sidiropoulou T, Cocco T, Sorge RP, Cocchiarale A, et al. Plasma 5alpha-androstane-3alpha,17betadiol, an endogenous steroid that positively modulates GABA(A) receptor function, and anxiety: a study in menopausal women. *Psychoneuroendocrinology*. (2000) 25:659–75. doi: 10.1016/S0306-4530(00)00017-2
39. Wu LJ, Kim SS, Zhuo M. Molecular targets of anxiety: from membrane to nucleus. *Neurochem Res*. (2008) 33:1925–32. doi: 10.1007/s11064-008-9679-8
40. Del Río JP, Alliende MI, Molina N, Serrano FG, Molina S, Vigil P. Steroid hormones and their action in women's brains: the importance of hormonal balance. *Front Public Health*. (2018) 6:141. doi: 10.3389/fpubh.2018.00141
41. Mitre M, Mariga A, Chao MV. Neurotrophin signalling: novel insights into mechanisms and pathophysiology. *Clin Sci*. (2017) 131:13–23. doi: 10.1042/CS20160044
42. Maranesi M, Parillo F, Leonardi L, Rebollar PG, Alonso B, Petrucci L, et al. Expression of nerve growth factor and its receptors in the uterus of rabbits: functional involvement in prostaglandin synthesis. *Domest Anim Endocrinol*. (2016) 56:20–8. doi: 10.1016/j.domaniend.2016.02.001
43. Wessels JM, Wu L, Leyland NA, Wang H, Foster WG. The brain-uterus connection: brain derived neurotrophic factor (BDNF) and its receptor (Ntrk2) are conserved in the mammalian uterus. *PloS ONE*. (2014) 9:e94036. doi: 10.1371/journal.pone.0094036
44. Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. *Pharmacol Ther*. (2013) 138:155–75. doi: 10.1016/j.pharmthera.2013.01.004
45. Chang HM, Wu HC, Sun ZG, Lian F, Leung PCK. Neurotrophins and glial cell line-derived neurotrophic factor in the ovary: physiological and pathophysiological implications. *Hum Reprod Update*. (2019) 25:224–42. doi: 10.1093/humupd/dmy047
46. Dolle L, Adriaenssens E, EI Yazidi-Belkoura I, Le Bourhis X, Nurcombe V, Hondermarck H. Nerve growth factor receptors and signaling in breast cancer. *Curr Cancer Drug Targets*. (2004) 4:463–70. doi: 10.2174/1568009043332853
47. Ding S, Zhu T, Tian Y, Xu P, Chen Z, Huang X, et al. Role of brain-derived neurotrophic factor in endometriosis pain. *Reprod Sci*. (2018) 25:1045–57. doi: 10.1177/1933719117732161
48. Pizot C, Boniol M, Mullie P, Koechlin A, Boniol M, Boyle P, et al. Physical activity, hormone replacement therapy and breast cancer risk: a meta-analysis of prospective studies. *Eur J Cancer*. (2016) 52:138–54. doi: 10.1016/j.ejca.2015.10.063
49. Sjogren LL, Morch LS, Lokkegaard E. Hormone replacement therapy and the risk of endometrial cancer: a systematic review. *Maturitas*. (2016) 91:25–35. doi: 10.1016/j.maturitas.2016.05.013
50. Crider A, Pillai A. Estrogen signaling as a therapeutic target in neurodevelopmental disorders. *J Pharmacol Exp Ther*. (2017) 360:48–58. doi: 10.1124/jpet.116.237412
51. Deroo BJ, Korach KS. Estrogen receptors and human disease. *J Clin Invest*. (2006) 116:561–70. doi: 10.1172/JCI27987
52. Vargas KG, Milic J, Zaciragic A, Wen KX, Jaspers L, Nano J, et al. The functions of estrogen receptor beta in the female brain: a systematic review. *Maturitas*. (2016) 93:41–57. doi: 10.1016/j.maturitas.2016.05.014
53. Morani A, Warner M, Gustafsson JA. Biological functions and clinical implications of oestrogen receptors alfa and beta in epithelial tissues. *J Intern Med*. (2008) 264:128–42. doi: 10.1111/j.1365-2796.2008.01976.x
54. Yamaguchi N, Yuri K. Estrogen-dependent changes in estrogen receptor-beta mRNA expression in middle-aged female rat brain. *Brain Res*. (2014) 1543:49–57. doi: 10.1016/j.brainres.2013.11.010

55. Jin M, Jin F, Zhang L, Chen Z, Huang H. Two estrogen replacement therapies differentially regulate expression of estrogen receptors alpha and beta in the hippocampus and cortex of ovariectomized rat. *Brain Res Mol Brain Res.* (2005) 142:107–14. doi: 10.1016/j.molbrainres.2005.09.013
56. Wilson ME, Rosewell KL, Kashon ML, Shughrue PJ, Merchenthaler I, Wise PM. Age differentially influences estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) gene expression in specific regions of the rat brain. *Mech Ageing Dev.* (2002) 123:593–601. doi: 10.1016/S0047-6374(01)00406-7
57. Olsen L, Rasmussen HB, Hansen T, Bagger YZ, Tanko LB, Qin G, et al. Estrogen receptor alpha and risk for cognitive impairment in postmenopausal women. *Psychiatr Genet.* (2006) 16:4. doi: 10.1097/01.ypg.0000194445.27555.71
58. Pereira LM, Bastos CP, de Souza JM, Ribeiro FM, Pereira GS. Estradiol enhances object recognition memory in Swiss female mice by activating hippocampal estrogen receptor alpha. *Neurobiol Learn Mem.* (2014) 114:1–9. doi: 10.1016/j.nlm.2014.04.001
59. Xu Y, Sheng H, Bao Q, Wang Y, Lu J, Ni X. NLRP3 inflammasome activation mediates estrogen deficiency-induced depression- and anxiety-like behavior and hippocampal inflammation in mice. *Brain Behav Immun.* (2016) 56:175–86. doi: 10.1016/j.bbi.2016.02.022
60. Yohn CN, Shifman S, Garino A, Diethorn E, Bokka L, Ashamalla SA, et al. Fluoxetine effects on behavior and adult hippocampal neurogenesis in female C57BL/6J mice across the estrous cycle. *Psychopharmacology*. (2020) 237:1281–90. doi: 10.1007/s00213-020-05456-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 27 November 2020
doi: 10.3389/fpsyt.2020.572533

Curcumin in Depression: Potential Mechanisms of Action and Current Evidence—A Narrative Review

Tahiana Ramaholimihaso, Fayçal Bouazzaoui and Arthur Kaladjian*

Psychiatry Department, University Hospital, Reims, France

Major depressive disorder (MDD) is one of the most prevalent and debilitating disorders. Current available treatments are somehow limited, so alternative therapeutic approaches targeting different biological pathways are being investigated to improve treatment outcomes. Curcumin is the main active component in the spice turmeric that has been used for centuries in Ayurvedic medicine to treat a variety of conditions, including anxiety and depressive disorders. In the past decades, curcumin has drawn researchers' attention and displays a broad range of properties that seem relevant to depression pathophysiology. In this review, we break down the potential mechanisms of action of curcumin with emphasis on the diverse systems that can be disrupted in MDD. Curcumin has displayed, in a number of studies, a potency in modulating neurotransmitter concentrations, inflammatory pathways, excitotoxicity, neuroplasticity, hypothalamic–pituitary–adrenal disturbances, insulin resistance, oxidative and nitrosative stress, and endocannabinoid system, all of which can be involved in MDD pathophysiology. To date, a handful of clinical trials have been published and suggest a benefit of curcumin in MDD. With evidence that is progressively growing, curcumin appears as a promising alternative option in the management of MDD.

Keywords: curcumin, turmeric, depression, mechanism of action, neuroprotective agent, inflammation, NLRP3, dietary supplement(s)

OPEN ACCESS

Edited by:
Zhang-Jin Zhang,
The University of Hong Kong,
Hong Kong

Reviewed by:
Kurt Leroy Hoffman,
Autonomous University of Tlaxcala, Mexico
Stefania Schiavone,
University of Foggia, Italy

****Correspondence:***
Tahiana Ramaholimihaso
tramaholimihaso@chu-reims.fr

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *14 June 2020*
Accepted: *28 October 2020*
Published: *27 November 2020*

Citation:
Ramaholimihaso T, Bouazzaoui F and Kaladjian A (2020) Curcumin in Depression: Potential Mechanisms of Action and Current Evidence—A Narrative Review. Front. Psychiatry 11:572533. doi: 10.3389/fpsyt.2020.572533

INTRODUCTION

Major depressive disorder (MDD) is the most common psychiatric disorder (1). In 2017, the World Health Organization announced that indeed depression was the leading cause of disability and ill health worldwide, with more than 300 million people living with depression (2).

Major depressive disorder is characterized, by the 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), by a depressed mood, loss of energy, a markedly diminished interest or pleasure, psychomotor retardation (or agitation), feelings of worthlessness or excessive or inappropriate guilt, insomnia or hypersomnia, significant weight loss, diminished ability to concentrate, and recurrent thoughts of death.

The MDD pathophysiology is recognized as being complex, ranging from genetic predispositions to interactions between environmental factors, and multiple biological systems. Among those, we can name disruption in the monoamine systems, which has been the prime hypothesis in MDD pathophysiology, as well as neurodegenerative processes, notably through hypothalamic–pituitary–adrenal (HPA) changes or inflammatory processes (3).

The management of MDD has long been based on psychological interventions, and although guidelines presently suggest to practitioners to combine psychological interventions and pharmacological treatments, the discovery of antidepressant medications has made them a staple in MDD management. However, as decades have gone by, the development of antidepressants somehow reached a plateau. Although they proved to be effective (4), they are still not optimal as we can see remission rates of around 30% after a first-line selective serotonin reuptake inhibitor therapy (5) and a cumulative remission rate of only 70% after a fourth line of pharmacological treatment (6). Moreover, lack of adherence to antidepressant medications is frequently observed, the presence of side effects being a major cause of this non-compliance.

The problem of treatment resistance has underscored the need for new management strategies, and as concepts and views on MDD pathophysiology have evolved, so have therapeutic alternatives. For this reason, we could see a growth of neurostimulation in the past decades (7) as well as the role of physical activity in the management of MDD (8, 9). Consequently, interest in complementary and alternative medicine has been growing.

As for alternative medicines, anti-inflammatory, antioxidants, and neuroprotective compounds thought to counteract the degenerative processes frequently associated with MDD have been a new concern of exploration for treatment or adjuvant therapy. Notably, the Canadian Network for Mood and Anxiety Treatments clinical guidelines recently inserted some alternative medicines such as omega 3, acetyl L carnitine, Lavandula, or saffron among adjunctive treatments for the management of MDD in adults (9).

Turmeric (*Curcuma longa*) is a yellow spice, part of the ginger family (*Zingiberacear*). It has been empirically used for centuries in Ayurvedic and traditional Chinese medicine in a wide variety of diseases and conditions (10). Research conducted in the last half century has revealed that the active compounds of turmeric were curcuminoids, which are polyphenolic pigments that give turmeric its yellowish color. Curcumin is the primary curcuminoid and main active component in turmeric and the compound for which most studies have been done.

In the past decades, there has been a surge of interest in curcumin as evidence about its efficacy in a wide variety of diseases is growing, including cardiovascular, autoimmune, and neurodegenerative diseases as well as diabetes and cancers (11, 12).

Thus, curcumin displays a broad range of properties that are relevant in the pathophysiology of depression. It has been demonstrated to possess an antidepressant activity in various animal models as well as in clinical trials. A dozen randomized controlled clinical trials have indeed been conducted (13), altogether suggesting that curcumin may be effective as a treatment (or adjunct treatment) of MDD *via* multiple mechanisms of action.

We will discuss in this review the potential mechanisms of action underlying the efficacy of curcumin in depression, giving us an overview of the current concepts in the pathophysiology of depression, and we will also discuss the existing evidence of the efficacy of curcumin in the treatment of depression.

CURCUMIN AND NEUROTRANSMITTERS

Curcumin and Monoamines

The monoamine deficiency theory has been the primal causative model of major depression and has accompanied the rise of antidepressants. The current pharmacologic arsenal is based on this theory as the main antidepressants, such as selective serotonin reuptake inhibitors, serotonin–noradrenaline reuptake inhibitors, and monoamine oxidase (MAO) inhibitors, are designed to increase the availability of monoamines (serotonin, noradrenaline, dopamine). According to this theory, it is thought that the depletion of these neurotransmitters in the central nervous system (CNS) constitutes the core of depression pathophysiology (14).

Evidence of curcumin being able to influence levels of monoamines in the central nervous system has emerged from animal and *in vitro* studies conducted over the past two decades. In a study, Bhutani et al. (15) showed that curcumin reversed the depressive-like behavior induced by chronic stress on mice and enhanced the serotoninergic and dopaminergic transmission alongside an inhibition of the MAO-A. Kulkarni et al. (16) had similar results in a study on rats in which curcumin dose-dependently increased serotonin and dopamine as well as inhibited the monoamine oxidase enzymes. More recent studies also showed that curcumin could elevate norepinephrine, serotonin, and dopamine in the frontal cortex, hippocampus, and striatum in rats (17–22).

Wang et al. (23), in an animal study on the effects of curcumin on serotonin (5-HT) receptors, stated that the antidepressant-like effect that they could notice was related to the serotoninergic system, possibly due to an interaction with 5-HT1A/1B and 5-HT2C receptors. Similarly, Xu et al. (24) conducted a study in which the antidepressant effect of curcumin seemed related to the expression of 5-HT1A receptors, as 5-HT1A receptor mRNA levels across all hippocampal subfields were increased after curcumin administration. These results have been replicated by more recent studies like by Li et al. (25) or Lian et al. (26) in which they noticed an upregulation of 5-HT1A receptor expression after curcumin administration in chronically stressed mice and a prevention of its antidepressant-like effect with the administration of a 5-HT1B receptor antagonist.

Taken together, these animal studies strongly support the hypothesis that curcumin can modulate monoaminergic systems in pre-clinical rodent models.

Curcumin and Glutamate

In 1959, Crane (27) made an observation that the anti-tuberculosis agent d-cycloserine, which acts as a glutamatergic modulator, could possess an antidepressant effect, yet this observation gained little attention during the following decades until 2000, when Berman *et al.* (28) reported that ketamine, an antiglutamatergic anesthetic agent, could induce a rapid antidepressant effect in severely depressed patients. This triggered vigorous research to understand how

modulating glutamate signaling could be beneficial in depression and to develop new antidepressants targeting glutamate neurotransmission. It is worth noting that ketamine has now proven its efficacy in treating depression (29).

Glutamate is the major excitatory neurotransmitter in the central nervous system and has a vital role in the regulation of synaptic plasticity. By binding to its receptors, notably NMDA receptors (NMDAR), glutamate will modulate post-synaptic plasticity as well as exert longer-term changes in synaptic strength and neuroplasticity. However, the abnormal elevation of NMDAR signaling leads to deleterious effects on neurons (30). As such, excitotoxicity is the phenomenon associated with excessive glutamate release and subsequent overactivation of NMDA receptors and has long been described (31).

A number of reports suggest that the glutamate system and excitotoxicity are involved in MDD pathophysiology. For example, glutamate levels have been shown to be elevated in the plasma, cerebrospinal fluid, and brains of patients with depression (32).

Chronic stress is believed to lead to detrimental changes within glutamate synapses, including reduced extracellular glutamate clearance by glia, especially in the pre-frontal cortex, leading to increased extrasynaptic glutamate levels and excitotoxicity, potentially contributing to synaptic loss (33). Hence, in a study conducted by Lin et al. (34), curcumin was shown to inhibit the liberation of glutamate in the rat pre-frontal cortex, counteracting this phenomenon in a similar way (yet greater) to that of fluoxetine (suggesting that "classic" antidepressants like fluoxetine also act on the glutamate pathway).

A study by Zhang et al. (35) suggested that the antiglutamatergic action of curcumin could be mediated by the GluN2B subunit of NMDA receptors. Also, they noted that the administration of a sub-effective (that did not produce antidepressant effect when administered alone) dose of curcumin produced an antidepressant-like effect when paired with a sub-effective dose of fluoxetine, leading to the hypothesis of a synergistic interaction between NMDA and 5-HT receptors. These results have been clarified by further *in vitro* studies showing that curcumin could reverse glutamate-induced neurotoxicity on hippocampal cells and downregulate the expression of the GluN2B subunit of NMDA receptors (36, 37). The antagonization of the GluN2B subunit seems to have a crucial role in the effect of anti-NMDAR drugs such as ketamine, as the activation of this subunit inhibits the synthesis of certain synaptic proteins, such as brain-derived neurotrophic factor (BDNF), thus altering synaptic function (38). Hence, the antagonization of GluN2B subunit permits an enhancement of synaptic function. For instance, memantine, which is also an antagonist of NMDA receptors, after acute administration, had no effect on hippocampal BDNF and failed to show immediate antidepressant behavioral effects in an animal depression model (39). However, the prolonged administration of memantine was associated with an increase in BDNF levels and hippocampal cell proliferation while displaying antidepressant-like effects in other animal studies (40, 41). In studies displaying the action of curcumin on the glutamatergic system, these effects were associated with increased levels of BDNF (23, 42) that might account for its antidepressant efficacy.

Collectively, those results suggest that an inhibitory effect of curcumin on excitotoxicity may be one of the mechanisms underlying its antidepressant effects.

DEPRESSION, INFLAMMATION, AND CURCUMIN

In the past decades, as the monoamine depletion theory has been the prime model of depression pathophysiology, other hypotheses have emerged. One of them implies that inflammation has a key role in depression pathophysiology. This hypothesis was prompted by the comparison we can make between "sickness behavior" and depression symptoms like anorexia, reduction of locomotor activity, anhedonia, and cognitive disturbances (43) which can be found in both conditions, and some studies showed that these types of symptoms in depression were positively correlated with C-reactive protein levels (CRP) (44, 45). Furthermore, some reports pointed the elevation of inflammatory cytokines [mainly interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α)] in depression (46–48). A meta-analysis displaying a reduction of inflammatory cytokine levels after antidepressant medication administration brought further support for the relationship between depression and inflammation (49). There have also been studies showing that cytokines or CRP levels could predict the antidepressant effect of the therapeutics used, including antidepressant medication (50, 51), physical exercise (52), or electroconvulsive therapy (53). While the meta-analysis performed did not show increased inflammation in every patient, its presence may be relevant to a subset of patients (45, 54), especially since it can guide the therapeutics.

Curcumin and Immunoinflammatory Pathways

As stated in the introduction, most of the first studies trying to unravel the beneficial effects of curcumin came from research on cancer and inflammatory diseases, and many anti-inflammatory effects of curcumin are now being acknowledged (55). A number of studies have indeed shown that curcumin could inhibit TNF production by macrophages and downregulate its expression by modulation of its transcription factors. Notably, it has been shown that curcumin was able to down-modulate the activation of nuclear factor kappa beta (NF-κβ) (56).

A study conducted by Wang et al. (57) in 2014 has reported the effects of curcumin on inflammation and depressive symptomatology in mice treated with lipopolysaccharide (LPS). This study showed that administrating curcumin reverted the depressive-like behavior and attenuated LPS-induced microglial activation and overproduction of pro-inflammatory cytokine (interleukin-1β and tumor necrosis factor-α). It could also inhibit LPS-induced NF-κβ activation in the hippocampus and pre-frontal cortex (PFC). In addition, curcumin could counteract the

increase in the levels of nitric oxide synthase and cyclooxygenase-2 mRNA in the hippocampus and pre-frontal cortex. Jangra et al. (58) reported similar results, as the administration of curcumin in LPS-treated mice improved the depressive-like symptoms, reversed the depletion of glutathione level in the hippocampus, and decreased the level of pro-inflammatory cytokines (IL-1β and TNF-α) in the hippocampus.

Fan et al. (59) showed that chronic unpredictable mild stress exposure in mice induced microglia activation and overexpression of the cytokines IL-1β, IL-6, and TNF-α within the medial pre-frontal cortex, effects which were paralleled with neuronal structural changes. They showed that curcumin administration produced antidepressant-like actions and reversed the inflammatory responses and neuronal structural abnormalities, thus concluding that inhibiting the IL-1β pathway could account for curcumin efficacy.

With regards to studies of human patients, a clinical trial conducted by Yu et al. (60) showed that curcumin decreased the inflammatory cytokines IL-1β and TNF-α in depressed patients in comparison with the placebo group. Moreover, it has been shown in other clinical trials that curcumin can lower TNF-α, IL-6, and CRP levels in patients' plasma (56, 61, 62).

All in all, what we can draw from these studies is that curcumin permits a decrease in inflammation, acting on pro-inflammatory cytokine pathways, notably TNF-α, IL-6, and IL-1β in the hippocampus and pre-frontal cortex.

Curcumin and Inflammasome Activation

Clinical depression is characterized by inflammatory cascades as indicated by elevated concentrations of pro-inflammatory cytokines in the serum and in the CNS tissue. Given the known action of curcumin on some immunoinflammatory pathways, the pivotal role of IL-1β and microglia activation in the pathophysiology of depression, and the relevance of these processes to the therapeutic properties of curcumin, we can highlight a probable role for the inflammasomes.

Inflammasomes are crucial components of the innate immune response that initiate immunological reactions against microbial infections, tissue injury, and other aggressions. The key inflammasome in depression is the NLRP3 inflammasome complex, which, upon activation, consists of a NOD-like receptor protein containing pyrin domain (NLRP3) associated with an adaptor protein ASC and an effector caspase-1 (63).

NLRP3 inflammasome activation is related to microglial activation. Upon initially recognizing an environmental stressor, the microglia can indeed enter in an active state and are thereafter more prone to induce a prolonged inflammatory response following the upcoming exposure to those stressors. NLRP3 inflammasome has drawn interest as an explanatory element as to why primed microglia cells display sensitivity to environmental stressors. In fact, NLRP3 inflammasome acts as a transducer of neuroinflammatory responses. A lowered threshold for NLRP3 activation induces an increased production of inflammatory cytokines, such as IL-1β and IL-18, and then causes a persistent neuroinflammation (64). This chronic inflammation results from chronic exposure to noxious threats that negatively affect the homeostatic feedback mechanisms. In sum, NLRP3 inflammasome is a molecular mechanism that translates psychological stressful stimuli into inflammatory responses.

The activation of the NLRP3 inflammasome relies on a two-step mechanism. It requires a priming signal to initiate the transcription of the NLRP3 inflammasome to above a certain threshold, while a second signal will then promote the formation of the NLRP3 inflammasome by recruiting ASC and pro-caspase-1 proteins.

The priming of NLRP3 transcription is mediated by NF-κβ activation, which can result from the stimulation of membrane-bound receptors by TNF or pathogen-associated molecular patterns like LPS (63) or damage-associated molecular patterns whose presence indicates cellular or metabolic stress. It is worth noting that there were some reports indicating that corticosterone may also stimulate the transcription of NLRP3 *via* glucocorticoid receptor activation, involving the NF-κβ pathway but also independently of NF-κβ activation (65, 66).

Another way of activating NLRP3 inflammasome is the reduction of intracellular potassium (K+) levels *via* the activation of the P2X7 purinergic receptors. Extracellular ATP is released by neurons and astrocytes following excitotoxicity. Activation of these receptors causes potassium efflux in microglia, which is crucial for the activation of the NLRP3 inflammasome (67). Besides that, P2X7 receptor is being investigated as a potential drug target in mood disorders (68). Reactive oxygen species (ROS) can also promote NLRP3 activation (69). An activated NLRP3 inflammasome will then cleave pro-IL-1β and pro-IL-18 into active IL-1β and IL-18.

There have been a few studies highlighting the role of NLRP3 inflammasome in depression. Animal studies investigating this theory showed that chronic stress could stimulate NLRP3 activation in rodent brains and that, accordingly, in the absence of the NLRP3 inflammasome activation, stress did not produce a depressive behavior, anhedonia, or social impairment in those mice (70–73). It has also been shown that NLRP3 is activated in mononuclear cells in patients with depressive disorder and that some antidepressants compounds seem to show a decrease in NLRP3 activation (74).

Concerning curcumin, in an *in vitro* study conducted by Li et al. (75) on rat hippocampal cells, excessive glutamate release induced IL-1β secretion and generation of ROS. Curcumin was able to inhibit the generation of ROS and NLRP3 inflammasome activation assessed by NLRP3 and caspase-1 expression, which resulted in a reduction of IL-1β secretion. In addition, curcumin could prevent glutamate-induced cell apoptosis. In the same vein, Fan et al. (76) showed that curcumin administration could prevent IL-1β-induced apoptosis in stressed rats in the ventromedial pre-frontal cortex and alleviate depressive behavior.

In a study conducted by Zhang et al. (77), rats were exposed to chronic unpredictable stress, and this exposure led to depressive-like behaviors. Curcumin successfully corrected the depressive-like behaviors in those stressed rats. Additionally, curcumin could effectively decrease the mRNA expression of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and suppress NF-κβ activation as well as inhibit the expression of

P2X7 receptors, thus preventing NLRP3 activation. These results were in accord with a study conducted by Wang et al. (78), in which curcumin could inhibit the activation of the P2X7 receptor and thus inhibit the inflammatory response and the microglial activation, thus preventing NLRP3 activation.

It is also worth quoting a recent study conducted by Ozkartal et al. (73) that highlighted the role of nitric oxide synthase (NOS) in the activation of NLRP3 inflammasome; in this study, the administration of NOS inhibitors in chronically stressed mice prevented the activation of NLRP3 inflammasome and the production of IL-1β.

In some reports, depression has been associated with an excessive activity of nitric oxide synthase (79). Also, some antidepressant medications in current use, such as paroxetine, inhibit NOS activity (80). In some other studies, suicidal and depressed patients had elevated levels of plasma NO and its metabolites (81, 82). The overactivation of NOS pathway and subsequently increased nitric oxide has been described as an important pathophysiological factor in neuroinflammation and neurotoxicity processes involved in stress and depression (79, 83) as well as an important modulating factor in the production of neurotransmitters such as noradrenaline, serotonin, and dopamine (79), thus explaining why NOS inhibitors can exert antidepressant and anxiolytic-like effects (73, 84).

In human cultured neurons exposed to quinolinic acid, quinolinic acid increased NOS activity and consequently increased the nitrite levels. In those neurons, curcumin could counteract this increase of NOS activity (85). Studies investigating the influence of curcumin on NOS pathway in animal models have shown that, in rat (86) or pig (87) stress models, curcumin could inhibit NOS hyperactivation and the subsequent increase in hippocampal NO. As previously cited in the study conducted by Wang et al. (57) on mice with LPS-induced depressive symptomatology, curcumin could attenuate the LPS-induced microglial activation and NF-κβ activation in the hippocampus and PFC as well as decreased the levels of NOS in the hippocampus and PFC.

These data suggest that NLRP3 activation can play a major role in inflammatory-related depressive symptoms and that its inhibition by curcumin might be a key feature of its effectiveness.

Curcumin and Kynurenine Pathway

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that is strongly activated by proinflammatory cytokines (such as TNF-α, IL-1, and IL-6) (88). This enzyme has an important role in depression by catabolizing tryptophan (the primary precursor of serotonin) into kynurenine pathway metabolites: IDO leads to tryptophan depletion and thus inhibits serotonin synthesis. As stated before, the role of serotonin in depression is already admitted. However, research shows that kynurenine pathway metabolites can impact several mechanisms associated with depression, such as pro-apoptotic changes in the CNS or generation of free radicals (89). Preclinical studies have shown that IDO activation results in depressive-like symptoms and that IDO inhibitors could relieve depression-like behaviors in mice (90).

There are two main kynurenine pathway metabolites studied in depression: one of them is quinolinic acid, an NMDA agonist, that is thought to be excitotoxic, thus leading to synaptic loss. It can also disrupt the oxidant/antioxidant balance by increasing the generation of free radicals and induce lipid peroxidation (88). Excess quinolinic acid levels were found in depression (91), however inconsistently (92). The levels of quinolinic acid were still shown to predict the response to antidepressant medications such as ketamine (93). The second main kynurenine pathway metabolite is kynurenic acid, considered to be neuroprotective. Its balance with quinolinic acid is considered to be protective against the excitotoxicity of the latter (94). Moreover, studies reported lower levels of kynurenic acid in depression (92).

In vitro studies demonstrated that curcumin could counteract the inflammation-induced overexpression of IDO (95), and more recently, a study conducted by Zhang et al. (77) showed that curcumin could inhibit the stress-induced overexpression of IDO in rats and normalize the quinolinic acid/tryptophane ratio.

Another important co-factor of the increase of IDO is cyclo-oxygenase 2 (COX-2) (96). COX-2 is an enzyme responsible for the production of prostaglandin E2 (PGE2), which is a pro-inflammatory chemical messenger. Studies have shown an increase in PGE2 production and COX-2 expression in depressed patients (97, 98). The peripheral blood cells of patients with recurrent depression also exhibited an increased expression of the genes encoding for COX-2 (99). A number of clinical trials have yielded promising results for the use of COX-2 inhibitors as an augmentation of antidepressant treatments (100). Their use could also help decrease inflammatory cytokine levels (101).

Regarding curcumin, studies have shown that it can downregulate COX-2 expression and PGE2 synthesis *in vitro* (102) and in animal models of depression (103), highlighting its potential role as an alternative natural COX-2 inhibitor option. It is relevant to mention that COX-2 can induce NOS activity and *vice versa* (104). The 2014 Wang et al. (57) study mentioned before (see the previous section) illustrates the interplay between NOS, IDO, and inflammatory cytokines.

Considering the possible role of COX-2 in depression and its role in IDO synthesis and the role of IDO in depression that we illustrated, these data suggest interesting mechanisms of action for the use of curcumin as an augmentation therapy.

Curcumin and Intestine Hyperpermeability

As the largest mucosal surface in the human body, the intestinal epithelium acts as an interface with the external environment (105). In some situations, the permeability of the intestinal epithelium can be altered in a state also called "leaky gut."

Lipopolysaccharides (LPS) are large molecules found in the outer membrane of gram-negative bacteria. In a state of "leaky gut," there will be an increased translocation of those gram-negative bacteria from the intestine into the systemic circulation and, with them, the LPS (106, 107). LPS can then stimulate toll-like receptors, generating an inflammatory process. This will result in pro-inflammatory cytokine secretion and neuroinflammation (108) and also activation of IDO with the previously described consequences (109–111).

Some studies have reported increased serum IgM and IgA levels directed against the LPS of gram-negative enterobacteria in depressed patients, reflecting an increased translocation

of LPS from those gram-negative enterobacteria, with the authors of such studies concluding that depressive disorder was accompanied with intestinal hyperpermeability (107, 112).

In animals, LPS induces depressive behaviors (110). Thus, a number of studies investigating the anti-inflammatory effects of curcumin were carried out using LPS models. In such models, curcumin reversed LPS-induced behavioral and molecular changes. Moreover, a handful of *in vitro* studies have shown that curcumin could increase the expression of tight junction proteins, thus preventing the disruption of tight junction organization and decreasing LPS increase due to paracellular permeability (113).

These data suggest that one antidepressant mechanism of action of curcumin could be its ability to counteract this state of gut permeability and subsequent LPS-induced inflammation.

DEPRESSION, METABOLISM, AND CURCUMIN

Curcumin and HPA Axis

The HPA axis is a central system in the body's stress response, and abnormalities in its activity have long been noted in MDD (114). Studies have shown that depression was associated with impairment in the responsiveness to glucocorticoids and a subsequent hypersecretion of CRH. This phenomenon is known as glucocorticoid resistance and can, in turn, prime the inflammatory response (114). We have indeed highlighted the role of corticosterone in the activation of NLRP3 inflammasome (65, 66). Depression is also associated with an increased size and activity of the pituitary and the adrenal glands (115).

Based on these assumptions, animal models of depression related to stress and elevated cortisol levels have been developed. In such models, curcumin has been shown to alleviate the depressive symptoms and other physiological alterations induced by cortisol.

Li et al. (25) showed that, in stressed rats, curcumin could restore the normal level of corticosterone. Rinwa and Kumar later found similar results (116). Researchers showed that curcumin could protect against corticosterone-induced neurotoxicity and downregulation of mRNA levels of serotoninergic receptors in a rodent model (5-HT1A, 5-HT2A, and 5-HT4) (117). Curcumin could also normalize the adrenal gland size in stressed rats (118). Furthermore, curcumin decreased the salivary cortisol concentrations in depressed patients compared with the placebo group in the clinical trial of Yu et al. (60).

Depression, Insulin Sensitivity, and Curcumin

Insulin receptors are present throughout the brain, and they play a central role in regulating its use of glucose for energy (119). Moreover, some of insulin's actions are specific for the CNS, like promoting neuronal survival or synaptic plasticity and regulation of brain functions including memory, cognition, learning as well as attention (120, 121). Insulin also inhibits norepinephrine and serotonin reuptake and downregulates alpha-2 receptor expression in hypothalamic neurons (122).

Consequently, there has been a hypothesis suggesting that insulin resistance played a role in depressive disorder pathophysiology, which was reinforced by epidemiological studies. For example, adults with insulin resistance are more at risk for the development of future depression (123), and a meta-analysis has shown an association between insulin resistance and depression incidence (124). Significantly, insulin resistance interacts with other depressogenic processes. Given the insulin functions in the brain, it has also been shown that reduced brain sensitivity to insulin can manifest as impaired neuroplasticity and disturbances in neurotransmitter's release and uptake (125). Insulin resistance has also been shown to be associated with altered dopamine signaling in rodents (126).

Inflammation and oxidative stress are both involved in the pathogenesis of insulin resistance (127, 128). In turn, insulin has antioxidant properties which become disrupted following insulin resistance (129). As described earlier, oxidative stress resulting from the disruption of insulin functions can, in turn, trigger the production of pro-inflammatory cytokines. The desensitization of glucocorticoid receptors that can be associated with insulin resistance (130) may potentiate this depressogenic cascade.

There is an emerging research field trying to reposition some antidiabetic medications as new means in depression management like GLP1 functional agonists, pioglitazone, or even metformin (131).

Concerning curcumin, Shen et al. (132) have conducted a study on mice with unpredictable chronic mild stress in which they showed that curcumin could attenuate insulin resistance alongside a decrease in depressive-like symptoms. There were also other studies showing the positive effects of curcumin on insulin resistance in *in vitro*, animal, or even human studies (133).

DEPRESSION AND NEUROPLASTICITY, CURCUMIN, AND NEUROPROTECTION

The 1990s witnessed the rise of the "neurotrophic" hypothesis of depression, which associated chronic stress and depression with a deficit in BDNF and demonstrated that traditional antidepressants increased BDNF expression (134). Hence, by the early 2000s, studies progressively exhibited increasing evidence of the presence of cellular atrophy and neuronal death in major depressive disorder. Studies also showed increased growth factor (such as BDNF) levels associated with antidepressant therapies such as physical exercise (135), antidepressants (136), electroconvulsive therapy (137), or lithium (138). This supports the hypothesis that the neurotrophic effects of antidepressants account for their efficacy in treating MDD, with some authors even viewing BDNF as an essential determinant of antidepressant efficacy (136).

We have already underlined the neuroprotective effect that curcumin could exert *via* its inhibition of inflammatory pathways and the mitigation of glutamate excitotoxicity (59, 76). This is particularly illustrated by a study conducted by Choi et al. (103) in which curcumin administration permitted an improvement of the depressive behavior induced by chronic unpredictable

stress in mice alongside reduced hippocampal neuronal cell death, attenuated long-term depression, increase in BDNF, and COX-2 inhibition, suggesting neuroprotection *via* anti-inflammatory effects. Curcumin can also exert neuroprotective effects by stimulating the production of neurotrophic factors, especially BDNF.

The extracellular signal-regulated kinase (ERK) is a subfamily of mitogen-activated protein kinase (MAPK), an essential family of serine/threonine kinase regulating cellular growth, differentiation, and survival in proliferative cells including brain cells (139). ERK, alongside BDNF, has shown to be downregulated in the PFC and hippocampus of depressed humans and animals, and antidepressants could, in turn, reverse the hypoactivity of ERK and alleviate depression-like behaviors. Reciprocally, in animal studies, blocking the BDNF pathway resulted in a loss of effects of antidepressants (140).

The transcription factor CREB is also a downstream target of ERK, and other studies found that CREB phosphorylation was decreased in the frontal cortex and the hippocampus of stressed humans and animals, accompanied with a decrease in ERK activity. Antidepressants reversed the reduction of CREB phosphorylation in stressed animals (140). In short, the activation of the ERK pathway by antidepressants increases the expression of nuclear CREB, which facilitates the expression of neurotrophic/neuroprotective proteins such as the BDNF.

In an adult male Wistar–Kyoto rat model of depression, Hurley et al. (141) showed that curcumin had an antidepressant activity and found an increase in hippocampal BDNF. Similarly, Xu et al. (24) showed that administrating curcumin to chronically stressed mice increased the hippocampal BDNF. Liu et al. (142) showed similar results in chronically stressed mice with a positive effect on stress-induced learning and memory deficits and an upregulation of BDNF and ERK in the hippocampus.

Some authors have also shown a neuroprotective effect of curcumin in the amygdala. For example, Zhang et al. (143) showed in a study that curcumin could increase BDNF levels in rat amygdala *via* ERK phosphorylation and alleviate depressive behavior. They later replicated those results (144). Similarly, Abd–Rabo (145) could show that the administration of curcumin in ovariectomized rats could alleviate depressive behavior and upregulate the BDNF mRNA in the limbic system. With regards to human studies, Yu et al. (60) showed, in a clinical trial, that curcumin increased the plasma BDNF levels compared to the placebo group.

These studies suggest that curcumin can alleviate depressive behavior through activation of the ERK/BDNF neurotrophic pathway, especially in the hippocampus, the pre-frontal cortex, or the amygdala that are involved in depression pathophysiology.

OXIDATIVE STRESS, DEPRESSION, AND CURCUMIN

As stated in the introduction, depression pathophysiology may involve several interconnected biochemical pathways including nitrosative stress and oxidative stress. Generally, oxidative stress is defined as an imbalance between the production of reactive oxygen and nitrogen species as well as the efficiency of enzymatic (like catalase, glutathione peroxidase, and superoxide dismutase) and non-enzymatic (like reduced glutathione and uric acid) antioxidative systems, so cells are said to be in a state of oxidative stress when the level of reactive oxygen species exceeds the endogenous antioxidant defense mechanisms (for e.g., the enzymes catalase, glutathione peroxidase, or superoxide dismutase) (146).

Studies to date indicate that patients with depression have lower levels of antioxidative systems, and at the same time, they display an increased amount of oxidative stress markers when compared with healthy individuals (147, 148). Some antioxidant therapeutics, like N-acetyl-cysteine, seem to show some effect in depression treatment (149).

Recently, Naqvi et al. (150) have shown that, on chronically stressed mice, curcumin administration could correct depressive behaviors and improve memory functions as well as improve oxidative stress as measured by the peroxidation of lipid and the antioxidant enzyme activities. Fidelis et al. (151) administrated curcumin in a model of oxidative stress induced by beta amyloid infusion in mice and noticed an antidepressant-like effect as well as a reduction of the Aβ-generated oxidative stress in the pre-frontal cortex, as evidenced by the reactive species levels and the superoxide dismutase and catalase activities. Rinwa and Kumar (116) exposed mice to a chronic unpredictable stress that significantly impaired the oxidative parameters (elevated malondialdehyde and nitrite concentrations and decreased glutathione and catalase levels) and mitochondrial enzyme complex activities. Those effects were reversed by the administration of curcumin; there was an improvement in the depressive-like behavior as well. Jangra et al. (58) were also previously able to show that curcumin could reverse glutathione depletion in the hippocampus induced by LPS administration. Concerning nitrogen species and nitrosative stress, we previously addressed this issue and how curcumin could oppose this noxious phenomenon by its action on NOS.

As we have stated before, oxidative stress and mitochondrial dysfunction leading to the formation of reactive oxygen species are involved with other pathophysiological mechanisms such as inflammation. As a consequence, tackling this pathogenic cascade could be an interesting way to improve treatment outcomes in depression.

CURCUMIN AND THE ENDOCANNABINOID SYSTEM

Two kinds of cannabinoid (CB) receptors have been found in the human body: CB1 and CB2 receptors. They can activate multiple cell signaling pathways to regulate the neurotransmitter release process (152). CB1 receptors are mainly distributed in the central nervous system and may be related to the cannabinoid functions of memory and cognition regulation as well as motor control and show relatively low expression levels in the peripheral nervous system (153). On the other hand, CB2 receptors are mostly distributed in peripheral immune cells, mainly affecting immune regulation (153).

Considering the regulatory function of CB receptors on mood (154) and their distribution in brain regions related to mood and reward (pre-frontal cortex, limbic system, raphe nuclei) (155), some authors proposed that the endocannabinoid signaling pathway may be involved in the formation and development of depression, as suggested by diverse animal studies showing the apparition of depressive behaviors with the blockade of CB1 receptors (156). We could also cite the example of rimonabant (a CB1 antagonist used to treat obesity) that has been forced out of the market due to anxiety and depression reported as frequent and important side effects (157) and studies reporting that the use of rimonabant could inhibit positive emotional memory as well as the reward system (158). Based on these observations, reports have also shown that the activation of CB1 receptors could result in similar behavior and biochemical changes as those caused by antidepressants (159).

He et al. (160) tested an association of curcumin and HU-122, a cannabinoid known for having no effect on CB receptors. They showed some efficacy in a corticosterone-induced model of depression. The administration of this dual drug could prevent corticosterone-induced neural cell apoptosis and improve the dopamine levels, especially in the hippocampus and the striatum. Alongside those effects, the authors noted an enhanced expression of CB1 receptors, which could have an important role in the antidepressant potency of curcumin. Hassanzadeh and Hassanzadeh (161) suggested that the endocannabinoid system could have a pivotal role in emotion regulation and neuroplasticity exerted by curcumin as they showed in their study that chronic curcumin administration in rats could increase endocannabinoid levels as well as nerve growth factor levels in key structures like the amygdala and the hippocampus. Witkin et al. (162) noted that curcumin had no effect on depression-like behavior on CB1 KO (–/–) mice, highlighting the role of CB1 receptors in curcumin efficacy.

In sum, these data highlight the role of CB1 receptors as a novel and multimodal target of curcumin.

TRANSLATION INTO CLINICAL PRACTICE

To date, clinical trials have yielded conflicting results regarding the efficacy of curcumin in depression; however, there has been two meta-analyses concluding that curcumin could be effective in depression: the first one was in 2017 (163), which included six studies with a total of 377 patients comparing the use of curcumin to placebo, supporting a significant clinical efficacy in depression, and the second one was conducted by Fusar-Poli et al. (13), in which curcumin was evaluated as an add-on therapeutic, that included 10 studies with a total of 531 patients, supporting the efficacy of curcumin as an add-on.

However, even though there were moderate to large effect sizes in those meta-analysis, it is important to note that not every study yielded significant results and that the studies were heterogeneous, especially with the formulations and the dosage of curcumin used. Yet a concern about curcumin has been its bioavailability, and thus diverse formulas are being developed to enhance its bioavailability, like the use of curcumin nanoparticles, liposomal encapsulation, emulsions, lipid complexes, use of piperine, or development of synthetic curcumin analogs (164, 165). However, no statistical analysis could be performed in those meta-analysis regarding a potential difference in efficacy between formulas due to the small number of studies, so further clinical trials with diverse curcumin formulations will be important since, in animal studies, different magnitudes of effects on the behavioral, molecular, and electrophysiological levels have been reported depending on the curcumin formula administered (20, 151). Nonetheless, the meta-analyses conducted did not show significant differences with respect to the dosage of curcumin used ($>$ or $\leq$500 mg/day). Moreover, it is important to note that the clinical trials conducted were generally small, with samples varying from 14 to 123 (13). Thus, enlarging samples in future studies would be desirable.

Curcumin generally appears to be well-tolerated, with only mild side effects like yellow stool, headache, or diarrhea noted in doses up to 12 g/day (166).

On another hand, most of the studies were conducted in Asian countries, where dietary regimens usually include curcuminoids, so it would be interesting to test the effects of curcumin in Western countries to see if the dietary regimen influences curcumin potency. Nonetheless, the most convincing effects of curcumin were shown in studies conducted in Australia (167, 168).

Due to the poor bioavailability of curcumin, turmeric ingestion as powder might instinctively be thought unlikely to have therapeutic potency, particularly as the curcumin content of pure turmeric powder is only around 3% (169). However, as we have previously highlighted the neuroprotective potency of curcumin, in some studies, dietary curcuminoid consumption was positively correlated with an overall better cognitive performance in the elderly (170). In fact, this lack of bioavailability could also account for curcumin efficacy *via* its direct effects in the intestine, as it can act on intestinal barrier dysfunction as described earlier and thus tackle the subsequent systemic inflammation. Gut microbiota can also, *via* demethylation, metabolize curcumin into active metabolites, such as methexylcurcumin that has been evaluated as a potential effective synthetic analog, so the metabolization by gut microbiota into active metabolites may also exert systemic effects (171). Primarily targeting enterocytes because of this lack of bioavailability and a subsequent extensive first-pass metabolism may also account for the increased efficacy of co-administered drugs (172), supporting the use of curcumin as an add-on agent. Giving further arguments to this hypothesis, Lopresti et al. (173) have conducted a clinical trial in which the basal levels of endothelin-1 were predictive of a greater effect of curcumin administration on depressive symptoms (assessed by the inventory of depressive symptomatology). Since endothelin-1 has shown in rat models that it had a role in bacterial translocation (174), this supports the fact that ameliorating intestinal permeability and bacterial translocation may play an important role in curcumin antidepressant efficacy. The basal levels of endothelin-1 may also reflect the systemic effects of curcumin as endothelin-1 influences oxidative stress (175), HPA activity (176), and cytokine production (177).

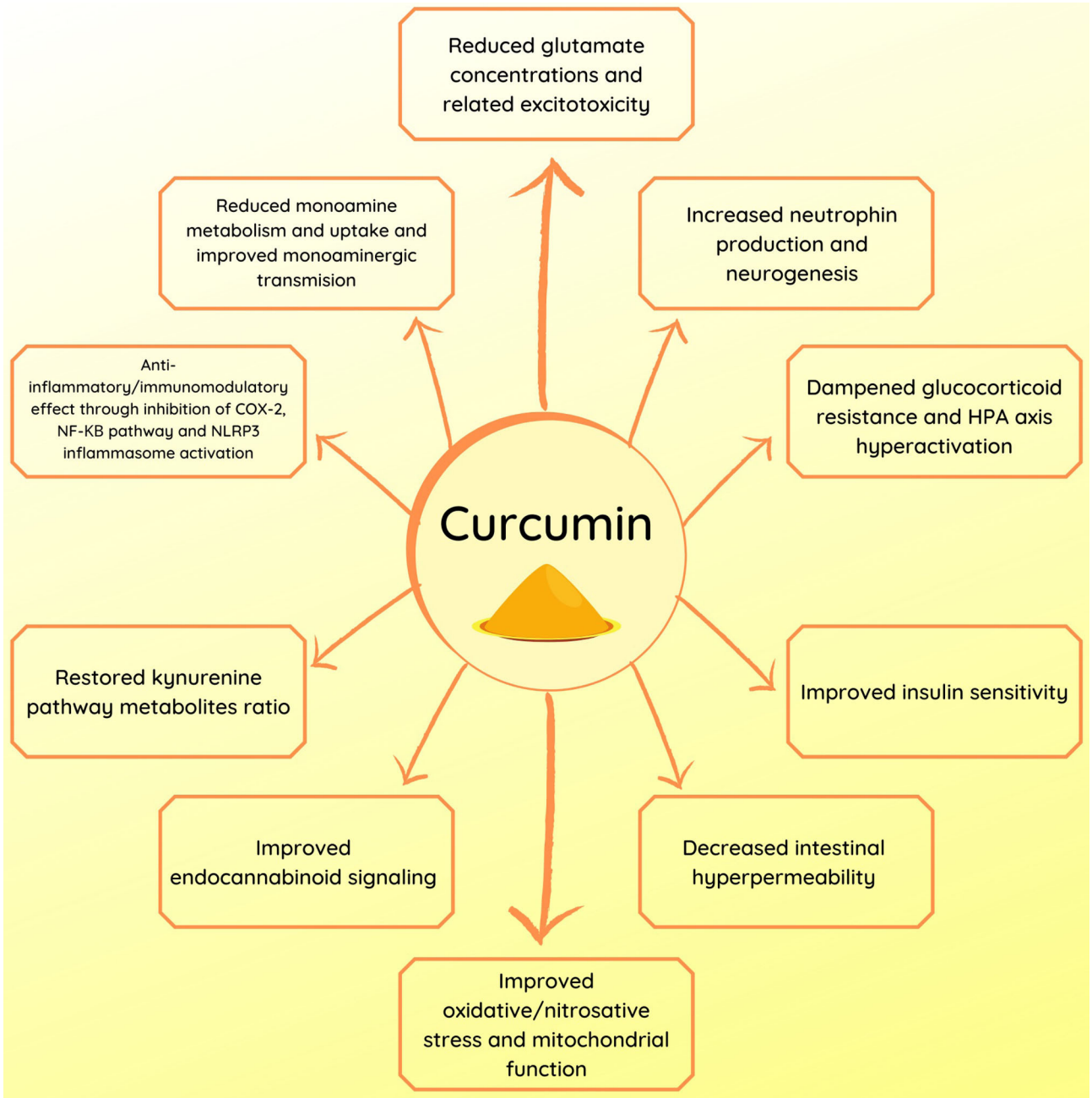

FIGURE 1 | Summary of the potential mechanisms of action of curcumin in depression.

Although curcumin has displayed an efficacy as monotherapy in placebo-controlled trials (163) or in antidepressant-controlled trials (178), this study of Lopresti et al. (173) raises the question of the subset of patients in which curcumin could be beneficial. Since curcumin has been studied in various diseases, especially inflammatory diseases, perhaps curcumin could be beneficial in patients with comorbidities. There have been two studies evaluating the use of curcumin in such conditions. The first was an 8-week randomized, double-blind, placebo-controlled trial conducted by Asadi et al. (179), testing the efficacy of curcumin on depression and anxiety in diabetic patients with polyneuropathy and showing a significant reduction of depression and anxiety in the curcumin group. The second was a 4-week randomized, double-blind, placebo-controlled trial (180) testing the antidepressant effects of curcumin in patients with systemic lupus erythematosus and that yielded positive results. There was also a positive correlation between TNF-α levels and depressive symptoms (assessed with the

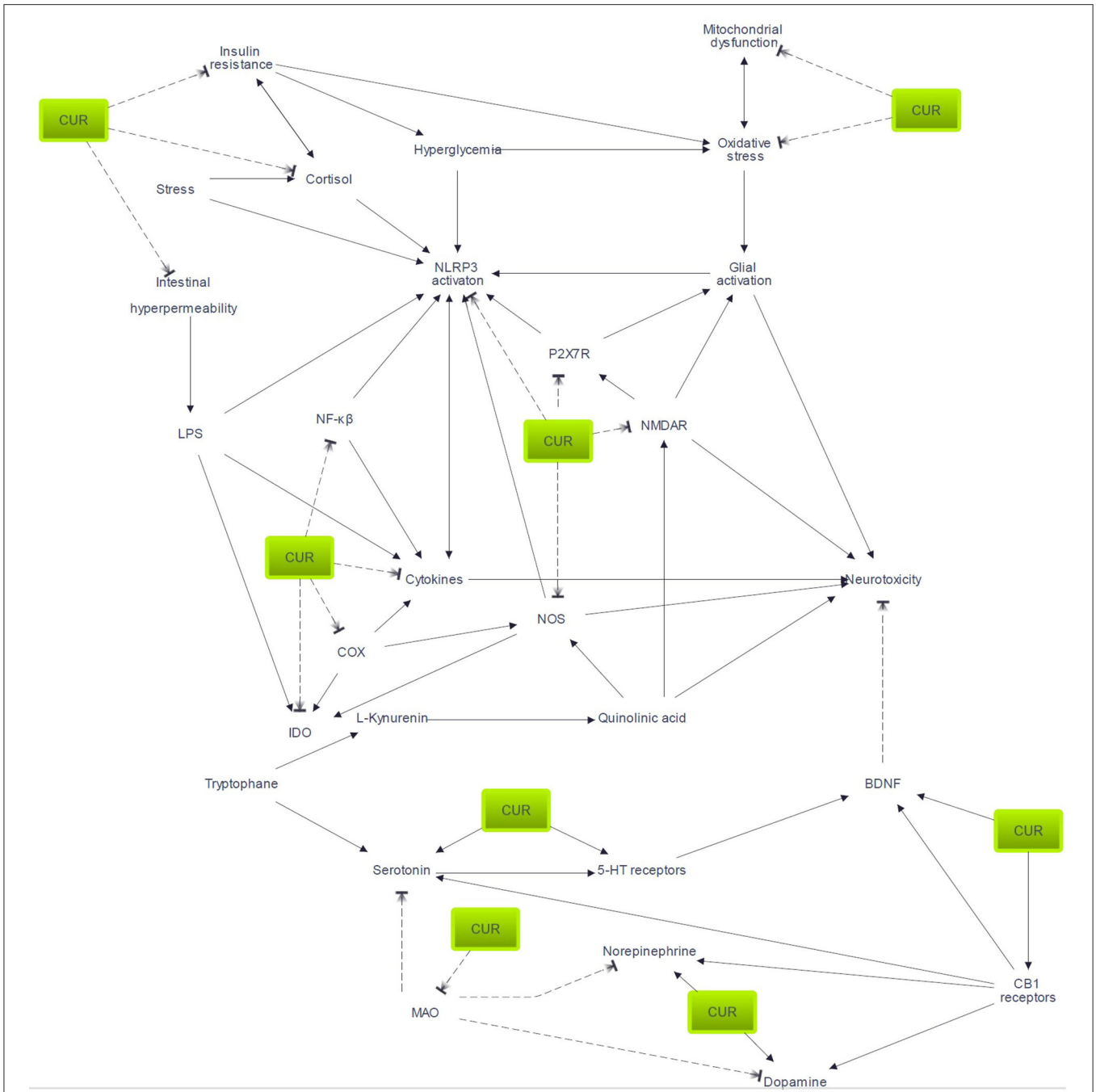

FIGURE 2 | Pathways potentially involved in curcumin efficacy. Arrow indicates activation or enhancement; broken line indicates inhibitory action. 5-HT, serotonin; BDNF, brain-derived neurotrophic factor; CUR, curcumin; COX, cyclo-oxygenase; IDO, indoleamine 2,3-dioxygenase; LPS, lipopolysaccharide; MAO, monoamine oxidase; NMDAR, N-methyl-D-aspartate receptors; P2X7R, P2X7 receptor.

Beck depression inventory) that both decreased with curcumin administration. By analogy, in a meta-analysis on patients with chronic inflammatory conditions, anti-cytokine agents exhibited an antidepressant effect, especially on severely depressed patients, that was not associated with improvement in primary physical illness (181). This highlights the fact that assessing the inflammatory status of patients may be of relevance when treating patients with curcumin since immuno-inflammatory dysregulation may account for the increased efficacy of curcumin in some patients due to its anti-inflammatory properties. This hypothesis is supported by a clinical trial in which curcumin showed a greater efficacy in patients displaying

"atypical" depressive symptoms (168). These symptoms are defined as leaden palsy, hypersomnia, and increased appetite, symptoms that are associated with higher levels of CRP (45). In sum, although further trials are needed, curcumin could be used in a personalized fashion in which using markers such as high-sensitive CRP could serve a biomarker-based personalized antidepressant treatment selection based on patient inflammatory status before treatment, as it has been described as a predictive factor of efficacy of certain treatments (50, 51, 182).

CONCLUSION AND PERSPECTIVES

In this review, we highlighted the wide range of targets and modes of action of curcumin. However, as many as they are (as summarized in **Figure 1**), they are also interconnected (as shown in **Figure 2**). This illustrates the complexity of depression pathophysiology that could be described as psychophysiopathologic processes. Given the diversity of pathways involved in depression, the unidimensional nature of existing pharmacologic therapeutics may be a cause for their limited efficacy. As a consequence, an enhancement of treatment efficacy is likely to occur from therapies that target multiple mechanisms. It is under this foregoing logic that curcumin may find a place as an augmentation treatment through diverse mechanisms that have been described in this review.

Given its major anti-inflammatory properties, curcumin could also be of use in a subset of patients. As inflammatory mechanisms are relevant to some patients, those differences could account for the lack of efficacy of classical antidepressants (181).

As we have discussed in the introduction, as society evolves, patients' demands do, too. As some patients are reluctant to take medication in fear of adverse effects, alternative medicine appears to be a seductive option. Thus, curcumin could embody the dawn of nutriceuticals as anti-inflammatory and antioxidant components appear to be a possible alternative in the treatment of depression. As curcumin displays neuroprotective effects, especially against stress-induced toxicity, it also suggests the use of such molecules as prophylactic agents.

All things considered, we have highlighted that curcumin is a promising molecule as it appears to be safe and displays positive results in studies, although more clinical trials on the antidepressant effect of curcumin are still required to determine its efficacy and optimal dosage.

AUTHOR CONTRIBUTIONS

TR: original idea, literature review, and redaction. FB: supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Institutional publishing funding.

REFERENCES

1. James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. *Lancet.* (2018) 392:1789–858. doi: 10.1016/S0140-6736(18)32279-7
2. World Health Orgazination. *Depression: Let's Talk.* (2017). Available online at: https://www.who.int/news-room/detail/30-03-2017--depression-let-s-talk-says-who-as-depression-tops-list-of-causes-of-ill-health (accessed May 14, 2020).
3. Malhi GS, Mann JJ. Depression. *Lancet.* (2018) 392:2299–312. doi: 10.1016/S0140-6736(18)31948-2
4. Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y, et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Focus.* (2018) 16:420–9. doi: 10.1176/appi.focus.16407
5. John Rush A, Jain S. Clinical implications of the STAR*D trial. In: Macaluso M, Preskorn SH, editors. *Antidepressants: From Biogenic Amines to New Mechanisms of Action*. Cham: Springer International Publishing (2019) p. 51–99.
6. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry.* (2006) 16:1905–17. doi: 10.1176/ajp.2006.163.11.1905
7. Milev RV, Giacobbe P, Kennedy SH, Blumberger DM, Daskalakis ZJ, Downar J, et al. Canadian network for mood and anxiety treatments (CANMAT) 2016. Clinical guidelines for the management of adults with major depressive disorder: section 4. neurostimulation treatments. *Can J Psychiatry.* (2016) 61:561–75. doi: 10.1177/0706743716660033
8. Stubbs B, Vancampfort D, Hallgren M, Firth J, Veronese N, Solmi M, et al. EPA guidance on physical activity as a treatment for severe mental illness: a meta-review of the evidence and position statement from the European psychiatric association (EPA), supported by the international organization of physical therapists in mental health (IOPTMH). *Eur Psychiatry.* (2018) 54:124–44. doi: 10.1016/j.eurpsy.2018.07.004
9. Ravindran AV, Balneaves LG, Faulkner G, Ortiz A, McIntosh D, Morehouse RL, et al. Canadian network for mood and anxiety treatments (CANMAT) 2016. Clinical guidelines for the management of adults with major depressive disorder. *Can J Psychiatry.* (2016) 61:576–87. doi: 10.1177/0706743716659417
10. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. *Adv Exp Med Biol.* (2007) 595:1–75. doi: 10.1007/978-0-387-46401-5_1
11. Shehzad A, Rehman G, Lee YS. Curcumin in inflammatory diseases. *Biofactors.* (2013) 39:69–77. doi: 10.1002/biof.1066
12. Slika L, Patra D. Traditional uses, therapeutic effects and recent advances of curcumin: a mini-review. *Mini Rev Med Chem.* (2020) 20:1072–82. doi: 10.2174/1389557520666200414161316
13. Fusar-Poli L, Vozza L, Gabbiadini A, Vanella A, Concas I, Tinacci S, et al. Curcumin for depression: a meta-analysis. *Crit Rev Food Sci Nutr.* (2019) 60:2643–53. doi: 10.1080/10408398.2019.1653260
14. Maletic V, Robinson M, Oakes T, Iyengar S, Ball SG, Russell J. Neurobiology of depression: an integrated view of key findings. *Int J Clin Pract.* (2007) 61:2030–40. doi: 10.1111/j.1742-1241.2007.01602.x
15. Bhutani MK, Bishnoi M, Kulkarni SK. Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. *Pharmacol Biochem Behav.* (2009) 92:39–43. doi: 10.1016/j.pbb.2008.10.007
16. Kulkarni SK, Bhutani MK, Bishnoi M. Antidepressant activity of curcumin: involvement of serotonin and dopamine system. *Psychopharmacology.* (2008) 201:435. doi: 10.1007/s00213-008-1300-y

17. Chang X, Wang L, Li J, Wu D. Analysis of anti-depressant potential of curcumin against depression induced male albino wistar rats. *Brain Res.* (2016) 1642:219–25. doi: 10.1016/j.brainres.2016.03.010
18. Lee B, Lee H. Systemic administration of curcumin affect anxiety-related behaviors in a rat model of posttraumatic stress disorder via activation of serotonergic systems. *Evid Based Complement Alternat Med.* (2018) 2018:9041309 doi: 10.1155/2018/9041309
19. Madiha S, Haider S. Curcumin restores rotenone induced depressive-like symptoms in animal model of neurotoxicity: assessment by social interaction test and sucrose preference test. *Metab Brain Dis.* (2019) 34:297–308. doi: 10.1007/s11011-018-0352-x
20. Mohammed HS, Khadrawy YA, El-Sherbini TM, Amer HM. Electrocortical and biochemical evaluation of antidepressant efficacy of formulated nanocurcumin. *Appl Biochem Biotechnol.* (2019) 187:1096–112. doi: 10.1007/s12010-018-2866-4
21. Qi XJ, Liu XY, Tang LMY, Li PF, Qiu F, Yang AH. Anti-depressant effect of curcumin-loaded guanidine-chitosan thermo-sensitive hydrogel by nasal delivery. *Pharm Dev Technol.* (2020) 25:316–25. doi: 10.1080/10837450.2019.1686524
22. Yohn SE, Gorka D, Mistry A, Collins S, Qian E, Correa M, et al. Oral ingestion and intraventricular injection of curcumin attenuates the effort-related effects of the VMAT-2 inhibitor tetrabenazine: implications for motivational symptoms of depression. *J Nat Prod.* (2017) 80:2839–44. doi: 10.1021/acs.jnatprod.7b00425
23. Wang R, Xu Y, Wu HL, Li YB, Li YH, Guo JB, et al. The antidepressant effects of curcumin in the forced swimming test involve 5-HT1 and 5-HT2 receptors. *Eur J Pharmacol.* (2008) 578:43–50. doi: 10.1016/j.ejphar.2007.08.045
24. Xu Y, Ku B, Cui L, Li X, Barish PA, Foster TC, et al. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. *Brain Res.* (2007) 1162:9–18. doi: 10.1016/j.brainres.2007.05.071
25. Li YC, Wang FM, Pan Y, Qiang LQ, Cheng G, Zhang WY, et al. Antidepressant-like effects of curcumin on serotonergic receptor-coupled AC-cAMP pathway in chronic unpredictable mild stress of rats. *Prog NeuroPsychopharmacol Biol Psychiatry.* (2009) 33:435–49. doi: 10.1016/j.pnpbp.2009.01.006
26. Lian L, Xu Y, Zhang J, Yu Y, Zhu N, Guan X, et al. Antidepressant-like effects of a novel curcumin derivative J147: involvement of 5-HT1A receptor. *Neuropharmacology.* (2018) 135:506–13. doi: 10.1016/j.neuropharm.2018.04.003
27. Crane GE. Cyloserine as an antidepressant agent. *AJP.* (1959) 115:1025–6. doi: 10.1176/ajp.115.11.1025
28. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry.* (2000) 47:351–4. doi: 10.1016/S0006-3223(99)00230-9
29. Singh JB, Daly EJ, Mathews M, Fedgchin M, Popova V, Hough D, et al. Approval of esketamine for treatment-resistant depression. *Lancet Psychiatry.* (2020) 7:232–5. doi: 10.1016/S2215-0366(19)30533-4
30. Hardingham GE, Bading H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat Rev Neurosci.* (2010) 11:682–96. doi: 10.1038/nrn2911
31. Olney JW, Price MT, Fuller TA, Labruyere J, Samson L, Carpenter M, et al. The anti-excitotoxic effects of certain anesthetics, analgesics and sedative-hypnotics. *Neurosci Lett.* (1986) 68:29–34. doi: 10.1016/0304-3940(86)90224-7
32. Sanacora G, Zarate CA, Krystal JH, Manji HK. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat Rev Drug Discov.* (2008) 7:426–37. doi: 10.1038/nrd2462
33. Sanacora G, Banasr M. From pathophysiology to novel antidepressant drugs: Glial contributions to the pathology and treatment of mood disorders. *Biol Psychiatry.* (2013) 73:1172–9. doi: 10.1016/j.biopsych.2013.03.032
34. Lin TY, Lu CW, Wang CC, Wang YC, Wang SJ. Curcumin inhibits glutamate release in nerve terminals from rat prefrontal cortex: Possible relevance to its antidepressant mechanism. *Prog Neuro Psychopharm Biol Psychiatry.* (2011) 35:1785–93. doi: 10.1016/j.pnpbp.2011.06.012
35. Zhang L, Xu T, Wang S, Yu L, Liu D, Zhan R, et al. NMDA GluN2B receptors involved in the antidepressant effects of curcumin in the forced swim test. *Prog Neuro Psychopharm Biol Psychiatry.* (2013) 40:12–7. doi: 10.1016/j.pnpbp.2012.08.017
36. Khalil RM, Khedr NF. Curcumin protects against monosodium glutamate neurotoxicity and decreasing NMDA2B and mGluR5 expression in rat hippocampus. *Neurosignals.* (2016) 24:81–7. doi: 10.1159/000442614
37. Xu Y, Lin D, Li S, Li G, Shyamala SG, Barish PA, et al. Curcumin reverses impaired cognition and neuronal plasticity induced by chronic stress. *Neuropharmacology.* (2009) 57:463–71. doi: 10.1016/j.neuropharm.2009.06.010
38. Miller OH, Yang L, Wang CC, Hargroder EA, Zhang Y, Delpire E, et al. GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. *eLife.* (2014) 3:e03581 doi: 10.7554/eLife.03581
39. Gideons ES, Kavalali ET, Monteggia LM. Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. *Proc Natl Acad Sci USA.* (2014) 111:8649–54. doi: 10.1073/pnas.1323920111
40. Takahashi K, Nakagawasai O, Nemoto W, Kadota S, Isono J, Odaira T, et al. Memantine ameliorates depressive-like behaviors by regulating hippocampal cell proliferation and neuroprotection in olfactory bulbectomized mice. *Neuropharmacology.* (2018) 137:141–55. doi: 10.1016/j.neuropharm.2018.04.013
41. Amidfar M, Kim YK, Wiborg O. Effectiveness of memantine on depression-like behavior, memory deficits and brain mRNA levels of BDNF and TrkB in rats subjected to repeated unpredictable stress. *Pharmacol Rep.* (2018) 70:600–6. doi: 10.1016/j.pharep.2017.12.007
42. Huang Z, Zhong XM, Li ZY, Feng CR, Pan AJ, Mao QQ. Curcumin reverses corticosterone-induced depressive-like behavior and decrease in brain BDNF levels in rats. *Neurosci Lett.* (2011) 493:145–8. doi: 10.1016/j.neulet.2011.02.030
43. Dunn AJ, Swiergiel AH. The role of cytokines in infection-related behavioral. *Ann N Y Acad Sci.* (1998) 840:577–85. doi: 10.1111/j.1749-6632.1998.tb09596.x
44. Fried EI, Stockert S von, Haslbeck JMB, Lamers F, Schoevers RA, Penninx BWJH. Using network analysis to examine links between individual depressive symptoms, inflammatory markers, and covariates. *Psychol Med.* (2019) 28:1–9 doi: 10.1017/S0033291719002770
45. Jokela M, Virtanen M, Batty GD, Kivimäki M. Inflammation and specific symptoms of depression. *JAMA Psychiatry.* (2016) 73:87–8. doi: 10.1001/jamapsychiatry.2015.1977
46. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. *Biol Psychiatry.* (2010) 67:446–57. doi: 10.1016/j.biopsych.2009.09.033
47. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: A meta-analysis. *Psychosom Med.* (2009) 71:171–186. doi: 10.1097/PSY.0b013e3181907c1b
48. Liu Y, Ho RCM, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression. *J Affect Disord.* (2012) 139:230–9. doi: 10.1016/j.jad.2011.08.003
49. Hannestad J, DellaGioia N, Bloch M. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology.* (2011) 36:2452–9. doi: 10.1038/npp.2011.132
50. Jha MK, Minhajuddin A, Gadad BS, Greer T, Grannemann B, Soyombo A, et al. Can C-reactive protein inform antidepressant medication selection in depressed outpatients? Findings from the CO-MED trial. *Psychoneuroendocrinology.* (2017) 78:105–13. doi: 10.1016/j.psyneuen.2017.01.023
51. Uher R, Tansey KE, Dew T, Maier W, Mors O, Hauser J, et al. An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. *Am J Psychiatry.* (2014) 171:1278–86. doi: 10.1176/appi.ajp.2014.14010094
52. Rethorst CD, Toups MS, Greer TL, Nakonezny PA, Carmody TJ, Grannemann BD, et al. Pro-inflammatory cytokines as predictors of antidepressant effects of exercise in major depressive disorder. *Mol Psychiatry.* (2013) 18:1119–24. doi: 10.1038/mp.2012.125

53. Kruse JL, Congdon E, Olmstead R, Njau S, Breen EC, Narr KL, et al. Inflammation and improvement of depression following electroconvulsive therapy in treatment resistant depression. *J Clin Psychiatry.* (2018) 79:17m11597. doi: 10.4088/JCP.17m11597
54. Raison CL, Miller AH. Is depression an inflammatory disorder? *Curr Psychiatry Rep.* (2011) 13:467–75. doi: 10.1007/s11920-011-0232-0
55. Deguchi A. Curcumin targets in inflammation and cancer. *Endocr Metab Immune Disord Drug Targets.* (2015) 15:88–96. doi: 10.2174/1871530315666150316120458
56. Aggarwal BB, Gupta SC, Sung B. Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *Br J Pharmacol.* (2013) 169:1672–92. doi: 10.1111/bph.12131
57. Wang Z, Zhang Q, Yuan L, Wang S, Liu L, Yang X, et al. The effects of curcumin on depressive-like behavior in mice after lipopolysaccharide administration. *Behav Brain Res.* (2014) 274:282–90. doi: 10.1016/j.bbr.2014.08.018
58. Jangra A, Kwatra M, Singh T, Pant R, Kushwah P, Sharma Y, et al. Piperine augments the protective effect of curcumin against lipopolysaccharide-induced neurobehavioral and neurochemical deficits in mice. *Inflammation.* (2016) 39:1025–38. doi: 10.1007/s10753-016-0332-4
59. Fan C, Song Q, Wang P, Li Y, Yang M, Liu B, et al. Curcumin protects against chronic stress-induced dysregulation of neuroplasticity and depression-like behaviors via suppressing IL-1β pathway in rats. *Neuroscience.* (2018) 392:92–106. doi: 10.1016/j.neuroscience.2018.09.028
60. Yu JJ, Pei LB, Zhang Y, Wen ZY, Yang JL. Chronic supplementation of curcumin enhances the efficacy of antidepressants in major depressive disorder: a randomized, double-blind, placebo-controlled pilot study. *J Clin Psychopharmacol.* (2015) 35:406–10. doi: 10.1097/JCP.0000000000000352
61. Sahebkar A. Are curcuminoids effective C-reactive protein-lowering agents in clinical practice? Evidence from a meta-analysis. *Phytother Res.* (2014) 28:633–42. doi: 10.1002/ptr.5045
62. Zhou H, Beevers CS, Huang S. Targets of curcumin. *Curr Drug Targets.* (2011) 12:332–47. doi: 10.2174/138945011794815356
63. Bauernfeind F, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, et al. NF-kB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J Immunol.* (2009) 183:787–91. doi: 10.4049/jimmunol.0901363
64. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int J Mol Sci.* (2019) 20:3328. doi: 10.3390/ijms20133328
65. Busillo JM, Azzam KM, Cidlowski JA. Glucocorticoids sensitize the innate immune system through regulation of the NLRP3 inflammasome. *J Biol Chem.* (2011) 286:38703–13. doi: 10.1074/jbc.M111.275370
66. Frank MG, Watkins LR, Maier SF. The permissive role of glucocorticoids in neuroinflammatory priming: mechanisms and insights. *Curr Opin Endocrinol Diabetes Obes.* (2015) 22:300–5. doi: 10.1097/MED.0000000000000168
67. Lamkanfi M, Mueller JL, Vitari AC, Misaghi S, Fedorova A, Deshayes K, et al. Glyburide inhibits the cryopyrin/Nalp3 inflammasome. *J Cell Biol.* (2009) 187:61–70. doi: 10.1083/jcb.200903124
68. Sperlágh B, Illes P. P2X7 receptor: an emerging target in central nervous system diseases. *Trends Pharmacol Sci.* (2014) 35:537–47. doi: 10.1016/j.tips.2014.08.002
69. Heid ME, Keyel PA, Kamga C, Shiva S, Watkins SC, Salter RD. Mitochondrial ROS induces NLRP3-dependent lysosomal damage and inflammasome activation. *J Immunol.* (2013) 191:5230–8. doi: 10.4049/jimmunol.1301490
70. Alcocer-Gómez E, Ulecia-Morón C, Marín-Aguilar F, Rybkina T, Casas-Barquero N, Ruiz-Cabello J, et al. Stress-induced depressive behaviors require a functional NLRP3 inflammasome. *Mol Neurobiol.* (2016) 53:4874–82. doi: 10.1007/s12035-015-9408-7
71. Lu M, Yang JZ, Geng F, Ding JH, Hu G. Iptakalim confers an antidepressant effect in a chronic mild stress model of depression through regulating neuro-inflammation and neurogenesis. *Int J Neuropsychopharmacol.* (2014) 17:1501–10. doi: 10.1017/S1461145714000285
72. Pan Y, Chen X-Y, Zhang Q-Y, Kong L-D. Microglial NLRP3 inflammasome activation mediates IL-1β-related inflammation in prefrontal cortex of depressive rats. *Brain Behav Immun.* (2014) 41:90–100. doi: 10.1016/j.bbi.2014.04.007
73. Ozkartal SC, Tuzun E, Kucukali CI, Ulusoy C, Giris M, Aricioglu F. Antidepressant-like effects of agmatine and NOS inhibitors in chronic unpredictable mild stress model of depression in rats: the involvement of NLRP inflammasomes. *Brain Res.* (2019) 1725:146438. doi: 10.1016/j.brainres.2019.146438
74. Alcocer-Gómez E, de Miguel M, Casas-Barquero N, Núñez-Vasco J, Sánchez-Alcazar JA, Fernández-Rodríguez A, et al. NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. *Brain Behav Immun.* (2014) 36:111–7. doi: 10.1016/j.bbi.2013.10.017
75. Li Y, Li J, Li S, Li Y, Wang X, Liu B, et al. Curcumin attenuates glutamate neurotoxicity in the hippocampus by suppression of ER stress-associated TXNIP/NLRP3 inflammasome activation in a manner dependent on AMPK. *Toxicol Appl Pharmacol.* (2015) 286:53–63. doi: 10.1016/j.taap.2015.03.010
76. Fan C, Song Q, Wang P, Li Y, Yang M, Yu SY. Neuroprotective effects of curcumin on IL-1β-induced neuronal apoptosis and depression-like behaviors caused by chronic stress in rats. *Front Cell Neurosci.* (2019) 12:516. doi: 10.3389/fncel.2018.00516
77. Zhang W, Guo Y, Han W, Yang M, Wen L, Wang K, et al. Curcumin relieves depressive-like behaviors via inhibition of the NLRP3 inflammasome and kynurenine pathway in rats suffering from chronic unpredictable mild stress. *Int Immunopharmacol.* (2019) 67:138–44. doi: 10.1016/j.intimp.2018.12.012
78. Wang Z, Ren W, Zhao F, Han Y, Liu C, Jia K. Curcumin amends Ca2+ dysregulation in microglia by suppressing the activation of P2X7 receptor. *Mol Cell Biochem.* (2020) 465:65–73. doi: 10.1007/s11010-019-03668-8
79. Dhir A, Kulkarni SK. Nitric oxide and major depression. *Nitric Oxide.* (2011) 24:125–31. doi: 10.1016/j.niox.2011.02.002
80. Wegener G, Volke V. Nitric oxide synthase inhibitors as antidepressants. *Pharmaceuticals.* (2010) 3:273–99. doi: 10.3390/ph3010273
81. Lee BH, Lee SW, Yoon D, Lee HJ, Yang JC, Shim SH, et al. Increased plasma nitric oxide metabolites in suicide attempters. *NPS.* (2006) 53:127–32. doi: 10.1159/000092542
82. Suzuki E, Yagi G, Nakaki T, Kanba S, Asai M. Elevated plasma nitrate levels in depressive states. *J Affect Disord.* (2001) 63:221–4. doi: 10.1016/S0165-0327(00)00164-6
83. Chen HJC, Spiers JG, Sernia C, Lavidis NA. Response of the nitrergic system to activation of the neuroendocrine stress axis. *Front Neurosci.* (2015) 9:3. doi: 10.3389/fnins.2015.00003
84. Gawali NB, Bulani VD, Gursahani MS, Deshpande PS, Kothavade PS, Juvekar AR. Agmatine attenuates chronic unpredictable mild stress-induced anxiety, depression-like behaviours and cognitive impairment by modulating nitrergic signalling pathway. *Brain Res.* (2017) 1663:66–77. doi: 10.1016/j.brainres.2017.03.004
85. Braidy N, Grant R, Adams S, Guillemin GJ. Neuroprotective effects of naturally occurring polyphenols on quinolinic acid-induced excitotoxicity in human neurons. *FEBS J.* (2010) 277:368–82. doi: 10.1111/j.1742-4658.2009.07487.x
86. Gilhotra N, Dhingra D. GABAergic and nitriergic modulation by curcumin for its antianxiety-like activity in mice. *Brain Res.* (2010) 1352:167–75. doi: 10.1016/j.brainres.2010.07.007
87. Wei S, Xu H, Xia D, Zhao R. Curcumin attenuates the effects of transport stress on serum cortisol concentration, hippocampal NO production, and BDNF expression in the pig. *Domest Anim Endocrinol.* (2010) 39:231–9. doi: 10.1016/j.domaniend.2010.06.004
88. Stone TW, Forrest CM, Darlington LG. Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection. *FEBS J.* (2012) 279:1386–97. doi: 10.1111/j.1742-4658.2012.08487.x
89. Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R. The new '5-HT' hypothesis of depression: Cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuro Psychopharmacol Biol Psychiatry.* (2011) 35:702–21. doi: 10.1016/j.pnpbp.2010.12.017
90. Qin Y, Wang N, Zhang X, Han X, Zhai X, Lu Y. IDO and TDO as a potential therapeutic target in different types of depression. *Metab Brain Dis.* (2018) 33:1787–800. doi: 10.1007/s11011-018-0290-7
91. Young KD, Drevets WC, Dantzer R, Teague TK, Bodurka J, Savitz J. Kynurenine pathway metabolites are associated with hippocampal activity

during autobiographical memory recall in patients with depression. *Brain Behav Immun.* (2016) 56:335–42. doi: 10.1016/j.bbi.2016.04.007
92. Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, et al. Kynurenine pathway in depression: A systematic review and meta-analysis. *Neurosci Biobehav Rev.* (2018) 90:16–25. doi: 10.1016/j.neubiorev.2018.03.023
93. Verdonk F, Petit AC, Abdel-Ahad P, Vinckier F, Jouvion G, de Maricourt P, et al. Microglial production of quinolinic acid as a target and a biomarker of the antidepressant effect of ketamine. *Brain Behav Immun.* (2019) 81:361–73. doi: 10.1016/j.bbi.2019.06.033
94. Myint AM. Kynurenines: from the perspective of major psychiatric disorders. *FEBS J.* (2012) 279:1375–85. doi: 10.1111/j.1742-4658.2012.08551.x
95. Jeong YI, Kim SW, Jung ID, Lee JS, Chang JH, Lee CM, et al. Curcumin suppresses the induction of indoleamine 2,3-dioxygenase by blocking the janus-activated kinase-protein kinase Cδ-STAT1 signaling pathway in interferon-γ-stimulated murine dendritic cells. *J Biol Chem.* (2009) 284:3700–8. doi: 10.1074/jbc.M807328200
96. Cesario A, Rutella BRS. The Iinterplay between indoleamine 2,3-dioxygenase 1 (IDO1) and cyclooxygenase (COX)-2 in chronic inflammation and cancer. *Curr nt Med Chem.* (2011) 18:2263–71. doi: 10.2174/092986711795656063
97. Calabrese JR, Skwerer RG, Barna B, Gulledge AD, Valenzuela R, Butkus A, et al. Depression, immunocompetence, and prostaglandins of the E series. *Psychiatry Res.* (1986) 17:41–7. doi: 10.1016/0165-1781(86)90040-5
98. Ohishi K, Ueno R, Nishino S, Sakai T, Hayaishi O. Increased level of salivary prostaglandins in patients with major depression. *Biol Psychiatry.* (1988) 23:326–34. doi: 10.1016/0006-3223(88)90283-1
99. Gałecki P, Talarowska M, Bobińska K, Szemraj J. COX-2 gene expression is correlated with cognitive function in recurrent depressive disorder. *Psychiatry Res.* (2014) 215:488–90. doi: 10.1016/j.psychres.2013.12.017
100. Faridhosseini F, Sadeghi R, Farid L, Pourgholami M. Celecoxib: a new augmentation strategy for depressive mood episodes. A systematic review and meta-analysis of randomized placebo-controlled trials. *Hum Psychopharmacol.* (2014) 29:216–23. doi: 10.1002/hup.2401
101. Abbasi SH, Hosseini F, Modabbernia A, Ashrafi M, Akhondzadeh S. Effect of celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: Randomized double-blind placebo-controlled study. *J Affect Disord.* (2012) 141:308–14. doi: 10.1016/j.jad.2012.03.033
102. Lee KH, Abas F, Alitheen NBM, Shaari K, Lajis NH, Ahmad S. A curcumin derivative, 2,6-bis(2,5-dimethoxybenzylidene)-cyclohexanone (BDMC33) attenuates prostaglandin E2 synthesis via selective suppression of cyclooxygenase-2 in IFN-γ/LPS-stimulated macrophages. *Molecules.* (2011) 16:9728–38. doi: 10.3390/molecules16119728
103. Choi GY, Kim HB, Hwang ES, Lee S, Kim MJ, Choi JY, et al. Curcumin alters neural plasticity and viability of intact hippocampal circuits and attenuates behavioral despair and COX-2 expression in chronically stressed rats. *Mediators Inflamm.* (2017) 2017:6280925. doi: 10.1155/2017/6280925
104. Sorokin A. Nitric oxide synthase and cyclooxygenase pathways: a complex interplay in cellular signaling. *Curr Med Chem.* (2016) 23:2559–78. doi: 10.2174/0929867323666160729105312
105. Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nat Rev Gastroenterol Hepatol.* (2005) 2:416–22. doi: 10.1038/ncpgasthep0259
106. Miller AL. The pathogenesis, clinical implications, and treatment of intestinal hyperpermeability. *Alternat Med Rev.* (1997) 2:16.
107. Maes M, Kubera M, Leunis JC. The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinol Lett.* (2008) 29:117–24.
108. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia.* (2007) 55:453–62. doi: 10.1002/glia.20467
109. Dobos N, de Vries EFJ, Kema IP, Patas K, Prins M, Nijholt IM, et al. The role of indoleamine 2,3-dioxygenase in a mouse model of neuroinflammation-induced depression. *J Alzheimer's Dis.* (2012) 28:905–15. doi: 10.3233/JAD-2011-111097
110. Fu X, Zunich SM, O'Connor JC, Kavelaars A, Dantzer R, Kelley KW. Central administration of lipopolysaccharide induces depressive-like behavior *in vivo* and activates brain indoleamine 2,3 dioxygenase in murine organotypic hippocampal slice cultures. *J Neuroinflammation.* (2010) 7:43. doi: 10.1186/1742-2094-7-43
111. O'Connor JC, Lawson MA, André C, Moreau M, Lestage J, Castanon N, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry.* (2009) 14:511–22. doi: 10.1038/sj.mp.4002148
112. Maes M, Kubera M, Leunis JC, Berk M. Increased IgA and IgM responses against gut commensals in chronic depression: Further evidence for increased bacterial translocation or leaky gut. *J Affect Disord.* (2012) 141:55–62. doi: 10.1016/j.jad.2012.02.023
113. Wang J, Ghosh SS, Ghosh S. Curcumin improves intestinal barrier function: modulation of intracellular signaling, and organization of tight junctions. *Am J Physiol Cell Physiol.* (2017) 312:C438–45. doi: 10.1152/ajpcell.00235.2016
114. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. *Trends Neurosci.* (2008) 31:464–8. doi: 10.1016/j.tins.2008.06.006
115. Nemeroff CB, Krishnan KRR, Reed D, Leder R, Beam C, Dunnick NR. Adrenal gland enlargement in major depression: a computed tomographic study. *Arch Gen Psychiatry.* (1992) 49:384–7. doi: 10.1001/archpsyc.1992.01820050048008
116. Rinwa P, Kumar A. Piperine potentiates the protective effects of curcumin against chronic unpredictable stress-induced cognitive impairment and oxidative damage in mice. *Brain Res.* (2012) 1488:38–50. doi: 10.1016/j.brainres.2012.10.002
117. Xu Y, Li S, Vernon MM, Pan J, Chen L, Barish PA, et al. Curcumin prevents corticosterone-induced neurotoxicity and abnormalities of neuroplasticity via 5-HT receptor pathway. *J Neurochem.* (2011) 118:784–95. doi: 10.1111/j.1471-4159.2011.07356.x
118. Xu Y, Ku B, Tie L, Yao H, Jiang W, Ma X, et al. Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. *Brain Res.* (2006) 1122:56–64. doi: 10.1016/j.brainres.2006.09.009
119. Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the brain: sources, localization and functions. *Mol Neurobiol.* (2013) 47:145–71. doi: 10.1007/s12035-012-8339-9
120. Stockhorst U, de Fries D, Steingrueber HJ, Scherbaum WA. Insulin and the CNS: effects on food intake, memory, and endocrine parameters and the role of intranasal insulin administration in humans. *Physiol Behav.* (2004) 83:47–54. doi: 10.1016/S0031-9384(04)00348-8
121. Zhao W-Q, Alkon DL. Role of insulin and insulin receptor in learning and memory. *Mol Cell Endocrinol.* (2001) 177:125–34. doi: 10.1016/S0303-7207(01)00455-5
122. Blázquez E, Velázquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the Brain: Its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front Endocrinol.* (2014) 5:161. doi: 10.3389/fendo.2014.00161
123. Ford AH, Flicker L, Hankey GJ, Yeap BB, Chubb SAP, Golledge J, et al. Insulin resistance and depressive symptoms in older men: the health in men study. *Am J Geriatr Psychiatry.* (2015) 23:872–80. doi: 10.1016/j.jagp.2014.10.010
124. Kan C, Silva N, Golden SH, Rajala U, Timonen M, Stahl D, et al. A systematic review and meta-analysis of the association between depression and insulin resistance. *Diabetes Care.* (2013) 36:480–9. doi: 10.2337/dc12-1442
125. Benedict C, Grillo CA. Insulin resistance as a therapeutic target in the treatment of Alzheimer's disease: A state-of-the-art review. *Front Neurosci.* (2018) 12:215. doi: 10.3389/fnins.2018.00215
126. Kleinridders A, Cai W, Cappellucci L, Ghazarian A, Collins WR, Vienberg SG, et al. Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. *Proc Natl Acad Sci USA.* (2015) 112:3463–8. doi: 10.1073/pnas.1500877112
127. Asghar A, Sheikh N. Role of immune cells in obesity induced low grade inflammation and insulin resistance. *Cell Immunol.* (2017) 315:18–26. doi: 10.1016/j.cellimm.2017.03.001

128. Tiganis T. Reactive oxygen species and insulin resistance: the good, the bad and the ugly. *Trends Pharmacol Sci.* (2011) 32:82–9. doi: 10.1016/j.tips.2010.11.006
129. Dandona P, Chaudhuri A, Ghanim H, Mohanty P. Proinflammatory effects of glucose and anti-inflammatory effect of insulin: relevance to cardiovascular disease. *Am J Cardiol.* (2007) 99:15–26. doi: 10.1016/j.amjcard.2006.11.003
130. Leonard BE, Wegener G. Inflammation, insulin resistance and neuroprogression in depression. *Acta Neuropsychiatr.* (2020) 32:1–9. doi: 10.1017/neu.2019.17
131. Yaribeygi H, Ashrafizadeh M, Henney NC, Sathyapalan T, Jamialahmadi T, Sahebkar A. Neuromodulatory effects of anti-diabetes medications: A mechanistic review. *Pharmacol Res.* (2020) 152:104611. doi: 10.1016/j.phrs.2019.104611
132. Shen JD, Wei Y, Li YJ, Qiao JY, Li YC. Curcumin reverses the depressive-like behavior and insulin resistance induced by chronic mild stress. *Metab Brain Dis.* (2017) 32:1163–72. doi: 10.1007/s11011-017-0017-1
133. Den Hartogh DJ, Gabriel A, Tsiani E. Antidiabetic properties of curcumin II: evidence from *in vivo* studies. *Nutrients.* (2019) 12:58. doi: 10.3390/nu12010058
134. Lee BH, Kim YK. The Roles of BDNF in the pathophysiology of major depression and in antidepressant treatment. *Psychiatry Investig.* (2010) 7:231–5. doi: 10.4306/pi.2010.7.4.231
135. Zoladz JA, Pilc A. The effect of physical activity on the brain derived neurotrophic factor: from animal to human studies. *J Physiol Pharmacol.* (2010) 61:533–41.
136. Björkholm C, Monteggia LM. BDNF - a key transducer of antidepressant effects. *Neuropharmacology.* (2016) 102:72–9. doi: 10.1016/j.neuropharm.2015.10.034
137. Rocha RB, Dondossola ER, Grande AJ, Colonetti T, Ceretta LB, Passos IC, et al. Increased BDNF levels after electroconvulsive therapy in patients with major depressive disorder: a meta-analysis study. *J Psychiatr Res.* (2016) 83:47–53. doi: 10.1016/j.jpsychires.2016.08.004
138. Malhi GS, Tanious M, Das P, Coulston CM, Berk M. Potential mechanisms of action of lithium in bipolar disorder. *CNS Drugs.* (2013) 27:135–53. doi: 10.1007/s40263-013-0039-0
139. Volmat V, Pouysségur J. Spatiotemporal regulation of the p42/p44 MAPK pathway. *Biol Cell.* (2001) 93:71–9. doi: 10.1016/S0248-4900(01)01129-7
140. Wang JQ, Mao L. The ERK pathway: molecular mechanisms and treatment of depression. *Mol Neurobiol.* (2019) 56:6197–205. doi: 10.1007/s12035-019-1524-3
141. Hurley LL, Akinfiresoye L, Nwulia E, Kamiya A, Kulkarni A, Tizabi Y. Antidepressant-like effects of curcumin in WKY rat model of depression is associated with an increase in hippocampal BDNF. *Behav Brain Res.* (2013) 239:27–30. doi: 10.1016/j.bbr.2012.10.049
142. Liu D, Wang Z, Gao Z, Xie K, Zhang Q, Jiang H, et al. Effects of curcumin on learning and memory deficits, BDNF, and ERK protein expression in rats exposed to chronic unpredictable stress. *Behav Brain Res.* (2014) 271:116–21. doi: 10.1016/j.bbr.2014.05.068
143. Zhang L, Xu T, Wang S, Yu L, Liu D, Zhan R, et al. Curcumin produces antidepressant effects via activating MAPK/ERK-dependent brain-derived neurotrophic factor expression in the amygdala of mice. *Behav Brain Res.* (2012) 235:67–72. doi: 10.1016/j.bbr.2012.07.019
144. Zhang L, Luo J, Zhang M, Yao W, Ma X, Yu SY. Effects of curcumin on chronic, unpredictable, mild, stress-induced depressive-like behaviour and structural plasticity in the lateral amygdala of rats. *Int J Neuropsychopharmacol.* (2014) 17:793–806. doi: 10.1017/S1461145713001661
145. Abd–Rabo MM, Georgy GS, Saied NM, Hassan WA. Involvement of the serotonergic system and neuroplasticity in the antidepressant effect of curcumin in ovariectomized rats: comparison with oestradiol and fluoxetine. *Phytother Res.* (2019) 33:387–96. doi: 10.1002/ptr.6232
146. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem Biol Interact.* (2014) 224:164–75. doi: 10.1016/j.cbi.2014.10.016
147. Bilici M, Efe H, Köroglu MA, Uydu HA, Bekaroglu M, Deger O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J Affect Disord.* (2001) 64:43–51. doi: 10.1016/S0165-0327(00)00199-3
148. Shafiee M, Ahmadnezhad M, Tayefi M, Arekhi S, Vatanparast H, Esmaeili H, et al. Depression and anxiety symptoms are associated with prooxidant-antioxidant balance: a population-based study. *J Affect Disord.* (2018) 238:491–8. doi: 10.1016/j.jad.2018.05.079
149. Fernandes BS, Dean OM, Dodd S, Malhi GS, Berk M. N-Acetylcysteine in depressive symptoms and functionality: a systematic review and meta-analysis. *J Clin Psychiatry.* (2016) 77:e457–466. doi: 10.4088/JCP.15r09984
150. Naqvi F, Saleem S, Naqvi F, Batool Z, Sadir S, Tabassum S, et al. Curcumin lessens unpredictable chronic mild stress-induced depression and memory deficits by modulating oxidative stress and cholinergic activity. *Pak J Pharm Sci.* (2019) 32:1893–900.
151. Fidelis EM, Savall ASP, da Luz Abreu E, Carvalho F, Teixeira FEG, Haas SE, et al. Curcumin-loaded nanocapsules reverses the depressant-like behavior and oxidative stress induced by β-amyloid in mice. *Neuroscience.* (2019) 423:122–30. doi: 10.1016/j.neuroscience.2019.09.032
152. Svízenská I, Dubový P, Sulcová A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures–a short review. *Pharmacol Biochem Behav.* (2008) 90:501–11. doi: 10.1016/j.pbb.2008.05.010
153. Mackie K. Cannabinoid receptors: where they are and what they do. *J Neuroendocrinol.* (2008) 20:10–4. doi: 10.1111/j.1365-2826.2008.01671.x
154. Williamson EM, Evans FJ. Cannabinoids in clinical practice. *Drugs.* (2000) 60:1303–14. doi: 10.2165/00003495-200060060-00005
155. Bisogno T, Berrendero F, Ambrosino G, Cebeira M, Ramos JA, Fernandez-Ruiz JJ, et al. Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem Biophys Res Commun.* (1999) 256:377–80. doi: 10.1006/bbrc.1999.0254
156. Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TTY, Gray JM, et al. Endogenous cannabinoid signaling is essential for stress adaptation. *Proc Natl Acad Sci USA.* (2010) 107:9406–11. doi: 10.1073/pnas.0914661107
157. Hill MN, Gorzalka BB. Impairments in endocannabinoid signaling and depressive illness. *JAMA.* (2009) 301:1165–6. doi: 10.1001/jama.2009.369
158. Horder J, Cowen PJ, Di Simplicio M, Browning M, Harmer CJ. Acute administration of the cannabinoid CB1 antagonist rimonabant impairs positive affective memory in healthy volunteers. *Psychopharmacology.* (2009) 205:85–91. doi: 10.1007/s00213-009-1517-4
159. Steffens M, Feuerstein T. Receptor-independent depression of DA and 5-HT uptake by cannabinoids in rat neocortex—Involvement of Na(+)/K(+)-ATPase. *Neurochem Int.* (2004) 44:529–38. doi: 10.1016/j.neuint.2003.08.009
160. He X, Zhu Y, Wang M, Jing G, Zhu R, Wang S. Antidepressant effects of curcumin and HU-211 coencapsulated solid lipid nanoparticles against corticosterone-induced cellular and animal models of major depression. *Int J Nanomedicine.* (2016) 11:4975–90. doi: 10.2147/IJN.S109088
161. Hassanzadeh P, Hassanzadeh A. The CB1 receptor-mediated endocannabinoid signaling and NGF: the novel targets of curcumin. *Neurochem Res.* (2012) 37:1112–20. doi: 10.1007/s11064-012-0716-2
162. Witkin JM, Leucke S, Thompson LK, Lynch RA, Ding C, Heinz B, et al. Further evaluation of the neuropharmacological determinants of the antidepressant-like effects of curcumin. *CNS Neurol Disord Drug Targets.* (2013) 12:498–505. doi: 10.2174/18715273113120 40008
163. Ng QX, Koh SSH, Chan HW, Ho CYX. Clinical use of curcumin in depression: a meta-analysis. *J Am Med Dir Assoc.* (2017) 18:503–8. doi: 10.1016/j.jamda.2016.12.071
164. Prasad S, Tyagi AK, Aggarwal BB. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice. *Cancer Res Treat.* (2014) 46:2–18. doi: 10.4143/crt.2014.46.1.2
165. Pulido-Moran M, Moreno-Fernandez J, Ramirez-Tortosa C, Ramirez-Tortosa Mc. Curcumin and health. *Molecules.* (2016) 21:264. doi: 10.3390/molecules21030264
166. Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, et al. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med.* (2006) 6:10. doi: 10.1186/1472-6882-6-10
167. Lopresti AL, Drummond PD. Efficacy of curcumin, and a saffron/curcumin combination for the treatment of major depression: a randomised,

double-blind, placebo-controlled study. *J Affect Disord.* (2017) 207:188–96. doi: 10.1016/j.jad.2016.09.047
168. Lopresti AL, Maes M, Maker GL, Hood SD, Drummond PD. Curcumin for the treatment of major depression: A randomised, double-blind, placebo controlled study. *J Affect Disord.* (2014) 167:368–75. doi: 10.1016/j.jad.2014.06.001
169. Tayyem RF, Heath DD, Al-Delaimy WK, Rock CL. Curcumin content of turmeric and curry powders. *Nutr Cancer.* (2006) 55:126–31. doi: 10.1207/s15327914nc5502_2
170. Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Curry consumption and cognitive function in the elderly. *Am J Epidemiol.* (2006) 164:898–906. doi: 10.1093/aje/kwj267
171. Burapan S, Kim M, Han J. Curcuminoid demethylation as an alternative metabolism by human intestinal microbiota. *J Agric Food Chem.* (2017) 65:3305–10. doi: 10.1021/acs.jafc.7b00943
172. Adiwidjaja J, McLachlan AJ, Boddy AV. Curcumin as a clinically-promising anti-cancer agent: pharmacokinetics and drug interactions. *Expert Opin Drug Metab Toxicol.* (2017) 13:953–72. doi: 10.1080/17425255.2017.1360279
173. Lopresti AL, Maes M, Meddens MJM, Maker GL, Arnoldussen E, Drummond PD. Curcumin and major depression: a randomised, double-blind, placebo-controlled trial investigating the potential of peripheral biomarkers to predict treatment response and antidepressant mechanisms of change. *European Neuropsychopharmacology.* (2015) 25:38–50. doi: 10.1016/j.euroneuro.2014.11.015
174. Xiao QC, Dui D, Lan TG. Effects of qingyi II granules on intestinal bacterial translocation in rats with acute necrotizing pancreatitis. *Chin J Integr Tradit West Med.* (2010) 26: 317–24. doi: 10.3978/j.issn.1005-6947.2017.03.008
175. Piechota A, Polańczyk A, Goraca A. Role of endothelin-1 receptor blockers on hemodynamic parameters and oxidative stress. *Pharmacol Rep.* (2010) 62:28–34. doi: 10.1016/S1734-1140(10)70240-1
176. Lange M, Pagotto U, Renner U, Arzberger T, Oeckler R, Stalla GK. The role of endothelins in the regulation of pituitary function. *Exp Clin Endocrinol Diabetes.* (2002) 110:103–12. doi: 10.1055/s-2002-29086
177. Giordano N, Papakostas P, Pecetti G, Nuti R. Cytokine modulation by endothelin-1 and possible therapeutic implications in systemic sclerosis. *J Biol Regul Homeost Agents.* (2011) 25:487–92.
178. Sanmukhani J, Satodia V, Trivedi J, Patel T, Tiwari D, Panchal B, et al. Efficacy and safety of curcumin in major depressive disorder: a randomized controlled trial. *Phytother Res.* (2014) 28:579–85. doi: 10.1002/ptr.5025
179. Asadi S, Gholami MS, Siassi F, Qorbani M, Sotoudeh G. Beneficial effects of nano-curcumin supplement on depression and anxiety in diabetic patients with peripheral neuropathy: a randomized, double-blind, placebo-controlled clinical trial. *Phytother Res.* (2020) 34:896–903. doi: 10.1002/ptr.6571
180. Setiawati MC, Ikawati Z, Kertia IN. Antiinflamatory and antidepressive activities of extract *Curcuma xanthorrhiza* roxb in systemic *lupus Erythematosus*. *Indones J Pharm.* (2017) 28:185. doi: 10.14499/indonesianjpharm28iss3pp185
181. Kappelmann N, Lewis G, Dantzer R, Jones PB, Khandaker GM. Antidepressant activity of anti-cytokine treatment: a systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. *Mol Psychiatry.* (2018) 23:335–43. doi: 10.1038/mp.2016.167
182. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, et al. A randomized controlled trial of the tumor necrosis factor-alpha antagonist infliximab in treatment resistant depression: role of baseline inflammatory biomarkers. *JAMA Psychiatry.* (2013) 70:31–41. doi: 10.1001/2013.jamapsychiatry.4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 28 January 2021
doi: 10.3389/fpsyt.2021.622204

The Antidepressant-Like Effects of Shen Yuan in a Chronic Unpredictable Mild Stress Rat Model

*Ning Jiang[1], Haixia Wang[1], Hong Huang[1], Jingwei Lv[1], Guirong Zeng[1], Qiong Wang[2], Yu Bao[1], Ying Chen[3] and Xinmin Liu[1]**

[1] Research Center for Pharmacology and Toxicology, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2] Affiliated Traditional Chinese Medicine Hospital/School of Pharmacy/Sino-Portugal TCM International Cooperation Center, Southwest Medical University, Luzhou, China, [3] Institute of Chinese Materia Medical, China Academy of Chinese Medical Sciences, Beijing, China

Depression is a common yet severe neuropsychiatric condition that causes imposes considerable personal, economic, and social burdens worldwide. Medicinal plant species (e.g., *Panax ginseng* and *Polygala tenuifolia*) demonstrate potent antidepressant-like effects with less toxicity and other side effects. Shen yuan prescription (SY), composed of *Panax ginseng* (GT) and *Polygala tenuifolia* (YT). The present study aimed to elucidate the effects of SY treatment on chronic unpredictable mild stress (CUMS) rats and study the underlying mechanism. Our results indicated that SY (67.5, 135, or 270 mg/kg) significantly reverses the depressive-like behaviors in rats with a 5-week CUMS exposure, as demonstrated by increased sucrose consumption in the sucrose preference test, and decreased immobility time in the tail suspension and forced swim test. Moreover, SY altered serum corticosterone levels, pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α), and oxidative markers (SOD, CAT, and MDA), and increased the levels of hippocampal neurotransmitters (5-HT, DA, and NE) in rats exposed to CUMS. Furthermore, rats treated with SY showed a reduction in the protein expression of BDNF, p-TrkB, p-Akt, and p-mTOR proteins induced by CUMS exposure in the hippocampus. In conclusion, SY prevented depressive-like behaviors in CUMS-exposed rats by preventing hypothalamus-pituitary-adrenal axis dysfunction, decreasing the levels of the neurotransmitters, minimizing oxidative stress, suppressing neuroinflammation, and activating the PI3K/Akt/mTOR-mediated BDNF/TrkB pathway, all of which are the key players in the pathological basis of depression.

Keywords: SY, depression, CUMS, neurotransmitters, oxidative stress

OPEN ACCESS

Edited by:
Elaine Elisabetsky, Federal University of Rio Grande Do Sul, Brazil

Reviewed by:
Tijen Utkan, Kocaeli University, Turkey
Marvin Rafael Diaz, Binghamton University, United States

****Correspondence:***
Xinmin Liu
liuxinmin@hotmail.com

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *28 October 2020*
Accepted: *04 January 2021*
Published: *28 January 2021*

Citation:
Jiang N, Wang H, Huang H, Lv J, Zeng G, Wang Q, Bao Y, Chen Y and Liu X (2021) The Antidepressant-Like Effects of Shen Yuan in a Chronic Unpredictable Mild Stress Rat Model. Front. Psychiatry 12:622204. doi: 10.3389/fpsyt.2021.622204

INTRODUCTION

The onset of depression, a neuropsychiatric disease, inflicts a significant global socioeconomic burden. With the ever-increasing pace of modern life, people experience increased stress in their professional and personal lives. The incidence of depression has increased throughout these years (1, 2). However, the pathogenic mechanisms underlying the onset of depression are unknown. Mounting evidence suggests that depression results from the dysfunction of the

hypothalamus-pituitary-adrenal (HPA) axis, reduced secretion of neurotransmitters (such as 5-hydroxy tryptophan, norepinephrine, and dopamine), neuro-inflammation, oxido-nitrosative stress, reduced cell proliferation, abnormal cytokine secretion, depleted levels of neurotrophic factors, and disordered neuroplasticity (3–5). At present, antidepressants such as selective serotonin reuptake inhibitors mainly target monoamine levels; however, these drugs have side effects and high failure rates (6). Thus, antidepressants with increased effectiveness and acceptable safety profiles should be developed.

In recent years, the search for effective low-toxicity antidepressants, mostly from natural products and underlying mechanisms, is a promising research area. *Panax ginseng* (GT) and *Polygala tenuifolia* (YT), the traditional Chinese herbal medicines, possess a broad spectrum of neurotrophic and neuroprotective effects and are widely used for treating several neurological disorders, including depression (7, 8). In one of our earlier studies, we demonstrated the superior antidepressant efficacy of SY prescription compared with GT or YT alone using tail suspension and forced swim tests (9). Recent studies have indicated that SY upregulates hippocampal BDNF and TrkB expression and induces antidepressant-like effects in learned helplessness (LH) and chronic mild stress rat models (10, 11). In the present study, we utilized the chronic unpredictable mild stress (CUMS)-induced depression animal model to explore SY's antidepressant effects using the sucrose preference test, tail suspension test, and forced swimming test. We also measured the neurotransmitters and corticosterone levels, inflammatory mediators, and oxidative stress levels and studied the PI3K/Akt/mTOR-mediated BDNF/TrkB pathway to elucidate the underlying mechanism of action.

MATERIALS AND METHODS

Chemicals and Reagents

Fluoxetine HCl (>98%) was procured from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Norepinephrine (NE, noradrenaline) was purchased from the National Institute for Food and Drug Control (Beijing, China); dopamine (DA), 5-hydroxy tryptamine (5-HT) and DOPAC (dihydroxy-phenyl acetic acid) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA); and commercial kits for CORT, IL-6, IL-1β, TNF-α, superoxide dismutase (SOD), (LPO) and malondialdehyde (MDA) were from Jiancheng Biological Technology Co., Ltd. (Nanjing, China).

SY Preparation

Ginseng was purchased from the Jilin Shennong Traditional Chinese Medicine Technology Development Co., Ltd. (Jilin, China), and *Polygala tenuifolia* Willd was purchased from Shanxi Yuncheng Tiandi Net Chinese Herbal Medicine Co., Ltd (Shanxi, China). SY was prepared by water extracts from the stems of *P. tenuifolia* and *P. ginseng*. Briefly, dried roots of *P. tenuifolia* and *P. ginseng* (crude drug quantity ratio, 3:2) were extracted three times with deionized water, each time for 1 h, and the solid-liquid ratio was 8, 6, and 6, respectively. The extract was filtered through a 100-mesh sieve and combined. The filtrate was concentrated to a relative density of 1.125 (1.10–1.15, 25°C), followed by alcohol precipitation [concentration at 70% (w/w)], and refrigerated for 12 h at 10°C. Finally, after filtration by a 200-mesh sieve, the filtrate was evaporated and concentrated by spray drying (air inlet temperature was 180°C, air outlet temperature was 90°C) to obtain dry extract powder, and the paste extraction rate relative to crude drug was 26.4–27%. The refined anhydrous SY contained 0.26% 3',6-disinapoyl sucrose (DISS), 0.12% ginsenoside Rg1 (Rg1), 0.12% ginsenoside Re (Re), and 0.30% ginsenoside Rb1 (Rb1), as determined by HPLC on an anhydrous basis (**Figure 1**).

Animals and Treatments

Sixty Sprague-Dawley (SD) rats (male; 180–200 g) (Institute of the Chinese Academy of Medical Science Center, Beijing, China) were housed under standard experimental conditions (20–22°C, 55% humidity, food and water *ad libitum*, 12:12 h light/dark cycle) and acclimatized. The animals had unrestricted access to food and water and were acclimatized to these conditions for 7 days. The animal ethics committee of the Institute of Medicinal Plant Development, Peking Union Medical College, sanctioned all animal experiments (approval no. SYXK 2017-0020).

Animals were randomly divided into six groups: the control group, the CUMS model group, the SY-treated groups (67.5, 135, and 270 mg/kg) and the fluoxetine group (10 mg/kg). Different doses of SY or fluoxetine were administered orally to CUMS rats for 35 consecutive days until the behavioral tests were completed. The experimental procedure is illustrated in **Figure 2**.

Chronic Unpredictable Mild Stress Model

The CUMS model was established following a modified procedure (9). The five test groups (excluding the control group) of rats were housed in individual cages and exposed to these stressors for 35 d: 24 h deprivation of food/water, 6 h restraint, 12 h illumination, 24 h light/dark alterations, 3 min tail pinch, predator sounds for 30 min, cold water (4°C) swimming for 5 min, electrical stimulation for 3 min, and hot water (40°C) swimming for 5 min. Rats were exposed to one or two different stressors daily for 5 weeks.

Behavioral Tests

Open Field Test (OFT)

We used a self-developed computer-aided controlling system to assess the locomotive activity of the experimental rats on day 34 via OFT (12). The system constituted round metal pools (d × h: 75 cm × 40 cm) with a video camera fixed at the top, and four rats were placed at the center to explore freely for 5 min in light condition at 100 lux.

Sucrose Preference Test (SPT)

Post-CUMS period, we performed the SPT following previously described method with slight modification (13, 14). All rats were housed in individual cages for a 2 day adaptation phase and could drink from two bottles *ad libitum* (first day: two 1% sucrose solutions; the second day: 1% sucrose solution or tap water). Next, SPT was performed post-24 h food/water deprivation. Every rat was allowed to drink the pre-weighed sugar water and pure water for 1 h in a quiet

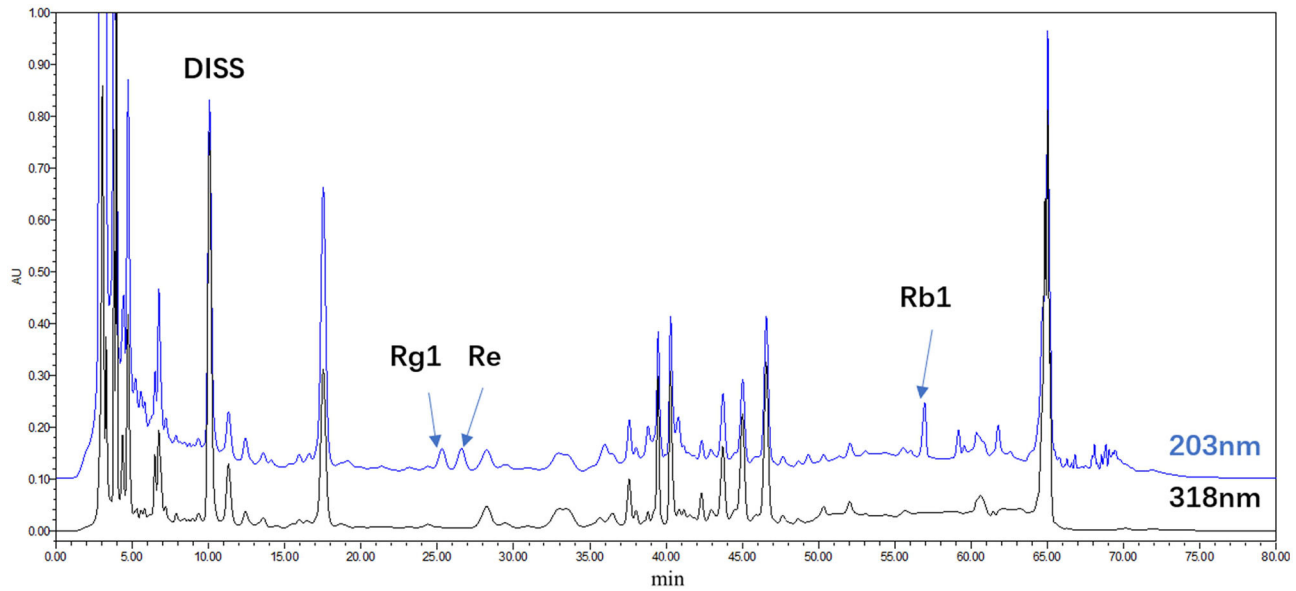

FIGURE 1 | HPLC chromatogram of the water extract of *Panax ginseng* and *Polygala tenuifolia* (SY); DISS; Rg1; Re; Rb1.

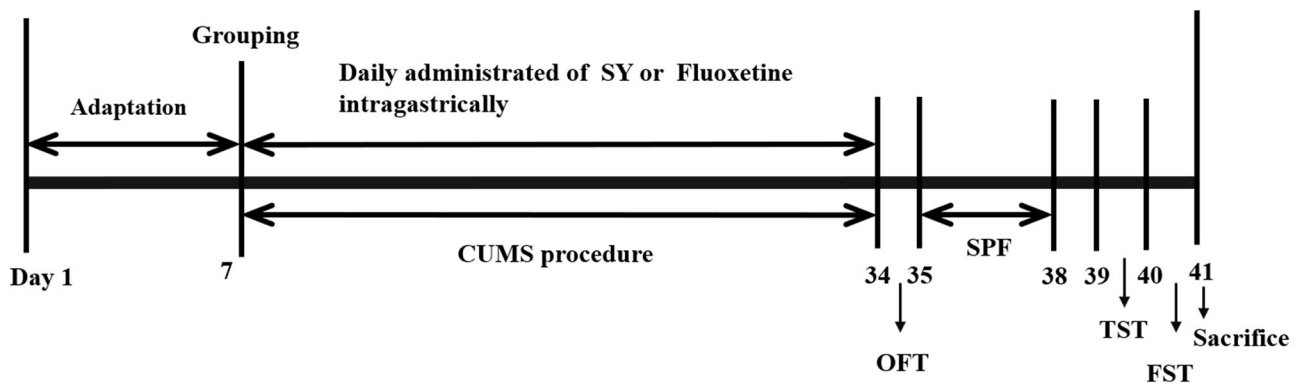

FIGURE 2 | Experimental design procedure.

and peaceful environment. Then, all the bottles were removed, weighed, and recorded. The following equation was used to calculate the sucrose preference: sucrose preference index (%) = (sucrose solution consumed/total solution consumed) × 100%.

Tail Suspension Test

The TST was performed following a previously described method (15, 16). Each rat was hung from a ledge for 6 min by the tail ∼70 cm above the floor using adhesive tape. The "despair behavior" was recorded as the period of immobility during the total 6 min test duration.

Forced Swimming Test

The FST was conducted following a previously described method (11, 17). On the first day, each rat was constrained to swim for 15 min at a depth of 30 cm in an acrylic cylinder (d × h: 18 × 40 cm) at a temperature of 25°C). After 24 h, we reintroduced the rats into the same cylinder for a 5 min swimming test. The total period of immobility during the 5 min test duration was noted.

Biochemical Analysis

Blood Sampling and Tissue Extraction

The rats were decapitated, and brain tissues were collected, followed by the isolation and weighing of the hippocampal tissue. After collection, blood was centrifuged (4°C; 15 min; 3,000 rpm). Both brain tissues and serum samples were stored at −80°C.

Measurement of the Neurotransmitter Levels in the Hippocampus

We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine the hippocampal levels of 5-HT, DA, and NE (18, 19). After homogenization, the hippocampal tissues

were added to the acetonitrile solution containing 5 μg/mL of 3,4-dihydroxybenzylamine (DHBA, the internal standard) and centrifuged (4°C; 20,000 rpm; 30 min). Next, we measured the levels of the neurotransmitters in the supernatant via LC-MS/MS. We used a TSKgel Amide-80 column, maintained at 35°C. The separation was done at a flow rate of 0.4 mL/min using an 15 mM ammonium formate-acetonitrile solution in a 60:40 ratio (pH 5.5). The system was operated in the positive ion ESI mode under the multiple reaction monitoring (MRM) mode, at the m/z 177.0→ 160.0, 170.0→ 152.0, 154.2→ 136.6, 140.0→ 123.0 for 5-HT, NE, DA, and DHBA, respectively. The ratio of the peak areas of the analyte and DHBA were used to determine the concentration of the neurotransmitters.

Determination of Serum Corticosterone, Pro-inflammatory Cytokines, and Oxidative Stress Levels

The serum levels of IL-1β, IL-6, and TNF-α (Pro-inflammatory markers); catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and corticosterone (CORT) (oxidative stress markers) were determined using commercial ELISA kits (Jiancheng, Nanjing, China).

Western Blot

After homogenization in RIPA lysis buffer (with protease/phosphatase inhibitors), the hippocampal homogenate was centrifuged (4°C; 12,000 g; 15 min), and quantified via the BCA protein assay. Next, the samples (30 μg) were electrophoresed (10% SDS-PAGE), and then transferred onto a PVDF membrane (Millipore, USA). The non-specific sites on the membrane were blocked by incubation for 1 h in 5% non-fat dry milk in TBS-T. Next, the membranes were kept in overnight incubation at 4°C with primary antibodies, including mTOR (1:1000, Cell Signaling); phospho-Akt (1:2000, Cell Signaling); phospho-TrkB (1:1000, Abcam); phospho-mTOR (1:1000, Cell Signaling); BDNF (1:5000, Abcam), and β-actin (1:1000, Cell Signaling). After washing, the membrane was incubated for 1 h with HRP-conjugated secondary antibody at room temperature. The enhanced chemiluminescence method was used to visualize the bands, which were captured using ChemiDoc XRS (Bio-Rad, USA). To eliminate variations in protein expression, three independent experiments were performed, and the data were adjusted to correspond to internal reference expression (β-actin), and the results are shown as a percentage of controls.

Data and Statistical Analyses

All results were analyzed using SPSS Statistics 21.0 (SPSS Inc., Illinois, Chicago, USA). The differences between the means were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's LSD *post hoc* test. Significance levels were considered at $p < 0.05$, and values are displayed as the mean ± standard error of the mean (SEM).

RESULTS

Effects of SY on Depression-Like Symptoms in the CUMS Rat Model

As per OFT results, we observed an insignificant difference in the total distance among all groups ($p > 0.05$; **Figure 3A**), which implies that SY treatment did not affect the locomotor activities of rats. Thus, the following behavioral tests could be used reliably to assess the animals.

Figure 3B shows the performance of rats in the sucrose preference test (SPT). Sucrose consumption was decreased in CUMS-exposed rats to the same extent as in the control rats [$F_{(5,54)} = 4.606$, $p < 0.01$]; the decreases in both the groups were significant. However, the decreases in sucrose consumption were considerably alleviated in all SY treatment groups ($p < 0.05$, $p < 0.01$, $p < 0.01$), consistent with the decrease in 10 $mgkg^{-1}$ fluoxetine group ($p < 0.01$).

In the tail suspension test (TST), CUMS-exposed rats exhibited behavioral despair, as shown by substantially enhanced immobility time as compared with no-stress control rats [**Figure 3C**; $F_{(5,54)} = 4.606$, $p < 0.01$]. However, treatment with SY (67.5, 135, and 270 $mgkg^{-1}$) considerably reduced the immobility time in CUMS-exposed rats, especially at 270 $mgkg^{-1}$ (all $p < 0.05$).

Figure 3D shows the results of the forced swimming test (FST). We observed a substantial increase in the immobility time in the CUMS group as compared with the control group after a 5 week exposure to the stressors [$F_{(5,54)} = 7.495$, $p < 0.01$], which was considerably reversed after administration of SY (67.5, 135, and 270 $mgkg^{-1}$) or fluoxetine (10 $mgkg^{-1}$) ($p < 0.01$, $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively).

Effects of SY on the Levels of Oxidative Stress Markers

The CUMS-exposed rats exhibited elevated levels of serum corticosterone (CORT) than the controls [**Figure 4A**; $F_{(5,54)} = 5.987$, $p < 0.01$]. However, 42 d treatment with SY (135 and 270 $mgkg^{-1}$) or fluoxetine (10 $mgkg^{-1}$) substantially reversed the CUMS-induced elevation of serum CORT levels ($p < 0.05$, each).

Figures 4B–D demonstrate a significant reduction in the hippocampal levels of NE, 5-HT, and DA post-CUMS induction as compared with the unstressed controls ($p < 0.01$ each). However, SY (270 $mgkg^{-1}$) or fluoxetine (10 $mgkg^{-1}$) considerably suppressed the decrease in the elevated levels of 5-HT [**Figure 4C**; $F_{(5,54)} = 4.425$, $p < 0.01$]. All SY-treated groups (67.5, 135, and 270 $mgkg^{-1}$) exhibited significantly increases in NE [**Figure 4C**; $F_{(5,54)} = 11.666$, $p < 0.01$ each] and DA levels [**Figure 4D**; $F_{(5,54)} = 4.220$, $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively].

Effects of SY on Serum Oxidative Stress Biomarkers and Pro-Inflammatory Levels

We measured the serum concentration of SOD, CAT, and MDA to assess the impact of the CUMS procedure and SY treatment on redox homeostasis. We observed a substantial reduction in SOD and CAT levels [**Figures 5A–C**; $F_{(5,54)} = 2.851$, $F_{(5,54)} = 10.940$, $F_{(5,54)} = 2.218$, $p < 0.01$ each] and a substantial increase in MDA

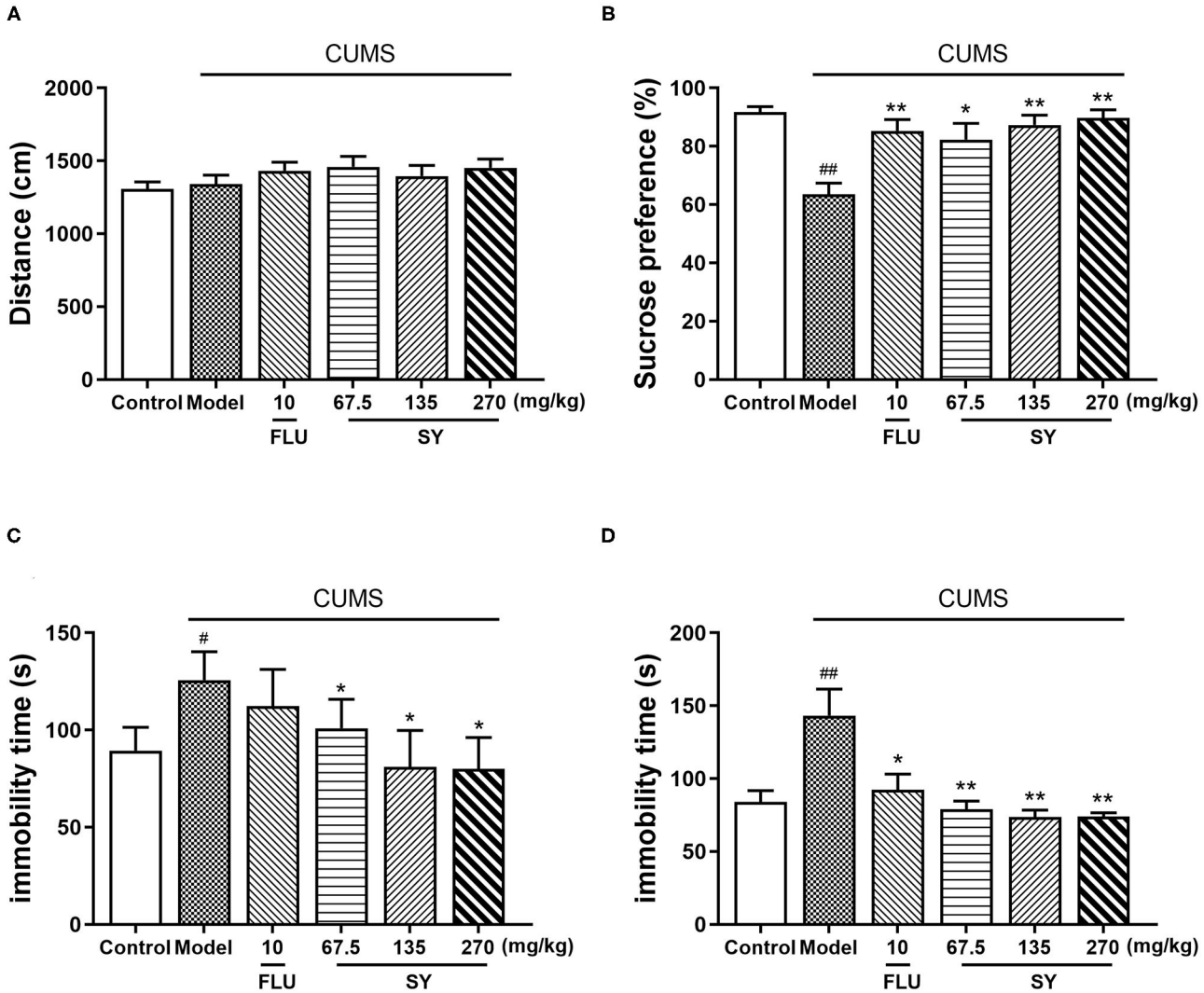

FIGURE 3 | Antidepressant-like effects of chronic SY treatment on CUMS rats. **(A)** Open field test. **(B)** Sucrose preference test. **(C)** Tail suspension test. **(D)** Forced swim test. Data represent the mean ± S.E.M. ($n = 10$ per group). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the CUMS group.

levels ($p < 0.01$) in CUMS group as compared with the control group. Serum levels of SOD and CAT increased after treatment with SY (270 $mgkg^{-1}$) ($p < 0.05$, $p < 0.01$); MDA levels decreased considerably post-treatment with SY (67.5, 135, and 270 $mgkg^{-1}$) as compared with the CUMS group (**Figure 4C**; $p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively).

Next, we estimated the hippocampal levels of IL-1β, IL-6, and TNF-α to confirm the protective response of SY on CUMS-induced neuroinflammation *in vivo*. CUMS procedure induced a substantial increase in the serum levels of IL-1β, IL-6, and TNF-α [**Figures 5D–F**; $F_{(5,54)} = 3.948$, $F_{(5,54)} = 2.294$, $F_{(5,54)} = 1.728$, all $p < 0.01$]. Additionally, there was a significant decrease in serum IL-1β levels after treatment with SY (135 and 270 $mgkg^{-1}$) in the CUMS group (**Figure 4D**; $p < 0.05$ and $p < 0.01$). Treatment with SY (67.5, 135, and 270 $mgkg^{-1}$) significantly reversed the elevation in the serum levels of IL-6 and TNF-α (**Figure 4E**; $p < 0.01$, **Figure 4F**; $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively).

Effects of SY on PI3K/Akt/mTOR-Mediated BDNF/TrkB Pathway-Related Protein Expression

We further evaluated the expression of PI3K/Akt/mTOR-mediated BDNF/TrkB pathway-related proteins to investigate the role of SY in depression. The western blotting results (**Figures 6A–D**) indicated that CUMS exposure significantly induced a decrease in hippocampal phosphorylated PI3K, phosphorylated Akt, phosphorylated mTOR, and BDNF expression as compared with control rats (all $p < 0.01$). However, varying degrees of increases were observed in the

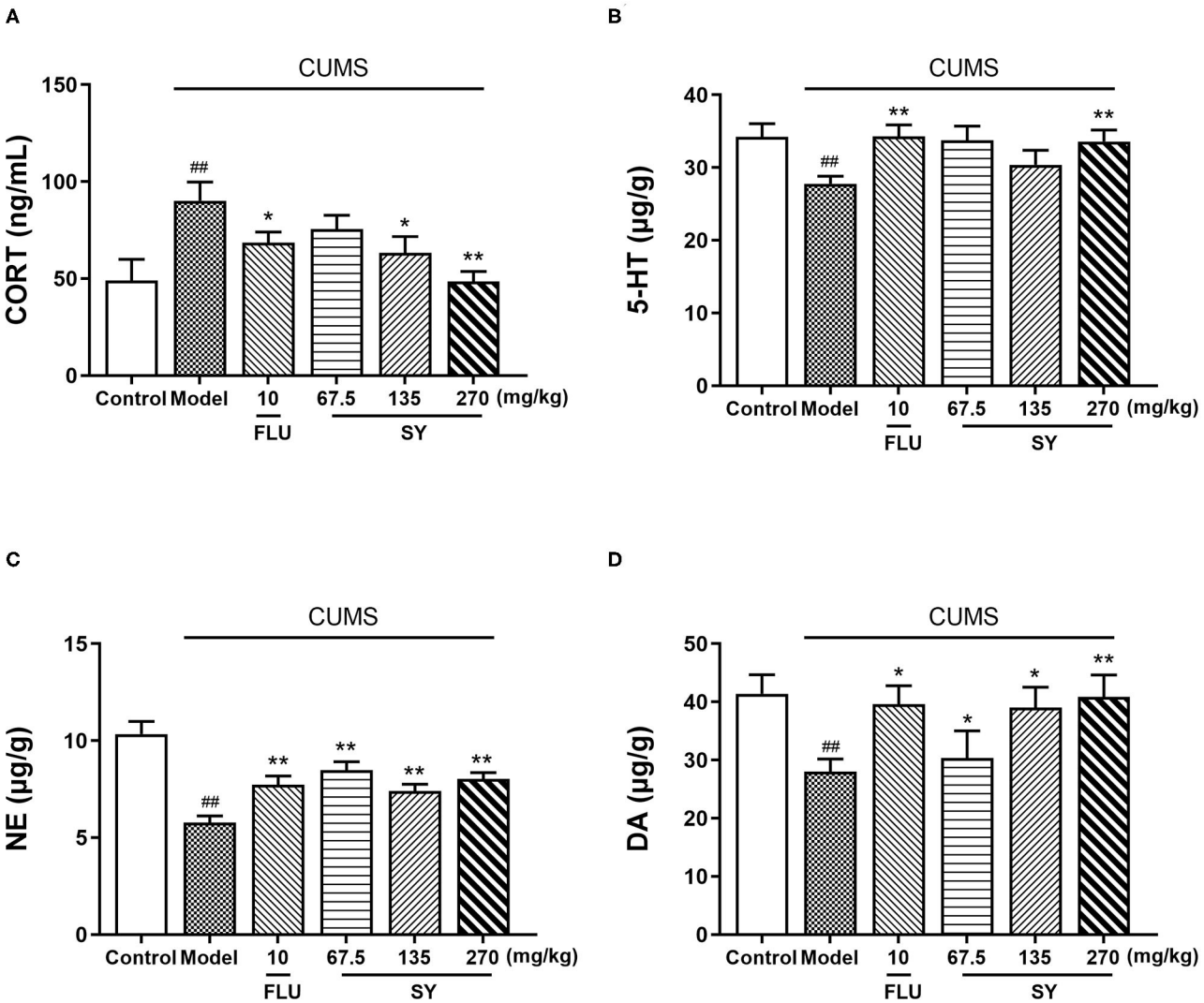

FIGURE 4 | Effects of SY on the levels of CORT, 5-HT, NE, and DA in CUMS rats. **(A)** Levels of corticosterone (CORT). **(B)** Levels of 5-hydroxy tryptamine (5-HT). **(C)** Levels of norepinephrine (NE). **(D)** Levels of dopamine (DA) in each group. Data represent the mean ± S.E.M. ($n = 10$ per group). $^{\#\#}p < 0.01$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the CUMS group.

expression of BDNF, phosphorylated PI3K, Akt, and mTOR in all the SY-treated groups ($p < 0.01$ each).

DISCUSSION

We observed that high concentrations of SY alleviated CUMS-induced depression symptoms, including anhedonia and despair, as demonstrated by significantly increased sucrose preference in SPT and reduced immobility in TST and FST in rats. At the same time, SY treatment significantly decreased the serum levels of CORT and increased hippocampal levels of neurotransmitters (NE, 5-HT, and DA) in CUMS-exposed rats. SY also alleviated oxidative stress and inhibited inflammation in CUMS-exposed rats, as evidenced by elevated SOD activity and CAT levels, lowered MAD content, and decreased IL-1β, IL-6, and TNF-α serum levels. Moreover, we found that SY-treated rats displayed enhanced hippocampal expression of BDNF, p-Akt, p-PI3K, and p-mTOR proteins.

Katz (20) observed that the rats showed reduced activity ability after getting exposed to a series of stress factors such as bright light stimulation, noise, and prolonged behavioral restriction. Willner (21) developed a CUMS depression model based on their observations. As compared with Katz's scheme, Willner's procedure had two changes: the reduced intensity of stimulants and the use of anhedonia as the measure of the model's success instead of the change in exercise ability. CUMS model simulates human beings' continuous acceptance of uncontrollable adverse events in social life and applies several different stress factors in random order throughout the

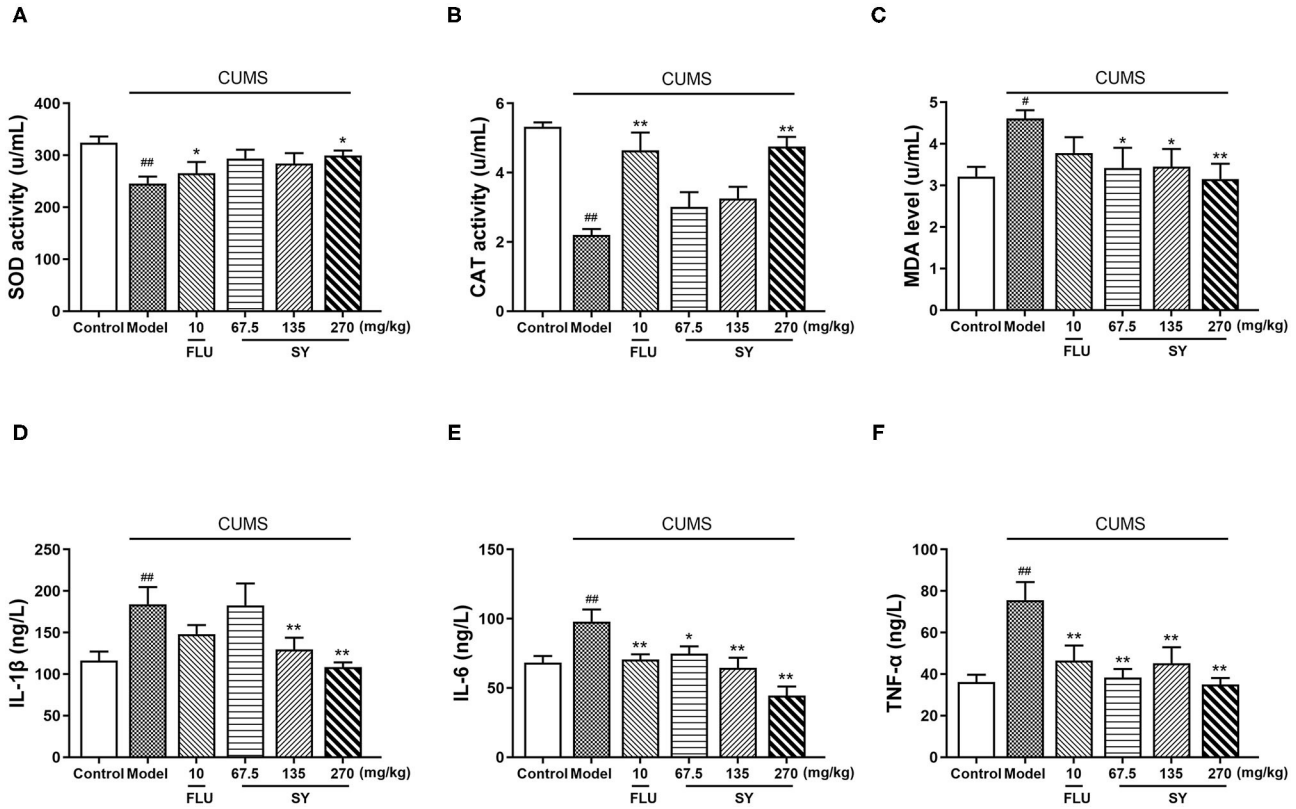

FIGURE 5 | Effects of SY on oxidative stress biomarkers and pro-inflammatory cytokines in CUMS rats. **(A)** SOD activity. **(B)** CAT activity **(C)** MDA level. **(D)** IL-1β level. **(E)** IL-6 level. **(F)** TNF-α level. Data represent the mean ± S.E.M. ($n = 10$ per group). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the CUMS group.

experiment so that the animals cannot anticipate the occurrence of stimuli. As compared with the animal model of depression caused by acute stress such as forced swimming or electric shock, CUMS model can better simulate the social and environmental stress caused by depression, with higher credibility, and is the most important animal model for screening antidepressants and their mechanism of action (22). Accumulating evidence has demonstrated that a 5-week CUMS exposure can elicit depression-like symptoms, including anhedonia and despair behavior, as shown by reduced sucrose intake in the SPT and enhanced immobility time in the TST and FST (23), which is consistent with the current study. As compared with treatment with classical antidepressants (fluoxetine and imipramine), SY treatment was found to significantly reverse the depression-like behaviors, consistent with a previous report (11). Therefore, this study proved that SY possesses significant antidepressant activity.

When the organism is in a state of stress, the hypothalamic-pituitary-adrenal (HPA) axis will improve its excitability and promote the organism's effective adaptive regulation, which is conducive to preserving life and adaptation to the environment. The HPA axis eventually releases CORT or cortisol that acts on corticosteroid receptors in the brain and is involved in negative feedback regulation in the hypothalamus and pituitary gland. Serum CORT or cortisol levels can largely reflect the status of the HPA axis. However, under long-term stress, the HPA axis is continuously excited and may even damage its negative feedback regulation mechanism; thus, the entire HPA axis cannot be suppressed. Excessively high CORT or cortisol levels for a long time are implicated in the pathophysiology of depression. Depressed patients have significantly high cortisol concentrations, and depression model animals also show sharply rising corticosterone levels (24). In the present study, CUMS exposure caused a significant increase in rats' serum CORT levels, reflecting HPA axis hyperfunction, which is in line with previous studies. SY significantly decreased these elevated CORT levels, indicating that SY might elicit its antidepressant effects by inhibiting the hyperactivation of the HPA axis. The monoamine hypothesis put forward in the last century is the classical hypothesis of depression, which has been widely accepted and verified. This hypothesis suggests that depression results from a deficiency in monoamine neurotransmitters in the brain, and the clinical antidepressants currently in use target the monoaminergic system (25). CUMS procedure reduces hippocampal and prefrontal cortex levels

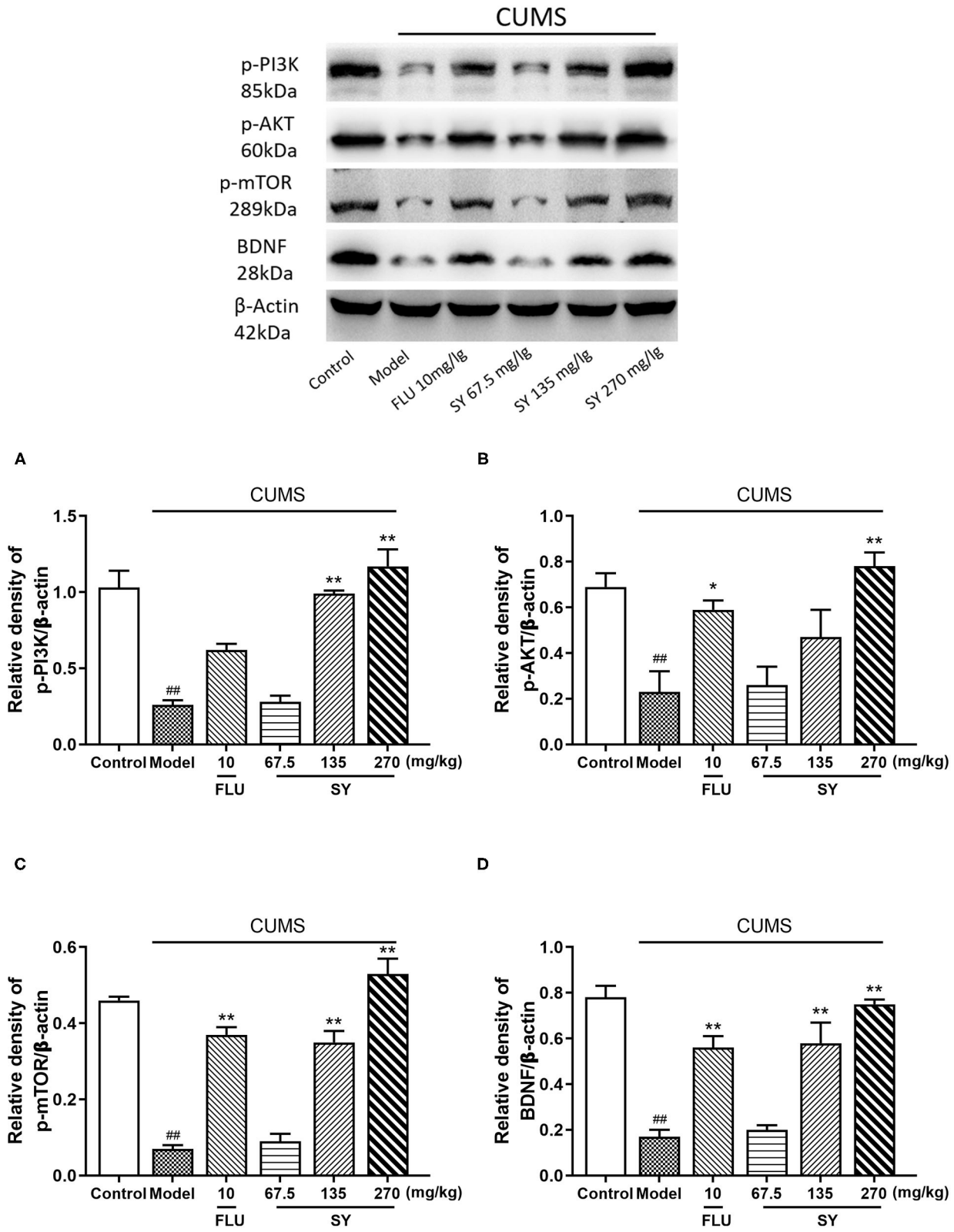

FIGURE 6 | Effects of SY on the hippocampal expression of p-PI3K, p-AKT, p-mTOR, and BDNF in CUMS rats. **(A)** Levels of p-PI3K. **(B)** Levels of p-AKT. **(C)** Levels of p-mTOR. **(D)** Levels of BDNF in each group. Data represent the mean ± S.E.M. ($n = 10$ per group). $^{\#\#}p < 0.01$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the CUMS group.

of monoamines, including DA, 5-HT, and NE, inducing monoamine deficiency (18). Our results also showed that NE, 5-HT, and DA levels in CUMS-exposed rats were significantly lower than those of non-stressed control rats. As expected, the administration of SY significantly reversed this effect for 5-HT, DA, and NE and increased their secretion to levels similar to those in rats that were given the antidepressant Flu. These observations indicated that this normalization of neurotransmitter expression might induce the antidepressant effects of SY.

Increasing evidence supports the view that depression and chronic stress-induced depression-like behavior are associated with inflammation (26). Excessive secretion of IL-1β, IL-6, and TNF-α triggers depression-like symptoms. Indeed, depressive patients exhibit upregulated levels of pro-inflammatory cytokines (27). Animal studies have also shown that some depressive-like symptoms are related to the chronic stress-mediated secretion of inflammatory cytokines (28). Accordingly, rats subjected to CUMS exhibited elevated levels of IL-1β, IL-6, and TNF-α; however, SY treatment successfully reversed this effect. Inflammation is closely related to oxidative stress. Under normal circumstances, there is a dynamic balance between the body's antioxidant capacity and oxidative capacity. During oxidative stress, a large number of free radicals are generated, causing an imbalance in the body's oxidation-antioxidant system, leading to mitochondrial damage, NO release, and MDA accumulation (29). The level of cytokines is increased, and the activity of antioxidants (SOD, CAT, and GSH) is decreased, causing damage and apoptosis of hippocampal neurons in the brain. These manifestations induce depression or aggravate the course of depression (30, 31). Similar to previous studies, our study also reports that a 5-week exposure to CUMS causes substantially reduced SOD and CAT levels and a notable increase in the serum concentration of MDA in rats. However, SY (67.5, 130, and 270 $mgkg^{-1}$) regulated the expression of these oxidative stress indicators in model rats to varying degrees and improved the depression-like symptoms. These findings suggest that SY exhibits its antidepressant effects by inhibiting immune-mediated inflammation and suppressing oxidative stress.

The hippocampus, which is a key brain area for learning, memory, and emotional disorders, is important in mediating the stress response and is also extremely vulnerable to damage, and it has been implicated in mood disorders (32).BDNF is one of the four major members of the neurotrophic factor family and is widely distributed in the mammalian brain. It is primarily expressed in the hippocampus and prefrontal cortex, among other areas. BDNF is vital for the pathogenesis of depression, cell plasticity regulation, inhibition of cell cascade death, increase of cell survival proteins related to neuron proliferation and maintenance, etc. Large evidence suggests that human and animal cases of depression exhibit downregulated hippocampal expression of BDNF (33). A variety of antidepressants (serotonin reuptake inhibitors and tricyclic antidepressants) can reverse the decrease in BDNF expression and significantly improve neuronal damage (7). Similar to other reports, we found that SY significantly improves depression-like symptoms and reduces CUMS-induced BDNF expression. This observation confirmed that SY exhibits its neuroprotective ability via the BDNF signaling pathway.

CUMS not only reduces BDNF expression but also results in significantly downregulated phosphorylation of Akt, PI3K, and mTOR in the hippocampus. These manifestations were reversed via SY treatment. The PI3K/Akt signaling pathway is the primary downstream signaling pathway in BDNF/TrkB signaling, regulating neuronal cell growth and survival in the hippocampus and mediating stress-induced depression and antidepressant effects (34, 35). Mammalian target of rapamycin (mTOR) is a downstream signaling molecule of PI3K/Akt pathway that regulates protein translation and synthesis. Depression is caused by synaptic protein defects induced by abnormal mTOR signaling (36). Recent studies have identified mTOR signaling as one of the targets involved in the rapid antidepressant response. Animal studies detected reduced phosphorylation of mTOR and Akt in the hippocampus of CUMS-exposed mice (37). Previous studies demonstrated that ketamine increases synaptic protein synthesis by activating the mTOR pathway, increases synaptic function, promotes synapse occurrence, and produces rapid antidepressant effects (38, 39). Similarly, the classical antidepressant fluoxetine modulates the mTOR signaling pathway in the hippocampus of mice exposed to chronically CUMS (40). These results suggest that the PI3K/Akt/mTOR signaling pathway plays an irreplaceable role in treating depression. We found that CUMS induced depression-like symptoms and reduced PI3K/Akt/mTOR phosphorylation in the hippocampus were ameliorated by long-term SY treatment. Overall, these neurochemical results suggest that SY upregulates the expression of BDNF/TrkB and the PI3K/Akt/mTOR signaling pathway to ameliorate CUMS-induced depression-like symptoms in rats.

Our study showed that SY prevents depression-like symptoms in CUMS-exposed rats by preventing HPA dysfunction, decreasing the neurotransmitter levels, minimizing oxidative stress, suppressing neuroinflammation, and activating the PI3K/Akt/mTOR-mediated BDNF/TrkB pathway, all of which are the key players in the pathological basis of depression. These findings suggest that SY can function as a potent therapeutic agent for preventing and treating stress-associated disorders, including depression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Institute of Medicinal Plant Development, Peking Union Medical College,

sanctioned all animal experiments (approval no. SYXK 2017-0020).

AUTHOR CONTRIBUTIONS

NJ, JL, and XL participated in the experiment design. NJ, HW, and HH conducted the experiments and performed the data analysis. NJ, JL, HW, HH, YB, GZ, and XL contributed to the writing and amendments of the manuscript. YC and QW were responsible for the supervision and project administration. All authors discussed, edited, and approved the final version.

FUNDING

This work was supported by the Ministry of Science and Technology of China (2017ZX09301029) and Space Medical Experiment Project of China Manned Space Program (HYZHXM05003).

REFERENCES

1. Yang L, Zhao Y, Wang Y, Liu L, Zhang X, Li B, et al. The effects of psychological stress on depression. *Curr Neuropharmacol*. (2015) 13:494–504. doi: 10.2174/1570159X1304150831150507
2. Yrondi A, Brauge D, LeMen J, Arbus C, Pariente J. Depression and sports-related concussion: a systematic review. *Presse Med*. (2017) 46:890–902. doi: 10.1016/j.lpm.2017.08.013
3. Ménard C, Hodes GE, Russo SJ. Pathogenesis of depression: insights from human and rodent studies. *Neuroscience*. (2016) 321:138–62. doi: 10.1016/j.neuroscience.2015.05.053
4. Li H, Lin S, Qin T, Li H, Ma Z, Ma S. Senegenin exerts anti-depression effect in mice induced by chronic un-predictable mild stress via inhibition of NF-κB regulating NLRP3 signal pathway. *Int Immunopharmacol*. (2017) 53:24–32. doi: 10.1016/j.intimp.2017.10.001
5. Zhong J, Li G, Xu H, Wang Y, Shi M. Baicalin ameliorates chronic mild stress-induced depression-like behaviors in mice and attenuates inflammatory cytokines and oxidative stress. *Braz J Med Biol Res*. (2019) 52:e8434. doi: 10.1590/1414-431x20198434
6. Zhang JJ, Gao TT, Wang Y, Wang JL, Guan W, Wang YJ, et al. Andrographolide exerts significant antidepressant-like effects involving the hippocampal BDNF system in mice. *Int J Neuropsychopharmacol*. (2019) 22:585–600. doi: 10.1093/ijnp/pyz032
7. Bo J, Zhe X, Jun Y, Wei W, Yue W, Zhuang-Li H, et al. Antidepressant-like effects of ginsenoside Rg1 are due to activation of the BDNF signalling pathway and neurogenesis in the hippocampus. *Br J Pharmacol*. (2012) 166:1872–87. doi: 10.1111/j.1476-5381.2012.01902.x
8. Wang L, Jin GF, Yu HH, Lu XH, Zou ZH, Liang JQ, et al. Protective effects of tenuifolin isolated from *Polygala tenuifolia* Willd roots on neuronal apoptosis and learning and memory deficits in mice with Alzheimer's disease. *Food Funct*. (2019) 10:7453–60. doi: 10.1039/C9FO00994A
9. Sun XP, Li TF, Shi Z, Liu J, Pan RL, Wang L, et al. Study of antidepressant-like effects of combination of ginseng total saponins and polygala tenuifolia total glycosiders in mice. *Chin J Comp Med*. (2012) 22. doi: 10.3969/j.issn.1671-7856.2012.06.008
10. Sun XP, Li SD, Shi Z, Li TF, Pan RL, Chang Q, et al. Antidepressant-like effects and memory enhancement of a herbal formula in mice exposed to chronic mild stress. *Neurosci Bull*. (2013) 29:737–44. doi: 10.1007/s12264-013-1378-z
11. Sun XP, Shi Z, Pan RL, Qin C, Zhang YL, Li YH, et al. Antidepressant-like effects and mechanism of action of SYG in depression model in rats. *Neuro Endocrinol Lett*. (2014) 35:129–36. doi: 10.1016/S2212-5671(14)00446-8
12. Walsh RN, Cummins RA. The Open-Field Test: a critical review. *Psychol Bull*. (1976) 83:482–504. doi: 10.1037/0033-2909.83.3.482
13. Forbes NF, Stewart CA, Matthews K, Reid IC. Chronic mild stress and sucrose consumption: validity as a model of depression. *Physiol Behav*. (1996) 60:1481–4. doi: 10.1016/S0031-9384(96)00305-8
14. Tacchi R, Ferrari A, Loche A, Bertolini A. Sucrose intake: increase in non-stressed rats and reduction in chronically stressed rats are both prevented by the gamma-hydroxybutyrate (GHB) analogue, GET73. *Pharmacol Res*. (2008) 57:464–8. doi: 10.1016/j.phrs.2008.05.004
15. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology*. (1985) 85:367–70. doi: 10.1007/BF00428203
16. Holmes A, Li Q, Koenig EA, Gold E, Stephenson D, Yang RJ, et al. Phenotypic assessment of galanin overexpressing and galanin receptor R1 knockout mice in the tail suspension test for depression-related behavior. *Psychopharmacology*. (2005) 178:276–85. doi: 10.1007/s00213-004-1997-1
17. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. (1977) 266:730–2. doi: 10.1038/266730a0
18. Jiang N, Zhang BY, Dong LM, Lv JW, Lu C, Wang Q, et al. Antidepressant effects of dammarane sapogenins in chronic unpredictable mild stress-induced depressive mice. *Phytother Res*. (2018) 32:1023–9. doi: 10.1002/ptr.6040
19. Wang LS, Tao X, Liu XM, Zhou YF, Zhang MD, Liao YH, et al. Cajaninstilbene acid ameliorates cognitive impairment induced by intrahippocampal injection of amyloid-β(1-42) oligomers. *Front Pharmacol*. (2019) 10:1084. doi: 10.3389/fphar.2019.01084
20. Katz RJ. Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacol Biochem Behav*. (1982) 16:965–8. doi: 10.1016/0091-3057(82)90053-3
21. Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology*. (1997) 134:319–29. doi: 10.1007/s002130050456
22. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability. *Neurosci Biobehav Rev*. (2019) 99:101–16. doi: 10.1016/j.neubiorev.2018.12.002
23. Xu P, Wang KZ, Lu C, Dong LM, Le ZJ, Liao YH, et al. Antidepressant-like effects and cognitive enhancement of the total phenols extract of *Hemerocallis citrina* Baroni in chronic unpredictable mild stress rats and its related mechanism. *J Ethnopharmacol*. (2016) 194:819–26. doi: 10.1016/j.jep.2016.09.023
24. Zahorodna A, Tokarski K, Hess G. Imipramine treatment ameliorates corticosterone-induced alterations in the effects of 5-HT1A and 5-HT4 receptor activation in the CA1 area of rat hippocampus. *Eur Neuropsychopharmacol*. (2006) 16:383–90. doi: 10.1016/j.euroneuro.2005.11.010
25. Dale E, Bang-Andersen B, Sánchez C. Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. *Biochem Pharmacol*. (2015) 95:81–97. doi: 10.1016/j.bcp.2015.03.011
26. Slavich GM, Irwin MR. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol Bull*. (2014) 140:774–815. doi: 10.1037/a0035302
27. Khairova RA, Machado-Vieira R, Du J, Manji HK. A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. *Int J Neuropsychopharmacol*. (2009) 12:561–78. doi: 10.1017/S1461145709009924
28. Song Y, Sun R, Ji Z, Li X, Fu Q, Ma S. Perilla aldehyde attenuates CUMS-induced depressive-like behaviors via regulating TXNIP/TRX/NLRP3 pathway in rats. *Life Sci*. (2018) 206:117–24. doi: 10.1016/j.lfs.2018.05.038
29. Lu C, Wang Y, Lv J, Jiang N, Fan B, Qu L, et al. Ginsenoside Rh2 reverses sleep deprivation-induced cognitive deficit in mice. *Behav Brain Res*. (2018) 349:109–15. doi: 10.1016/j.bbr.2018.03.005
30. Lee SY, Lee SJ, Han C, Patkar AA, Masand PS, Pae CU. Oxidative/nitrosative stress and antidepressants: targets for novel antidepressants.

Prog Neuropsychopharmacol Biol Psychiatry. (2013) 46:224–35. doi: 10.1016/j.pnpbp.2012.09.008
31. Banagozar Mohammadi A, Torbati M, Farajdokht F, Sadigh-Eteghad S, Fazljou SMB, Vatandoust SM, et al. Sericin alleviates restraint stress induced depressive- and anxiety-like behaviors via modulation of oxidative stress, neuroinflammation and apoptosis in the prefrontal cortex and hippocampus. *Brain Res*. (2019) 1715:47–56. doi: 10.1016/j.brainres.2019.03.020
32. Sheline YI, Liston C, McEwen BS. Parsing the hippocampus in depression: chronic stress, hippocampal volume, and major depressive disorder. *Biol Psychiatry*. (2019) 85:436–8. doi: 10.1016/j.biopsych.2019.01.011
33. Deyama S, Bang E, Kato T, Li XY, Duman RS. Neurotrophic and antidepressant actions of brain-derived neurotrophic factor require vascular endothelial growth factor. *Biol Psychiatry*. (2019) 86:143–52. doi: 10.1016/j.biopsych.2018.12.014
34. Luo L, Liu XL, Li J, Mu RH, Liu Q, Yi LT, et al. Macranthol promotes hippocampal neuronal proliferation in mice via BDNF-TrkB-PI3K/Akt signaling pathway. *Eur J Pharmacol*. (2015) 762:357–63. doi: 10.1016/j.ejphar.2015.05.036
35. Wu Y, Wang L, Hu K, Yu C, Zhu Y, Zhang S, et al. Mechanisms and therapeutic targets of depression after intracerebral hemorrhage. *Front Psychiatry*. (2018) 9:682. doi: 10.3389/fpsyt.2018.00682
36. Abelaira HM, Réus GZ, Neotti MV, Quevedo J. The role of mTOR in depression and antidepressant responses. *Life Sci*. (2014) 101:10–4. doi: 10.1016/j.lfs.2014.02.014
37. Liu S, Li T, Liu H, Wang X, Bo S, Xie Y, et al. Resveratrol exerts antidepressant properties in the chronic unpredictable mild stress model through the regulation of oxidative stress and mTOR pathway in the rat hippocampus and prefrontal cortex. *Behav Brain Res*. (2016) 302:191–9. doi: 10.1016/j.bbr.2016.01.037
38. Walker DL, Davis M. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacol Biochem Behav*. (2002) 71:379–92. doi: 10.1016/S0091-3057(01)00698-0
39. Ding ML, Ma H, Man YG, Lv HY. Protective effects of a green tea polyphenol, epigallocatechin-3-gallate, against sevoflurane-induced neuronal apoptosis involve regulation of CREB/BDNF/TrkB and PI3K/Akt/mTOR signalling pathways in neonatal mice. *Can J Physiol Pharmacol*. (2017) 95:1396–405. doi: 10.1139/cjpp-2016-0333
40. Liu XL, Luo L, Mu RH, Liu BB, Geng D, Liu Q, et al. Fluoxetine regulates mTOR signalling in a region-dependent manner in depression-like mice. *Sci Rep*. (2015) 5:16024. doi: 10.1038/srep16024

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 11 February 2021
doi: 10.3389/fpsyt.2021.638032

From an Alternative Medicine to a New Treatment for Refractory Epilepsies: Can Cannabidiol Follow the Same Path to Treat Neuropsychiatric Disorders?

Rafael M. Bitencourt[1]*, *Reinaldo N. Takahashi*[2] and *Elisaldo A. Carlini*[3†]

[1] Laboratory of Behavioral Neuroscience, Graduate Program in Health Sciences, University of Southern Santa Catarina, University of Southern Santa Catarina (UNISUL), Tubarão, Brazil, [2] Post Graduate Program in Pharmacology, Department of Pharmacology, Federal University of Santa Catarina, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil, [3] Centro Brasileiro de Informações Sobre Drogas Psicotrópicas (CEBRID), Department of Preventive Medicine, Federal University of São Paulo, UNIFESP, São Paulo, Brazil

OPEN ACCESS

Edited by:
Elaine Elisabetsky,
Federal University of Rio Grande do Sul, Brazil

Reviewed by:
Pasquale Striano,
University of Genoa, Italy
Gary Stephens,
University of Reading, United Kingdom

***Correspondence:**
Rafael M. Bitencourt
rafael.bitencourt2@unisul.br

†In Memoriam

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: 04 December 2020
Accepted: 12 January 2021
Published: 11 February 2021

Citation:
Bitencourt RM, Takahashi RN and Carlini EA (2021) From an Alternative Medicine to a New Treatment for Refractory Epilepsies: Can Cannabidiol Follow the Same Path to Treat Neuropsychiatric Disorders? Front. Psychiatry 12:638032. doi: 10.3389/fpsyt.2021.638032

Although cannabis has been known for ages as an "alternative medicine" to provide relief from seizures, pain, anxiety, and inflammation, there had always been a limited scientific review to prove and establish its use in clinics. Early studies carried out by Carlini's group in Brazil suggested that cannabidiol (CBD), a non-psychotropic phytocannabinoid present in *Cannabis sativa*, has anticonvulsant properties in animal models and reduced seizure frequency in limited human trials. Over the past few years, the potential use of cannabis extract in refractory epilepsy, including childhood epilepsies such as Dravet's syndrome and Lennox-Gastaut Syndrome, has opened a new era of treating epileptic patients. Thus, a considerable number of pre-clinical and clinical studies have provided strong evidence that phytocannabinoids has anticonvulsant properties, as well as being promising in the treatment of different neuropsychiatric disorders, such as depression, anxiety, post-traumatic stress disorder (PTSD), addiction, neurodegenerative disorders and autism spectrum disorder (ASD). Given the advances of cannabinoids, especially CBD, in the treatment of epilepsy, would the same expectation regarding the treatment of other neuropsychiatric disorders be possible? The present review highlights some contributions from Brazilian researchers and other studies reported elsewhere on the history, pre-clinical and clinical data underlying the use of cannabinoids for the already widespread treatment of refractory epilepsies and the possibility of use in the treatment of some neuropsychiatric disorders.

Keywords: cannabis, phytocannabinoids, cannabidiol, epilepsy, neuropsychiatric disorders

INTRODUCTION

Cannabis sativa, a plant popularly known for giving rise to marijuana, has in its composition more than 140 compounds called phytocannabinoids. In addition to the phytocannabinoids present in the plant, endocannabinoids (eCB) are produced endogenously through physiological stimulation and cannabinoids of synthetic origin, all called cannabinoids. Both together and

isolated, cannabinoids have a wide variety of effects on the nervous system, making these compounds promising psychopharmacological alternatives in treating many neuropsychiatric disorders (1–3). Among the possibilities for pharmacotherapeutic use, stand-depression, anxiety, post-traumatic stress disorder (PTSD), addiction, neurodegenerative disorders, autism spectrum disorder (ASD), and especially refractory epilepsy, among others (4–12).

Concerning the treatment of refractory epilepsies, the last few years have shown a significant increase in studies evaluating the risks and benefits of using cannabinoids in this context (13, 14). Epilepsy is a pathological condition that affects about 65 million people worldwide, and its principal characteristic is recurrent seizures, and its etiology can be varied, ranging from genetic syndromes to brain damage (15–18). It is also a condition that often does not respond to the pharmacotherapy used, and, in this sense, cannabinoids appear as a promising alternative. The two phytocannabinoids most researched for the treatment of epilepsies are delta-9-tetrahydrocannabinol (THC—main psychoactive compound) and especially cannabidiol (CBD—main non-psychoactive compound), which are useful in preventing seizures and reducing mortality, with low toxicity and high tolerability (11, 17–20). The path to the safe and effective use of cannabinoids in treating epilepsy seems to be unraveled by science; however, the next question: would the same expectation regarding the treatment of other neuropsychiatric disorders be possible? To shed light on this issue, this review, in addition to emphasizing the use of CBD in the treatment of epilepsy, examines the possibility of using this compound as an alternative to the treatment of some neuropsychiatric disorders. For more details about the botany, psychobiology, and the medical potential of cannabis, the readers can examine the various reviews available in the literature or direct toward an elegant review by Solymosi and Köfalvi (21).

"FROM AN ALTERNATIVE MEDICINE:" FIRST EVIDENCE AND CARLINI'S GROUP CONTRIBUTION

The use of Cannabis for the treatment of epilepsy has been going on for a long time, with evidence found in Sumerian tablets more than 3,800 years ago (14). The most recent reports started in the middle of the nineteenth century when the Irish surgeon William O'Shaughnessy announced the plant's therapeutic effects in the treatment of epilepsy, a fact that was soon reinforced by two other renowned English neurologists, J. R. Reynolds and W. Gowers (22, 23). Scientific publications from the 1940s, both in animal models (24) and in children with epilepsy (25), were the first reports of the therapeutic use of Cannabis for this condition. A significant step in the study of cannabinoids was taken by Mechoulam in the 1960s, when he isolated, clarified the structure, and synthesized THC and CBD, the most abundant and most studied phytocannabinoids in Cannabis to date, including for epilepsy (26–28).

In the sequence, and even before the discovery of the eCB system (which occurred only in the '90s), the Brazilian researcher Elisaldo Carlini started studies using CBD in animal models of epilepsy, suggesting the first scientific evidence about the therapeutic potential of CBD in treatment of this pathology (29, 30). Next, in partnership with the Mechoulam group, Carlini et al. conducted the first placebo-controlled study of CBD in patients with refractory epilepsy. At the time, the authors showed that two of the four epileptic patients treated with 200 mg of CBD daily showed an improvement in their epileptic status, without having any seizures within 3 months of treatment. The third patient had a partial improvement, while the fourth patient treated with CBD, and the other five patients in the placebo group showed no improvement. No toxic effects were observed, and this was the first study in humans from the "modern scientific era" to consider the possibility of CBD's therapeutic potential, isolated, in the treatment of refractory epilepsies (31).

Continuing the investigations, Carlini et al. published a series of studies that confirmed CBD's therapeutic potential in the treatment of seizures. In the early 1980s, a double-blind controlled trial was performed with CBD 200–300 mg/kg or placebo administered daily over more than 4 months in 15 patients with generalized epilepsy. Of the eight patients treated with CBD, four of them had practically no seizures throughout the experiment, and three had partial improvement, while the seven patients in the placebo group showed no improvement in the clinical picture of the seizures (32).

Subsequently, other studies by Carlini et al. have reinforced CBD's therapeutic potential, a non-psychoactive phytocannabinoid and, therefore, with fewer side effects than THC, in the treatment of epileptic conditions (33–35). Since then, different researchers have confirmed the pioneering studies of Carlini et al. since the 1970s, that is, that CBD can be a safe and effective therapeutic alternative for the treatment of epilepsy, a condition that affects millions of people across the world (11, 36–40). This contribution made an extensive article published recently (2020) in The NY Times about CBD, which considers Carlini as the "discoverer" of the use of this compound in epilepsy (41). A recent and simple search made based on scientific articles PubMed, using the descriptors "cannabidiol" and "epilepsy" lists ∼470 publications addressing this topic. Although there is now much more evidence from studies in animals than in humans, in addition to few randomized controlled trials (28, 42), many clinical observations have suggested cannabinoids, not just CBD, as a new treatment for refractory epilepsies (for better visualization of the contribution of Carlini et al., see **Figure 1**).

Abbreviations: eCB, endocannabinoid; PTSD, post-traumatic stress disorder; ASD, autism spectrum disorder; THC, delta-9-tetrahydrocannabinol; CBD, cannabidiol; QOLCE, quality of life in childhood epilepsy; THCV, delta-9-tetrahydrocannabivarin; CBDV, cannabidivarin; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; AEA, anandamide; 2-AG, 2-arachidonylglycerol; GABA, gamma-aminobutyric Acid; SSRIs, selective serotonin reuptake inhibitors; DSM-5, Diagnostic and Statistical Manual; CPP, conditioned place preference; NDDs, neurodegenerative disorders; PD, Parkinson disease; AD, Alzheimer disease; ANVISA, National Health Surveillance Agency.

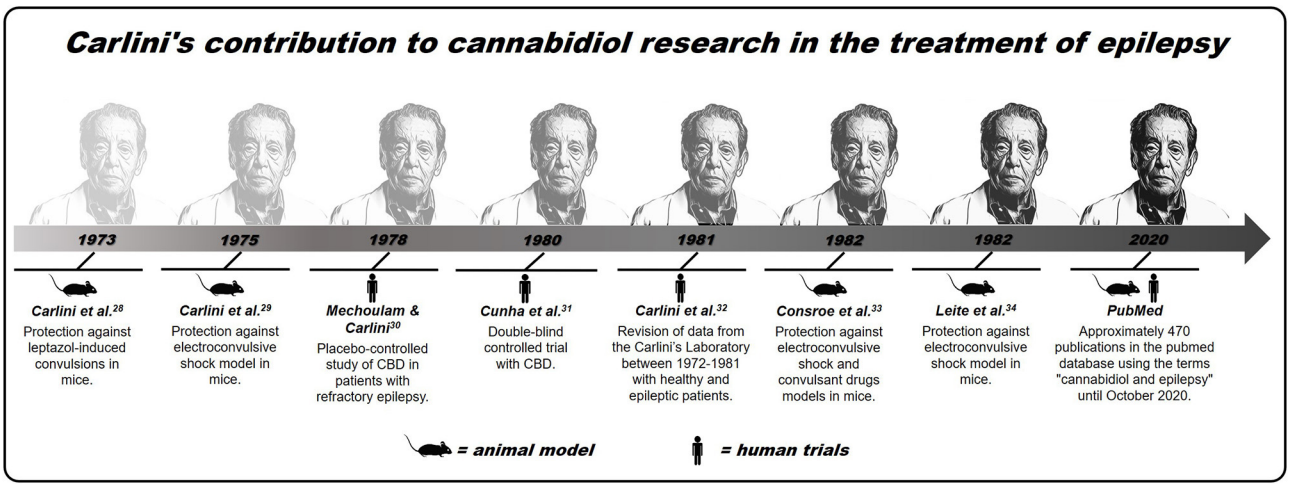

FIGURE 1 | Brief history of Carlini's contribution to cannabidiol research in the treatment of epilepsy.

"TO A NEW TREATMENT FOR REFRACTORY EPILEPSIES:" CLINICAL CONFIRMATION AND THE ROLE OF ANECDOTAL REPORTS

The fact that medical cannabis is still illegal in several countries, coupled with the high financial cost for patients, when available, ends up favoring the use of artisanal extracts. In turn, these extracts do not always have strict quality control over the quantity and quality of the components present in the formulation. This situation makes it difficult to obtain reliable scientific data regarding the efficiency and safety of the drug use of cannabinoids. However, it is precisely anecdotal reports, mostly obtained through the use of cannabis herbal extract oil, that have provided a considerable amount of evidence for the use of cannabis and CBD in isolation as a treatment for epilepsies (43).

In a study of 74 children resistant to traditional treatment of epilepsy, treatment with CBD-rich herbal cannabis extract (20: 1 THC; ∼10 mg/kg CBD per day) reduced the frequency of seizures by 89% of the studied population, with 43% of these children had a reduction that surpassed 50%. Only 6.7% of these children worsened in seizures, with treatment discontinue (44). In another prospective open-label study, using CBD-rich herbal cannabis extract (50: 1 THC; ∼13 mg/kg CBD per day) to treat 20 children with Dravet's syndrome for 20 weeks, there was a reduction of more than 70% in seizures (45). Also, about the effectiveness of treatments using artisanal cannabis preparations, a retrospective study investigating the effects of CBD oil in 108 children with epilepsy is highlighted. In this study, 10% of children treated with CBD oil had no seizures, and 39% had a reduction more significant than 50% of those seizures, showing promising potential for this type of preparation. Less than 4% had sedation as an adverse effect (46). These studies, together, point to a direction and show that controlled clinical tests using different cannabinoid compounds, including standardized ones, are necessary, as well as they are promising in the treatment of epilepsy.

In this sense, some clinical studies have been done with standardized cannabinoid compounds, therefore, with better possibilities for the treatment's efficacy and safety. Among these, we highlight the open-label trial by Devinsky et al., which showed the effectiveness of Epidiolex in the control of refractory epilepsy in a study involving 162 patients. These patients received CBD 2 at 50 mg/kg per day, in stages, for 12 weeks. There was a reduction of ∼36% in seizures compared to baseline. Mild adverse effects involving drowsiness, reduced appetite, diarrhea, fatigue, and seizures were reported in 79% of patients. Another 12% of patients had serious adverse effects (47). Besides, quality of life was measured in 48 of the 162 initial participants. There was an improvement in the scores obtained through the Quality of Life in Childhood Epilepsy (QOLCE) (48). In short, treatment with CBD proved to be relatively safe, reducing seizures and promoting an improvement in patients' quality of life (47, 48).

Facing the need for a double-blind/randomized/placebo-controlled trials, Devinsky et al. developed a study that reinforced the effectiveness of Epidiolex in the control of epilepsy in patients with Dravet's syndrome. The study included 120 subjects, including children and young adults, who were randomized to receive CBD (Epidiolex) at a dose of 20 mg/kg per day or placebo over 14 weeks. After treatment, 5% of participants who received CBD were free from seizures than the placebo group (0%). Also, 43% of patients treated with CBD had a 50% reduction in seizures, against 27% in the placebo group. It is also important to note that 93% of patients who received CBD treatment had an adverse effect, with the majority (89%) of these effects being considered mild or moderate (e.g., diarrhea, vomiting, drowsiness, etc.) (49). These results reinforce the evidence from the same group in previous studies (47, 48).

In another double-blind, placebo-controlled trial, two groups, one with 86 and 85 patients with Lennox-Gastaut syndrome, were treated, respectively, with CBD or placebo for 14 weeks.

In this study, the group that received CBD, 20 mg/kg per day, had an average monthly reduction in epileptic seizures of around 43%, while the placebo group had an average of 21%. Mild and moderate adverse effects occurred in 86% of patients treated with CBD vs. 69% of patients in the placebo group, with diarrhea, drowsiness, decreased appetite, pyrexia, and vomiting being the most frequent. This study points to CBD as useful in treating seizures associated with Lennox-Gastaut syndrome and being relatively well-tolerated, as it has not caused severe adverse effects (50).

A point that has been discussed in the clinic and, therefore, deserves to be highlighted concerns the possible interactions of CBD with other anticonvulsant agents through the so-called polytherapy, so common in patients with refractory epilepsies. A better understanding of this adjunctive therapy is necessary so that there is greater clarification concerning the adverse effects, quality of life of the patient, as well as the effectiveness of the doses used. This understanding should involve, for example, genetic, pharmacodynamic, and pharmacokinetic issues that influence the effects of CBD in the presence of other drugs (and vice versa), thus providing greater security in choosing an appropriate pharmacotherapeutic strategy [for a more detailed review, see (51)]. One of these possibilities that have been documented is the interaction of CBD with benzodiazepine clobazam. Both are metabolized by the cytochrome P450 (CYP) pathway, and CBD could be potentiating the effects of clobazam by inhibiting this metabolism pathway. Likewise, clobazam could also be potentiating the antiepileptic effects of CBD by inhibiting its degradation pathway. This possibility raised a question about the effectiveness of CBD in the treatment of epilepsy, which was: would CBD have effects *per se* in the treatment of epilepsy, or would it need to be associated with clobazam? (52, 53) Although this answer is not yet clear, CBD in Europe has been approved only as an adjunctive treatment with clobazam. However, a study carried out with Lennox-Gastaut syndrome patients and Dravet syndrome who received CBD in the absence of clobazam (54), in addition to other studies (55, 56), strongly point to the fact that this phytocannabinoid exerts its therapeutic effects independently of its interaction with the mentioned benzodiazeinic. Therefore, the evidence suggests that the European Medicines Agency Public Assessment Report's prescription restriction is not supported. The lack of randomization for studies involving CBD interaction with clobazam may have contributed to some misconceptions (52).

In recent years, there have also been some systematic reviews of clinical trials, including meta-analysis, which have reinforced the effectiveness and safety of CBD or CBD-Rich Cannabis Extracts in the treatment of epilepsies (18, 40, 57). After a long time where anecdotal reports predominated, the evidence through well-conducted clinical studies indicates a safe use of cannabinoids, especially CBD, in the treatment of epilepsies. Further studies are needed to understand better the benefits to the possible risks of using cannabinoids in this situation. Studies are needed to better elucidate another prevalent issue among anecdotal reports, which concerns the interaction between CBD and THC influencing antiepileptic effects' effectiveness (20, 58). Would CBD alone be more effective, or would it need to interact with THC and other constituents of *Cannabis sativa*? Such an issue will be further discussed in the next section of this article.

THE POSSIBILITY OF THE ENTOURAGE EFFECT: WHAT IS KNOWN ABOUT?

"One plus one is always more than two," phrase of the song "O sal da terra" by Beto Guedes, a singer of Brazilian popular music. Obviously, he was not referring to cannabinoids but to the need to bring people together to build a better world. The same phrase can also make sense when speculating about the entourage effect observed in many studies that address the therapeutic application of cannabinoids.

The entourage effect is a term suggested referring to a situation where a group of endogenous compounds similar to eCB, when acting together, potentiate the effects mediated by cannabinoid receptors. This term was first mentioned by Bem-Shabat et al. (59), and soon expanded to also define the synergistic effects observed through the use of mixtures of plants in general, including concerning the different compounds present in cannabis. It is worth mentioning in this topic another pioneering aspect of Carlini and his group. Long before the advent of this terminology on cannabinoid effects, the Brazilian group published studies showing the relevance of the interaction between the different phytocannabinoids present in cannabis samples (60–62). Thus, evidence indicates that many of the therapeutic effects observed through the use of phytocannabinoids occur, in fact, much more from the complex and poorly understood interaction of all the compounds present in the plant (mainly THC and CBD) rather than the isolated action of a single compound (63, 64). In this case, can one plus one also be more than two?

Studies, mainly in animals but also in humans, have shown that the answer is yes. A recent meta-analysis study published by Pamplona et al. (40), which searched 199 articles (with 11 validated references) with a total of 670 patients, showed that CBD-rich extracts seem to present a better therapeutic profile than purified CBD. In this meta-analysis, 71% of patients treated with CBD-rich extracts reported some improvement in their epileptic condition, compared to 46% of patients treated with purified CBD. Patients treated with CBD-rich extracts also required lower doses (6 mg/kg/day) than patients who used only CBD (25.3 mg/kg/day), that is, an effect four times greater on the part of the unpurified extract. Still, in the same study, it was also possible to notice that patients treated with CBD-rich extracts had fewer side effects, both mild (33%) and severe (7%) when compared to those who received purified CBD (76% mild and 26% serious).

In addition to the main constituents of cannabis CBD and THC, another possibility that favors the entourage effect is the findings of the anticonvulsant potential of other phytocannabinoids, for example, delta-9-tetrahydrocannabivarin (THCV) and cannabidivarin (CBDV). These phytocannabinoids also proved useful anticonvulsants, with THCV having its effects *via* CB1 receptors, while CBDV did not (65–67). CBDV also had their anticonvulsant effects enhanced by CBD (66). Additional

contributions to these specific topics through Carlini-trained researchers can be found in recent reviews (68–71). Considering the spectrum of possibilities of cannabis expanded to more than 200 terpenes present in the plant, the findings can be even more promising. Some terpenes are known to have pharmacological activities in the central nervous system, although they have not been tested in patients with epilepsy (63, 72). Although such interactions do seem to occur, more controlled clinical studies proving such a possibility are needed. However, regardless of whether they are better together or apart, one thing is sure, phytocannabinoids are proving to be increasingly influential in the treatment of epilepsies.

IF CANNABIDIOL WORKS, HOW DOES IT WORK?

To speculate about the neurobiological mechanisms involved in the antiepileptic activity of cannabinoids, including cannabidiol, it is necessary to understand a little more about the eCB system's physiology. This fact has been well-elucidated since the early 90s, when the discovery of this system occurred, which revolutionized the understanding of many neurophysiological responses. The eCB system consists of two receptors (CB1 and CB2), endogenous ligands (anandamide/AEA and 2-arachidonylglycerol/2-AG), and enzymes involved the synthesis and degradation of these ligands. It is a system with neuromodulatory functions, which regulate the presynaptic release of both excitatory and inhibitory neurotransmitters (73, 74). These neuromodulatory functions appear to play an essential role in controlling epilepsies, for example, through the activation of CB1 type cannabinoid receptors (75).

These receptors are located presynaptically, and their activation, either by endogenous ligands (e.g., AEA) or exogenous (e.g., THC), results in a transient hyperpolarization of the presynaptic membrane that, consequently, inhibits the release of excitatory neurotransmitters like glutamate (76). This fact agrees with the evidence that shows the downregulation of CB1 receptors in axial glutamatergic terminals extracted from the brain tissue of patients with epilepsy. On the other hand, the evidence also points to the upregulation of the same receptors at the GABAergic axonal terminals. In both possibilities, there is a loss of control over neuronal hyperexcitability, favoring epilepsy. The antiepileptic effects obtained from manipulating the eCB system or using exogenous phytocannabinoids may be related to the reestablishment of control over this hyperexcitability (19, 77–79).

In the case of phytocannabinoids, THC has a high affinity for the CB1 receptor and, through this receptor, can regulate neuronal excitability. This compound was the first phytocannabinoid to have its anticonvulsant properties evaluated, which must result from a reduction in the levels of excitatory neurotransmitters caused by its agonist action on CB1 receptors (19, 20). While CBD, the plant's non-psychoactive phytocannabinoid and possibly the most studied when it comes to antiepileptic properties, has a mechanism of action that has not yet been elucidated. This phytocannabinoid has a low affinity for CB1 receptors and may have its antiepileptic effect related to neuronal excitability's modulation through changes in the influx of Ca and Na ions, as well as actions in vanilloid receptors, adenosinergic and serotonergic systems (80–82). Another possibility to explain CBD's antiepileptic effects would be an eventual ability to inhibit both uptake/hydrolysis of the eCB and, thus, indirectly, to decrease neuronal excitability (83) by potentiating this system (84, 85). The fact is that this issue of the neurobiological mechanisms involved in the antiepileptic action of cannabinoids is not entirely defined and still requires a better understanding [for a complete review of the possible mechanisms of action of cannabinoids in epilepsy, see (80)]. As science grasps these mechanisms, this will result in more efficient pharmacotherapeutic approaches for the treatment of epilepsy and make it possible to expand the medicinal use of cannabinoids, including CBD, for other neuropsychiatric diseases.

CAN THE SUCCESS OF CANNABIDIOL IN THE TREATMENT OF EPILEPSY PREDICT THE SAME PATH FOR THE TREATMENT OF NEUROPSYCHIATRIC DISORDERS?

It is not yet possible to say whether the use of CBD and other cannabinoids to treat different neuropsychiatric disorders will follow the same route observed for the treatment of epilepsy. However, significant steps have also been taken for these other possibilities. From depression to anxiety, including PTSD, addiction, neurodegenerative diseases, and ASD, these are disorders that, according to some studies, can use cannabinoids, especially CBD, as a pharmacotherapeutic alternative (4, 8, 86–88).

Depression

Regarding depression and anxiety, it is known that many cannabis users report its use for its relaxing effects; therefore, as a way to reduce the symptoms of these disorders (87, 89, 90). Additionally, several studies have pointed to the potentization of the eCB system or the use of exogenous ligands as promising possibilities in treating depression (5, 7, 91–97). Reinforcing this possibility, the blockade of this system, whether through the use of antagonists or genetic deletion, seems to lead to depressive and anxiety symptoms, which caused the withdrawal of the CB1 antagonist rimonabant, proposed for the treatment of obesity, from the market (98–100). According to this perspective, patients with major depression had reduced serum levels of eCBs, in addition to a lower density of CB1 receptors in the glial cells of the brain gray matter (101, 102). In this sense, a proposal to reestablish the eCB system's functions by inhibiting the degradation of its endogenous ligands can be explored as an antidepressant potential (103). Remembering that CBD, a phytocannabinoid with few side effects, may be acting in this way, potentiating the eCB system (84), even being reported in different studies as an effective antidepressant (6, 104, 105). Considering that traditional antidepressants (serotonin and/or noradrenaline reuptake inhibitors) have relatively low efficiency and still need

weeks for their effects (106), it is suggested that the manipulation of the eCB system, which even has a response rate faster, can be an alternative for the treatment of depressive disorders [for a more detailed review, see (7, 91, 93, 96)].

Anxiety

About anxiety, CBD has also been shown to be a more exciting alternative, given the potentially anxiogenic effects of THC (107, 108). Several pre-clinical studies using different animal models (109–116), as well as some clinical studies (117–121), confirm the anxiolytic effects of CBD (122). In this research area, it is worth mentioning the vital participation of groups from the Faculty of Medicine of Rib. Preto—University São Paulo, BR, led by Zuardi and Guimaraes. In addition to the use of CBD, manipulation of the eCB system is an alternative in treating anxiety. This system is located in brain regions important for modulating responses related to fear and anxiety (123), with increased anandamide *via* inhibition of its degradation, promoting anxiolytic effects (109, 124–127). Considering the high abuse potential of benzodiazepines and the slow response of selective serotonin reuptake inhibitors (SSRIs), both CBD and potentiation of the eCB system are promising alternatives in pharmacotherapy of anxiety disorders [for a mor detailed review, see (87, 122, 127, 128)].

PTSD

Until recently considered as an anxiety disorder, post-traumatic stress disorder (PTSD), which from the DSM-5 was included in a new category called "trauma and stress-related disorders," has also responded very well to research that involves cannabinoid treatment, especially CBD (8). The speculations started from the work of Marsicano et al. (129), showing the eCB system's role in the extinction of aversive memories. From then on, a series of pre-clinical studies started to indicate that the potentiation of the eCB system (130), the use of exogenous agonists for the CB1 receptor (131) or even the CBD (109, 132) could facilitate the extinction of aversive memories. In addition to facilitating the extinction process, different studies have shown the effect of cannabinoids impairing the processes of retrieval and consolidation of these memories, that is, more possibilities for intervention in the remembrance of traumatic events (133–135). In the face of so many reports of pre-clinical studies, it was not long before evidence also emerged from clinical studies (119, 136–140) and thus reinforced the potential of cannabinoids, including CBD, as a therapeutic alternative for the treatment of this disorder [for a more detailed review, see (8, 141–145)].

Addiction

In addition to PTSD, another neuropsychiatric condition where memories play a fundamental role, and that there is also evidence for the use of cannabinoids, is addiction/relapse to drugs of abuse. Although it seems to be a paradoxical variant, understanding the action of cannabinoids in the breakdown of hedonic or reinforcing memories can provide up-and-coming therapeutic alternatives. In this perspective, de Carvalho and Takahashi showed in a pioneering way the inhibitory effect of CBD in reactivation sessions in animals that previously had conditioned place preference induced by morphine or cocaine (88). This finding suggests CBD's disruptive effect on the reconsolidation of memories associated with drugs of abuse, thus reducing the risk of relapse (146). A similar result was reported by Luján et al. (147), showing that the CBD attenuated cocaine-induced conditioned place preference, in addition to reducing voluntary consumption by mice. Besides, this work showed that CBD increased the expression of CB1 receptors and neural cell proliferation in the hippocampus, reinforcing the ability of this cannabinoid to modulate both behavioral and molecular manifestations related to cocaine reinforcement (147). Cannabinoid receptors CB1 and CB2 even seem to perform opposite functions, and the antagonism of CB1 receptors has the same inhibitory effects seen in the activation of CB2 receptors concerning the modulation of cocaine-induced sensitization and conditioned place preference (CPP). These effects probably occur due to a block in neuronal activation of the hippocampus (148). Other studies have also shown the CBD's ability to also reduce alcohol consumption in animal models of an alcohol use disorder, in addition to reducing alcohol-related steatosis and fibrosis in the liver, and alcohol-related brain damage, preventing neuronal loss (149). From a clinical perspective, promising results showed that the voluntary use of cannabis caused a decrease in crack use and also promoted an improvement in the quality of life in individuals dependent on this substance (150–152). The evidence from CBD as a treatment for drug abuse disorders is still discreet but deserves a closer look [for a more detailed review, see (10, 149, 153)].

NDDs

One possibility that is increasingly attracting researchers' attention to the use of cannabinoids is related to the application of these compounds in neurodegenerative disorders (NDDs). These NDDs are strongly related to oxidative damage and a series of neuroinflammatory responses that ultimately lead to cell death (154). Among the NDDs, the most common are Parkinson's disease (PD) and Alzheimer's disease (AD), conditions in which the potentiation of the eCB system (155) or even the use of phytocannabinoids, especially CBD (156, 157), can play an auspicious role as neuroprotectants (4). This promising possibility on CBD is pointed out *in vitro* and *in vivo* studies (158–160), and even in clinical studies (161). Taking into account that the current classic treatments for NDDs do not stop and/or slow the progression of the disease, alternatives such as CBD or any other substances that target the eCB system can be good candidates as prototypes for the development of neuroprotective drugs [for a more detailed review, see (154–157, 161)].

ASD

Another neuropathology that appears to be associated with inflammatory processes and, therefore, can also be a target for cannabinoids is an autism spectrum disorder (ASD) (86, 162). This disorder is characterized by constant communication and social interaction deficits and restricted and repetitive behavior patterns, which still have unknown etiopathogenesis (163). One of the possibilities may be an imbalance in the

eCB system, responsible for regulating some typically impaired functions in the ASD (164–168). This fact, associated with the anti-inflammatory activity of cannabinoids, has encouraged pre-clinical (169–171) and clinical (12, 172, 173) research to investigate the therapeutic potential of cannabinoids for the treatment of ASD. Among the possibilities, CBD seems to be the safest and most promising alternative (12, 172, 173), although other phytocannabinoids like CBDV also present themselves as candidates (171). The reestablishment of the balance of the eCB system and the anti-neuroinflammatory activity seems to support these compounds' activities as a treatment to ASD [for a more detailed review, see (86, 163, 164, 166, 174)].

There are still many other possibilities for neuropsychiatric disorders that can find cannabinoids as a possible therapeutic option. In this case, CBD, the main focus of this review, and other phytocannabinoids (e.g., THC, THCV, CBDV) appear to present quite promising pharmacotherapeutic alternatives for an increasingly broad number of neuropsychiatric disorders. However, for all these possibilities, including those mentioned here, further prospective, double-blind, placebo-controlled studies must be needed (for a summary, see **Table 1**). These studies are essential to ensure the effectiveness and safety of these compounds in each specific situation. In any case, this is a promising field of study where many pharmacotherapeutic alternatives may be revealed.

CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, we emphasize promising pre-clinical and clinical findings with cannabinoids, mostly from Brazilian-based researchers and other researchers worldwide. Specific studies have focused on the multifunctional phytocannabinoid, CBD, showing remarkable benefits, mainly for refractory epilepsy in children. These data contributed to the considered "prohibited substance" to enter the list of medicines for controlled use by the National Health Surveillance Agency (ANVISA), the regulatory agency responsible for the approval of new drugs in Brazil. Besides, one may anticipate other phytocannabinoid-based preparations and even new drugs acting at the endocannabinoid system as a promising therapeutic advance for other neuropsychiatric disorders, represented here by depression, anxiety-related disorders, PTSD, drug addiction and drug-induced relapse, neurodegenerative disorders, and ASD. If CBD (or other cannabinoids) with regard to

TABLE 1 | CBD as a promising psychopharmacological alternative in the treatment of neuropsychiatric disorders.

Neuropsychiatry Disorders	Briefly, What Is Known About The Use of Cannabidiol in This Condition*	For a More Detailed Review, See:
Depression	Several studies, preclinical and clinical, suggest that the eCB system's blockade induces depressive-like responses, while the potentiation of this system produces an antidepressant action. Therefore, it is suggested that substances that directly activate cannabinoid receptors or promote the increase of their endogenous ligands, such as CBD, may represent good therapeutic alternatives for the treatment of depression.	(7, 91, 93, 96)
Anxiety	For the treatment of anxiety, CBD is the most promising alternative from the plant, given THC's anxiogenic effects. Another alternative is the potentiation of the eCB system by inhibiting the degradation and reuptake of anandamide. Both preclinical and clinical studies have pointed to the possibility that cannabinoids can be used as anxiolytics.	(87, 122, 127, 128)
Post-traumatic stress disorder (PTSD)	The eCB system plays a fundamental role in the extinction of aversive memories; therefore, its enhancement facilitates this process. Additionally, this system's enhancement can still block the retrieval and reconsolidation processes of this type of memory. In all possibilities, the result seems to be the improvement of symptoms related to PTSD, both in preclinical and clinical studies. In this sense, CBD stands out as an up-and-coming alternative.	(8, 141–145)
Addiction	Although it seems paradoxical, cannabinoids act by causing a breakdown in hedonic or reinforcing memories related to drugs and can be an alternative in treating addiction/relapse. Pre-clinical studies have shown CBD's effects in reducing drug-seeking behavior in models involving morphine, cocaine, and alcohol. Studies show that cannabis use can lead to reduced consumption and improved quality of life in crack-dependent individuals from a clinical perspective.	(10, 149, 153)
Neurodegenerative diseases (NDDs)	Both the enhancement of the eCB system and phytocannabinoids have effects in reducing oxidative stress and neuroinflammation, conditions present in NDDs. *In vitro*, *in vivo*, and clinical studies suggest a neuroprotective action of CBD, making this phytocannabinoids a therapeutic possibility for treating diseases such as Parkinson's and Alzheimer's.	(154–157, 161)
Autism spectrum disorder (ASD)	Although the etiopathogenesis of ASD is unknown, evidence points to an imbalance of the eCB system and the presence of a neuroinflammatory process. From this perspective, phytocannabinoids, especially CBD, appear with good preclinical and clinical evidence to improve symptoms in ASD, possibly through reestablishment of the eCB system and already confirmed anti-neuroinflammatory activities.	(86, 163, 164, 166, 174)

**Important Clinical studies regarding Cannabidiol as a treatment for these neuropsychiatric disorders are still very preliminary or even non-existent. However, the evidence found is promising and suggests that investing in larger-scale placebo-controlled clinical studies is necessary and worthwhile.*

neuropsychiatric disorders, will follow the same path observed for refractory epilepsies-from alternative medicine to a new treatment-, only advances in research can respond. While the definitive answers do not arrive, the fact is what we have so far allows us to glimpse a promising path.

AUTHOR CONTRIBUTIONS

RB and RT: final form of the manuscript. All authors: conceived the review and prepared the first draft.

ACKNOWLEDGMENTS

First and foremost, the present review is dedicated to the memory of EC. We would thank EC who contributed to our knowledge and to his passionate support of the medicinal use of cannabis and its derivates. The authors wish to thank students Marina G. da Silva and Guilherme C. Darós at the Laboratory of Behavioral Neuroscience and all past students at the Laboratory of Psychopharmacology, UFSC, for their technical contributions.

REFERENCES

1. Hanuš LO, Meyer SM, Muñoz E, Taglialatela-Scafati O, Appendino G. Phytocannabinoids: a unified critical inventory. *Nat Prod Rep*. (2016) 33:1357–92. doi: 10.1039/C6NP00074F
2. Howard P, Twycross R, Shuster J, Mihalyo M, Wilcock A. Cannabinoids. *J Pain Symptom Manage*. (2013) 46:142–9. doi: 10.1016/j.jpainsymman.2013.05.002
3. Mechoulam R, Hanuš LO, Pertwee R, Howlett AC. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat Rev Neurosci*. (2014) 15:757–64. doi: 10.1038/nrn3811
4. Campos AC, Fogaça MV, Sonego AB, Guimarães FS. *Cannabidiol, Neuroprotection and Neuropsychiatric Disorders*. Academic Press (2016). Available online at: https://www.sciencedirect.com/science/article/pii/S1043661816000396?via%3Dihub (accessed October 26, 2017).
5. Jiang W, Zhang Y, Xiao L, Van Cleemput J, Ji SP, Bai G, et al. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *J Clin Invest*. (2005) 115:3104–16. doi: 10.1172/JCI25509
6. Schier A, Ribeiro N, Coutinho D, Machado S, Arias-Carrion O, Crippa J, et al. Antidepressant-like and anxiolytic-like effects of cannabidiol: a chemical compound of *Cannabis sativa*. *CNS Neurol Disord Drug Targets*. (2014) 13:953–60. doi: 10.2174/18715273136661406121148 38
7. Poleszak E, Wośko S, Sławińska K, Szopa A, Wróbel A, Serefko A. Cannabinoids in depressive disorders. *Life Sci*. (2018) 213:18–24. doi: 10.1016/j.lfs.2018.09.058
8. Bitencourt RM, Takahashi RN. Cannabidiol as a therapeutic alternative for post-traumatic stress disorder: from bench research to confirmation in human trials. *Front Neurosci*. (2018) 12:502. doi: 10.3389/fnins.2018.00502
9. De Carvalho CR, Pamplona FA, Cruz JS, Takahashi RN. Endocannabinoids underlie reconsolidation of hedonic memories in Wistar rats. *Psychopharmacology (Berl)*. (2014) 231:1417–25. doi: 10.1007/s00213-013-3331-2
10. Stern CAJ, de Carvalho CR, Bertoglio LJ, Takahashi RN. Effects of cannabinoid drugs on aversive or rewarding drug-associated memory extinction and reconsolidation. *Neuroscience*. (2018) 370:62–80. doi: 10.1016/j.neuroscience.2017.07.018
11. Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, et al. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia*. (2014) 55:791–802. doi: 10.1111/epi.12631
12. Fleury-Teixeira P, Caixeta FV, da Silva LCR, Brasil-Neto JP, Malcher-Lopes R. Effects of cbd-enriched *Cannabis sativa* extract on autism spectrum disorder symptoms: an observational study of 18 participants undergoing compassionate use. *Front Neurol*. (2019) 10:1145. doi: 10.3389/fneur.2019.01145
13. Elliott J, DeJean D, Clifford T, Coyle D, Potter BK, Skidmore B, et al. Cannabis-based products for pediatric epilepsy: an updated systematic review. *Seizure*. (2020) 75:18–22. doi: 10.1016/j.seizure.2019.12.006
14. O'Connell BK, Gloss D, Devinsky O. Cannabinoids in treatment-resistant epilepsy: a review. *Epilepsy Behav*. (2017) 70:341–8. doi: 10.1016/j.yebeh.2016.11.012
15. Fisher RS, Van Emde Boas W, Blume W, Elger C, Genton P, Lee P, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*. (2005) 46:470–2. doi: 10.1111/j.0013-9580.2005.66104.x
16. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the "common" neurologic disorders? *Neurology*. (2007) 68:326–37. doi: 10.1212/01.wnl.0000252807.38124.a3
17. Lattanzi S, Cagnetti C, Matricardi S, Silvestrini M. Palliative non-resective surgery for drug-resistant epilepsy. *Brain Dev*. (2018) 40:512–3. doi: 10.1016/j.braindev.2017.12.012
18. Lattanzi S, Brigo F, Trinka E, Zaccara G, Cagnetti C, Del Giovane C, et al. Efficacy and safety of Cannabidiol in epilepsy: a systematic review and meta-analysis. *Drugs*. (2018) 78:1791–804. doi: 10.1007/s40265-018-0992-5
19. Huntsman RJ, Tang-Wai R, Shackelford AE. Cannabis for pediatric epilepsy. *J Clin Neurophysiol*. (2020) 37:2–8. doi: 10.1097/WNP.0000000000000641
20. Rosenberg EC, Tsien RW, Whalley BJ, Devinsky O. Cannabinoids and epilepsy. *Neurotherapeutics*. (2015) 12:747–68. doi: 10.1007/s13311-015-0375-5
21. Solymosi K, Kofalvi A. Cannabis: a treasure trove or pandora's box? *Mini Rev Med Chem*. (2016) 17:1223–91. doi: 10.2174/1389557516666161004162133
22. O'Shaughnessy WB. On the preparations of the Indian hemp, or Gunjah: cannabis indica their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases. *BMJ*. (1843) s1-5:363–9. doi: 10.1136/bmj.s1-5.123.363
23. Reynolds JR. Epilepsy: its symptoms, treatment, and relation to other chronic convulsive diseases. *Br Foreign Med Chir Rev*. (1862) 30:309–12.
24. Loewe S, Goodman LS. Anticonvulsant action of marihuana-active substances. *Fed Proc*. (1947) 6:352.
25. Davis J, Proc HR-F. *Antiepileptic Action of Marihuana Active Substances*. (1949). calgarycmmc.com Available online at: https://www.calgarycmmc.com/Anti-epileptic-Action-of-Marijuana-Active-Substances-1947.pdf (accessed October 16, 2020).
26. Mechoulam R, Shvo Y. Hashish-I. The structure of cannabidiol. *Tetrahedron*. (1963) 19:2073–8. doi: 10.1016/0040-4020(63)85022-X
27. Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of Hashish. *J Am Chem Soc*. (1964) 86:1646–7. doi: 10.1021/ja01062a046
28. Hill AJ, Hill TDM, Whalley BJ. The development of cannabinoid based therapies for epilepsy. In: Murillo-Rodríguez, editor. *Endocannabinoids: Molecular, Pharmacological, Behavioral and Clinical Features*. Oak Park, IL: Bentham Science Publishers (2013). p. 164–204.
29. Carlini EA, Leite JR, Tannhauser M, Berardi AC. Cannabidiol and *Cannabis sativa* extract protect mice and rats against convulsive agents. *J Pharm Pharmacol*. (1973) 25:664–5. doi: 10.1111/j.2042-7158.1973.tb10660.x
30. Carlini EA, Mechoulam R, Lander N. Anticonvulsant activity of four oxygenated cannabidiol derivatives. *Res Commun Chem Pathol Pharmacol*. (1975) 12:1–15.
31. Mechoulam R, Carlini EA. Toward drugs derived from cannabis. *Naturwissenschaften*. (1978) 65:174–9. doi: 10.1007/BF00450585
32. Cunha JM, Carlini EA, Pereira AE, Ramos OL, Pimentel C, Gagliardi R, et al. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology*. (1980) 21:175–85. doi: 10.1159/000137430

33. Carlini EA, Cunha JM. Hypnotic and antiepileptic effects of cannabidiol. *J Clin Pharmacol*. (1981) 21:417S−27S. doi: 10.1002/j.1552-4604.1981.tb02622.x
34. Consroe P, Benedito MAC, Leite JR, Carlini EA, Mechoulam R. Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. *Eur J Pharmacol*. (1982) 83:293–8. doi: 10.1016/0014-2999(82)90264-3
35. Leite JR, Carlini EA, Lander N, Mechoulam R. Anticonvulsant effects of the (-) and (+)isomers of cannabidiol and their dimethylheptyl homologs. *Pharmacology*. (1982) 24:141–6. doi: 10.1159/000137588
36. Leo A, Russo E, Elia M. Cannabidiol and epilepsy: rationale and therapeutic potential. *Pharmacol Res*. (2016) 107:85–92. doi: 10.1016/j.phrs.2016.03.005
37. Samanta D. Cannabidiol: a review of clinical efficacy and safety in epilepsy. *Pediatr Neurol*. (2019) 96:24–9. doi: 10.1016/j.pediatrneurol.2019.03.014
38. Silvestro S, Mammana S, Cavalli E, Bramanti P, Mazzon E. Use of cannabidiol in the treatment of epilepsy: efficacy and security in clinical trials. *Molecules*. (2019) 24:1459. doi: 10.3390/molecules24081459
39. Franco V, Perucca E. Pharmacological and therapeutic properties of cannabidiol for epilepsy. *Drugs*. (2019) 79:1435–54. doi: 10.1007/s40265-019-01171-4
40. Pamplona FA, Da Silva LR, Coan AC. Potential clinical benefits of CBD-rich cannabis extracts over purified CBD in treatment-resistant epilepsy: observational data meta-analysis. *Front Neurol*. (2018) 9:759. doi: 10.3389/fneur.2018.00759
41. *A Hidden Origin Story of the CBD Craze*. Available online at: https://www-nytimes-com.cdn.ampproject.org/c/s/www.nytimes.com/2020/05/23/sunday-review/coronavirus-cbd-oil.amp.html (accessed October 16, 2020).
42. Szaflarski JP, Martina Bebin E. Cannabis, cannabidiol, and epilepsy—From receptors to clinical response. *Epilepsy Behav*. (2014) 41:277–82. doi: 10.1016/j.yebeh.2014.08.135
43. Cilio MR, Thiele EA, Devinsky O. The case for assessing cannabidiol in epilepsy. *Epilepsia*. (2014) 55:787–90. doi: 10.1111/epi.12635
44. Tzadok M, Uliel-Siboni S, Linder I, Kramer U, Epstein O, Menascu S, et al. CBD-enriched medical cannabis for intractable pediatric epilepsy: the current Israeli experience. *Seizure*. (2016) 35:41–4. doi: 10.1016/j.seizure.2016.01.004
45. McCoy B, Wang L, Zak M, Al-Mehmadi S, Kabir N, Alhadid K, et al. A prospective open-label trial of a CBD/THC cannabis oil in dravet syndrome. *Ann Clin Transl Neurol*. (2018) 5:1077–88. doi: 10.1002/acn3.621
46. Porcari GS, Fu C, Doll ED, Carter EG, Carson RP. Efficacy of artisanal preparations of cannabidiol for the treatment of epilepsy: practical experiences in a tertiary medical center. *Epilepsy Behav*. (2018) 80:240–6. doi: 10.1016/j.yebeh.2018.01.026
47. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol*. (2016) 15:270–8. doi: 10.1016/S1474-4422(15)00379-8
48. Rosenberg EC, Louik J, Conway E, Devinsky O, Friedman D. Quality of life in childhood epilepsy in pediatric patients enrolled in a prospective, open-label clinical study with cannabidiol. *Epilepsia*. (2017) 58:e96–100. doi: 10.1111/epi.13815
49. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of cannabidiol for drug-resistant seizures in the dravet syndrome. *N Engl J Med*. (2017) 376:2011–20. doi: 10.1056/NEJMoa1611618
50. Thiele EA, Marsh ED, French JA, Mazurkiewicz MB, Benbadis SR, Joshi C, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*. (2018) 391:1085–96. doi: 10.1016/S0140-6736(18)30136-3
51. Raucci U, Pietrafusa N, Paolino MC, Di Nardo G, Villa MP, Pavone P, et al. Cannabidiol treatment for refractory epilepsies in pediatrics. *Front Pharmacol*. (2020) 11:1. doi: 10.3389/fphar.2020.586110
52. Lattanzi S, Trinka E, Striano P, Zaccara G, Del Giovane C, Nardone R, et al. Cannabidiol efficacy and clobazam status: a systematic review and meta-analysis. *Epilepsia*. (2020) 61:1090–8. doi: 10.1111/epi.16546
53. Geffrey AL, Pollack SF, Bruno PL, Thiele EA. Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. *Epilepsia*. (2015) 56:1246–51. doi: 10.1111/epi.13060
54. Bialer M, Perucca E. Does cannabidiol have antiseizure activity independent of its interactions with clobazam? An appraisal of the evidence from randomized controlled trials. *Epilepsia*. (2020) 61:1082–9. doi: 10.1111/epi.16542
55. Savage TE, Sourbron J, Bruno PL, Skirvin LA, Wolper ES, Anagnos CJ, et al. Efficacy of cannabidiol in subjects with refractory epilepsy relative to concomitant use of clobazam. *Epilepsy Res*. (2020) 160:106263. doi: 10.1016/j.eplepsyres.2019.106263
56. Gaston TE, Bebin EM, Cutter GR, Ampah SB, Liu Y, Grayson LP, et al. Drug-drug interactions with cannabidiol (CBD) appear to have no effect on treatment response in an open-label expanded access program. *Epilepsy Behav*. (2019) 98:201–6. doi: 10.1016/j.yebeh.2019.07.008
57. Stockings E, Zagic D, Campbell G, Weier M, Hall WD, Nielsen S, et al. Evidence for cannabis and cannabinoids for epilepsy: a systematic review of controlled and observational evidence. *J Neurol Neurosurg Psychiatry*. (2018) 89:741–53. doi: 10.1136/jnnp-2017-317168
58. Berkovic SF. Cannabinoids for epilepsy—real data, at last. *N Engl J Med*. (2017) 376:2075–6. doi: 10.1056/NEJMe1702205
59. Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee M-H, Vogel Z, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol*. (1998) 353:23–31. doi: 10.1016/S0014-2999(98)00392-6
60. Karniol IG, Carlini EA. The content of (-)Δ9-trans-tetrahydrocannabinol (Δ9-THC) does not explain all biological activity of some Brazilian marihuana samples. *J Pharm Pharmacol*. (1972) 24:833–5. doi: 10.1111/j.2042-7158.1972.tb08897.x
61. Karniol IG, Carlini EA. Pharmacological interaction between cannabidiol and δ9-tetrahydrocannabinol. *Psychopharmacologia*. (1973) 33:53–70. doi: 10.1007/BF00428793
62. Takahashi RN, Zuardi AW, Karniol IG. Composicao quimica e importancia dos diversos constituintes na atividade farmacologica de amostras de *Cannabis sativa* brasileiras. *Brazilian J Med Biol Res*. (1977) 10:379–85.
63. Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br J Pharmacol*. (2011) 163:1344–64. doi: 10.1111/j.1476-5381.2011.01238.x
64. Russo E, Guy GW. A tale of two cannabinoids : the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med Hypotheses*. (2006) 66:234–46. doi: 10.1016/j.mehy.2005.08.026
65. Hill AJ, Weston SE, Jones NA, Smith I, Bevan SA, Williamson EM, et al. 9-Tetrahydrocannabivarin suppresses *in vitro* epileptiform and *in vivo* seizure activity in adult rats. *Epilepsia*. (2010) 51:1522–32. doi: 10.1111/j.1528-1167.2010.02523.x
66. Hill TDM, Cascio MG, Romano B, Duncan M, Pertwee RG, Williams CM, et al. Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat *via* a CB1 receptor-independent mechanism. *Br J Pharmacol*. (2013) 170:679–92. doi: 10.1111/bph.12321
67. Hill AJ, Mercier MS, Hill TDM, Glyn SE, Jones NA, Yamasaki Y, et al. Cannabidivarin is anticonvulsant in mouse and rat. *Br J Pharmacol*. (2012) 167:1629–42. doi: 10.1111/j.1476-5381.2012.02207.x
68. Vilela LR, Lima IV, Kunsch ÉB, Pinto HPP, de Miranda AS, Vieira ÉLM, de Oliveira ACP, et al. Anticonvulsant effect of cannabidiol in the pentylenetetrazole model: pharmacological mechanisms, electroencephalographic profile, and brain cytokine levels. *Epilepsy Behav*. (2017) 75:29–35. doi: 10.1016/j.yebeh.2017.07.014
69. Asth L, Iglesias LP, De Oliveira AC, Moraes MFD, Moreira FA. Exploiting cannabinoid and vanilloid mechanisms for epilepsy treatment. *Epilepsy Behav*. (in press) 106832. doi: 10.1016/j.yebeh.2019.106832
70. dos Santos RG, Hallak JEC, Zuardi AW, de Souza Crippa AC, de Souza Crippa JA. Cannabidiol for the treatment of epilepsy: an overview of possible mechanisms of action and preclinical and human studies. In: Preedy VR, editor. *Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment*. Amsterdam: Elsevier Inc. (2017). p. 795–801
71. Vilela LR, de Oliveira ACP, Moraes MF, Moreira FA, Takahashi RN. The endocannabinoid system as a target for new antiseizure drugs. In: Preedy VR, editor. *Handbook of Cannabis and Related Pathologies: Biology,*

Pharmacology, Diagnosis, and Treatment. Amsterdam: Elsevier Inc. (2017). p. 605–15.
72. Russo EB, Marcu J. Cannabis pharmacology: the usual suspects and a few promising leads. In: Kendall D, Alexander SPH, editors. *Advances in Pharmacology*. Cambridge: Academic Press Inc. (2017). p. 67–134. (2019).
73. Di Marzo V. The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol Res*. (2009) 60:77–84. doi: 10.1016/j.phrs.2009.02.010
74. Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y. Endocannabinoid signaling and synaptic function. *Neuron*. (2012) 76:70–81. doi: 10.1016/j.neuron.2012.09.020
75. Sugaya Y, Kano M. Control of excessive neural circuit excitability and prevention of epileptic seizures by endocannabinoid signaling. *Cell Mol Life Sci*. (2018) 75:2793–811. doi: 10.1007/s00018-018-2834-8
76. Bitencourt RM, Alpár A, Cinquina V, Ferreira SG, Pinheiro BS, Lemos C, et al. Lack of presynaptic interaction between glucocorticoid and CB1 cannabinoid receptors in GABA- and glutamatergic terminals in the frontal cortex of laboratory rodents. *Neurochem Int*. (2014) 90:72–84. doi: 10.1016/j.neuint.2015.07.014
77. Soltesz I, Alger BE, Kano M, Lee SH, Lovinger DM, Ohno-Shosaku T, et al. Weeding out bad waves: towards selective cannabinoid circuit control in epilepsy. *Nat Rev Neurosci*. (2015) 16:264–77. doi: 10.1038/nrn3937
78. Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*. (2003) 302:84–8. doi: 10.1126/science.1088208
79. Lupica CR, Hu Y, Devinsky O, Hoffman AF. Cannabinoids as hippocampal network administrators. *Neuropharmacology*. (2017) 124:25–37. doi: 10.1016/j.neuropharm.2017.04.003
80. Ligresti A, De Petrocellis L, Di Marzo V. From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. *Physiol Rev*. (2016) 96:1593–659. doi: 10.1152/physrev.00002.2016
81. Jones NA, Glyn SE, Akiyama S, Hill TDM, Hill AJ, Weston SE, et al. Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. *Seizure*. (2012) 21:344–52. doi: 10.1016/j.seizure.2012.03.001
82. Gaston TE, Friedman D. Pharmacology of cannabinoids in the treatment of epilepsy. *Epilepsy Behav*. (2017) 70:313–8. doi: 10.1016/j.yebeh.2016.11.016
83. Sylantyev S, Jensen TP, Ross RA, Rusakov DA. Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proc Natl Acad Sci USA*. (2013) 110:5193–8. doi: 10.1073/pnas.1211204110
84. Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol*. (2001) 134:845–52. doi: 10.1038/sj.bjp.0704327
85. Elmes MW, Kaczocha M, Berger WT, Leung KN, Ralph BP, Wang L, et al. Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). *J Biol Chem*. (2015) 290:8711–21. doi: 10.1074/jbc.M114.618447
86. Poleg S, Golubchik P, Offen D, Weizman A. Cannabidiol as a suggested candidate for treatment of autism spectrum disorder. *Prog Neuro-Psychopharmacology Biol Psychiatry*. (2019) 89:90–6. doi: 10.1016/j.pnpbp.2018.08.030
87. Yin A qi, Wang F, Zhang X. Integrating endocannabinoid signaling in the regulation of anxiety and depression. *Acta Pharmacol Sin*. (2019) 40:336–41. doi: 10.1038/s41401-018-0051-5
88. de Carvalho CR, Takahashi RN. Cannabidiol disrupts the reconsolidation of contextual drug-associated memories in Wistar rats. *Addict Biol*. (2017) 22:742–51. doi: 10.1111/adb.12366
89. Volkow ND, Hampson AJ, Baler RD. Don't worry, be happy: endocannabinoids and cannabis at the intersection of stress and reward. *Annu Rev Pharmacol Toxicol*. (2017) 57:285–308. doi: 10.1146/annurev-pharmtox-010716-104615
90. Feingold D, Weiser M, Rehm J, Lev-Ran S. The association between cannabis use and mood disorders: a longitudinal study. *J Affect Disord*. (2015) 172:211–8. doi: 10.1016/j.jad.2014.10.006
91. Bambico FR, Gobbi G. The cannabinoid CB1 receptor and the endocannabinoid anandamide: possible antidepressant targets. *Expert Opin Ther Targets*. (2008) 12:1347–66. doi: 10.1517/14728222.12.11.1347
92. Hillard C, Liu Q. Endocannabinoid signaling in the etiology and treatment of major depressive illness. *Curr Pharm Des*. (2014) 20:3795–811. doi: 10.2174/13816128113196660735
93. Hill MN, Hillard CJ, Bambico FR, Patel S, Gorzalka BB, Gobbi G. The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. *Trends Pharmacol Sci*. (2009) 30:484–93. doi: 10.1016/j.tips.2009.06.006
94. Griebel G, Stemmelin J, Lopez-Grancha M, Fauchey V, Slowinski F, Pichat P, et al. The selective reversible FAAH inhibitor, SSR411298, restores the development of maladaptive behaviors to acute and chronic stress in rodents. *Sci Rep*. (2018) 8:2416. doi: 10.1038/s41598-018-20895-z
95. Micale V, Di Marzo V, Sulcova A, Wotjak CT, Drago F. Endocannabinoid system and mood disorders: priming a target for new therapies. *Pharmacol Ther*. (2013) 138:18–37. doi: 10.1016/j.pharmthera.2012.12.002
96. Micale V, Tabiova K, Kucerova J, Drago F. Role of the endocannabinoid system in depression: from preclinical to clinical evidence. In: *Campolongo P, Fattore L, editors. Cannabinoid Modulation of Emotion, Memory, and Motivation*. New York, NY: Springer (2015). p. 97–129.
97. Patel S, Hillard CJ. Role of endocannabinoid signaling in anxiety and depression. *Curr Top Behav Neurosci*. (2009) 1:347–71. doi: 10.1007/978-3-540-88955-7_14
98. Van Gaal L, Pi-Sunyer X, Després JP, McCarthy C, Scheen A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the Rimonabant in Obesity (RIO) program. *Diabetes Care*. (2008) 31(Suppl. 2):229–40. doi: 10.2337/dc08-s258
99. Aso E, Ozaita A, Serra MÀ, Maldonado R. Genes differentially expressed in CB1 knockout mice: involvement in the depressive-like phenotype. *Eur Neuropsychopharmacol*. (2011) 21:11–22. doi: 10.1016/j.euroneuro.2010.06.007
100. Valverde O, Torrens M. CB1 receptor-deficient mice as a model for depression. *Neuroscience*. (2012) 204:193–206. doi: 10.1016/j.neuroscience.2011.09.031
101. Hill MN, Miller GE, Ho WSV, Gorzalka BB, Hillard CJ. Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. *Pharmacopsychiatry*. (2008) 41:48–53. doi: 10.1055/s-2007-993211
102. Koethe D, Llenos IC, Dulay JR, Hoyer C, Torrey EF, Leweke FM, et al. Expression of CB1 cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. *J Neural Transm*. (2007) 114:1055–63. doi: 10.1007/s00702-007-0660-5
103. Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs. low CRH/NE states. *Mol Psychiatry*. (2002) 7:254–75. doi: 10.1038/sj.mp.4001032
104. Elsaid S, Kloiber S, Le Foll B. Effects of cannabidiol (CBD) in neuropsychiatric disorders: a review of pre-clinical and clinical findings. In: Rahman S, editor. *Progress in Molecular Biology and Translational Science*. Amsterdam: Elsevier B.V. (2019). p. 25–75.
105. Sales AJ, Fogaça MV, Sartim AG, Pereira VS, Wegener G, Guimarães FS, et al. Cannabidiol induces rapid and sustained antidepressant-like effects through increased BDNF signaling and synaptogenesis in the prefrontal cortex. *Mol Neurobiol*. (2019) 56:1070–81. doi: 10.1007/s12035-018-1143-4
106. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry*. (2006) 163:28–40. doi: 10.1176/appi.ajp.163.1.28
107. Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by δ9-THC in normal subjects. *Psychopharmacology (Berl)*. (1982) 76:245–50. doi: 10.1007/BF00432554
108. Martin-Santos R, Crippa JA, Batalla A, Bhattacharyya S, Atakan Z, Borgwardt S, et al. Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC)

and cannabidiol (CBD) administration in healthy volunteers. *Curr Pharm Des*. (2012) 18:4966–79. doi: 10.2174/138161212802884780
109. Bitencourt RM, Pamplona FA, Takahashi RN. Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur Neuropsychopharmacol*. (2008) 18:849–59. doi: 10.1016/j.euroneuro.2008.07.001
110. Fogaça MV, Campos AC, Coelho LD, Duman RS, Guimarães FS. The anxiolytic effects of cannabidiol in chronically stressed mice are mediated by the endocannabinoid system: role of neurogenesis and dendritic remodeling. *Neuropharmacology*. (2018) 135:22–33. doi: 10.1016/j.neuropharm.2018.03.001
111. Shallcross J, Hámor P, Bechard AR, Romano M, Knackstedt L, Schwendt M. The divergent effects of cdppb and cannabidiol on fear extinction and anxiety in a predator scent stress model of ptsd in rats. *Front Behav Neurosci*. (2019) 13:91. doi: 10.3389/fnbeh.2019.00091
112. De Gregorio D, McLaughlin RJ, Posa L, Ochoa-Sanchez R, Enns J, Lopez-Canul M, et al. Cannabidiol modulates serotonergic transmission and reverses both allodynia and anxiety-like behavior in a model of neuropathic pain. *Pain*. (2019) 160:136–50. doi: 10.1097/j.pain.0000000000001386
113. Zieba J, Sinclair D, Sebree T, Bonn-Miller M, Gutterman D, Siegel S, et al. Cannabidiol (CBD) reduces anxiety-related behavior in mice *via* an FMRP-independent mechanism. *Pharmacol Biochem Behav*. (2019) 181:93–100. doi: 10.1016/j.pbb.2019.05.002
114. Granjeiro ÉM, Gomes FV, Guimarães FS, Corrêa FMA, Resstel LBM. Effects of intracisternal administration of cannabidiol on the cardiovascular and behavioral responses to acute restraint stress. *Pharmacol Biochem Behav*. (2011) 99:743–8. doi: 10.1016/j.pbb.2011.06.027
115. Fogaça MV, Reis FMCV, Campos AC, Guimarães FS. Effects of intra-prelimbic prefrontal cortex injection of cannabidiol on anxiety-like behavior: involvement of 5HT1A receptors and previous stressful experience. *Eur Neuropsychopharmacol*. (2014) 24:410–9. doi: 10.1016/j.euroneuro.2013.10.012
116. Guimarães FS, Aguiar JCD, Mechoulam R, Breuer A. Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze. *Gen Pharmacol*. (1994) 25:161–4. doi: 10.1016/0306-3623(94)90027-2
117. Zuardi AW, Cosme RA, Graeff FG, Guimarães FS, Guimaraes FS. Effects of ipsapirone and cannabidiol on human experimental anxiety. *J Psychopharmacol*. (1993) 7:82–8. doi: 10.1177/026988119300700112
118. Shannon S, Lewis N, Lee H, Hughes S. Cannabidiol in anxiety and sleep: a large case series. *Perm J*. (2019) 23:18–41. doi: 10.7812/TPP/18-041
119. Shannon S, Opila-Lehman J. Effectiveness of cannabidiol oil for pediatric anxiety and insomnia as part of posttraumatic stress disorder: a case report. *Perm J*. (2016) 20:108–11. doi: 10.7812/TPP/16-005
120. Crippa JAS, Nogueira Derenusson G, Borduqui Ferrari T, Wichert-Ana L, Duran FLS, Martin-Santos R, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J Psychopharmacol*. (2011) 25:121–30. doi: 10.1177/0269881110379283
121. Bergamaschi MM, Queiroz RHC, Chagas MHN, De Oliveira DCG, De Martinis BS, Kapczinski F, et al. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-nave social phobia patients. *Neuropsychopharmacology*. (2011) 36:1219–26. doi: 10.1038/npp.2011.6
122. Blessing EM, Steenkamp MM, Manzanares J, Marmar CR. Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics*. (2015) 12:825–36. doi: 10.1007/s13311-015-0387-1
123. Demers CH, Drabant Conley E, Bogdan R, Hariri AR. Interactions between anandamide and corticotropin-releasing factor signaling modulate human amygdala function and risk for anxiety disorders: an imaging genetics strategy for modeling molecular interactions. *Biol Psychiatry*. (2016) 80:356–62. doi: 10.1016/j.biopsych.2015.12.021
124. Duan T, Gu N, Wang Y, Wang F, Zhu J, Fang Y, et al. Fatty acid amide hydrolase inhibitors produce rapid anti-anxiety responses through amygdala long-term depression in male rodents. *J Psychiatry Neurosci*. (2017) 42:230–41. doi: 10.1503/jpn.160116
125. Bluett RJ, Gamble-George JC, Hermanson DJ, Hartley ND, Marnett LJ, Patel S. Central anandamide deficiency predicts stress-induced anxiety: behavioral reversal through endocannabinoid augmentation. *Transl Psychiatry*. (2014) 4:e408. doi: 10.1038/tp.2014.53
126. Lomazzo E, Bindila L, Remmers F, Lerner R, Schwitter C, Hoheisel U, et al. Therapeutic potential of inhibitors of endocannabinoid degradation for the treatment of stress-related hyperalgesia in an animal model of chronic pain. *Neuropsychopharmacology*. (2015) 40:488–501. doi: 10.1038/npp.2014.198
127. Gaetani S, Dipasquale P, Romano A, Righetti L, Cassano T, Piomelli D, et al. Chapter 5 the endocannabinoid system as a target for novel anxiolytic and antidepressant drugs. *Int Rev Neurobiol*. (2009) 85:57–72. doi: 10.1016/S0074-7742(09)85005-8
128. Papagianni EP, Stevenson CW. Cannabinoid regulation of fear and anxiety: an update. *Curr Psychiatry Rep*. (2019) 21:1–10. doi: 10.1007/s11920-019-1026-z
129. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, et al. The endogenous cannabinoid system controls extinction of aversive memories. *Nature*. (2002) 418:530–4. doi: 10.1038/nature00839
130. Chhatwal JP, Davis M, Maguschak KA, Ressler KJ. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology*. (2005) 30:516–24. doi: 10.1038/sj.npp.1300655
131. Pandolfo P, Takahashi RN. The cannabinoid receptor agonist WIN 55, 212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. *Psychopharmacology (Berl)*. (2006) 188:641–9. doi: 10.1007/s00213-006-0514-0
132. Do Monte FH, Souza RR, Bitencourt RM, Kroon JA, Takahashi RN. Infusion of cannabidiol into infralimbic cortex facilitates fear extinction *via* CB1 receptors. *Behav Brain Res*. (2013) 250:23–7. doi: 10.1016/j.bbr.2013.04.045
133. Lemos JI, Resstel LB, Silveira Guimarães F. Involvement of the prelimbic prefrontal cortex on cannabidiol-induced attenuation of contextual conditioned fear in rats. *Behav Brain Res*. (2010) 207:105–11. doi: 10.1016/j.bbr.2009.09.045
134. Stern CAJ, Gazarini L, Takahashi RN, Guimarães FS, Bertoglio LJ. On disruption of fear memory by reconsolidation blockade: evidence from cannabidiol treatment. *Neuropsychopharmacology*. (2012) 37:2132–42. doi: 10.1038/npp.2012.63
135. Stern CAJ, Gazarini L, Vanvossen AC, Zuardi AW, Galve-Roperh I, Guimaraes FS, et al. Δ9-Tetrahydrocannabinol alone and combined with cannabidiol mitigate fear memory through reconsolidation disruption. *Eur Neuropsychopharmacol*. (2015) 25:958–65. doi: 10.1016/j.euroneuro.2015.02.001
136. LaFrance EM, Glodosky NC, Bonn-Miller M, Cuttler C. Short and long-term effects of cannabis on symptoms of post-traumatic stress disorder. *J Affect Disord*. (2020) 274:298–304. doi: 10.1016/j.jad.2020.05.132
137. Elms L, Shannon S, Hughes S, Lewis N. Cannabidiol in the treatment of post-traumatic stress disorder: a case series. *J Altern Complement Med*. (2019) 25:392–7. doi: 10.1089/acm.2018.0437
138. Passie T, Emrich HM, Karst M, Brandt SD, Halpern JH. Mitigation of post-traumatic stress symptoms by Cannabis resin: a review of the clinical and neurobiological evidence. *Drug Test Anal*. (2012) 4:649–59. doi: 10.1002/dta.1377
139. Das RK, Kamboj SK, Ramadas M, Curran HV, Morgan CJA, Yogan K, et al. Cannabidiol enhances consolidation of explicit fear extinction in humans. *Psychopharmacology (Berl)*. (2013) 226:781–92. doi: 10.1007/s00213-012-2955-y
140. Greer GR, Grob CS, Halberstadt AL. PTSD symptom reports of patients evaluated for the New Mexico medical cannabis program. *J Psychoactive Drugs*. (2014) 46:73–7. doi: 10.1080/02791072.2013.873843
141. Lisboa SF, Vila-Verde C, Rosa J, Uliana DL, Stern CAJ, Bertoglio LJ, et al. Tempering aversive/traumatic memories with cannabinoids: a review of evidence from animal and human studies. *Psychopharmacology (Berl)*. (2019) 236:201–26. doi: 10.1007/s00213-018-5127-x
142. Papini S, Sullivan GM, Hien DA, Shvil E, Neria Y. Toward a translational approach to targeting the endocannabinoid system in posttraumatic stress disorder: a critical review of preclinical research. *Biol Psychol*. (2015) 104:8–18. doi: 10.1016/j.biopsycho.2014.10.010
143. Sbarski B, Akirav I. Cannabinoids as therapeutics for PTSD. *Pharmacol Ther*. (2020) 211:107551. doi: 10.1016/j.pharmthera.2020.107551
144. Zer-Aviv TM, Segev A, Akirav I. Cannabinoids and post-traumatic stress disorder: clinical and preclinical evidence for treatment and prevention. *Behav Pharmacol*. (2016) 27:561–9. doi: 10.1097/FBP.0000000000000253

145. Hill MN, Campolongo P, Yehuda R, Patel S. Integrating endocannabinoid signaling and cannabinoids into the biology and treatment of posttraumatic stress disorder. *Neuropsychopharmacology*. (2018) 43:80–102. doi: 10.1038/npp.2017.162
146. *Drug Memories: CBD & Addiction|Project CBD*. Available online at: https://www.projectcbd.org/medicine/drug-memories-cbd-addiction (accessed November 16, 2020).
147. Luján MÁ, Castro-Zavala A, Alegre-Zurano L, Valverde O. Repeated cannabidiol treatment reduces cocaine intake and modulates neural proliferation and CB1R expression in the mouse hippocampus. *Neuropharmacology*. (2018) 143:163–75. doi: 10.1016/j.neuropharm.2018.09.043
148. Lopes JB, Bastos JR, Costa RB, Aguiar DC, Moreira FA. The roles of cannabinoid CB1 and CB2 receptors in cocaine-induced behavioral sensitization and conditioned place preference in mice. *Psychopharmacology (Berl)*. (2020) 237:385–94. doi: 10.1007/s00213-019-05370-5
149. De Ternay J, Naassila M, Nourredine M, Louvet A, Bailly F, Sescousse G, et al. Therapeutic prospects of cannabidiol for alcohol use disorder and alcohol-related damages on the liver and the brain. *Front Pharmacol*. (2019) 10:627. doi: 10.3389/fphar.2019.00627
150. Labigalini E, Rodrigues LR, DaSilveira DX. Therapeutic use of cannabis by crack addicts in Brazil. *J Psychoactive Drugs*. (1999) 31:451–5. doi: 10.1080/02791072.1999.10471776
151. Socías ME, Kerr T, Wood E, Dong H, Lake S, Hayashi K, et al. Intentional cannabis use to reduce crack cocaine use in a Canadian setting: a longitudinal analysis. *Addict Behav*. (2017) 72:138–43. doi: 10.1016/j.addbeh.2017.04.006
152. Goncalves JR, Nappo SA. Factors that lead to the use of crack cocaine in combination with marijuana in Brazil: a qualitative study health behavior, health promotion and society. *BMC Public Health*. (2015) 15:706. doi: 10.1186/s12889-015-2063-0
153. Lee JLC, Bertoglio LJ, Guimarães FS, Stevenson CW. Cannabidiol regulation of emotion and emotional memory processing: relevance for treating anxiety-related and substance abuse disorders. *Br J Pharmacol*. (2017) 174:3242–56. doi: 10.1111/bph.13724
154. Basavarajappa BS, Shivakumar M, Joshi V, Subbanna S. Endocannabinoid system in neurodegenerative disorders. *J Neurochem*. (2017) 142:624–48. doi: 10.1111/jnc.14098
155. Ren S-Y, Wang Z-Z, Zhang Y, Chen N-H. Potential application of endocannabinoid system agents in neuropsychiatric and neurodegenerative diseases-focusing on FAAH/MAGL inhibitors. *Acta Pharmacol Sin*. (2020) 41:1263–71. doi: 10.1038/s41401-020-0385-7
156. Cassano T, Villani R, Pace L, Carbone A, Bukke VN, Orkisz S, et al. From *Cannabis sativa* to cannabidiol: promising therapeutic candidate for the treatment of neurodegenerative diseases. *Front Pharmacol*. (2020) 11:124. doi: 10.3389/fphar.2020.00124
157. Watt G, Karl T. *In vivo* evidence for therapeutic properties of cannabidiol (CBD) for alzheimer's disease. *Front Pharmacol*. (2017) 8:20. doi: 10.3389/fphar.2017.00020
158. Lastres-Becker I, Molina-Holgado F, Ramos JA, Mechoulam R, Fernández-Ruiz J. Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity *in vivo* and *in vitro*: relevance to Parkinson's disease. *Neurobiol Dis*. (2005) 19:96–107. doi: 10.1016/j.nbd.2004.11.009
159. Esposito G, Scuderi C, Valenza M, Togna GI, Latina V, De Filippis D, et al. Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS ONE*. (2011) 6:e28668. doi: 10.1371/journal.pone.0028668
160. Esposito G, De Filippis D, Maiuri MC, De Stefano D, Carnuccio R, Iuvone T. Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in β-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-κB involvement. *Neurosci Lett*. (2006) 399:91–5. doi: 10.1016/j.neulet.2006.01.047
161. Crippa JAS, Hallak JEC, Zuardi AW, Guimarães FS, Tumas V, dos Santos RG. Is cannabidiol the ideal drug to treat non-motor Parkinson's disease symptoms? *Eur Arch Psychiatry Clin Neurosci*. (2019) 269:121–33. doi: 10.1007/s00406-019-00982-6
162. Siniscalco D, Schultz S, Brigida A, Antonucci N. Inflammation and neuro-immune dysregulations in autism spectrum disorders. *Pharmaceuticals*. (2018) 11:56. doi: 10.3390/ph11020056
163. Fusar-Poli L, Cavone V, Tinacci S, Concas I, Petralia A, Signorelli MS, et al. Cannabinoids for people with asd: a systematic review of published and ongoing studies. *Brain Sci*. (2020) 10:1–18. doi: 10.20944/preprints202007.0373.v1
164. Yeh ML, Levine ES. Perspectives on the role of endocannabinoids in autism spectrum disorders. *OBM Neurobiol*. (2017) 1:005. doi: 10.21926/obm.neurobiol.1702005
165. Aran A, Eylon M, Harel M, Polianski L, Nemirovski A, Tepper S, et al. Lower circulating endocannabinoid levels in children with autism spectrum disorder. *Mol Autism*. (2019) 10:2. doi: 10.1186/s13229-019-0256-6
166. Pietropaolo S, Bellocchio L, Bouzón-Arnáiz I, Yee BK. The role of the endocannabinoid system in autism spectrum disorders: evidence from mouse studies. In: Ilieva M, Kwok-Wai Lau W, editors. *Progress in Molecular Biology and Translational Science*. Amsterdam: Elsevier B.V. (2020). p. 183–208. doi: 10.1016/bs.pmbts.2020.04.016
167. Karhson DS, Krasinska KM, Dallaire JA, Libove RA, Phillips JM, Chien AS, et al. Plasma anandamide concentrations are lower in children with autism spectrum disorder. *Mol Autism*. (2018) 9:18. doi: 10.1186/s13229-018-0203-y
168. Kerr DM, Downey L, Conboy M, Finn DP, Roche M. Alterations in the endocannabinoid system in the rat valproic acid model of autism. *Behav Brain Res*. (2013) 249:124–32. doi: 10.1016/j.bbr.2013.04.043
169. Kerr DM, Gilmartin A, Roche M. Pharmacological inhibition of fatty acid amide hydrolase attenuates social behavioural deficits in male rats prenatally exposed to valproic acid. *Pharmacol Res*. (2016) 113:228–35. doi: 10.1016/j.phrs.2016.08.033
170. Wu HF, Lu TY, Chu MC, Chen PS, Lee CW, Lin HC. Targeting the inhibition of fatty acid amide hydrolase ameliorate the endocannabinoid-mediated synaptic dysfunction in a valproic acid-induced rat model of Autism. *Neuropharmacology*. (2020) 162:107736. doi: 10.1016/j.neuropharm.2019.107736
171. Zamberletti E, Gabaglio M, Woolley-Roberts M, Bingham S, Rubino T, Parolaro D. Cannabidivarin treatment ameliorates autism-like behaviors and restores hippocampal endocannabinoid system and glia alterations induced by prenatal valproic acid exposure in rats. *Front Cell Neurosci*. (2019) 13:367. doi: 10.3389/fncel.2019.00367
172. Barchel D, Stolar O, De-Haan T, Ziv-Baran T, Saban N, Fuchs DO, et al. Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and Co-morbidities. *Front Pharmacol*. (2019) 9:1521. doi: 10.3389/fphar.2018.01521
173. Aran A, Cassuto H, Lubotzky A, Wattad N, Hazan E. Brief report: cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems-a retrospective feasibility study. *J Autism Dev Disord*. (2019) 49:1284–8. doi: 10.1007/s10803-018-3808-2
174. Agarwal R, Burke SL, Maddux M. Current state of evidence of cannabis utilization for treatment of autism spectrum disorders. *BMC Psychiatry*. (2019) 19:328. doi: 10.1186/s12888-019-2259-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 26 February 2021
doi: 10.3389/fphar.2021.620926

Bulleyaconitine A Inhibits Morphine-Induced Withdrawal Symptoms, Conditioned Place Preference, and Locomotor Sensitization Via Microglial Dynorphin A Expression

*Meng-Jing Zhao, Mi-Ya Wang, Le Ma, Khalil Ali Ahmad and Yong-Xiang Wang**

King's Lab, Shanghai Jiao Tong University School of Pharmacy, Shanghai, China

OPEN ACCESS

Edited by:
Elaine Elisabetsky, Federal University of Rio Grande do Sul, Brazil

Reviewed by:
Wen-Lung Ma, China Medical University, Taiwan
Elisabet Jerlhag, University of Gothenburg, Sweden

***Correspondence:**
Yong-Xiang Wang
yxwang@sjtu.edu.cn

Specialty section:
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

Received: *24 October 2020*
Accepted: *12 January 2021*
Published: *26 February 2021*

Citation:
Zhao M-J, Wang M-Y, Ma L, Ahmad KA and Wang Y-X (2021) Bulleyaconitine A Inhibits Morphine-Induced Withdrawal Symptoms, Conditioned Place Preference, and Locomotor Sensitization Via Microglial Dynorphin A Expression. Front. Pharmacol. 12:620926. doi: 10.3389/fphar.2021.620926

Bulleyaconitine A (BAA), a C19-diterpenoid alkaloid, has been prescribed as a nonnarcotic analgesic to treat chronic pain over four decades in China. The present study investigated its inhibition in morphine-induced withdrawal symptoms, conditioned place preference (CPP) and locomotor sensitization, and then explored the underlying mechanisms of actions. Multiple daily injections of morphine but not BAA up to 300 μg/kg/day into mice evoked naloxone-induced withdrawal symptoms (i.e., shakes, jumps, genital licks, fecal excretion and body weight loss), CPP expression, and locomotor sensitization. Single subcutaneous BAA injection (30–300 μg/kg) dose-dependently and completely attenuated morphine-induced withdrawal symptoms, with ED_{50} values of 74.4 and 105.8 μg/kg in shakes and body weight loss, respectively. Subcutaneous BAA (300 μg/kg) also totally alleviated morphine-induced CPP acquisition and expression and locomotor sensitization. Furthermore, subcutaneous BAA injection also specifically stimulated dynorphin A expression in microglia but not astrocytes or neurons in nucleus accumbens (NAc) and hippocampal, measured for gene and protein expression and double immunofluorescence staining. In addition, subcutaneous BAA-inhibited morphine-induced withdrawal symptoms and CPP expression were totally blocked by the microglial metabolic inhibitor minocycline, dynorphin A antiserum, or specific KOR antagonist GNTI, given intracerebroventricularly. These results, for the first time, illustrate that BAA attenuates morphine-induced withdrawal symptoms, CPP expression, and locomotor sensitization by stimulation of microglial dynorphin A expression in the brain, suggesting that BAA may be a potential candidate for treatment of opioids-induced physical dependence and addiction.

Keywords: bulleyaconitine A, dynorphin A, microglia, nucleus accumbens, hippocampus, physical dependence, conditioned place preference, locomotor sensitization 3

INTRODUCTION

Opioid addiction is the accumulated results of tolerance and dependence, mainly including physical and psychological dependence (Wang et al., 2019). Physical dependence seeks drug repeatedly and increases gradually doses of the drug to avoid any withdrawal symptoms (Nestler et al., 1993). Psychological dependence refers to drug craving and euphoria achieved by repeated medication, which is difficult to eliminate. Drug relapse can persist for a long time after drug cessation in human being (Robbins et al., 2008) and the high rate of relapse after detoxification is a major clinical problem and becomes a severe challenge to treat drug abuse (Aguilar et al., 2009). Morphine and related opioids are the most potent and widely used analgesics in treating moderate to severe pain. However, development of tolerance and dependence are the important limitation to use of opioid drugs in chronic pain management (Thorn et al., 2016; Ruzza et al., 2019). Opioid addiction in Western countries, particularly in the United States, has become a serious health and social problem in recent years, which requires to be urgently addressed (Wingo et al., 2016; Ezard et al., 2018).

Dynorphin A is widely distributed in the central nervous system to bind to three opioid receptor subtypes with different affinities, especially to κ-opioid receptors (KORs) (Fallon and Leslie, 1986; Schwarzer, 2009). KORs are also widely distributed in the brain (Yuferov et al., 2004; Shippenberg et al., 2007; Bruchas et al., 2010), and the dynorphin/KOR system plays an important role in pain/analgesia, temperature, emotions, and neuroendocrine functions (Pfeiffer et al., 1986; Bodnar, 2010). The dynorphin/KOR pathway is also a major anti-reward system and participates in development of drug addiction. There is growing evidence showing that administration of dynorphin A and other related opioid peptides alleviates withdrawal symptoms of morphine physical dependence (Takemori et al., 1993; Hooke et al., 1995). The KOR agonists, unlike the μ-opioid receptor agonists, do not produce any reinforcing effects but reduce drug abuse under certain conditions. Indeed, it was reported that the KOR agonists ethylketocyclazocine and U50,488 attenuated cocaine behavioral sensitization, conditioned place preference (CPP) acquisition, and self-administration in rhesus monkeys by repressing the release of dopamine (Maisonneuve et al., 1994). It was also reported that the dynorphin/KOR system antagonized the rewarding effects in drug abuse and inhibited the brain reward function by suppressing dopamine release from the mesolimbic reward pathway (Chartoff et al., 2008; Mysels and Sullivan, 2009). On the contrary, it was also reported that the KOR antagonists nor-BNI and arodyn blocked stress-induced reinstatement of cocaine-induced self-administration or CPP acquisition (Beardsley et al., 2005; Carey et al., 2007). These data suggest a complex role of the dynorphin/KOR system in the drug abuse development.

Bulleyaconitine A (BAA), isolated from the rhizomes of *Aconitum bulleyanum*, is a C19-diterpenoid alkaloid without activities of binding to opioid receptors (Wang et al., 2007). As it is a nonnarcotic analgesic and has lower toxicity and wider treatment window than aconitine, BAA has been widely prescribed in China to treat various forms of chronic pain over four decades (Bello-Ramirez and Nava-Ocampo, 2004; Xie et al., 2018). Accumulated evidence demonstrated that BAA and its analogs aconitine (C19-diterpenoid), bullatine A (C20-diterpenoid), and lappaconitine (C18-diterpenoid) produced antinociception without inducing antinociceptive tolerance in various rodent models of pain hypersensitivity, including neuropathic pain, bone cancer pain, inflammatory pain, diabetic pain, and visceral pain (Li et al., 2016a; Li et al., 2016b; Huang et al., 2016; Sun et al., 2018; Huang et al., 2020a; Huang et al., 2020b). Our recent studies further uncovered that BAA, aconitines, bullatine A, and lappaconitine alleviated pain directly through stimulating spinal microglial dynorphin A expression and subsequently activating KORs (Huang et al., 2016; Li et al., 2016a; Li et al., 2017; Sun et al., 2018). In addition, BAA and bullatine A injection blocked chronic morphine-induced antinociceptive tolerance in rats and mice (Li et al., 2016a; Huang et al., 2017a). These studies led to our hypothesis that aconitines including BAA may have a therapeutic potential in treatment of morphine withdrawal symptoms and compulsive drug-seeking and abuse.

In this study, we assessed the inhibitory effects of BAA on regulation of morphine-induced withdrawal symptoms, CPP acquisition and expression, and locomotor sensitization. We first tested whether a subcutaneous BAA injection attenuated naloxone-induced withdrawal symptom in chronic morphine-treated mice. We then assessed whether subcutaneous BAA inhibited morphine-induced CPP acquisition and expression and locomotor sensitization. Thereafter, we explored the involvement of microglial dynorphin A expression and subsequent KOR activation in BAA-induced anti-addictive effects. Our results uncover that BAA inhibits morphine-induced withdrawal symptoms, CPP acquisition and expression, and locomotor sensitization through microglial expression of dynorphin A, suggesting that stimulation of microglial expression of dynorphin A is a potential strategy in treatment of opioid addiction and abuse.

MATERIALS AND METHODS

Drugs and Reagents

BAA was purchased from Zelang Bio-Pharmaceutical (Nanjing, China) with a purity no less than 98% determined by manufacturer with high performance liquid chromatography. Morphine hydrochloride, minocycline, and pentobarbital sodium were obtained from the Northeast Pharmaceuticals Group (Shenyang, China), Yuanye Biotech (Shanghai, China), and Sinopharm Chemical Reagent Co., (Shanghai, China), respectively. Both 5′-guanidinonaltrindole (GNTI) and naloxone hydrochloride were from Sigma-Aldrich (St. Louis, MO, United States). Furthermore, the rabbit polyclonal antiserum neutralizing dynorphin A was purchased from Phoenix Pharmaceuticals (Burlingame, CA, United States). The antiserum was specific to dynorphin A (100%), but not to dynorphin B (0%), β-endorphin (0%), α-neo-endorphin (0%) or leu-enkephalin (0%) according to the manufacturer′s datasheet. Its specificity was also validated by the antigen absorption test from other laboratories (Wakabayashi et al., 2010; Yamada et al., 2013). All the drugs and reagents were dissolved or diluted in 0.9% normal saline.

Experimental Animals

Adult male Swiss mice (8–9 weeks and 20–25 g bodyweight) were purchased from the Shanghai Experimental Animal Institute for Biological Sciences (Shanghai, China). The animals were maintained in a 12-h light/dark cycle (light period 7:00 a.m.—7:00 p.m.) with free access to food and water at standard room temperature (22 ± 2°C) in the Shanghai Jiao Tong University Experimental Animal Center (Shanghai, China). All mice were acclimatized 3–5 days before the experiments. Mice (n = 10–12 per group) were randomly assigned and the behavior tests were performed in a blind manner. All housing conditions and experimental procedures were approved by the Animal Care and Welfare Committee of Shanghai Jiao Tong University (Shanghai, China).

Induction of Morphine-Induced Withdrawal Symptoms in Mice

The paradigm in induction of morphine-induced withdrawal symptoms was performed as established previously (Goeldner et al., 2011; Bobzean et al., 2019). Briefly, morphine was administered in mice with escalating doses (5, 10, 20, 40, 80, and 100 mg/kg) by twice-daily subcutaneous injections at 10:00 a.m. and 4:00 p.m. for six consecutive days. On the seventh day, mice received a single subcutaneous injection of morphine (100 mg/kg) at 10:00 a.m., followed by an intraperitoneal injection of naloxone (5 mg/kg) 4 h later. Naloxone, by blocking opioid receptors, can expedite morphine withdrawal symptoms and is widely applied in the morphine addiction studies. The withdrawal symptoms, including shakes, jumps, genital licks, fecal excretion and loss of body weight, were observed and recorded for 30 min immediately after naloxone injection. To test the effect of BAA on morphine-induced withdrawal symptoms, mice received a single bolus BAA injection (30, 100, or 300 μg/kg) 40 min prior to the intraperitoneal injection of naloxone.

Conditioned Place Preference Apparatus and Paradigm

CPP is a widely used model to assess the reinforcing effect of drug abuse in laboratories (Wu et al., 2016). As like other addictive drugs, morphine-induced CPP expression is considered to constitute a part of the addiction process associated with the opioid reinforcing properties. The apparatus in the CPP test consists of three compartments: two equal-sized chambers (25 × 25 × 40 cm) with a connecting white protruded chamber (null compartment, 25 × 5 × 40 cm) separated by a removable door. To distinguish each other, one of the main chambers was decorated with black walls and a striped floor, while the other one was with black and white striped wall and round dot floor. The environmental lighting was adjusted to exclude baseline preference. The apparatus was kept in a quiet room and dim 40 lx illumination (Marszalek-Grabska et al., 2018).

The 10-day scheduled CPP paradigm included three distinct phases: preconditioning, conditioning, and post-conditioning (Khaleghzadeh-Ahangar and Haghparast, 2015; Khaleghzadeh-Ahangar and Haghparast, 2017). The preconditioning phase started with a 3-day twice-daily (10:00 a.m. and 4:00 p.m.) mouse handling with the cupping open gloved hand method (Gouveia and Hurst, 2017). On Day 4, each mouse was placed into the null compartment with full access to the entire apparatus for 15 min. The time spent in each chamber was recorded by a 3CCD camera (Panasonic Inc., Japan) and analyzed using the EthoVision XT 8.0 (Noldus Information Technology Co., China) to determine the baseline preference. Animals that spent more than 450 s in any of the three chambers were excluded from the experiment. During the conditioning phase, mice underwent 5 days of morphine (10 mg/kg) or saline (10 ml/kg) alternatively subcutaneous injections, with a 6-h interval (between 10:00 a.m. and 4:00 p.m.) and included ten 45-min sessions in a five-day schedule. On day 5, 7, and 9 of the conditioning phase, mice were treated with morphine in the morning and immediately confined to the morphine-paired chamber for 45 min and received saline in the afternoon and then put into the saline-paired chamber for 45 min. On day 6 and 8, the injection sequence of morphine and saline was changed. Morphine-induced CPP in mice was tested by being allowed with free access to all three compartments for 15 min in the post-conditioning phase (on Day 10). The conditioning score was expressed by the time spent in the drug-paired chamber minus that in the saline-paired chamber. To determine the influence of BAA on morphine-induced CPP acquisition and expression, BAA was administration 30 min prior to morphine injection during the conditioning phase and 50 min prior to the post-conditioning phase, respectively.

Morphine-Induced Locomotor Sensitization

The locomotor sensitization is a phenomenon that repeated administration of opioids can induce a progressive and long-lasting enhancement in behavioral response, which is associated with relapse and compulsive drug-seeking (Zhang et al., 2003). The methods for the behavioral sensitization in mice were described previously (Cordonnier et al., 2007). Mice were placed into a locomotor detection chamber (40 × 40 × 35 cm) under a video tracking system, and the data were analyzed automatically using ANY-maze. The procedure of the development of morphine-induced behavioral sensitization included habituation phase (Day 1–3) and morphine-induced behavioral sensitization phase (Day 4–8). In the habituation phase (Day 1–3), all mice were injected normal saline (10 ml/kg) and placed into the test apparatus for 3 days (1 h per session). In the morphine-induced behavioral sensitization phase (Day 4–8), mice were injected with saline (10 ml/kg) or morphine (10 mg/kg), and then placed into the test apparatus, where their locomotion was recorded for 1 h/day for 5 days. Mice received subcutaneous BAA injection (300 μg/kg) 20 min before morphine injection for 5 days (Day 4–8).

Intracerebroventricular Catheterization and Injection in Mice

For intracerebroventricular catheterization, mice were anesthetized by intraperitoneal injection of 1.5% pentobarbital

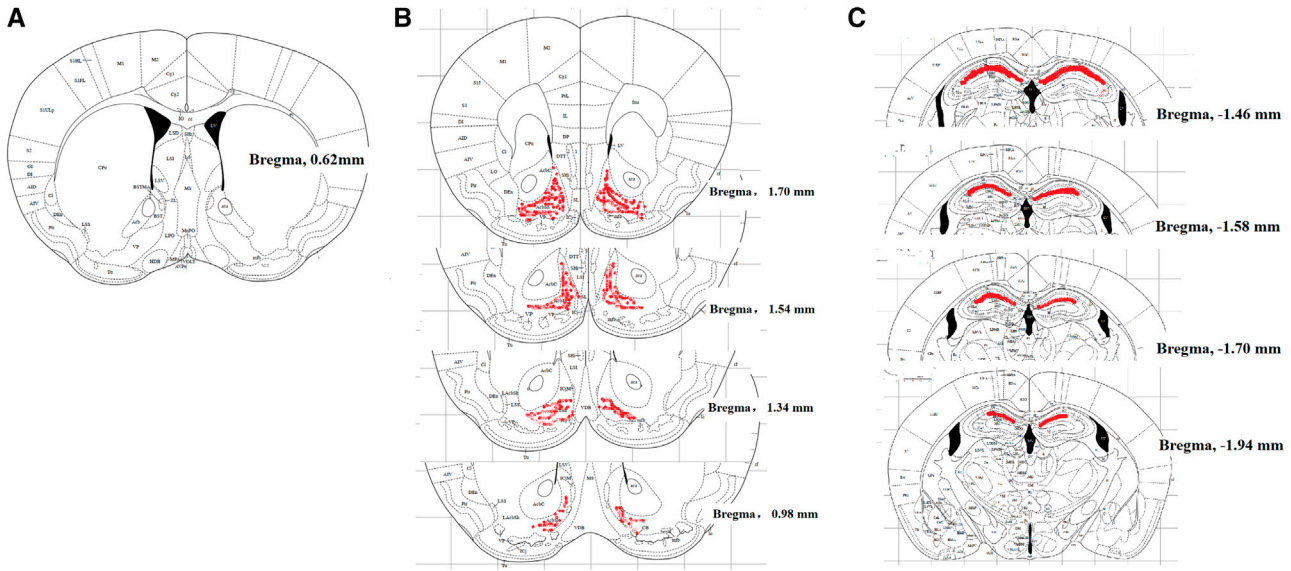

FIGURE 1 | Localization of mouse cerebroventricle [in blank, **(A)**], nucleus accumbens shell [NAcSh, in red, **(B)**] and hippocampal CA1 [in red, **(C)**], displayed in the coronal plane. The cerebroventricle is approximately 1.0 mm lateral to the sagittal suture and 0.6 mm caudal from the bregma; the NAcSh is 1.70 to 0.98 mm from the bregma; hippocampal CA1 is −1.46 to −1.94 mm from the bregma.

sodium and positioned in a stereotaxic instrument (Stoelting Company, Wood Dale, IL, United States). The surgical site was shaved and sterilized with 70% ethanol and a 1.5 cm incision was made to expose the skull. A 22-gauge stainless steel cannula was directed to 1.0 mm lateral and 0.6 mm caudal to bregma and inserted 3 mm deep according to the mouse brain stereotaxic coordinates (**Figure 1A**). Dental cement was applied to adhere the cannula to the skull. The incision was sutured and the cap of cannula was covered. Animals were returned to their cages and allowed recovery at least for three days. The drug was administrated slowly over 3 min in a 6-μL volume through the planted cannula, using an insulin needle mated with a 10-μL microsyringe via a polyethylene tube (Kim et al., 1998; Lenard and Roerig, 2005; Glascock et al., 2011; Kim et al., 2016). It is noted that the ventricular injection may be a limit as its injection volume more than 2 μL could affect the behaviors of the animals. To verify the causal relationship between the microglial expression of dynorphin A in the brain and BAA-inhibited withdrawal signs in morphine-treated mice, the microglial metabolic inhibitor minocycline (10 μg) (Neigh et al., 2009), dynorphin A antiserum (1:30 dilution) (Li et al., 2016a) and KOR antagonist GNTI (5 μg) (Loh et al., 2017) were intracerebroventricularly injected into mice separately.

RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction

The total RNA was isolated from mouse nucleus accumbens (NAc) and hippocampus using the TRIzol reagent (Invitrogen, Carlsbad, CA, United States) and reversely transcribed into cDNA using the ReverTraAce RT-qPCR kit (Toyobo Co., Osaka, Japan) according to the manufacturers' instructions. qPCR was performed with a Mastercycler ep realplex (Eppendorf, Hamburg, Germany) using the Realmaster Mix (SYBR Green I, Toyobo, Japan). The forward and reverse primer sequences were 5′-ATG ATG AGA CGC CAT CCT TC-3′ and 5′-TTA ATG AGG GCT GTG GGA AC-3′ for prodynorphin, which was designed by Premier 6 (version 6.0, Premier Biosoft, San Francisco, United States); and 5′-CCA AGG TCA TCC ATG ACG AC-3′ and 5′-TCC ACA GTC TTC TGA GTG GC-3′ for GAPDH (Reiss et al., 2017). The fold change was calculated using the $2^{-\triangle\triangle Ct}$ method after normalization to level of GAPDH mRNA (Huang et al., 2017b).

Measurement of Dynorphin A

Mouse NAc and hippocampus were obtained and immediately frozen in liquid nitrogen and stored an −80°C until use. Tissues were homogenized at 4,000 rpm for 15 s with a homogenizer (Fluko Equipment Co., Shanghai, China) in 10 mM Tris-HCl (pH 7.4) and centrifuged at 1,500 rpm at 4°C for 15 min. The total protein concentrations in NAc and hippocampus were determined by a standard bicinchoninic acid protein assay (Beyotime Institute of Biotechnology, Jiangsu, China) and dynorphin A was assayed using a commercialized fluorescence enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals, Burlingame, CA, United States) according to the operation manual (Leitermann et al., 2004; Nocjar et al., 2012).

Immunofluorescence Staining

Double immunofluorescence labeling of dynorphin A and cellular biomarkers of microglia, astrocytes, and neurons in mouse NAc and hippocampus was carried out using a TCS SP8 confocal microscope (Leica Microsystems, Wetzlar,

Germany) according to the previously published method with minor modifications. Mice were deeply anesthetized by intraperitoneal 1.5% pentobarbital sodium (5 ml/kg), and intracardially perfusion with 20 ml of 0.9% saline, followed by 20 ml of 4% paraformaldehyde. The brain was dissected and fixed in the 4% paraformaldehyde for 12 h at 4°C. Paraformaldehyde was then removed with phosphate buffered saline (PBS) and the brain was dehydrated with the gradient sucrose solutions (10%, 20% and 30% diluted with PBS) at 4°C. The dehydrated brain was embedded in the optimal cutting temperature embedding agent (Leica Microsystems) and cut into 30-μm-thick transverse sections with a sliding microtome. The frozen sections were incubated in 10% goat serum (v/v) and 0.5% Triton X-100 (v/v) for 1 h at the room temperature and then incubated at 4°C for 24 h with different primary antibodies. The primary antibodies included an anti-dynorphin A antibody (1:100; rabbit polyclonal; Phoenix Pharmaceuticals) and cellular markers, i.e., anti-Iba-1 (1:100; mouse monoclonal; Millipore, Darmstadt, Germany) for microglia, anti-GFAP (1:100; mouse monoclonal) for astrocytes, and anti-NeuN (1:60; mouse polyclonal; Millipore) for neurons. After washing with PBS, the sections were incubated for 1 h at 37°C with the Alexa-555-conjugated goat anti-rabbit secondary antibody for dynorphin A and the Alexa-488-conjugated goat anti-mouse secondary antibody for microglia, astrocytes or neurons (Qi et al., 2018). Expression of dynorphin A, Iba-1, GFAP, and NeuN was visualized in the shell of nucleus accumbens (NAcSh) (from bregma 1.70 mm to 0.98 mm, according to the mouse brain stereotaxic coordinates, **Figure 1B**) and hippocampal CA1 (from bregma −1.46 to −1.94 mm, according to the mouse brain stereotaxic coordinates, **Figure 1C**) under a confocal microscope. To quantify the relative intensity of dynorphin A in Iba-1-, GFAP- or NeuN-immunopositive cells in NAcSh and hippocampal CA1, the images were acquired at a 10× or 30× magnification. The background fluorescence was normalized and only immunofluorescent intensity from positively stained areas were included using the low and high thresholds. A co-localization analysis was performed using the ImageJ software with a co-localization finder to generate images in which the co-localized pixels appeared as white. All surface areas in each group were measured following the same setup configurations at the same time. The averaged value of the immunolabeled surface area was recorded as the positive immunofluorescence area from three nonadjacent sections of NAcSh or hippocampal CA1. Data were calculated from six mice of each group.

Statistical analysis

For the dose-response curve analysis, the parameters, i.e., the minimum effect, half-effective dose (ED_{50}), E_{max} and Hill coefficient (n), were calculated by fitting nonlinear least-squares curves to the relation $Y = a + bx$, where $x = [D]^n/(ED_{50}^n + [D]^n)$. The values of ED_{50} and b (E_{max}) were projected by yielding a minimum residual sum of squares of deviations from the theoretical curve (Zhang et al., 2013).

The data were summarized as means ± standard error of the mean (S.E.M.). The statistical significance was evaluated by unpaired and two-tailed Student *t*-test, one-way or repeated-measures two-way analysis of variance (ANOVA) using the Prism (version 7.00, GraphPad Software Inc., San Diego, CA, United States). The ANOVA analysis was performed based on the assumptions of normal distribution and variance consistency verified by residual plots. The post-hoc Student-Newman-Keuls test was used when the effect of the drug (for the one-way ANOVA, the factor was drug; for the two-way ANOVA, the factors were drug, time and their interaction) was observed to be statistically significant. The probability values were two-tailed and the statistical significance criterion value was 0.05.

RESULTS

Bulleyaconitine A Attenuates Naloxone-Induced Withdrawal Symptoms in Morphine-Treated Mice

Six groups of mice (n = 10 per group) were subjected to bi-daily subcutaneous injections of normal saline (10 ml/kg), BAA (300 μg/kg) or morphine (escalating doses of 5, 10, 20, 40, 80, and 100 mg/kg) for 7 days. On Day 7, mice received an intraperitoneal injection of naloxone (5 mg/kg) 4 h post last injection of saline, BAA, or morphine (100 mg/kg), and their withdrawal symptoms were observed immediately for 30 min. For the BAA inhibitory effects, mice received a single injection of saline (10 ml/kg) or BAA (30, 100, or 300 μg/kg) 40 min prior to the intraperitoneal naloxone injection. The experiment procedure is shown in **Figure 2A**. Intraperitoneal naloxone injection did not induce any abnormal behaviors in bi-daily saline- or BAA-treated mice. In contrast, naloxone in bi-daily morphine injected mice induced significant withdrawal symptoms, including shakes [$F(5, 54) = 23.13$, $p < 0.05$; **Figure 2B**], jumps [$F(5, 54) = 13.50$, $p < 0.05$; **Figure 2C**], genital licks [$F(5, 54) = 6.578$, $p < 0.05$; **Figure 2D**], fecal excretion [$F(5, 54) = 7.284$, $p < 0.05$; **Figure 2E**], and body weight loss [$F(5, 54) = 2.356$, $p < 0.05$; **Figure 2F**]. In addition, pretreatment with a single subcutaneous BAA injection (30, 100 and 300 μg/kg) dose-dependently attenuated naloxone-induced withdrawal signs in bi-daily morphine-treated mice, with a maximal inhibition of around 70–100% in each sign. The dose-response analyses were performed after data transformation, yielding ED_{50} values of 74.4 μg/kg in shakes (**Figure 2B**), and 105.8 μg/kg in body weight loss, respectively (**Figure 2F**).

Bulleyaconitine A Attenuates Morphine-Induced Conditioned Place Preference Acquisition and Expression

To assess the inhibitory effect of BAA on morphine-induced CPP acquisition, four groups of mice (n = 12 per group) were subjected to the preconditioning phase of three days and conditioning phase of five days. The alternative daily subcutaneous injections of saline (10 ml/kg) or BAA (300 μg/kg) 30 min were prior to each morphine (10 mg/kg) or saline injection (10 ml/kg) during the conditioning phase (**Figure 3A**). There was no significant difference between the

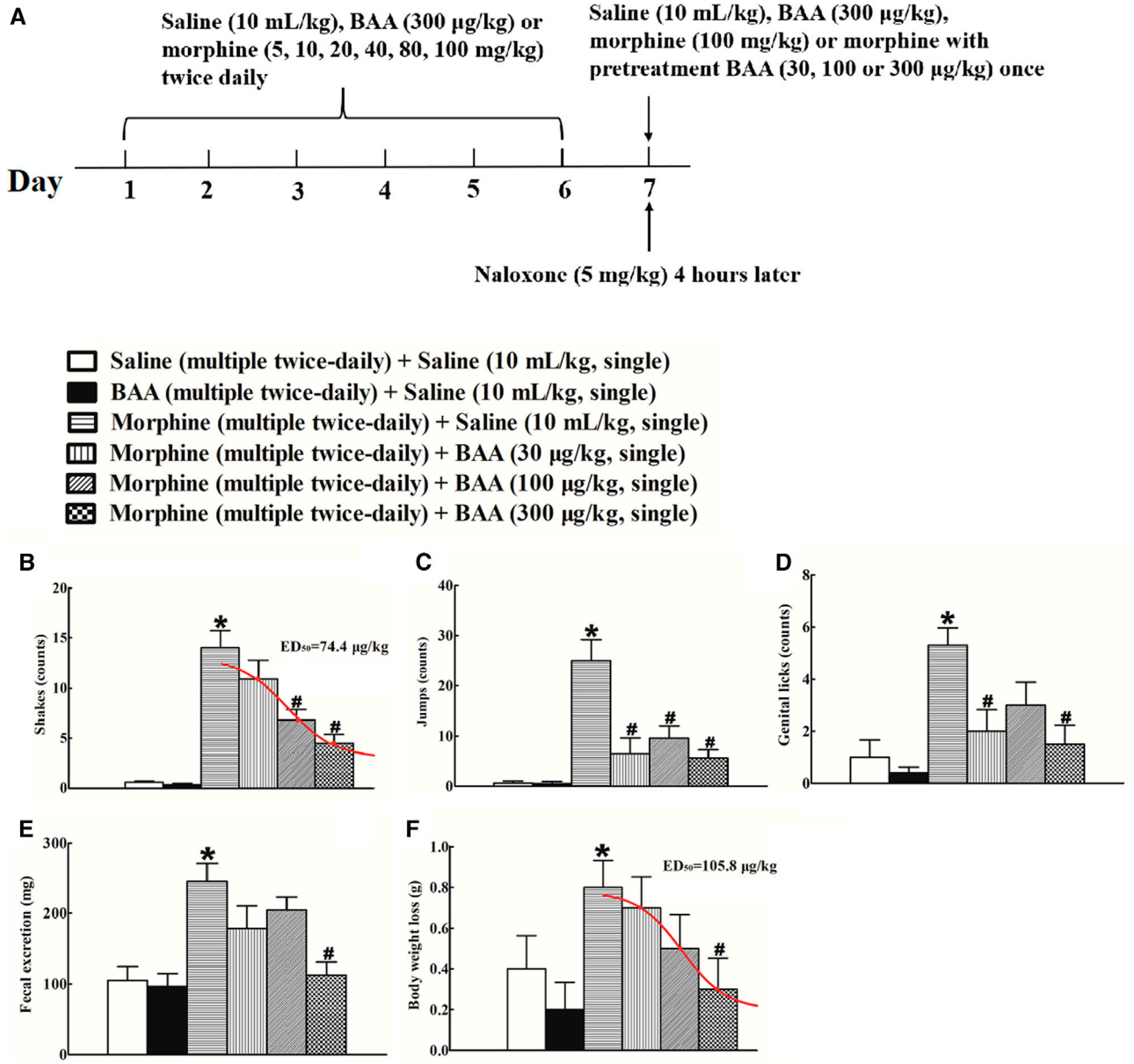

FIGURE 2 | Inhibitory effects of subcutaneous (sc) injection of bulleyaconitine A on naloxone-induced withdrawal symptoms in morphine-treated mice **(A),** the timeline of the experiment procedure), including shakes **(B)**, jumps **(C)**, genital licks **(D)**, fecal excretion **(E)** and body weight loss **(F)**. Mice were subjected to bi-daily subcutaneous injections of normal saline (10 ml/kg), BAA (300 μg/kg) or morphine (an escalating doses of 5, 10, 20, 40, 80 and 100 mg/kg) for 6 days. On Day 7, mice received an intraperitoneal injection of naloxone (5 mg/kg) 4 h post last injection of morphine (100 mg/kg) to induce withdrawal signs, which were observed immediately for 30 min. For the BAA inhibitory effects, mice received a single injection of saline (10 ml/kg) or BAA (30, 100 or 300 μg/kg) 40 min prior to naloxone intraperitoneal injection. The dose-response data were best projected by the nonlinear least squares methods in **B** and **F**. The data are presented as means ± S.E.M (n = 10 per group). $^{*,\#}p < 0.05$ compared with the saline control mice and morphine-treated mice, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test.

time spent in morphine-paired and saline-paired compartments during the preconditioning phase. Repeated morphine subcutaneous injections during the conditioning phase produced significant CPP acquisition, whereas saline or BAA did not show any CPP responses. Co-administrations of BAA (300 μg/kg) completely inhibited morphine-induced CPP acquisition [$F(3, 40) = 21.42$, $p < 0.05$, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test; **Figure 3B**].

To further determine the influence of BAA on morphine-induced CPP expression, four groups of mice (n = 12 per group) were subjected to alternative daily subcutaneous injections of

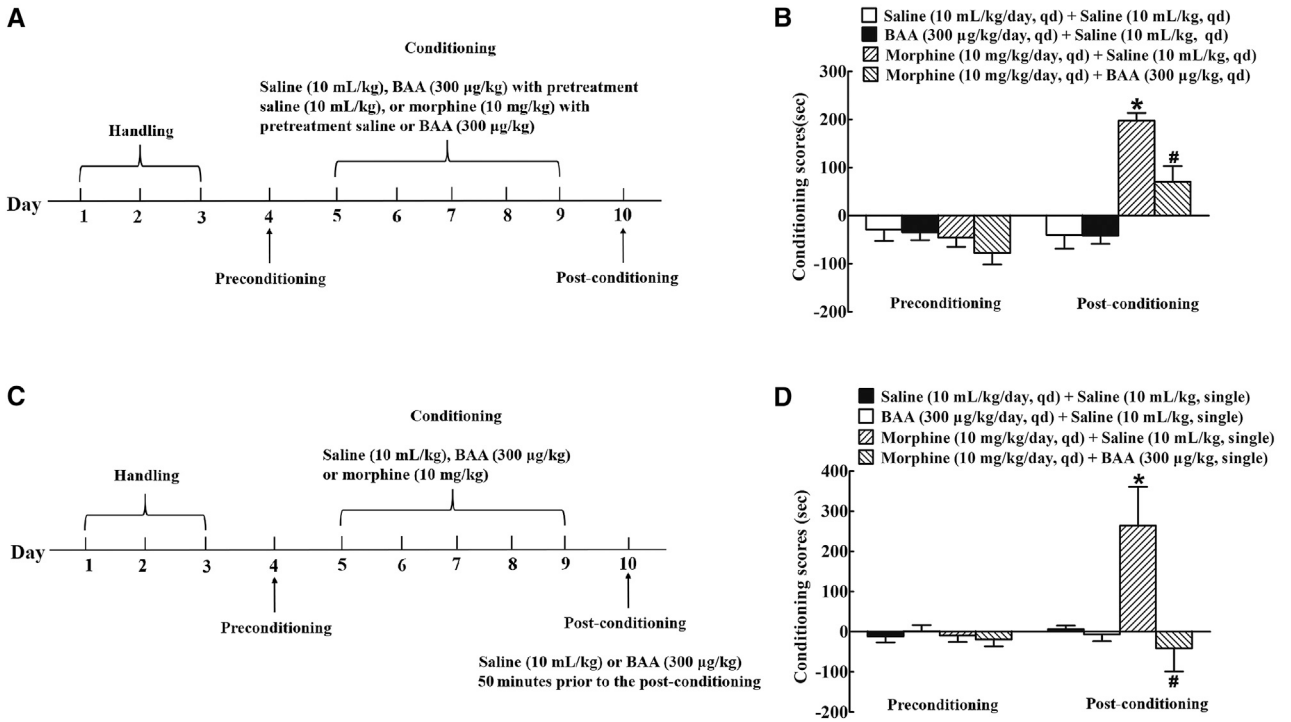

FIGURE 3 | Inhibitory effects of subcutaneous (sc) injection of bulleyaconitine A (BAA, 300 μg/kg) on morphine-induced conditioned place preference (CPP) acquisition **(A,B)** and expression **(C,D)** in mice. For morphine-induced CPP acquisition, mice were treated with saline (10 ml/kg) or BAA (300 μg/kg) 30 min before each normal saline (10 ml/kg/day), BAA (300 μg/kg/day) or morphine (10 mg/kg/day) treatment during the conditioning phase. For morphine-induced CPP expression, mice were subjected to alternatively daily subcutaneous injections of normal saline (10 ml/kg/day), BAA (300 μg/kg/day), or morphine (10 mg/kg/day) for 5 days followed by a single subcutaneous injection of saline (10 ml/kg) or BAA (300 μg/kg) 50 min before the post-conditioning phase. Results are presented as means ± S.E.M. (n = 11 or 12 per group). *$p < 0.05$, compared to the saline control and morphine CPP control groups, respectively, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test.

normal saline (10 ml/kg), BAA (300 μg/kg) or morphine (10 mg/kg) for 5 days after the preconditioning phase of three days. On the 10th day, mice received a single subcutaneous injection of saline (10 ml/kg) or BAA (300 μg/kg) 50 min prior to the post-conditioning and the place preference test was conducted immediately afterward (**Figure 3C**). Bi-daily subcutaneous injections of morphine but not saline or BAA showed remarkable CPP expression, while pretreatment with a single subcutaneous BAA injection completely attenuated morphine-induced CPP expression [$F(3, 44) = 6.043$, $p < 0.05$, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test; **Figure 3D**].

Bulleyaconitine A Suppresses Morphine-Induced Locomotor Sensitization Development

To investigate the inhibitory effect of BAA on morphine-induced locomotor sensitization development, four groups of mice (n = 12 per group) were subjected to the habituation phase (Day 1–3). After that, mice received a subcutaneous injection of saline (10 ml/kg) or BAA (300 μg/kg) 20 min before normal saline (10 ml/kg), BAA (300 μg/kg) or morphine (10 mg/kg) injection for 5 days (Day 4–8). The locomotion activity was then recorded for 1 h in each time point (**Figure 4A**). The 5-day BAA treatment did not significantly influence the locomotion activity compared to the saline control group. However, morphine treatment significantly increased the locomotion activity and the multi-daily treatment further significantly increased the travel distance [$F(3, 155)_{Day} = 119.3$, $p < 0.05$]. BAA co-treatment completely attenuated development of morphine-induced locomotor sensitization [$F(4, 155)_{Treatment} = 3{,}398$, $p < 0.05$, by repeated-measures two-way ANOVA followed by the post-hoc Student-Newman-Keuls test; **Figure 4B**].

Bulleyaconitine A Specifically Stimulates Microglial Dynorphin A Expression in NAc and Hippocampus in Morphine-Multi-Daily Treated Mice

Two groups of mice (n = 10 per group) treated daily with morphine (10 mg/kg) for 5 days received a subcutaneous injection of normal saline (10 ml/kg) or BAA (300 μg/kg). Mice were sacrificed 1 h after the subcutaneous injection and NAc and hippocampus were obtained for the prodynorphin mRNA detection using qRT-PCR analysis. As shown, BAA treatment compared to the saline control group significantly increased prodynorphin gene expression by 1.9-fold in NAc **Figure 5A**) and 1.7- fold in hippocampus ($p < 0.05$,

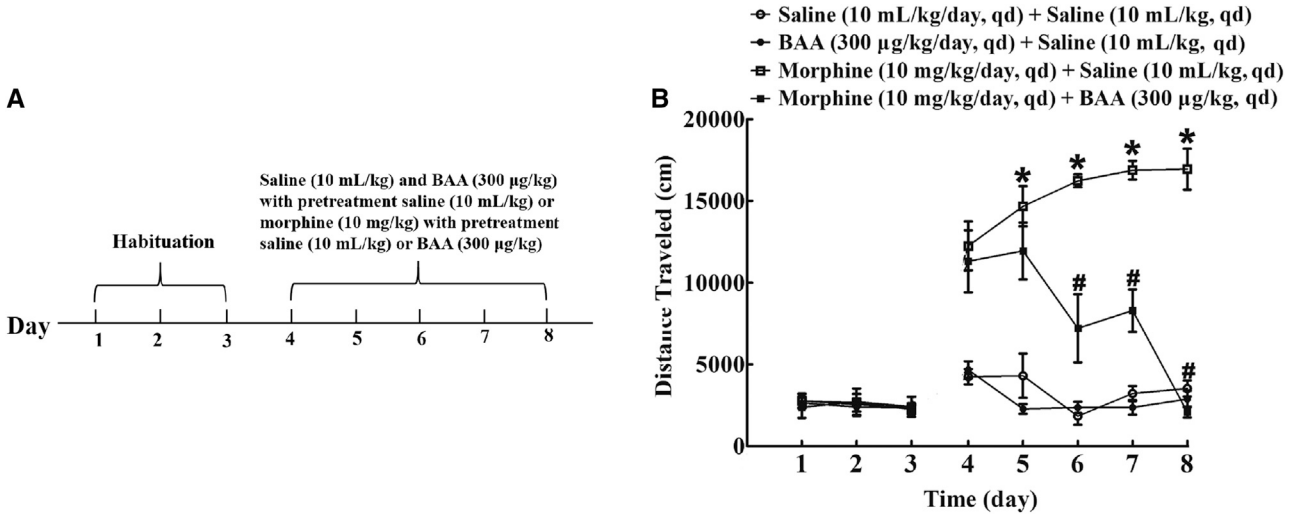

FIGURE 4 | Inhibitory effects of subcutaneous (sc) injection of bulleyaconitine A (BAA) on morphine-induced locomotor sensitization **(A,B)**. Mice received subcutaneous injection of saline (10 ml/kg) or BAA (300 μg/kg) 20 min before normal saline (10 ml/kg), BAA (300 μg/kg) or morphine (10 mg/kg) injection for 5 days (Day 4–8) and the locomotor activity was recorded for 1 h each time point. Results are presented as means ± S.E.M. (n = 12 per group). $^{*,\#}p < 0.05$ compared to the Day 4 and morphine group by repeated-measures two-way ANOVA followed by the post-hoc Student-Newman-Keuls test.

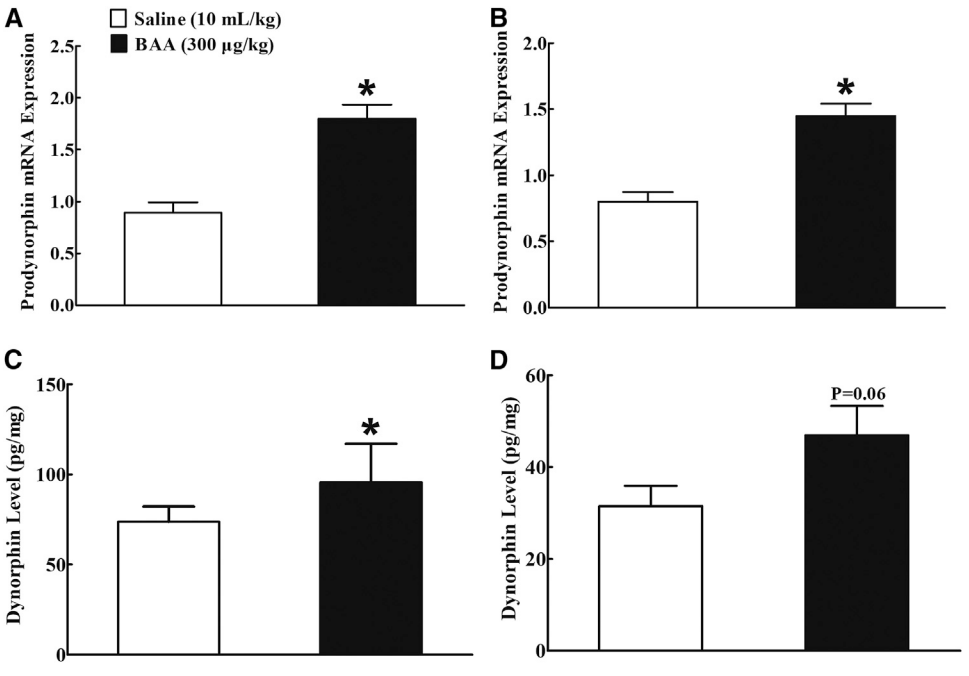

FIGURE 5 | Stimulatory effects of subcutaneous (sc) injection of bulleyaconitine A (BAA, 300 μg/kg) on dynorphin A mRNA **(A,B)** and protein **(C,D)** levels in nucleus accumbens (NAc) and hippocampus in multiple daily morphine (10 mg/kg)-treated mice. Levels of dynorphin A mRNA and protein were measured by using qRT-PCR and fluorescent ELISA kit, respectively. The data are presented as mean ± S.E.M (n = 8–12 per group). $^{*}p < 0.05$, compared to the saline control group, by unpaired and two-tailed Student t-test.

by unpaired and two-tailed Student t-test; **Figure 5B**). The stimulatory effects of BAA on expression of dynorphin A protein were also measured in NAc and hippocampus in the same mice using the commercial fluorescent ELISA kit. As exhibited, subcutaneous BAA significantly increased dynorphin A expression in NAc (**Figure 5C**) and hippocampus, compared to the saline control group ($p < 0.05$, by unpaired and two-tailed Student t-test; **Figure 5D**).

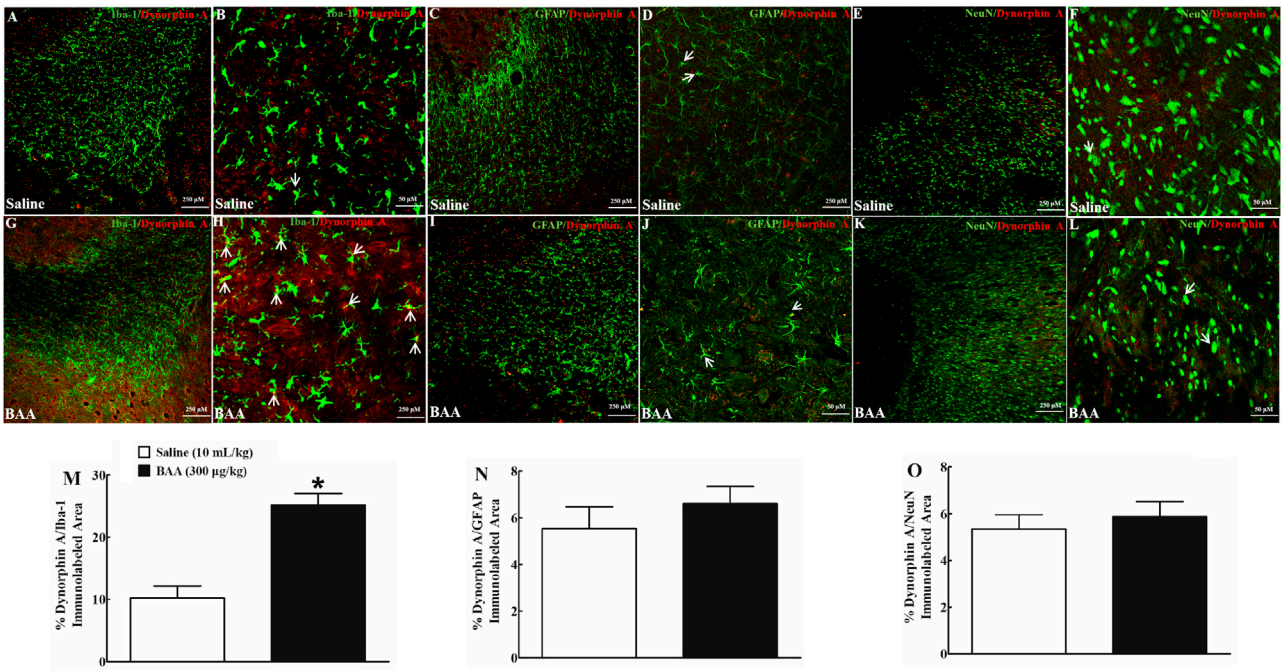

FIGURE 6 | Specific stimulatory effects of subcutaneous (sc) injection of bulleyaconitine A (BAA, 300 μg/kg) on dynorphin A expression in microglia, but not in astrocytes or neurons in the shell of nucleus accumbens (NAcSh) in multiple daily morphine (10 mg/kg)-treated mice. Frozen sections of the NAcSh were obtained 1 h after a subcutaneous injection of saline (10 ml/kg) or BAA (300 μg/kg) and subjected to double immunofluorescence staining with dynorphin A/microglial marker Iba-1 **(A,B,G,H)**, dynorphin A/astrocytic marker GFAP **(C,D,I,J)** and dynorphin A/neuronal marker NeuN **(E,F,K,L)** under 10× magnification (The scale bar, 250 μm) and 30× magnification (The scale bar, 50 μm), respectively. The arrowheads indicate co-localization of dynorphin A with microglia, astrocytes or neurons. The co-localized areas of dynorphin A/Iba-1 (**M**), dynorphin A/GFAP (**N**) and dynorphin A/NeuN (**O**) were quantified at 10× magnification using the ImageJ software. The data are presented as mean ± S.E.M. (n = 6 per group). *$p < 0.05$, compared to the saline control group, by unpaired and two-tailed Student t-test.

Dynorphin A is localized in neurons, astrocytes and microglia in the central nervous system (Wahlert et al., 2013; Ayrout et al., 2019). To verify cell types that specifically upregulate dynorphin A expression in NAcSh and hippocampal CA1 following BAA treatment, dynorphin A was immunofluorescence-labeled with the microglial cellular marker Iba-1, astrocytic cellular marker GFAP, and neuronal cellular marker NeuN. Two groups of mice (n = 6 per group) treated daily with morphine (10 mg/kg) for 5 days received a subcutaneous injection of saline (10 ml/kg) or BAA (300 μg/kg). Mice were then sacrificed 1 h after the subcutaneous injection and NAcSh and hippocampal CA1 were obtained for fluorescence immunostaining. As shown, dynorphin A was co-localized with Iba-1, GFAP and NeuN in NAcSh of saline-treated mice (**Figures 6A–F**). Subcutaneous BAA specifically increased co-labeling of dynorphin A/Iba-1 (**Figures 6G,H**) but not dynorphin A/GFAP (**Figures 6I,J**) or dynorphin A/NeuN (**Figures 6K,L**) at 10× and 30× magnification. Furthermore, the ImageJ software was used to quantify immunofluorescence intensity of dynorphin A with Iba-1, GFAP or NeuN at 10× magnification. Treatment with subcutaneous BAA significantly increased dynorphin A/Iba-1 by 2.4-fold compared to the saline control group ($p < 0.05$, by unpaired and two-tailed Student t-test; **Figure 6M**), but not dynorphin A/GFAP (**Figure 6N**) or dynorphin A/NeuN (**Figure 6O**). In addition, the same specific stimulatory effects of BAA on microglial dynorphin A expression were observed in hippocampal CA1 from the same mice as above (**Figures 7A–L**), with an increase in immunofluorescence intensity of dynorphin A/Iba-1 by 1.9-fold ($p < 0.05$, by unpaired and two-tailed Student t-test; **Figure 7M**), but not dynorphin A/GFAP (**Figure 7N**) or dynorphin A/NeuN (by unpaired and two-tailed Student t-test; **Figure 7O**).

Microglial dynorphin a Expression Mediates Bulleyaconitine A-Inhibited Morphine Dependence

To verify the causal relationship between the microglial expression of dynorphin A in the brain and the BAA-inhibited withdrawal signs in morphine-treated mice, the microglial metabolic inhibitor minocycline (Wu et al., 2002; Neigh et al., 2009; Kobayashi et al., 2013), dynorphin A antiserum (Li et al., 2016a), and KOR antagonist GNTI (Zhang et al., 2007; Liu et al., 2013) were intracerebroventricularly injected individually. Four groups of morphine-treated mice (n = 10 per group) received the first intracerebroventricular injection followed by the second subcutaneous injection of saline (6 μL) + saline (10 ml/kg), minocycline (10 μg) + saline (10 ml/kg), saline (6 μL) + BAA (300 μg/kg), or minocycline (10 μg) + BAA (300 μg/kg). The second subcutaneous injection was delivered 4 h post the first intracerebroventricular injection. Withdrawal signs were precipitated by the intraperitoneal injection of naloxone

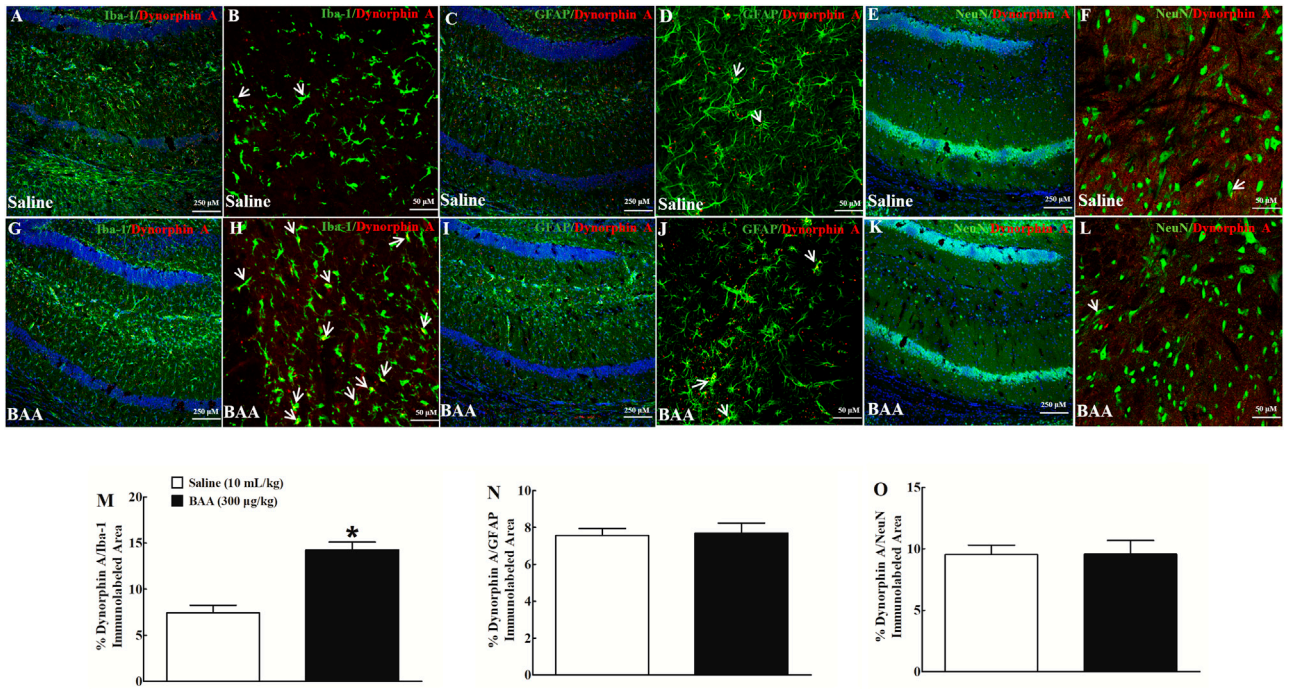

FIGURE 7 | Specific stimulatory effects of subcutaneous (sc) injection of bulleyaconitine A (BAA, 300 μg/kg) on dynorphin A expression in microglia, but not in astrocytes or neurons in hippocampal CA1 in multiple twice-daily morphine (10 mg/kg)-treated mice. Frozen sections of hippocampal CA1 were obtained 1 h after a subcutaneous injection of saline (10 ml/kg) or BAA (300 μg/kg) and then subjected to double immunofluorescence staining with dynorphin A/microglial marker Iba-1 **(A,B,G,H)**, dynorphin A/astrocytic marker GFAP **(C,D,I,J)**, and dynorphin A/neuronal marker NeuN (**E,F,K,L**) under 10× magnification (The scale bar, 250 μm, DAPI was also co-labeled with the nucleus in blue) and 30× magnification (The scale bar, 50 μm), respectively. The arrowheads indicate co-localization of dynorphin A with microglia, astrocytes or neurons. The co-localized areas of dynorphin A/Iba-1 (**M**), dynorphin A/GFAP (**N**), and dynorphin A/NeuN (**O**) were quantified at 10× magnification using the ImageJ software. The data are presented as mean ± S.E.M. (n = 6 per group). *$p < 0.05$, compared to the saline control group, by unpaired and two-tailed Student t-test.

(5 mg/kg) 40 min after the subcutaneous injection. As shown in **Figures 8A–E**, subcutaneous BAA injection into morphine-treated mice significantly attenuated naloxone-induced withdrawal signs, including shakes, jumps, genital licks, fecal excretions, and body weight loss, whereas the intracerebroventricular minocycline failed to influence naloxone-induced morphine withdrawal responses in mice. However, pretreatment with intracerebroventricular minocycline nearly completely restored the systemic BAA-suppressed withdrawal symptoms.

Additional four groups of morphine-treated mice (n = 10 per group) received the first intracerebroventricular injection followed by the second subcutaneous injection of saline (6 μL) + saline (10 ml/kg), dynorphin A antiserum (1:30 dilution, 6 μL) + saline (10 ml/kg), saline (6 μL) + BAA (300 μg/kg), or dynorphin A antiserum (1:30 dilution, 6 μL) + BAA (300 μg/kg). The second subcutaneous injection was 30 min post the first intracerebroventricular injection. Withdrawal symptoms were precipitated by intraperitoneal injection of naloxone (5 mg/kg) 40 min after the subcutaneous injection. Subcutaneous BAA injection into morphine-treated mice attenuated naloxone-induced withdrawal signs. Intracerebroventricular injection of the dynorphin A antiserum did not significantly affect baseline morphine withdrawal symptoms, but reemerged naloxone-induced withdrawal symptoms from BAA inhibition (**Figures 8F–J**).

Further four groups of morphine-treated mice (n = 10 per group) received the same treatments as above except that the dynorphin A antiserum was replaced with GNTI (5 μg). As shown in **Figures 8K–O**, intracerebroventricular GNTI injection predominantly restored BAA-suppressed withdrawal symptoms in morphine-treated mice, although it did not significantly alter naloxone-induced withdrawal signs in saline-treated morphine-treated mice.

Microglial Dynorphin A Expression Mediates Bulleyaconitine A-Inhibited Conditioned Place Preference Expression

Minocycline, dynorphin A antiserum, and GNTI, given intracerebroventricularly, were applied to further determine whether microglial expression of dynorphin A in the brain contributed to BAA-inhibited morphine-induced CPP expression. Four groups of morphine-treated mice (n = 10 per group) were first intracerebroventricularly injected followed 4 h later by subcutaneously injected with saline (6 μL) + saline (10 ml/kg), minocycline (10 μg) + saline (10 ml/kg), saline (6 μL) + BAA (300 μg/kg), or minocycline (10 μg) + BAA (300 μg/kg). The place preference test was assessed 50 min subsequent to the subcutaneous injection. As shown in **Figure 9A**, subcutaneous

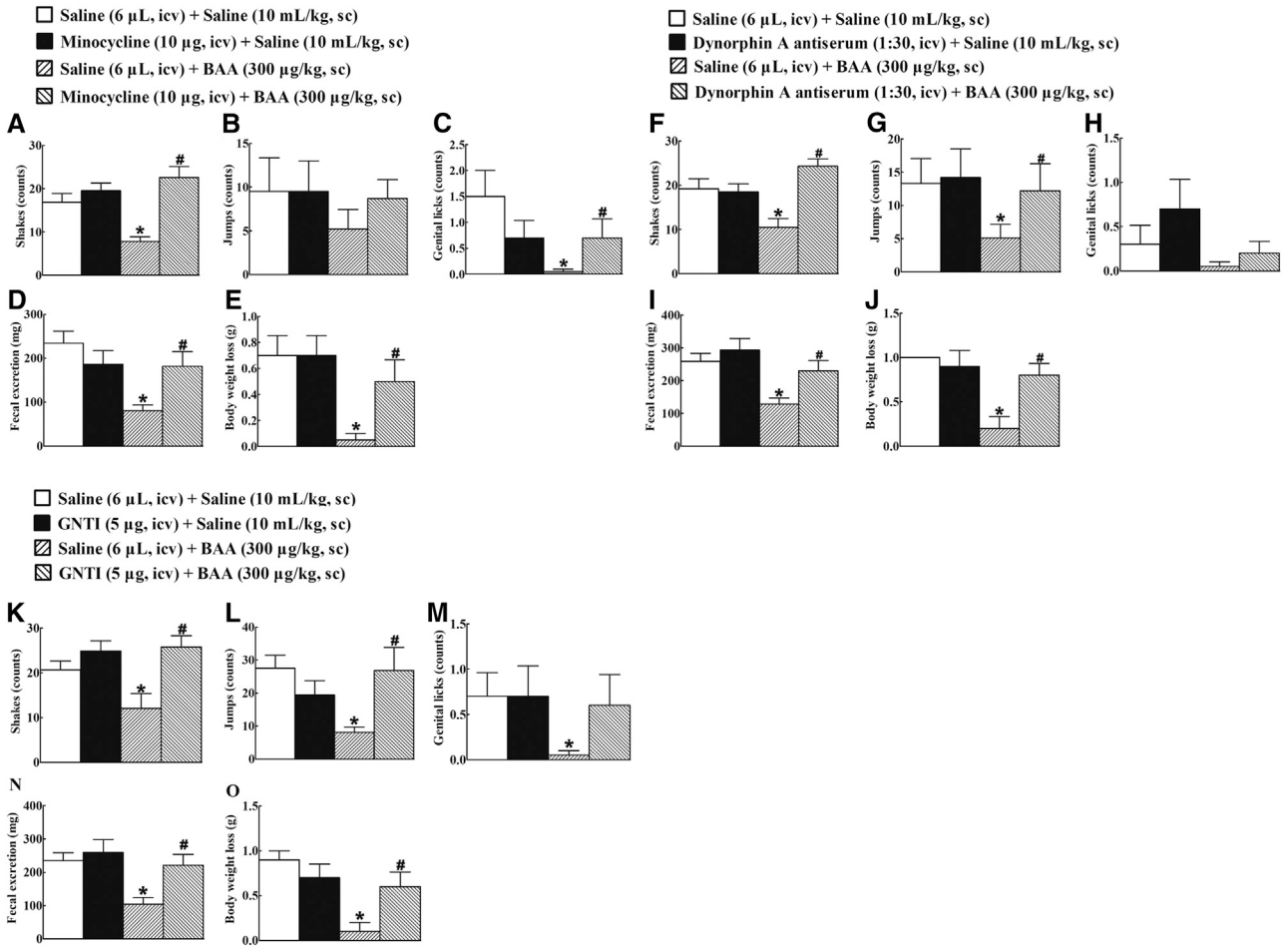

FIGURE 8 | Blockade effects of intracerebroventricular (icv) injection of the microglial metabolic inhibitor minocycline **(A–E)**, specific dynorphin A antiserum **(F–J)**, and selective KOR antagonist GNTI (**K-O**) on subcutaneous (sc) injection of bulleyaconitine A (BAA)-attenuated withdrawal signs in morphine-treated mice. Naloxone-induced withdrawal symptoms included shakes **(A,F,K)**, jumps **(B,G,L)**, genital licks **(C,H,M)**, fecal excretion **(D,I,N)**, and body weight loss **(E,J,O)**. The data are presented as means ± S.E.M. (n = 10 per group). $^{*,\#}p < 0.05$, compared to the saline control group and BAA group, respectively, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test.

BAA injection but not intracerebroventricular minocycline completely attenuated morphine-induced CPP expression. However, pretreatment with intracerebroventricular minocycline entirely restored BAA-suppressed CPP expression [F (3, 36) = 3.829, $p < 0.05$, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test].

Furthermore, four groups of morphine CPP mice (n = 10 per group) were first intracerebroventricularly injected 30 min later followed by subcutaneously injected with saline (6 μL) + saline (10 ml/kg), dynorphin A (1:30 dilution, 10 μL) + saline (10 ml/kg), saline (6 μL) + BAA (300 μg/kg), or dynorphin A (1:30 dilution, 10 μL) + BAA (300 μg/kg). The place preference test was assessed 50 min subsequent to the subcutaneous injection. Subcutaneous BAA injection but not intracerebroventricular dynorphin A antiserum totally inhibited expression of morphine-induced CPP. However, pretreatment with intracerebroventricular injection of dynorphin A antiserum completely attenuated BAA-suppressed morphine-induced CPP expression [F (3, 36) = 4.459, $p < 0.05$, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test; **Figure 9B**].

In addition, other four groups of morphine CPP mice (n = 10 per group) received the same treatment regimen as above except that the dynorphin A antiserum was replaced with GNTI (5 μg). As shown in **Figure 9C**, intracerebroventricular GNTI injection did not have any significantly inhibitory effects on morphine-induced CPP expression, but almost or totally restored the systemic BAA-suppressed withdrawal signs in morphine-treated mice (F (3, 36) = 1.330, $p = 0.09$, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test; **Figure 9C**).

DISCUSSION

Long-term use of morphine and other opioids induces addiction, including both physical and psychological dependences. In the present study, withdrawal symptoms were developed after bi-daily

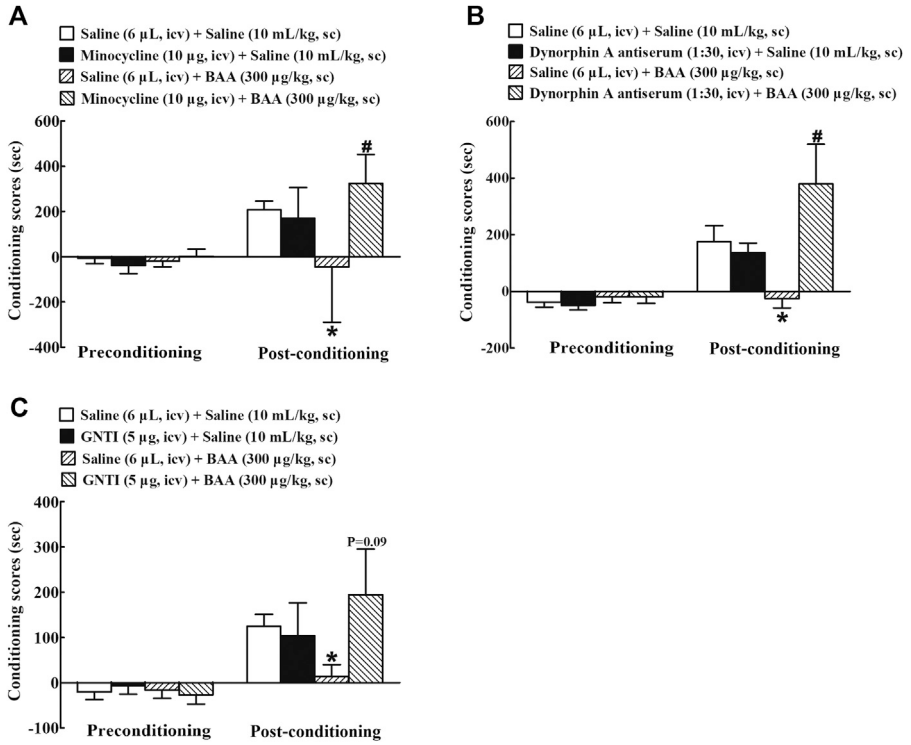

FIGURE 9 | Blockade effects of intracerebroventricular (icv) injection of the microglial metabolic inhibitor minocycline **(A)**, specific dynorphin A antiserum **(B)**, and selective KOR antagonist GNTI **(C)** on a subcutaneous (sc) injection of bulleyaconitine A (BAA)-attenuated morphine conditioned place preference (CPP) expression in mice. The data are presented as means ± S.E.M. (n = 10 per group). $^{*,\ \#}p < 0.05$, compared to the saline control group and BAA group, respectively, by one-way ANOVA followed by the post-hoc Student-Newman-Keuls test.

subcutaneous morphine injections into mice for 7 consecutive days, which was reflected by withdrawal signs (i.e., shakes, jumps, genital licks, fecal excretion, and body weight loss) following application of naloxone. In contrast, bi-daily subcutaneous BAA injections with a dose up to 300 μg/kg/day for 7 days did not induce any withdrawal symptoms, which is consistent with the previous finding in which daily subcutaneous BAA did not induce jumping responses following nalorphine challenge (Tang et al., 1986). Furthermore, a single subcutaneous BAA injection alleviated naloxone-induced withdrawal signs in morphine-treated mice. In a dose ranging between 30 and 300 μg/kg, BAA injection into morphine-treated mice caused a dose-related inhibition of abrupt withdrawal symptoms, typically shakes and body weight loss with ED_{50} values of 74.4 and 105.8 μg/kg respectively. Consistently, BAA analog lappaconitine was reported to alleviate morphine and cocaine physical dependence (Qu and Qu, 1994). On the other respect, a daily subcutaneous morphine injection but not BAA (300 μg/kg/day) for 5 consecutive days acquired remarkable CPP response with high conditioning scores. Co-administration of BAA significantly inhibited morphine-induced CPP acquisition during the conditioning phase. In addition, single subcutaneous BAA injection entirely abolished morphine-induced CPP expression in the post-conditioning phase. Furthermore, co-treatment with BAA entirely attenuated development of morphine-induced locomotor sensitization. All these results suggest that BAA does not induce physical or psychological dependence, but markedly alleviates morphine-induced withdrawal symptoms, CPP acquisition and expression, and locomotor sensitization. However, the hypothesis may be compromised because we just used male mice in this study and previous studies revealed that degrees of morphine-induced physical and psychological dependences varied with gender (Cicero et al., 2002; Mohammadian and Miladi-Gorji, 2019). Thus, future studies are needed to evaluate the anti-addictive effects of BAA in female animals.

Opiates, such as morphine and heroin, act at the mesolimbic dopamine pathway projecting from the ventral tegmental (VTA) to NAc (Patyal et al., 2012). Opiates drugs effectively stimulate dopamine release in the NAc within 1 h after intracerebroventricular injection or local administration into VTA (Murphy et al., 1996; Sebastian et al., 2016). Hippocampal input to NAcSh is important in rewarding behaviors (LeGates et al., 2018). Thus, it can be speculated that NAc and hippocampus, located around the cerebroventricular area, are important sites for development of drug addiction. Dynorphin A regulates the activity of dopamine neurons by acting on KORs in mesolimbic, NAcSh, prefrontal cortex and VTA that have been implicated in drug abuse liability (Meshul and McGinty, 2000; Volkow et al., 2009). In the current study, we explored whether the dynorphin A/KOR system in NAc and hippocampus was closely associated with BAA-attenuated naloxone-induced morphine withdrawal symptoms and CPP expression. Subcutaneous BAA injection into

morphine-treated mice stimulated expression of dynorphin A in NAc and hippocampus at 1 h after injection, which was in agreement with the time-course of its anti-addictive effects. The results are parallel to the previous findings in which intrathecal and subcutaneous injection of BAA, bullatine A, and lappaconitine stimulated expression of dynorphin but not β-endorphin in the spinal cord (Li et al., 2016a; Li et al., 2016b).

The notion is further supported by the following interventional injections through the intracerebroventricular route, which is localized around NAc and hippocampus. It was previously reported that a single intravenous dynorphin A injection attenuated withdrawal symptoms of morphine dependence (Takemori et al., 1993). The present study demonstrated that intracerebroventricular dynorphin A antiserum injection totally eliminated systemic BAA-inhibited morphine withdrawal symptoms and CPP expression, although the injection volume more than 2 μL could affect the behaviors of the animals and might be a limitation. Moreover, the highly selective KOR antagonist GNTI, given intracerebroventricularly, also entirely eliminated the systemic BAA-inhibited morphine-induced withdrawal symptoms and CPP expression. These data are consistent with the previous studies showing that the KOR agonist salvinorin A punished self-administration of cocaine and remifentanil in monkeys (Freeman et al., 2014), and that addition of the KOR agonist U69,593 to fentanyl produced a proportion-dependent decrease in fentanyl self-administration in rats (Negus et al., 2008).

Drug abuse activates microglia to produce a large amount of inflammatory factors and affect synapse reconstruction, chemical changes in the neural signal transduction and phagocytosis of apoptotic neurons, and ultimately to regulate the dopamine reward-signaling pathway and enhance drug dependence and addiction (Kovacs, 2012; Garaschuk and Verkhratsky, 2019). However, recent studies also showed that microglia had an alternative activation or protective state to activate the anti-inflammatory cascades and exhibited neuroprotection and antinociception (Hu et al., 2012; Fan et al., 2015; Wu et al., 2017; Wu et al., 2018). Our present data provide additional evidence showing that BAA stimulated microglia to express dynorphin A for attenuation of the drug dependence and addiction. Subcutaneous BAA injection stimulated dynorphin A expression only in microglia and not in astrocytes and neurons in NAcSh and hippocampal CA1, similar to the previous findings in which injection of a BAA analog bullatine A specifically stimulated microglial (but not astrocytic or neural) expression of dynorphin A in the spinal cords of neuropathic rats (Huang et al., 2016). We further demonstrated that intracerebroventricular injection of the microglial metabolic inhibitor minocycline entirely blocked the systemic BAA-inhibited morphine-induced withdrawal symptoms and CPP expression. Consistently, the antinociceptive effects of BAA and its analogs bullatine A and lappaconitine were also blocked by the intrathecal injection of minocycline in the rat models of pain hypersensitivity (Huang et al., 2016; Sun et al., 2018; Huang et al., 2020b). These results highlight that stimulation of microglia expression and release of dynorphin A, in contrast to proinflammatory cytokines and neurotrophins, inhibited morphine-induced withdrawal symptoms and CPP expression as well as blocked pain hypersensitivity.

The main treatment option of the opioid addiction is detoxification and relieves withdrawal symptoms; for this purpose, methadone substitution is most commonly used. This treatment, however, is just provided to inpatients in well-equipped management institutions due to its own physical dependence and other adverse reactions (Kampman and Jarvis, 2015; Tran et al., 2017). On the other respect, psychotherapy of addiction should be emphasized conceptually. Only few patients are unfortunately willing to undergo drug addiction program, while many patients are still carving for addictive substances after improving withdrawal symptoms, which eventually leads to relapse (Arevalo et al., 2008). Thus, approaches at the current time to solve opioid addiction, especially psychological dependence, are limited and treatment strategy with novel mechanisms of actions is therefore, urgently needed. Our current study did provide a solid pharmacological base for BAA to alleviate morphine-induced withdrawal symptoms, CPP acquisition and expression, and locomotor sensitization in mice through the mechanism of microglial expression of dynorphin A. Moreover, oral administration of lappaconitine over 6 days reduced or eliminated withdrawal symptoms, like yawing, shedding tears, chilliness, mydriasis and restlessness in drug addict patients whose duration of heroin or opium addiction ranged from 1 to 4 years (Qu and Qu, 1994). Taken together, all these findings suggest that BAA is a promising clinical development candidate for treatment of opioid addiction, especially the psychological dependence. The data also indicate that targeting of microglial expression and secretion of dynorphin A is a potentially novel strategy in treatment of opioid drug addiction.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care and Welfare Committee of Shanghai Jiao Tong University (Shanghai, China).

AUTHOR CONTRIBUTIONS

YW and MZ conceived and designed the experiments; MW, LM and KA performed the experiments; MZ, LM and YW analyzed the data; and YW and MZ prepared the manuscript. All authors read and approved the final version of this manuscript.

FUNDING

This study was supported in part by a grant from the National Natural Science Foundation of China (#81673403).

REFERENCES

Aguilar, M. A., Rodríguez-Arias, M., and Miñarro, J. (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res. Rev.* 59, 253–277. doi:10.1016/j.brainresrev.2008.08.002

Arévalo, S., Prado, G., and Amaro, H. (2008). Spirituality, sense of coherence, and coping responses in women receiving treatment for alcohol and drug addiction. *Eval. Progr. Plann.* 31, 113–123. doi:10.1016/j.evalprogplan.2007.05.009

Ayrout, M., Le Billan, F., Grange-Messent, V., Mhaouty-Kodja, S., Lombès, M., and Chauvin, S. (2019). Glucocorticoids stimulate hypothalamic dynorphin expression accounting for stress-induced impairment of GnRH secretion during preovulatory period. *Psychoneuroendocrinology* 99, 47–56. doi:10.1016/j.psyneuen.2018.08.034

Beardsley, P. M., Howard, J. L., Shelton, K. L., and Carroll, F. I. (2005). Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology (Berlin)* 183, 118–126. doi:10.1007/s00213-005-0167-4

Bello-Ramírez, A. M., and Nava-Ocampo, A. A. (2004). A QSAR analysis of toxicity of Aconitum alkaloids. *Fundam. Clin. Pharmacol.* 18, 699–704. doi:10.1111/j.1472-8206.2004.00280.x

Bobzean, S. A. M., Kokane, S. S., Butler, B. D., and Perrotti, L. I. (2019). Sex differences in the expression of morphine withdrawal symptoms and associated activity in the tail of the ventral tegmental area. *Neurosci. Lett.* 705, 124–130. doi:10.1016/j.neulet.2019.04.057

Bodnar, R. J. (2010). Endogenous opiates and behavior: 2009. *Peptides* 31, 2325–2359. doi:10.1016/j.peptides.2010.09.016

Bruchas, M. R., Land, B. B., and Chavkin, C. (2010). The dynorphin/kappa opioid system as a modulator of stress-induced and pro-addictive behaviors. *Brain Res.* 1314, 44–55. doi:10.1016/j.brainres.2009.08.062

Carey, A. N., Borozny, K., Aldrich, J. V., and Mclaughlin, J. P. (2007). Reinstatement of cocaine place-conditioning prevented by the peptide kappa-opioid receptor antagonist arodyn. *Eur. J. Pharmacol.* 569, 84–89. doi:10.1016/j.ejphar.2007.05.007

Chartoff, E. H., Potter, D., Damez-Werno, D., Cohen, B. M., and Carlezon, W. A., Jr (2008). Exposure to the selective kappa-opioid receptor agonist salvinorin A modulates the behavioral and molecular effects of cocaine in rats. *Neuropsychopharmacology* 33, 2676–2687. doi:10.1038/sj.npp.1301659

Cicero, T. J., Nock, B., and Meyer, E. R. (2002). Gender-linked differences in the expression of physical dependence in the rat. *Pharmacol. Biochem. Behav.* 72, 691–697. doi:10.1016/s0091-3057(02)00740-2

Cordonnier, L., Sanchez, M., Roques, B. P., and Noble, F. (2007). Blockade of morphine-induced behavioral sensitization by a combination of amisulpride and RB101, comparison with classical opioid maintenance treatments. *Br. J. Pharmacol.* 151, 94–102. doi:10.1038/sj.bjp.0707195

Ezard, N., Cecilio, M. E., Clifford, B., Baldry, E., Burns, L., Day, C. A., et al. (2018). A managed alcohol program in Sydney, Australia: acceptability, cost-savings and non-beverage alcohol use. *Drug Alcohol Rev.* 37 (Suppl 1), S184–S194. doi:10.1111/dar.12702

Fallon, J. H., and Leslie, F. M. (1986). Distribution of dynorphin and enkephalin peptides in the rat brain. *J. Comp. Neurol.* 249, 293–336. doi:10.1002/cne.902490302

Fan, H., Li, T. F., Gong, N., and Wang, Y. X. (2015). Shanzhiside methylester, the principle effective iridoid glycoside from the analgesic herb Lamiophlomis rotata, reduces neuropathic pain by stimulating spinal microglial β-endorphin expression. *Neuropharmacology* 101, 98–109. doi:10.1016/j.neuropharm.2015.09.010

Freeman, K. B., Naylor, J. E., Prisinzano, T. E., and Woolverton, W. L. (2014). Assessment of the kappa opioid agonist, salvinorin A, as a punisher of drug self-administration in monkeys. *Psychopharmacology (Berlin)* 231, 2751–2758. doi:10.1007/s00213-014-3436-2

Garaschuk, O., and Verkhratsky, A. (2019). Physiology of microglia. *Meth. Mol. Biol.* 2034, 27–40. doi:10.1007/978-1-4939-9658-2_3

Glascock, J. J., Osman, E. Y., Coady, T. H., Rose, F. F., Shababi, M., and Lorson, C. L. (2011). Delivery of therapeutic agents through intracerebroventricular (ICV) and intravenous (IV) injection in mice. *J. Vis. Exp.* 2011 (56), 2968. doi:10.3791/2968

Goeldner, C., Lutz, P. E., Darcq, E., Halter, T., Clesse, D., Ouagazzal, A. M., et al. (2011). Impaired emotional-like behavior and serotonergic function during protracted abstinence from chronic morphine. *Biol. Psychiatr.* 69, 236–244. doi:10.1016/j.biopsych.2010.08.021

Gouveia, K., and Hurst, J. L. (2017). Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Sci. Rep.* 7, 44999. doi:10.1038/srep44999

Hooke, L. P., He, L., and Lee, N. M. (1995). Dynorphin A modulates acute and chronic opioid effects. *J. Pharmacol. Exp. Therapeut.* 273, 292–297.

Hu, X., Li, P., Guo, Y., Wang, H., Leak, R. K., Chen, S., et al. (2012). Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke* 43, 3063–3070. doi:10.1161/STROKEAHA.112.659656

Huang, Q., Mao, X. F., Wu, H. Y., Li, T. F., Sun, M. L., Liu, H., et al. (2016). Bullatine A stimulates spinal microglial dynorphin A expression to produce anti-hypersensitivity in a variety of rat pain models. *J. Neuroinflammation* 13, 214. doi:10.1186/s12974-016-0696-2

Huang, Q., Mao, X. F., Wu, H. Y., Liu, H., Sun, M. L., Wang, X., et al. (2017b). Cynandione A attenuates neuropathic pain through p38β MAPK-mediated spinal microglial expression of β-endorphin. *Brain Behav. Immun.* 62, 64–77. doi:10.1016/j.bbi.2017.02.005

Huang, Q., Sun, M. L., Chen, Y., Li, X. Y., and Wang, Y. X. (2017a). Concurrent bullatine A enhances morphine antinociception and inhibits morphine antinociceptive tolerance by indirect activation of spinal κ-opioid receptors. *J. Ethnopharmacol.* 196, 151–159. doi:10.1016/j.jep.2016.12.027

Huang, S. N., Wei, J., Huang, L. T., Ju, P. J., Chen, J., and Wang, Y. X. (2020a). Bulleyaconitine A inhibits visceral nociception and spinal synaptic plasticity through stimulation of microglial release of dynorphin A. *Neural Plast.* 2020, 1484087. doi:10.1155/2020/1484087

Huang, S. N., Yang, B., Ma, L., Huang, L. T., Ju, P. J., Wei, J., et al. (2020b). Bulleyaconitine A exerts antianxiety and antivisceral hypersensitivity effects. *Front. Pharmacol.* 11, 328. doi:10.3389/fphar.2020.00328

Kampman, K., and Jarvis, M. (2015). American society of addiction medicine (ASAM) national practice guideline for the use of medications in the treatment of addiction involving opioid use. *J. Addiction Med.* 9, 358–367. doi:10.1097/ADM.0000000000000166

Khaleghzadeh-Ahangar, H., and Haghparast, A. (2017). Intra-accumbal cannabinoid agonist attenuated reinstatement but not extinction period of morphine-induced conditioned place preference; evidence for different characteristics of extinction period and reinstatement. *Neurochem. Res.* 42, 3321–3330. doi:10.1007/s11064-017-2374-x

Khaleghzadeh-Ahangar, H., and Haghparast, A. (2015). Intra-accumbal CB1 receptor blockade reduced extinction and reinstatement of morphine. *Physiol. Behav.* 149, 212–219. doi:10.1016/j.physbeh.2015.06.005

Kim, D. H., Jung, J. S., Song, D. K., Suh, H. W., Huh, S. O., and Kim, Y. H. (1998). Intracerebroventricular injection-induced increase in plasma corticosterone levels in the mouse: a stress model. *J. Pharmacol. Toxicol. Methods* 39, 71–73. doi:10.1016/s1056-8719(97)00105-6

Kim, H. Y., Lee, D. K., Chung, B. R., Kim, H. V., and Kim, Y. (2016). Intracerebroventricular injection of amyloid-β peptides in normal mice to acutely induce alzheimer-like cognitive deficits. *J. Vis. Exp.* 2016 (109), 53308. doi:10.3791/53308

Kobayashi, K., Imagama, S., Ohgomori, T., Hirano, K., Uchimura, K., Sakamoto, K., et al. (2013). Minocycline selectively inhibits M1 polarization of microglia. *Cell Death Dis.* 4, e525. doi:10.1038/cddis.2013.54

Kovacs, K. J. (2012). Microglia and drug-induced plasticity in reward-related neuronal circuits. *Front. Mol. Neurosci.* 5, 74. doi:10.3389/fnmol.2012.00074

LeGates, T. A., Kvarta, M. D., Tooley, J. R., Francis, T. C., Lobo, M. K., Creed, M. C., et al. (2018). Reward behaviour is regulated by the strength of hippocampus-nucleus accumbens synapses. *Nature* 564, 258–262. doi:10.1038/s41586-018-0740-8

Leitermann, R. J., Terashvili, M., Mizoguchi, H., Wu, H. E., Chen, F., Clithero, A., et al. (2004). Increased release of immunoreactive dynorphin A1-17 from the spinal cord after intrathecal treatment with endomorphin-2 in anesthetized rats. *Eur. J. Pharmacol.* 504, 177–183. doi:10.1016/j.ejphar.2004.10.006

Lenard, N. R., and Roerig, S. C. (2005). Development of antinociceptive tolerance and physical dependence following morphine i.c.v. infusion in mice. *Eur. J. Pharmacol.* 527, 71–76. doi:10.1016/j.ejphar.2005.10.031

Li, T. F., Fan, H., and Wang, Y. X. (2016a). Aconitum-derived bulleyaconitine A exhibits antihypersensitivity through direct stimulating dynorphin A expression in spinal microglia. *J. Pain* 17, 530–548. doi:10.1016/j.jpain.2015.12.015

Li, T. F., Gong, N., and Wang, Y. X. (2016b). Ester hydrolysis differentially reduces aconitine-induced anti-hypersensitivity and acute neurotoxicity: involvement of spinal microglial dynorphin expression and implications for aconitum processing. *Front. Pharmacol.* 7, 367. doi:10.3389/fphar.2016.00367

Li, T. F., Wu, H. Y., Wang, Y. R., Li, X. Y., and Wang, Y. X. (2017). Molecular signaling underlying bulleyaconitine A (BAA)-induced microglial expression of prodynorphin. *Sci. Rep.* 7, 45056. doi:10.1038/srep45056

Liu, R., Huang, X-P., Yeliseev, A., Xi, J., and Roth, B. (2013). Novel molecular targets of dezocine and their clinical implications. *Anesthesiology* 120 (3), 714–723. doi:10.1097/ALN.0000000000000076

Loh, R., Chau, L., Aijaz, A., Wu, K., and Galvez, R. (2017). Antagonizing the different stages of kappa opioid receptor activation selectively and independently attenuates acquisition and consolidation of associative memories. *Behav. Brain Res.* 323, 1–10. doi:10.1016/j.bbr.2017.01.032

Maisonneuve, I. M., Archer, S., and Glick, S. D. (1994). U50,488, a kappa opioid receptor agonist, attenuates cocaine-induced increases in extracellular dopamine in the nucleus accumbens of rats. *Neurosci. Lett.* 181, 57–60. doi:10.1016/0304-3940(94)90559-2

Marszalek-Grabska, M., Gibula-Bruzda, E., Bodzon-Kulakowska, A., Suder, P., Gawel, K., Filarowska, J., et al. (2018). Effects of the positive allosteric modulator of metabotropic glutamate receptor 5, VU-29, on impairment of novel object recognition induced by acute ethanol and ethanol withdrawal in rats. *Neurotox. Res.* 33, 607–620. doi:10.1007/s12640-017-9857-z

Meshul, C. K., and McGinty, J. F. (2000). Kappa opioid receptor immunoreactivity in the nucleus accumbens and caudate-putamen is primarily associated with synaptic vesicles in axons. *Neuroscience* 96, 91–99. doi:10.1016/s0306-4522(99)90481-5

Mohammadian, J., and Miladi-Gorji, H. (2019). Age- and sex-related changes in the severity of physical and psychological dependence in morphine-dependent rats. *Pharmacol. Biochem. Behav.* 187, 172793. doi:10.1016/j.pbb.2019.172793

Murphy, N. P., Ly, H. T., and Maidment, N. T. (1996). Intracerebroventricular orphanin FQ/nociceptin suppresses dopamine release in the nucleus accumbens of anaesthetized rats. *Neuroscience* 75, 1–4. doi:10.1016/0306-4522(96)00322-3

Mysels, D., and Sullivan, M. A. (2009). The kappa-opiate receptor impacts the pathophysiology and behavior of substance use. *Am. J. Addict.* 18, 272–276. doi:10.1080/10550490902925862

Negus, S. S., Schrode, K., and Stevenson, G. W. (2008). Micro/kappa opioid interactions in rhesus monkeys: implications for analgesia and abuse liability. *Exp. Clin. Psychopharmacol* 16, 386–399. doi:10.1037/a0013088

Neigh, G. N., Karelina, K., Glasper, E. R., Bowers, S. L., Zhang, N., Popovich, P. G., et al. (2009). Anxiety after cardiac arrest/cardiopulmonary resuscitation: exacerbated by stress and prevented by minocycline. *Stroke* 40, 3601–3607. doi:10.1161/STROKEAHA.109.564146

Nestler, E. J., Hope, B. T., and Widnell, K. L. (1993). Drug addiction: a model for the molecular basis of neural plasticity. *Neuron* 11, 995–1006. doi:10.1016/0896-6273(93)90213-b

Nocjar, C., Zhang, J., Feng, P., and Panksepp, J. (2012). The social defeat animal model of depression shows diminished levels of orexin in mesocortical regions of the dopamine system, and of dynorphin and orexin in the hypothalamus. *Neuroscience* 218, 138–153. doi:10.1016/j.neuroscience.2012.05.033

Patyal, R., Woo, E. Y., and Borgland, S. L. (2012). Local hypocretin-1 modulates terminal dopamine concentration in the nucleus accumbens shell. *Front. Behav. Neurosci.* 6, 82. doi:10.3389/fnbeh.2012.00082

Pfeiffer, A., Brantl, V., Herz, A., and Emrich, H. M. (1986). Psychotomimesis mediated by kappa opiate receptors. *Science* 233, 774–776. doi:10.1126/science.3016896

Qi, C., Guo, B., Ren, K., Yao, H., Wang, M., Sun, T., et al. (2018). Chronic inflammatory pain decreases the glutamate vesicles in presynaptic terminals of the nucleus accumbens. *Mol. Pain* 14, 1744806918781259. doi:10.1177/1744806918781259

Qu, Y., and Qu, P. (1994). *Aconitane derivatives used as a medication to treat addiction*. US-5290784-A

Reiss, D., Ceredig, R. A., Secher, T., Boué, J., Barreau, F., Dietrich, G., et al. (2017). Mu and delta opioid receptor knockout mice show increased colonic sensitivity. *Eur. J. Pain* 21, 623–634. doi:10.1002/ejp.965

Robbins, T. W., Ersche, K. D., and Everitt, B. J. (2008). Drug addiction and the memory systems of the brain. *Ann. N. Y. Acad. Sci.* 1141, 1–21. doi:10.1196/annals.1441.020

Ruzza, C., Holanda, V. A., Gavioli, E. C., Trapella, C., and Calo, G. (2019). NOP agonist action of cebranopadol counteracts its liability to promote physical dependence. *Peptides* 112, 101–105. doi:10.1016/j.peptides.2018.12.001

Schwarzer, C. (2009). 30 years of dynorphins--new insights on their functions in neuropsychiatric diseases. *Pharmacol. Ther.* 123, 353–370. doi:10.1016/j.pharmthera.2009.05.006

Sebastian, T., Peters, M. T., Bohrer, K., and Iain, S. (2016). Oxytocin inhibits ethanol consumption and ethanol-induced dopamine release in the nucleus accumbens. *Addiction Biol.* 22 (3), 702–711. doi:10.1111/adb.12362

Shippenberg, T. S., Zapata, A., and Chefer, V. I. (2007). Dynorphin and the pathophysiology of drug addiction. *Pharmacol. Ther.* 116, 306–321. doi:10.1016/j.pharmthera.2007.06.011

Sun, M. L., Ao, J. P., Wang, Y. R., Huang, Q., Li, T. F., Li, X. Y., et al. (2018). Lappaconitine, a C18-diterpenoid alkaloid, exhibits antihypersensitivity in chronic pain through stimulation of spinal dynorphin A expression. *Psychopharmacology (Berlin)* 235, 2559–2571. doi:10.1007/s00213-018-4948-y

Takemori, A. E., Loh, H. H., and Lee, N. M. (1993). Suppression by dynorphin A and [des-Tyr1]dynorphin A peptides of the expression of opiate withdrawal and tolerance in morphine-dependent mice. *J. Pharmacol. Exp. Therapeut.* 266, 121–124.

Tang, X. C., Liu, X. J., Wei-Hua, L. U., Wang, M. D., and Ai-Ling, L. I. (1986). Studies on the analgesic action and physical dependence of bulleyaconitine A. *Acta Pharm. Sin.* 21 (12), 886–891.

Thorn, D. A., Zhang, Y., and Li, J. X. (2016). Effects of the imidazoline I2 receptor agonist 2-BFI on the development of tolerance to and behavioural/physical dependence on morphine in rats. *Br. J. Pharmacol.* 173, 1363–1372. doi:10.1111/bph.13435

Tran, T. H., Griffin, B. L., Stone, R. H., Vest, K. M., and Todd, T. J. (2017). Methadone, buprenorphine, and naltrexone for the treatment of opioid use disorder in pregnant women. *Pharmacotherapy* 37, 824–839. doi:10.1002/phar.1958

Volkow, N. D., Fowler, J. S., Wang, G. J., Baler, R., and Telang, F. (2009). Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology* 56 (Suppl 1), 3–8. doi:10.1016/j.neuropharm.2008.05.022

Wahlert, A., Funkelstein, L., Fitzsimmons, B., Yaksh, T., and Hook, V. (2013). Spinal astrocytes produce and secrete dynorphin neuropeptides. *Neuropeptides* 47, 109–115. doi:10.1016/j.npep.2012.10.006

Wakabayashi, Y., Nakada, T., Murata, K., Ohkura, S., Mogi, K., Navarro, V. M., et al. (2010). Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. *J. Neurosci.* 30, 3124–3132. doi:10.1523/JNEUROSCI.5848-09.2010

Wang, C. F., Gerner, P., Wang, S. Y., and Wang, G. K. (2007). Bulleyaconitine A isolated from aconitum plant displays long-acting local anesthetic properties *in vitro* and *in vivo*. *Anesthesiology* 107, 82–90. doi:10.1097/01.anes.0000267502.18605.ad

Wang, S. C., Chen, Y. C., Lee, C. H., and Cheng, C. M. (2019). Opioid addiction, genetic susceptibility, and medical treatments: a review. *Int. J. Mol. Sci.* 20. doi:10.3390/ijms20174294

Wingo, T., Nesil, T., Choi, J. S., and Li, M. D. (2016). Novelty seeking and drug addiction in humans and animals: from behavior to molecules. *J. Neuroimmune Pharmacol.* 11, 456–470. doi:10.1007/s11481-015-9636-7

Wu, D. C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., et al. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J. Neurosci.* 22, 1763. doi:10.1523/JNEUROSCI.22-05-01763.2002

Wu, H. Y., Mao, X. F., Tang, X. Q., Usman, A., Evhy, A., Hao, L., et al. (2018). Spinal interleukin-10 produces antinociception in neuropathy through microglial β-endorphin expression, separated from antineuroinflammation. *Brain Behav Immun* 73, 504–519. doi:10.1016/j.bbi.2018.06.015

Wu, H. Y., Tang, X. Q., Mao, X. F., and Wang, Y. X. (2017). Autocrine interleukin-10 mediates glucagon-like peptide-1 receptor-induced spinal microglial

β-endorphin expression. *J. Neurosci.* 37, 11701–11714. doi:10.1523/JNEUROSCI.1799-17.2017

Wu, X., Zhao, N., Bai, F., Li, C., Liu, C., Wei, J., et al. (2016). Morphine-induced conditioned place preference in rhesus monkeys: resistance to inactivation of insula and extinction. *Neurobiol. Learn. Mem.* 131, 192–200. doi:10.1016/j.nlm.2016.04.005

Xie, M. X., Zhu, H. Q., Pang, R. P., Wen, B. T., and Liu, X. G. (2018). Mechanisms for therapeutic effect of bulleyaconitine A on chronic pain. *Mol. Pain* 14, 1744806918797243. doi:10.1177/1744806918797243

Yamada, K., Ono, Y., Kubo, K. Y., Yamamoto, T., and Onozuka, M. (2013). Occlusal disharmony transiently impairs learning and memory in the mouse by increasing dynorphin A levels in the amygdala. *Tohoku J. Exp. Med.* 230, 49–57. doi:10.1620/tjem.230.49

Yuferov, V., Fussell, D., LaForge, K. S., Nielsen, D. A., Gordon, D., Ho, A., et al. (2004). Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. *Pharmacogenetics* 14, 793–804. doi:10.1097/00008571-200412000-00002

Zhang, H., Shi, Y. G., Woods, J. H., Watson, S. J., and Ko, M. C. (2007). Central kappa-opioid receptor-mediated antidepressant-like effects of nor-Binaltorphimine: behavioral and BDNF mRNA expression studies. *Eur. J. Pharmacol.* 570, 89–96. doi:10.1016/j.ejphar.2007.05.045

Zhang, J. Y., Gong, N., Huang, J. L., Guo, L. C., and Wang, Y. X. (2013). Gelsemine, a principal alkaloid from *Gelsemium sempervirens* Ait., exhibits potent and specific antinociception in chronic pain by acting at spinal α3 glycine receptors. *Pain* 154, 2452–2462. doi:10.1016/j.pain.2013.07.027

Zhang, Q., Li, J. X., Zheng, J. W., Liu, R. K., and Liang, J. H. (2003). L-type Ca(2+) channel blockers inhibit the development but not the expression of sensitization to morphine in mice. *Eur. J. Pharmacol.* 467, 145–150. doi:10.1016/s0014-2999(03)01567-x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 26 March 2021
doi: 10.3389/fpsyt.2021.638328

Dammarane Sapogenins Improving Simulated Weightlessness-Induced Depressive-Like Behaviors and Cognitive Dysfunction in Rats

Qiong Wang[1,2†], Li Dong[1†], Mengdi Wang[1,3†], Shanguang Chen[4], Shanshan Li[1,3], Yongbing Chen[5], Wenlu He[1], Hong Zhang[3], Yongliang Zhang[4], Alberto Carlos Pires Dias[1,6], Sijin Yang[1*] and Xinmin Liu[1,7*]*

[1] Affiliated (T.C.M) Hospital, Sino-Portugal Traditional Chinese Medicine (TCM) International Cooperation Center, Southwest Medical University, Luzhou, China, [2] Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [3] Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou, China, [4] National Key Laboratory of Human Factors Engineering, China Astronaut Research and Training Center, Beijing, China, [5] Department of General Surgery, Beijing Shijitan Hospital, Capital Medical University, Beijing, China, [6] Department of Biology, University of Minho, Braga, Portugal, [7] Research Center for Pharmacology and Toxicology, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Background: Our studies demonstrated that the space environment has an impact on the brain function of astronauts. Numerous ground-based microgravity and social isolation showed that the space environment can induce brain function damages in humans and animals. Dammarane sapogenins (DS), an active fraction from oriental ginseng, possesses neuropsychic protective effects and has been shown to improve depression and memory. This study aimed to explore the effects and mechanisms of DS in attenuating depressive-like behaviors and cognitive deficiency induced by simulated weightlessness and isolation [hindlimb suspension and isolation (HLSI)] in rats.

Methods: Male rats were orally administered with two different doses of DS (37.5, 75 mg/kg) for 14 days, and huperzine-A (1 mg/kg) served as positive control. Rats were subjected to HLSI for 14 days except the control group during drug administration. The depressive-like behaviors were then evaluated by the open-field test, the novel object recognition test, and the forced swimming test. The spatial memory and working memory were evaluated by the Morris water maze (MWM) test, and the related mechanism was further explored by analyzing the activity of choline acetyltransferase (ChAT), acetylcholinesterase (AChE), and superoxide dismutase (SOD) in the hippocampus of rats.

Results: The results showed that DS treatment significantly reversed the HLSI-induced depressive-like behaviors in the open-field test, the novel object recognition test, and the forced swimming test and improved the HLSI-induced cognitive impairment in the MWM test. Furthermore, after DS treatment, the ChAT and SOD activities of HLSI rats were increased while AChE activity was significantly suppressed.

OPEN ACCESS

Edited by:
Gary T. Hardiman,
Queen's University Belfast,
United Kingdom

Reviewed by:
Cong Lu,
Chinese Academy of Agricultural Sciences (CAAS), China
Saida Haider,
University of Karachi, Pakistan

***Correspondence:**
Xinmin Liu
liuxinmin@hotmail.com
Shanguang Chen
shanguang_chen@126.com
Sijin Yang
ysjimn@sina.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: 06 December 2020
Accepted: 18 February 2021
Published: 26 March 2021

Citation:
Wang Q, Dong L, Wang M, Chen S, Li S, Chen Y, He W, Zhang H, Zhang Y, Pires Dias AC, Yang S and Liu X (2021) Dammarane Sapogenins Improving Simulated Weightlessness-Induced Depressive-Like Behaviors and Cognitive Dysfunction in Rats. Front. Psychiatry 12:638328. doi: 10.3389/fpsyt.2021.638328

Conclusions: These findings clearly demonstrated that DS might exert a significant neuropsychic protective effect induced by spaceflight environment, driven in part by the modulation of cholinergic system and anti-oxidation in the hippocampus.

Keywords: Dammarane sapogenins, simulated weightlessness, depression, cognitive dysfunction, hindlimb suspension and isolation, rats

INTRODUCTION

In long-term space missions, astronauts are exposed to complex and extreme environmental conditions (microgravity, circadian dys-synchrony, isolation, and confinement) which can negatively affect their physiological and psychological state and performance (1). Several studies have confirmed that the microgravity environment is an underlying factor for cognitive disorder. Cognitive impairment caused by weightlessness has also been reported earlier. For example, participants' reaction time of working memory is significantly decreased in head-down bed rest (HDBR) (2). Pavy-Le et al. (3) demonstrated that the balance between functional input and response output is disrupted, and HDBR detrimentally influence cognitive function under prolonged exposure to increased cephalic fluid distribution under simulated weightlessness (4). A human study of simulated weightlessness with 15° head-down tilt and 45° head-up tilt profoundly affects the brain function and mental arithmetic with impairment of memory processes (5). Additionally, simulated weightlessness increased cognitive impairment in rats (6). Effective countermeasures need to be explored regarding the health and performance of astronauts both during in- and post-flight conditions.

There is substantial evidence that Chinese herbs may provide a potential treatment of cognitive impairment induced by microgravity. *Panax ginseng* has been widely used as a tonic for over 2,000 years in China. It contains a diverse group of saponins known as ginsenosides with significant effects on cognition and depression. Dammarane sapogenins (DS), a kind of extracts derived from the *P. ginseng*, contains two main ingredients: 20(s)-protopanaxatriol (PPT) and 20(s)-protopanaxadiol (PPD) with neuroprotective effect (7, 8). In addition, DS caused improvement in cognitive impairment induced by sleep interruption in mice in a dose-dependent manner (9) and also has antidepressant-like properties (10). Head-down bed rest in human and hindlimb suspension (HLS) are popularly used in animals to simulate microgravity on earth. Liu et al. (2) reported that healthy young men ($n = 16$) who participated in the two-back task exhibited significantly lower reaction time, suggesting that the prolonged HDBR may have a detrimental effect on working memory. HLS has been recognized as a cost-effective ground-based rodent model mimicking weightlessness in microgravity research (11), which is acceptable by the National Aeronautics and Space Administration.

In the present study, the effect of DS on improving depressive-like behaviors and cognitive deficits induced by simulated weightlessness and isolation was addressed, as potential candidate for counteracting depression and memory deficiency induced in space environment.

METHODS

Drugs and Reagents

The DS was provided by Panagin Pharmaceuticals Incorporated (Canada, US patent 6888014B2, May 3, 2005, Huang D and Qi DF). It was prepared by alkaline hydrolysis of total ginsenosides derived from the stem and leaf of *Panax ginseng* C. A. Mayer and identified as described in our previous study. In brief, it contained 33% PPT and 16% PPD on the anhydrous basis (12) (**Figure 1**). Huperzine-A tablets were purchased from Shanghai Fudan Forward Pharmaceutical Co. Ltd. The acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and superoxide dismutase (SOD) kits were purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

Apparatus

The computer-aided open-field test, the novel object recognition test, the computer-aided forced swimming test, and the computer-aided Morris water maze apparatus were developed jointly by the China Astronaut Research and Training Center and the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.

Animals

Fifty SPF male Sprague-Dawley rats (weighing 180–200 g) were purchased from Beijing Hua Fukang Biological Polytron Technologies Inc. (Beijing, China). They were housed individually in specific pathogen-free animal house with free access to standard diet and sterilized drinking water. Rats were maintained in a room with temperature of 22–25°C, relative humidity of 50–65%, and a 12-h light-dark cycle (lights on at 08:00 AM). Prior to experiments, rats were acclimatized for 3 consecutive days. All experiments were conducted according to the "Principles of Laboratory Animal Care" (NIH publication No. 86-23, 1996) and P.R. China legislation for the use and care of laboratory animals. The protocols were approved by the committee for the Care and Use of Laboratory Animals of IMPLAD, CAMS & PUMC, China (No. 2016515).

Experimental Design and Treatment

The rats were randomly divided into five groups with 10 rats per group: control, hindlimb suspension and isolation (HLSI), HLSI + huperzine-A 1 mg/kg (positive control), HLSI + DS 37.5 mg/kg, and HLSI + DS 75 mg/kg, with the latter two serving as DS treated groups. Rats of the control group were housed as five per cage, while rats with the HLSI groups were kept alone per cage. All rats were housed in the same environment with a natural light-dark cycle. The open-field test, the novel object recognition

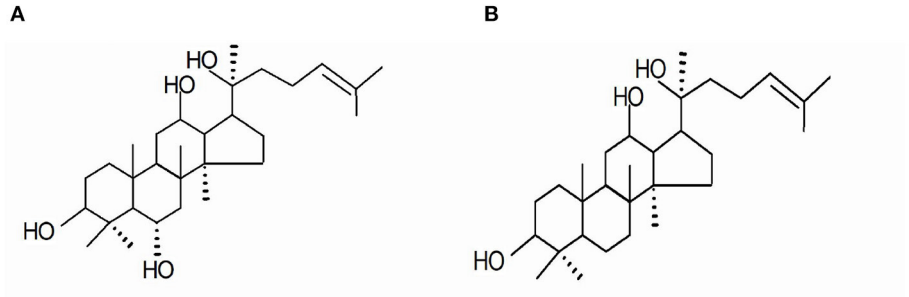

FIGURE 1 | Main components residing in DS include the following: **(A)** 20(S)-protopanaxatrriol (PPT) and **(B)** 20(S)-protopanxadiol (PPD).

FIGURE 2 | Rats in HLSI and the instrument for HLSI.

test, the forced swimming test, and the Morris water maze test were performed after 14 days of HLSI.

DS and huperzine-A were dissolved in distilled water and orally administered at 10 ml/kg to rats in the HLSI + DS group and HLSI + huperzine-A group. The control animals and the HLSI group received distilled water (10 ml/kg). Drugs and water were administered daily to rats. Behavioral test was performed 1 h after drugs or water administration in rats.

Hindlimb Suspension and Isolation Procedures

The hindlimb suspension is a classical method of simulating weightlessness. In order to reduce spontaneous activity and the damage to tail, a simulated weightlessness apparatus was developed (Chinese patent No. 201310228949.2) (**Figure 2**). In brief, rats were placed in a 26 × 26 × 30 cm black plexiglass box with animals isolated from each other. Rat tails were bound using medical adhesive tape and fixed with a small hook in a stainless chain mounted at the top of the cage, allowing free access to water and food. Rats were maintained at an angle of about 30° suspension between the floor and the body of the animal for 14 days, followed by behavior tests. After behavioral tests, rats were maintained HLSI again.

The Open-Field Test

To assess the locomotor activity and the response to a novel environment, rats were placed into an open box (100 cm diameter, 50 cm height) with two 60 lx lighting on the ceiling and allowed to explore freely for 10 min. The computer-aided image analysis system for real-time detection test was employed during the test. After every experiment, the rats were returned to the cage and the open-field boxes were cleaned with 70% alcohol solution and dried. The arena was divided into two areas, the center area (central diameter 40 cm), and the peripheral area (left diameter). The total locomotor activity and the performance in the two arenas (with four paws) were evaluated. The parameters recorded included: total movement distance, average speed, duration of movement, movement distance in the central area, duration of movement in the central area, average speed in the central area, number of rearings during former 5 min (animal's double forelimbs leave the ground at the same time, or the two forelimbs are placed on the wall of the cage) (13).

The Novel Object Recognition Test

After the open-field test, the novel object recognition test (NORT) was performed as described earlier (14). Firstly, the rats were placed in the box for 2 min for adaptation. After acclimatization period, a blue cylindrical object (5 cm × 5 cm in height and diameter) was introduced in the middle of the box. The time rats explored it for the first time was recorded for 10 min representing the exploration latency. The numbers of sniffing the object and the total time of sniffing were recorded as the numbers of exploring and the total time of exploration. The exploratory behavior is defined when the animals' mouth and nose are <1 cm away from the object or directly touching the object, but animals standing on objects are not considered explorations (15).

The Forced Swimming Test

The force swimming test (FST) is a behavioral test used in rodents to assess anti-depressant-like behavior and performed as previously described (10). All experimental animals were first habituated to the testing room 30 min prior to testing. A pre-swimming (10 min) was conducted initially 24 h prior to the experiment. On the test day, all animals were subjected to the plexiglass cylinder (40 cm tall × 20 cm in diameter) filled with water (24°C) to a depth of 18 cm. Test sessions (5 min) were recorded by a video camera positioned directly above the cylinder. Animals were removed from their home cage and placed into the tank for a 5-min test. After drying, they were returned to their home cages. Water was replaced between animal experiments. Behaviors such as immobility, swimming, and climbing in the tank were conducted. Climbing was defined by the rat presenting its forepaws along the edge of the cylinder in an upward movement. Any horizontal movement was classified as swimming. Finally, immobility was defined as no additional movement required by the animal to maintain its head above water (16).

The Morris Water Maze Test

This test was used to evaluate spatial learning ability that relies on distal cues to navigate from start locations around the pool to locate a submerged platform for escaping. The apparatus consisted of a circular pool (160 cm diameter, 50 cm height), filled with 22 ± 2°C water. In order to hide the platform, black ink was added to the water. In the pool, a platform (9 cm diameter) was placed at the center of a quadrant and adjusted 1.5 cm below the water level. Rats would start from two different starting points. It is worth mentioning that the position of the laboratory operator and the cues around the maze remained unchanged throughout the test.

Escape Acquisition (Days 1–5)

Each rat was tested in two independent trials per day for 5 days, each trial with a period of 90 s. Before each training, the rat was left on the platform for 15 s to be familiarized with the environment around the maze. Once the rat climbed the platform, they stayed there for 15 s. After that, it was removed from water, dried, and returned in its cage until the next trial. For each trial, latency to locate the hidden platform and the total swimming distances were measured representing spatial learning performance.

Probe Trial (Day 6)

After 24 h of training, a probe trial was conducted on day 6 without the absence of the platform. During 90 s test, rat was placed into the water in the opposite quadrant and the number of target crossing and the time spent in the target quadrant were recorded by the tracking system to assess the spatial memory ability.

Working Memory (Days 7–10)

After the probe trial test, the working memory evaluation was conducted. The procedure similar to the escape acquisition method was used, but the position of the hidden platform was changed daily. The starting position was randomly changed from trial to trial within a given day. Rats were submitted to two trials per day and were allowed to swim freely to search for the hidden platform for up to 90 s. The experimenters should guide the rat to stay on the platform for 15 s if it failed to find the platform. All rats were tested twice daily for 4 days, and the escape latency and the distance spent in the target quadrant were calculated for the evaluation of the working memory.

Tissue Preparation

After final behavioral tests, rats were anesthetized and killed. The hippocampus tissues were collected rapidly and stored at −80°C until further assays.

Biochemical Assays

In the hippocampus, AChE, ChAT, and SOD activities were measured using commercially available assay kits. Briefly, the hippocampus was homogenized in 10 × saline on ice, centrifuged (3,500 rpm/min) at 4°C for 15 min, and the supernatant was collected. AChE activity was determined using a thiol agent to form trinitrobenzene at 412 nm. ChAT activity was determined by using a commercial kit. All procedures completely complied with the manufacturer's instructions. The mixture was incubated at 37°C for 20 min and boiling water (100°C) was added to stop the reaction. It was centrifuged (4,000 r/m) for 10 min, and the suspension was used for color reaction. The SOD activity was based on its ability to inhibit the oxidation by superoxide anion free radical produced from the xanthine–xanthine oxidase system, measured at 560 nm.

Statistical Analysis

All data analyses were carried out using SPSS (version 19.0). Values are expressed as mean ± standard error of the mean (SEM). Group differences in the escape latency and swimming distance during acquisition of Morris water maze test (MWM) trials were analyzed using repeated-measure two-way ANOVA. Other data were analyzed by one-way ANOVA and the least significant difference required after ANOVA. The value of $p < 0.05$ was considered to be significant.

RESULTS

Body Weight

The body weight in the HLSI group decreased significantly ($p < 0.001$). However, after treatment with DS-1227 75 mg/kg, it was significantly reversed as compared with the control groups (**Figure 3**).

Effect of DS on the Open-Field Test in Simulated Microgravity Induced by HLSI in Rats

In the open-field test (**Figure 4**), rats of the HLSI group covered less total movement distance, accompanied by less duration of movement and slower average speed compared with the control group (**Figures 4A–C**, $p < 0.05$ or $p < 0.01$). The DS treatment at 75 mg/kg reversed HLSI-induced decrease duration of movement. The huperzine-A demonstrated positive effects

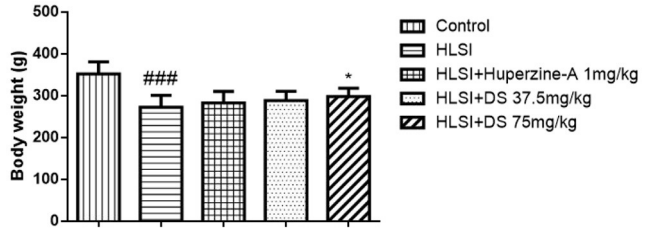

FIGURE 3 | The effects of DS on the body weight after HLSI in rats. All values are means ± SEM ($n = 8$–10). The symbols represent: $^{###}p < 0.0001$ vs. the control group; $^{*}p < 0.05$ vs. the HLSI group.

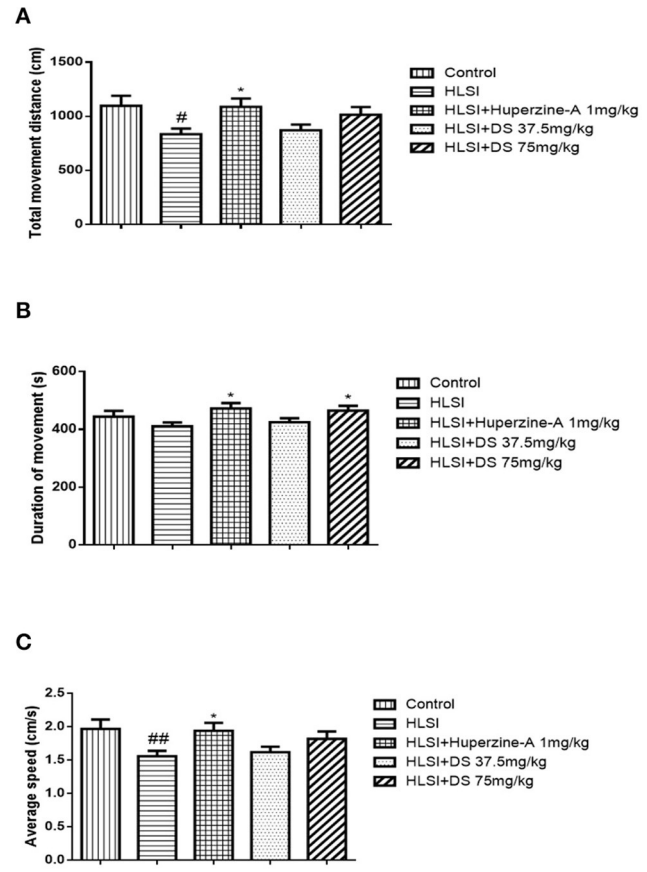

FIGURE 4 | The effects of DS on the locomotor activity in the open-field test after HLSI in rats. The values are mean ± SEM ($n = 8$–9) for the following: **(A)** total movement distance, **(B)** duration of movement, and **(C)** average speed. The symbols represent: $^{#}p < 0.05$ and $^{##}p < 0.01$ as compared with the control group; $^{*}p < 0.05$ as compared with the HLSI group.

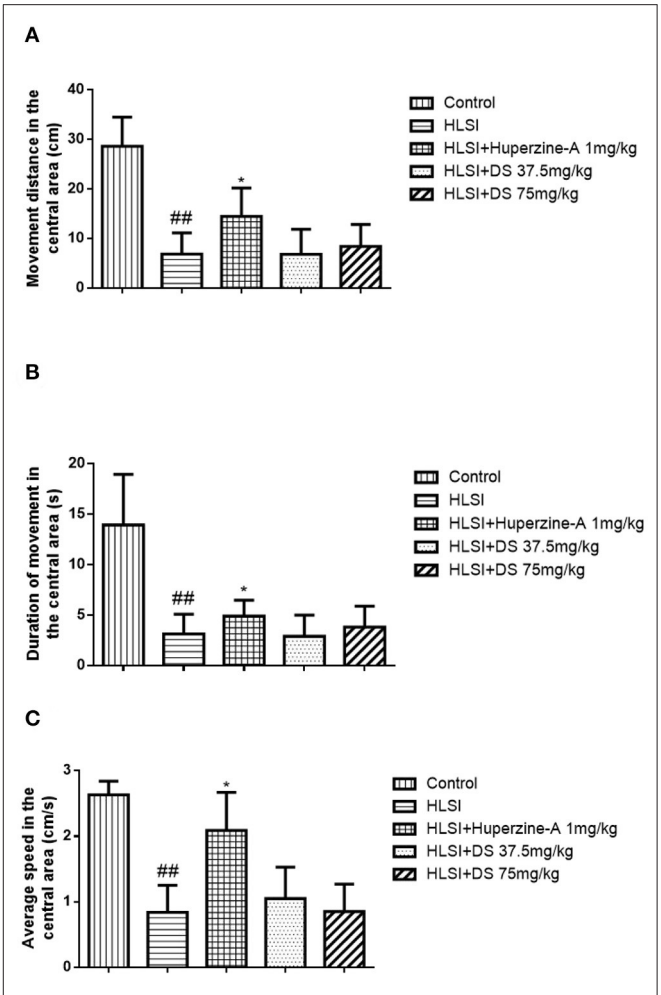

FIGURE 5 | The effects of DS on the locomotor activity in the open-field test after HLSI in rats. The values are mean ± SEM ($n = 8$–9) for the following: **(A)** movement distance in the central area, **(B)** duration of movement in the central area, and **(C)** average speed in the central area. The symbols represent: $^{##}p < 0.01$ as compared with the control group; $^{*}p < 0.05$ as compared with the HLSI group.

on HLSI-induced decrease in the total movement distance, less duration of movement, and slower average speed in rats ($p < 0.05$). In the center area of the open-field test, rats in the HLSI group covered shorter total movement distance, less duration of movement, and with slower average speed compared with the control group ($p < 0.01$) (**Figures 5A–C**). The huperzine-A treatment elicited positive effects by improving the locomotor activity in the HLSI rats in the center area ($p < 0.05$). Whereas, DS treatment showed a weak effect in enhancing the visit of the HLSI rats in the center area. The number of rearings in the HLSI group decreased significantly; however, DS or huperzine-A treatment increased the number of rearings in the open-field test, showing their protective effects on the HLSI rats (**Figure 6**; $p < 0.05$ or $p < 0.01$).

Effect of DS on the Novel Object Recognition Test in Simulated Microgravity Induced by HLSI in Rats

As presented in **Figure 7**, the latency to nose poke increased while it decreased in the HLSI rats compared with the control rats ($p < 0.01$). Both DS or huperzine-A treatment could significantly reversed the HLSI-induced damage to rats ($p < 0.01$).

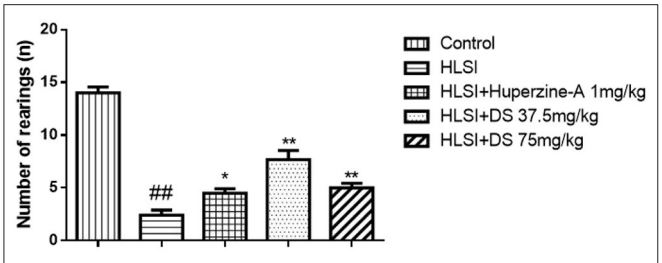

FIGURE 6 | The effects of DS on the number of rearings in the open-field test after HLSI in rats. The values are mean ± SEM ($n = 7$). The symbols represent: $^{\#\#}p < 0.01$ as compared with the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with the HLSI group.

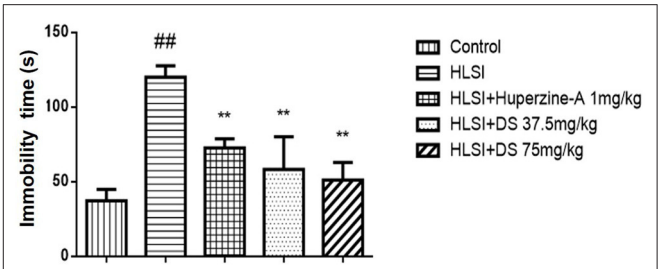

FIGURE 8 | The effects of DS on the immobility time in the forced swimming test after HLSI in rats. The values are mean ± SEM ($n = 8$–9). The symbols represent: $^{\#\#}p < 0.01$ as compared with the control group; $^{**}p < 0.01$ as compared with the HLSI group.

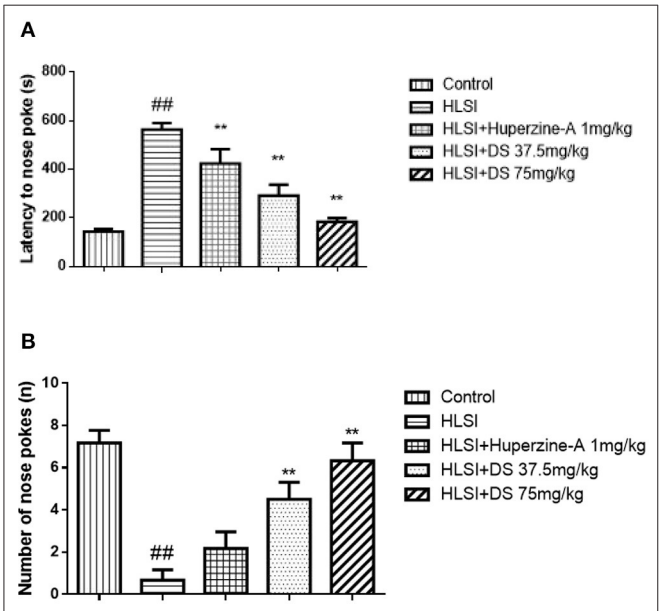

FIGURE 7 | The effects of DS on the novel object recognition test after HLSI in rats. The values are mean ± SEM ($n = 8$–9) for the following: **(A)** latency of nose poke and **(B)** number of nose pokes. The symbols represent: $^{\#\#}p < 0.01$ as compared with the control group; $^{**}p < 0.01$ as compared with the HLSI group.

Effect of DS on the Forced Swimming Test in Simulated Microgravity Induced by HLSI in Rats

The immobility time was increased in the HLSI rats as compared with the control rats ($p < 0.01$); however, DS or huperzine-A treatment significantly reversed the HLSI-induced despair in the force swimming test in rats ($p < 0.01$, **Figure 8**).

Effect of DS on Spatial Learning and Memory in the MWM Test in Simulated Microgravity Induced by HLSI in Rats

In the MWM test, our results revealed significant differences on the swimming distance and escape latency between training days and among groups. The rats in the HLSI group showed significantly higher values regarding the total swimming distance and the escape latency as compared with the control group from days 3 to 5 (**Figures 9A,B**). Administration of DS (37.5 and 75 mg/kg) in rats reversed the HLSI-induced increase in the total swimming distance and the escape latency in the MWM test ($p < 0.05$ or $p < 0.01$). Whereas, administration of huperzine-A (1 mg/kg) in rats induced a significant reduction in the total swimming distance and the escape latency as compared with the HLSI group ($p < 0.05$).

In the probe trial, rats of the HLSI group spent less time and swam the least distances in target quadrant ($p < 0.05$) (**Figures 10A,B**). The DS showed non-significant reversal of this decline. Likewise, non-significant differences in speed and number of target crossings between control and HLSI rats were evident (**Figures 10C,D**). However, the rats after treatment with DS (75 mg/kg) demonstrated a significant rise in the number of target crossing as compared with the HLSI group ($p < 0.05$). Huperzine-A had no effect on either the swimming speed or the number of target crossing in the target quadrant in the probe test.

In the MWM test during 7–10 days, all rats were subjected to the working memory test, to assess working or trial-dependent learning and memory (**Figures 11A,B**). On day 7, DS (75 mg/kg) revealed a shorter distance and time to find the platform in novel position ($p < 0.01$). Likewise, on days 8–10, the latency still showed a shorter total swimming distance and escape latency than that of the HLSI group (day 8, $p < 0.01$; day 9, $p < 0.01$; day 10, $p < 0.01$). However, the total swimming distance and escape latency of the HLSI rats exhibited a tendency toward an increase during the MWM test.

Effect of DS on Acetylcholine-Related Enzymes in Simulated Microgravity Induced by HLSI in Rats

Figures 12A,B depicts the effect of DS on acetylcholine-related enzyme changes on the HLSI-induced cognitive deficit. Results showed that in rat hippocampus, AChE activity ($p < 0.05$) was significantly higher whereas ChAT activity ($p < 0.01$) decreased in the HLSI group compared with the control. After treatment with DS (75 mg/kg) for 2 weeks, AChE activity ($p < 0.01$) was reduced whereas the ChAT activity ($p < 0.05$) elevated

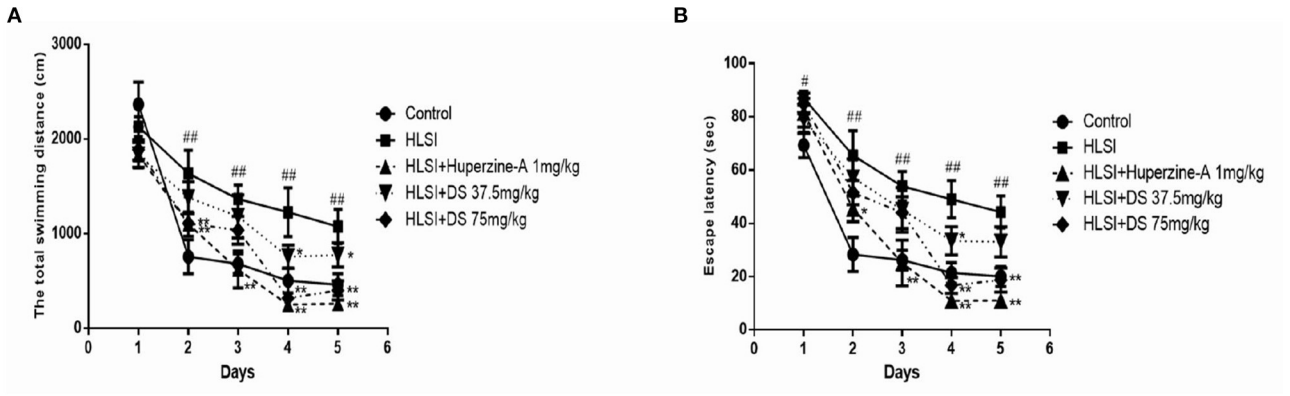

FIGURE 9 | The effects of DS on the spatial memory in the MWM test after HLSI in rats. All values are mean ± SEM ($n = 8$–10) for the following: **(A)** total swimming distance and **(B)** escape latency. The symbols represent: $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the HLSI group.

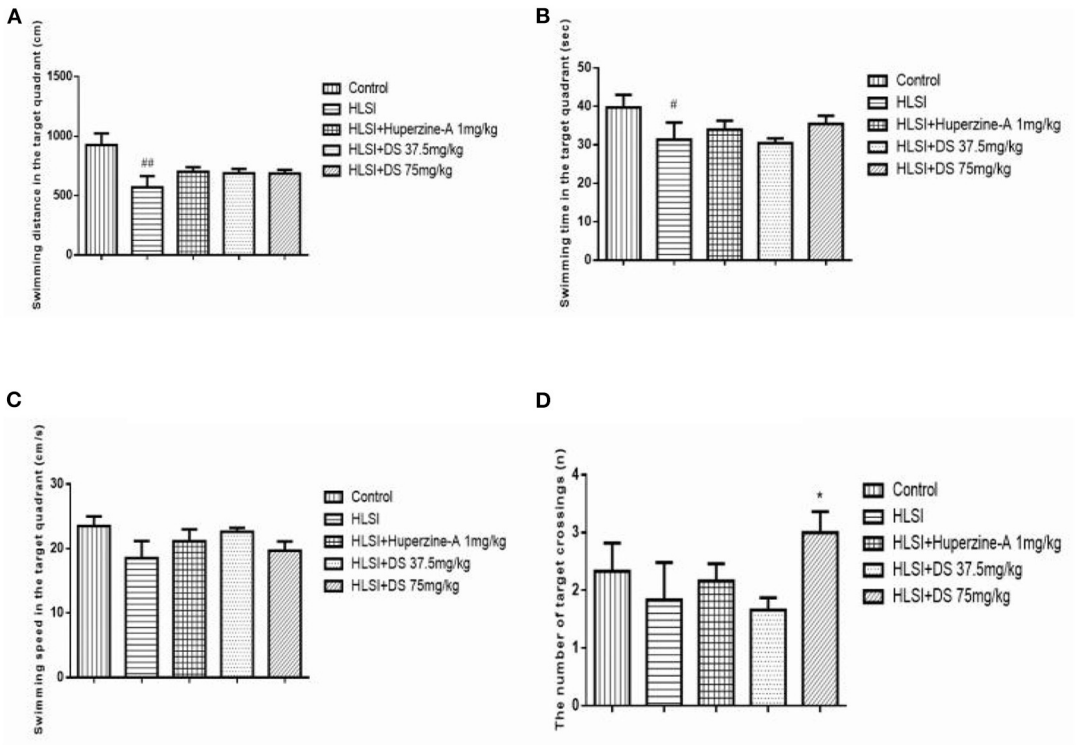

FIGURE 10 | The effect of DS on memory of rats in probe trial of the MWM test. All values are means ± SEM ($n = 7$–8). In the target quadrant, parameters noted were as follows: **(A)** swimming time, **(B)** swimming distance, **(C)** swimming speed, and **(D)** the number of target crossings. The symbols represent: $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. the control group; $^{*}p < 0.05$ vs. the HLSI group.

significantly ($p < 0.05$ or $p < 0.01$). Huperzine-A (1 mg/kg) also produced an increase in ChAT activity ($p < 0.05$).

Effect of DS on SOD Activity in Simulated Microgravity Induced by HLSI in Rats

As verified in **Figure 13**, SOD activity in the hippocampus of the HLSI group was significantly lower than the control group ($p < 0.05$). After treatment with DS (75 mg/kg), SOD activity was significantly increased in rats submitted to HLSI insult, up to levels similar to the control ($p < 0.05$). The SOD activity of the huperzine-A group also increased significantly ($p < 0.05$).

DISCUSSION

In our study, protective effects of DS were evident in depressive-like behavior and cognitive dysfunction in rats after 14 days to HLSI exposure. Moreover, the protective effect of DS was highly significant at higher dose.

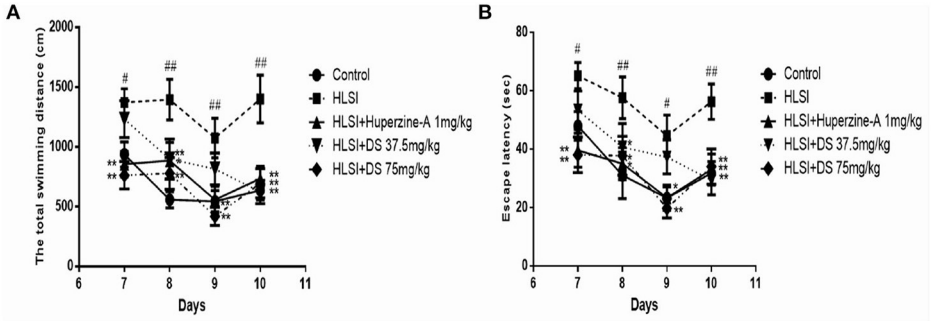

FIGURE 11 | The effect of DS on the working memory of rats in the MWM test. All values are means ± SEM ($n = 8$–9) for the following: **(A)** the total swimming distance, **(B)** the escape latency. The symbols represent: $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the HLSI group.

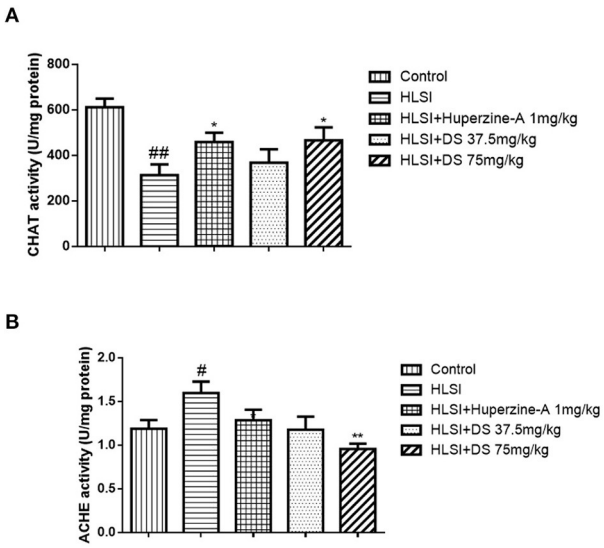

FIGURE 12 | The effect of DS on acetylcholine-related enzymes in the hippocampus of rats. All values are means ± SEM ($n = 6$–8) for the following: **(A)** the AChE activity in the hippocampus and **(B)** the ChAT activity. The symbols represent: $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the HLSI group.

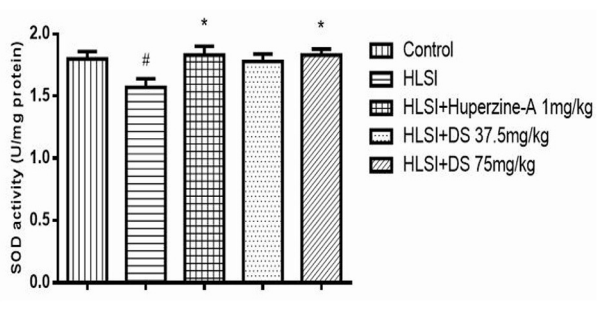

FIGURE 13 | The effect of DS on SOD activity in the hippocampus of rats. All values are means ± SEM ($n = 6$). The symbols represent: $^{\#}p < 0.05$ vs. the control group; $^{*}p < 0.05$ vs. the HLSI group.

The DS is acquired from *Panax ginseng* C. A. Mey and predominantly contains PPT and PPD. Earlier studies demonstrated that they possesses various pharmacological activities, including anti-inflammatory, antitumor, and neuroprotective effects (17). Our research also showed that ginsenoside Rg1 and Rb1, PPD, and PPT were able to ameliorate memory impairments in mice induced by scopolamine (18) and chronic sleep deprivation (8). Ginsenoside Rg1 significantly reversed the low Bcl-2 with concomitant increase in Bax expression in the prefrontal cortex (PFC) in rats induced by chronic restraint stress (13), indicating its biological effect on anti-oxidation. Wu Xiaorui et al. showed that DS improved the cognition dysfunction in rats after 42 days of simulated long-duration spaceflight environment exposure (19). Thus, in the present study, we aimed to explore the beneficial effects of DS on depressive-like behaviors and memory dysfunction in rats after 14 days of simulated microgravity.

HLS in animals provides a popular animal model to simulate microgravity on earth. Rats exposed to HLS for 2 weeks showed a significantly lower performance in the water maze test, and Nissls and Golgi-Cox staining showed significant changes in hippocampus CA1 neuronal cytomorphometry (20). Our previous studies also strongly supported poor performance of rats in the Morris water maze and shuttle box test after encountering HLS for 2 weeks (21, 22). Simultaneously, isolation, as a kind of psychosocial stress (23), has a serious influence on emotion and cognition function. Gaskin et al. showed that 40 days of post-weaning social isolation caused novel object recognition impairment in isolation-reared rats compared with the group-housed rats (24). Likewise, in our study, HLSI-induced significant damage in novel object recognition test. Thus, a combination of hindlimb suspension and isolation provides an appropriate method to simulate the extreme environment of space.

It is known that simulated weightlessness can cause depressive-like behavior in rats (25). The simulated weightlessness led to weight loss, decreased locomotor activity, prolonged latency to nose poke, decreased number of nose poke, and increased immobility time in rats, indicating that the rats have depressive-like symptoms. The administration of

DS (75 mg/kg) improved the weight loss caused by simulated weightlessness in rats. The open-field test, mainly used to study the exploratory behavior of animals and to test the ability of animals to perform in a relatively open and unfamiliar environment (26). Rodents suffering from depression, due to avoidance and fear of unfamiliar environment, mainly reduce their activities in the central area of the open field and increase their peripheral activities (27). In the open-field test, DS (37.5, 75 mg/kg) antagonized the depressive-like behavior caused by simulated weightlessness, as reflected by increasing duration of movement, and number of rearing, of which 75 mg/kg dose of DS was more effective. Huperzine-A also improved the locomotor ability of rats. The novel object recognition test is suitable to study the behavior of animals exploring novel things in a familiar environment, and its latency of nose poke reflects the exploration ability of rodents (15). The present experiment showed that HLSI can reduced the ability of mice to explore new things, while DS 37.5 and 75 mg/kg and huperzine-A can reduce the latency of nose poke thereby increasing the number of nose pokes. The effect of DS 75 mg/kg appeared to be better than its lower dose. The forced swimming test based on animals escaping from the water and struggle constantly. Depressed animals will show less struggling or even giving up struggling activity (28). In FST, DS (37.5, 75 mg/kg) and huperzine-A also reversed the increase in immobility time induced by HLSI. These behavioral experiments have indicated that DS possesses appreciable antidepressant effect.

The MWM is designed to test spatial learning and working memory and is a widely accepted method for assessing the therapeutic potential of new drugs for cognitive dysfunction (29). When the test is repeated several times, the changes in latency and distance covered to reach the platform are suitable indicators for the learning and memory abilities of the animals (29, 30). In our study, the HLSI rats demonstrated longer latency and swimming distance in the escape acquisition test. Moreover, the number in target crossings, the time, and the swimming distance toward the target quadrant were decreased in the HLSI group in the probe trail test, meaning that HLSI for 2 weeks possibly impairs spatial memory. After treatment with DS, the performance over the two trials of rats in MWM was significantly improved. It is very interesting to note that the HLSI rats required longer time to find the platform as compared with the control rats in the working memory task in the MWM test. The term working memory, defined as the capacity to maintain a limited amount of information through active rehearsal (1), provides temporary storage and manipulation of the information (31). It is well-established that working memory is crucial for arithmetic problem solving (32), language learning (33), and long-term memory consolidation (34). Moreover, spatial working memory is necessary for actions to guide thought processes. Previous research showed that participants' accuracy and reaction time decreased significantly in head-down bed rest, especially the Thurston's card rotation and cube mental rotation tasks, which usually measure the working memory. Our study showed for the first time that DS could improve spatial learning and working memory impairment induced by HLSI.

Indeed, cholinergic nervous system plays a vital role in the learning and memory processes. Both ChAT and AChE are the most specific indicators for monitoring the functional state of cholinergic neurons in the central nervous system. Reduced ChAT activity and increased AChE activity have been reported in the brains of patients with AD (35). Protecting central cholinergic neurons from functional degenerative disorders and maintaining the activities of ChAT and AChE in the neurons may be valuable for the prevention of the development or progression of AD and/or other chronic brain degenerative diseases (36). Thus, the activities of ChAT and AChE are popularly used to appraise the cognitive function in animals. Our results showed that HLSI did affect these two enzymes, especially ChAT activity which was reduced in the hippocampus of the HLSI group. The DS ameliorated the increased AChE activity and increased ChAT activity in the hippocampus. These results suggest that cognitive damage caused by HLSI is closely associated with dysfunction of the cholinergic system. In the brain of AD patients, changes in free radical-induced damages and SOD activity or expression have been reported (37). Simulated microgravity induced an oxidative imbalance in astronauts and other animal models. Our study confirmed that SOD activity was significantly decreased in the hippocampus of rats submitted to HLSI insult, indicating the activation of the oxidative stress system after exposure to microgravity.

Based on our findings, HLSI significantly induced the loss of locomotor rearings and exploration to novel object, despaired to survival in forced swimming, impaired reference, and working memory. Intragastric administration of DS could improve these depressive-like behaviors and cognitive impairments. The mechanisms were associated with inhibition of AChE activity and activation of ChAT and SOD activities in the hippocampus of rats. DS might be a potential agent for preventing and treating depressive-like behaviors and memory loss, especially for the deficiency of working memory under spaceflight.

CONCLUSIONS

In summary, our study provides evidence that DS from *Panax ginseng* significantly alleviated the depressive-like behaviors and cognitive impairment induced by HLSI in rats, and the psychotic and neuroprotective effects may be related to mediate the cholinergic system and oxidative stress, including activation of ChAT and SOD activities and inhibition of AChE activity. Further investigations on the protective activity of DS in depressive-like behaviors and cognitive impairments induced by weightlessness are needed to illustrate deep mechanisms and may provide a useful candidate during spaceflight.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by The committee for the Care and Use of Laboratory Animals of IMPLAD, CAMS & PUMC, China (No. 2016515).

AUTHOR CONTRIBUTIONS

QW designed the project and wrote this manuscript. MW and YC helped in writing the manuscript. LD, SL, and YZ performed the experiments. SC, SY, and XL helped in conducting the research. WH and HZ helped in analyzing the data and contributed to the discussion. AP helped in editing the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

We would like to thank the opening foundation of the State Key Laboratory of Space Medicine Fundamentals and Application, Chinese Astronaut Research and Training Center (SMFA18K06), the National Key Research and Development Program (No. 2016YFE0131800), Science & Technology department of Sichuan province (2019YFH0023), and the High-end Talents Recruitment Program (XL group) of Luzhou Municipal People's Government.

ACKNOWLEDGMENTS

We thank Prof. Elizabeth M. Williamson from Reading University and Prof. Farooq Ahsana Dar from Hamdard University for their help on English editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.638328/full#supplementary-material

REFERENCES

1. Koppelmans V, Erdeniz B, Dios YED, Wood SJ, Reuter-Lorenz PA, Kofman I, et al. Study protocol to examine the effects of spaceflight and a spaceflight analog on neurocognitive performance: extent, longevity, and neural bases. *BMC Neurology*. (2013) 13:205. doi: 10.1186/1471-2377-13-205
2. Liu Q, Zhou R, Zhao X, Oei TPS. Effects of prolonged head-down bed rest on working memory. *Neuropsychiatr Dis Treat*. (2015) 11:835–42. doi: 10.2147/NDT.S76292
3. Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006). *Eur J Appl Physiol*. (2007) 101:143–94. doi: 10.1007/s00421-007-0474-z
4. Messerotti BS, Bianchin M, Angrilli A. Effects of simulated microgravity on brain plasticity: a startle reflex habituation study. *Physiol Behav*. (2011) 104:503–6. doi: 10.1016/j.physbeh.2011.05.019
5. Wei J, Yan G, Guan Z. Changes of brain response induced by simulated weightlessness. *Acta Astronautica*. (1992) 27:31–6. doi: 10.1016/0094-5765(92)90171-E
6. Sun XQ, Xu ZP, Zhang S, Cao XS, Liu TS. Simulated weightlessness aggravates hypergravity-induced impairment of learning and memory and neuronal apoptosis in rats. *Behav Brain Res*. (2009) 199:197–202. doi: 10.1016/j.bbr.2008.11.035
7. Lu C, Lv J, Dong L, Jiang N, Wang Y, Fan B, et al. The protective effect of 20(S)-protopanaxadiol (PPD) against chronic sleep deprivation (CSD)-induced memory impairments in mice. *Brain Res Bulletin*. (2017) 137:249–56. doi: 10.1016/j.brainresbull.2017.12.012
8. Lu C, Dong L, Lv J, Wang Y, Fan B, Wang F, et al. 20(S)-protopanaxadiol (PPD) alleviates scopolamine-induced memory impairment *via* regulation of cholinergic and antioxidant systems, and expression of Egr-1, c-Fos and c-Jun in mice. *Chem Biol Interactions*. (2018) 279:64–72. doi: 10.1016/j.cbi.2017.11.008
9. Lan-Lan BU, Zhe S, Hong-Wei WU, Cong LU, Ke-Zhu W, Ying-Hui LI, et al. Improving effects of dammarane sapogenins on sleep interruption-induced learning and memory impairment in mice. *Chin J Comparative Med*. (2014) 24:48–53.
10. Jiang N, Zhang BY, Dong LM, Lv JW, Lu C, Wang Q, et al. Antidepressant effects of dammarane sapogenins in chronic unpredictable mild stress-induced depressive mice. *Phytotherapy ResPtr*. (2018) 55:1–7. doi: 10.1002/ptr.6040
11. White RJ, Averner M. Humans in space. *Nature*. (2001) 409:1115–8. doi: 10.1038/35059243
12. Yang Y, Xu S, Xu Q, Liu X, Gao Y, Steinmetz A, et al. Protective effect of dammarane sapogenins against chemotherapy-induced myelosuppression in mice. *Exp Biol Med*. (2011) 236:729. doi: 10.1258/ebm.2011.010369
13. Jiang N, Lv J-W, Wang H-X, Huang H, Wang Q, Chen S-G, et al. Antidepressant-like effects of ginsenoside Rg1 in the chronic restraint stress-induced rat model. *Digital Chin Med*. (2019) 2:207–18. doi: 10.1016/j.dcmed.2020.01.002
14. Mathiasen JR, DiCamillo A. Novel object recognition in the rat: a facile assay for cognitive function. *Curr Protoc Pharmacol*. (2010) 5:59. doi: 10.1002/0471141755.ph0559s49
15. Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *J Vis Exp*. (2017) 2017:126. doi: 10.3791/55718
16. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther*. (1977) 229:327–36.
17. Liu DQ, Cheng ZQ, Feng QJ, Li HJ, Ye SF, Teng B, et al. Polycaprolactone nanofibres loaded with 20(S)-protopanaxadiol for *in vitro* and *in vivo* anti-tumour activity study. *Royal Soc Open Sci*. (2018) 5:180137. doi: 10.1098/rsos.180137
18. Wang Q, Sun L-H, Jia W, Liu X-M, Dang H-X, Mai W-L, et al. Comparison of ginsenosides Rg1 and Rb1 for their effects on improving scopolamine-induced learning and memory impairment in mice. *Phytotherapy Res*. (2010) 24:1748–54. doi: 10.1002/ptr.3130
19. Wu X, Li D, Liu J, Diao L, Ling S, Li Y, et al. Dammarane sapogenins ameliorates neurocognitive functional impairment induced by simulated long-duration spaceflight. *Front Pharmacol*. (2017) 8:315. doi: 10.3389/fphar.2017.00315
20. Ranjan A, Behari J, Mallick BN. Cytomorphometric changes in hippocampal ca1 neurons exposed to simulated microgravity using rats as model. *Front Neurol*. (2014) 5:77. doi: 10.3389/fneur.2014.00077
21. Wang Q, Zhang Y-L, Li Y-H, Chen S-G, Gao J-H, Chen Y-X, et al. The memory enhancement effect of Kai Xin San on cognitive deficit induced by simulated weightlessness in rats. *J Ethnopharmacol*. (2016) 187:9–16. doi: 10.1016/j.jep.2016.03.070
22. Zhang Y-L, Wang Q, Chen H-l, Liu X, Lv K, Wang T, et al. Involvement of cholinergic dysfunction and oxidative damage in the effects of simulated weightlessness on learning and memory in rats. *BioMed Res Int*. (2018) 2018:2547532. doi: 10.1155/2018/2547532
23. Friedler B, Crapser J, Mccullough L. One is the deadliest number: the detrimental effects of social isolation on cerebrovascular diseases and cognition. *Acta Neuropathologica*. (2015) 129:493–509. doi: 10.1007/s00401-014-1377-9

24. Gaskin PL, Toledo-Rodriguez M, Alexander SP, Fone KC. Down-regulation of hippocampal genes regulating dopaminergic, gabaergic, and glutamatergic function following combined neonatal phencyclidine and post-weaning social isolation of rats as a neurodevelopmental model for schizophrenia. *Int J Neuropsychopharmacol*. (2016) 19:pyw062. doi: 10.1093/ijnp/pyw062
25. Morey-Holton ER, Wronski TJ. Animal models for simulating weightlessness. *Physiologist*. (1982) 24:45–6.
26. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol*. (2003) 463:3–33. doi: 10.1016/S0014-2999(03)01272-X
27. Paulus M, Dulawa S, Ralph R, Geyer MA. Behavioral organization is independent of locomotor activity in 129 and C57 mouse strain. *Alcohol*. (1999) 19:43–9.
28. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology*. (2005) 177:245–55. doi: 10.1007/s00213-004-2048-7
29. Faes C, Aerts M, Geys H, De Schaepdrijver D. Modeling spatial learning in rats based on Morris water maze experiments. *Pharm Stat*. (2010) 9:10–20. doi: 10.1002/pst.361
30. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods. (1984) 11:47–60. doi: 10.1016/0165-0270(84)90007-4
31. Baddeley A. Working memory. *Science*. (1992) 255:556–9. doi: 10.1126/science.1736359
32. Ashkenazi S, Rosenberg-Lee M, Metcalfe AW, Swigart AG, Menon V. Visuo-spatial working memory is an important source of domain-general vulnerability in the development of arithmetic cognition. *Neuropsychologia*. (2013) 51:2305–17. doi: 10.1016/j.neuropsychologia.2013.06.031
33. Martin RC, Shelton JR, Yaffee LS. Language processing and working memory: neuropsychological evidence for separate phonological and semantic capacities. *J Memory Language*. (1994) 33:83–111. doi: 10.1006/jmla.1994.1005
34. Andrews SC, Hoy KE, Enticott PG, Daskalakis ZJ, Fitzgerald PB. Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimulation*. (2011) 4:84–9. doi: 10.1016/j.brs.2010.06.004
35. Zhang R, Zhang J, Fang L, Li X, Zhao Y, Shi W, et al. Neuroprotective effects of sulforaphane on cholinergic neurons in mice with Alzheimer' s disease-like lesions. *Int J Mol Sci*. (2014) 15:14396–410. doi: 10.3390/ijms150814396
36. Fibiger HC. Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. *Trends Neurosci*. (1991) 14:220–3. doi: 10.1016/0166-2236(91)90117-D
37. Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W, Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer' s disease. *Neurosci Lett*. (2010) 469:6–10. doi: 10.1016/j.neulet.2009.11.033

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 08 April 2021
doi: 10.3389/fpsyt.2021.638981

Melatonin and Depression: A Translational Perspective From Animal Models to Clinical Studies

André C. Tonon[1,2]*, *Luísa K. Pilz*[1,2], *Regina P. Markus*[3], *Maria Paz Hidalgo*[1,2] and *Elaine Elisabetsky*[4]

[1] Laboratório de Cronobiologia e Sono, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [2] Graduate Program in Psychiatry and Behavioral Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [3] Laboratório de Cronofarmacologia, Departamento de Fisiologia, Instituto de Biociência, Universidade de São Paulo, São Paulo, Brazil, [4] Programa de Pós-Graduação em Ciências Biológicas-Bioquímica, Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Daily rhythm of melatonin synchronizes the body to the light/dark environmental cycle. Several hypotheses have been raised to understand the intersections between melatonin and depression, in which changes in rest-activity and sleep patterns are prominent. This review describes key experimental and clinical evidence that link melatonin with the etiopathology and symptomatology of depressive states, its role in the follow up of therapeutic response to antidepressants, as well as the clinical evidence of melatonin as MDD treatment. Melatonin, as an internal temporal cue contributing to circadian organization and best studied in the context of circadian misalignment, is also implicated in neuroplasticity. The monoaminergic systems that underly MDD and melatonin production overlap. In addition, the urinary metabolite 6-sulfatoxymelatonin (aMT6) has been proposed as biomarker for antidepressant responders, by revealing whether the blockage of noradrenaline uptake has taken place within 24 h from the first antidepressant dose. Even though animal models show benefits from melatonin supplementation on depressive-like behavior, clinical evidence is inconsistent vis-à-vis prophylactic or therapeutic benefits of melatonin or melatonin agonists in depression. We argue that the study of melatonin in MDD or other psychiatric disorders must take into account the specificities of melatonin as an integrating molecule, inextricably linked to entrainment, metabolism, immunity, neurotransmission, and cell homeostasis.

Keywords: psychiatry, mood disorder, neuropsychaitric disorders, behavior, biological rhythms, chronobiology, 6-sulfatoxymelatonin (aMT6s), biomarker

OPEN ACCESS

Edited by:
Mirko Manchia, University of Cagliari, Italy

Reviewed by:
Anna Brancato, University of Palermo, Italy
Elena Martín-García, Pompeu Fabra University, Spain

***Correspondence:**
André C. Tonon
labcronoesono@hcpa.edu.br

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *08 December 2020*
Accepted: *15 March 2021*
Published: *08 April 2021*

Citation:
Tonon AC, Pilz LK, Markus RP, Hidalgo MP and Elisabetsky E (2021) Melatonin and Depression: A Translational Perspective From Animal Models to Clinical Studies. Front. Psychiatry 12:638981. doi: 10.3389/fpsyt.2021.638981

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is an amphiphilic indoleamine conserved from unicellular organisms to plants and animals. In mammals, pineal and extra-pineal synthesis were described (1). Darkness at night triggers a polysynaptic pathway that begins with the retino-hypothalamic tract projecting to the suprachiasmatic nuclei (SCN) of the hypothalamus and ends as a sympathetic input to the pineal gland. Melatonin synthesis is prompted by norepinephrine and requires serotonin as a precursor (2). Pineal melatonin, a primary output of the circadian pacemaker (SCN), transduces light-dark information to the whole body, coordinating daily physiological functions and behaviors.

Biological rhythms and melatonin represent recent frameworks in the investigation of the etiology, prognostic improvement, and treatment approach in the clinical course of depression. The somatic, cognitive, and affective symptoms of depressive states that occur in association with neurochemical/hormonal imbalance, may result, among other factors, from the misalignment of biological rhythms (3–5). A summary of the physiological regulation and organization of biological rhythms is shown in **Figure 1**.

Considering the cyclic nature of mood disorders, the shared monoaminergic involvement, the data from seasonal depression, and the marked sleep and circadian changes in depressive disorders, several hypotheses have been raised to understand the intersections between melatonin with depression. Experimental and clinical studies were designed to understand possible pathophysiological connections and devise therapeutic strategies to optimize the pharmacological management of depressive patients. This review aims to discuss the interconnections of melatonin production pathways and its physiological actions with the etiopathology and clinical approaches to depression. We will initially describe the framework of depression relevant for the understanding its association with biological rhythms and melatonin. We proceed by discussing the role of melatonin as an internal temporal cue, its production and physiological actions, and its role as a neurohormone sharing common neuro-endocrine inputs with the pathways associated with depression. Finally, we describe the current knowledge from clinical research in the field, including the use of exogenous melatonin and urinary 6-sulfatoxymelatonin as treatment and response biomarker, respectively.

DEPRESSION AND BIOLOGICAL RHYTHMS

What Do we Mean by Depression?

Depression is a term with multiple meanings. As a state, a depressive mood may be an adaptive stress response (6). Depressed mood states manifest as typical behavioral phenomena (e.g., anhedonia, psychomotor disturbance) and dysregulation in neurovegetative functions (e.g., altered appetite, disturbed sleep), which may be a result of a great variety of physiological events. As part of a mental disturbance, depressive symptoms can manifest in several conditions such as unipolar or bipolar mood disorder, substance abuse, and general medical disease. On the other hand, Major Depressive Disorder (MDD), as defined by international guidelines such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Diseases ICD, is a syndromic construct assembled as a distinct clinical condition (7, 8). According to the fifth edition of the DSM, at least one of the two cardinal symptoms must be manifested to reach a positive diagnosis: depressed mood and/or loss of interest or pleasure in daily activities. Of the nine cognitive (e.g., impaired concentration), emotional (e.g., feelings of worthlessness) and somatic symptoms (e.g., psychomotor agitation or retardation, changes in appetite, sleep disturbances) listed as diagnostic criteria, at least five must be present during the same 2-week period, with the symptoms representing a change from previous baseline. Of note, other medical conditions that can cause the same symptoms must be excluded. MDD is one of the most burdensome disorders and the leading cause of disability worldwide (9, 10). Therefore, the assessment of the severity of the depressive syndrome (as a continuous feature) in clinical and research settings is fundamental, which typically implies the use of validated self-reported instruments and structured interviews of cognitive, physical, and psychomotor changes. Though sadness and anhedonia are the cardinal symptoms of MDD, the multivariate features of depression, together with its multifactorial etiology and the lack of a reliable biomarker, can explain the individual differences in pharmacological response.

Clinical response is usually noticeable after 2–5 weeks (11–13). The poor or absent response to antidepressants that is associated with the morbidity and mortality of MDD justifies the search for improvement in diagnosis, while searching for subtypes of depression that show treatment idiosyncrasies. In addition, preventing the relapse and recurrence of clinically significant symptoms is essential for new treatment approaches and/or the identification of markers of response to established treatments. In fact, several studies document a vast heterogeneity of clinical features of depressive disorders, indicating that specific symptoms represent different physiological phenomena, boosting the search for specific diagnostic and treatment biomarkers and unraveling new therapeutic strategies (14). In this context, despite inherent limitations, animal models have been instrumental in the investigation of subjacent neuronal mechanisms associated with the genesis, trigger factors, and maintenance of depressive states, as well as potential treatments.

The difficulties in modeling depression and neuropsychiatric disorders in experimental non-human species may, in part, be attributed to the variability of depressive symptoms (across and within individuals), and the sheer impossibility to reproduce in non-human animals some important features, such as guilt and suicidality. This somehow mirrors the challenges of Psychiatry as a field in categorizing debilitating disorders (with overlapping symptoms and varied underlying mechanisms) as opposed to normal behavior. Chronic stress, maternal deprivation, and olfactory bulbectomy are among the models that present higher degrees of face validity (i.e., animal behavior correlates with MDD symptomatology), predictive validity (i.e., good chances of replicating the positive or negative results in human patients), and construct validity (i.e., consistent underlying pathology biomarkers, triggering factors, time course, and response to antidepressants). The majority of animal models only replicate anhedonia (with varying degrees of face value), which is certainly one of the core symptoms of depression. The widely used tail suspension and forced swimming tests have no pathophysiological or undisputed behavioral manifestations equivalent to those seen in depression but possess some degree of translation predictability. It is of utmost importance to select the model appropriate for a given question, and interpret results considering specific limitations.

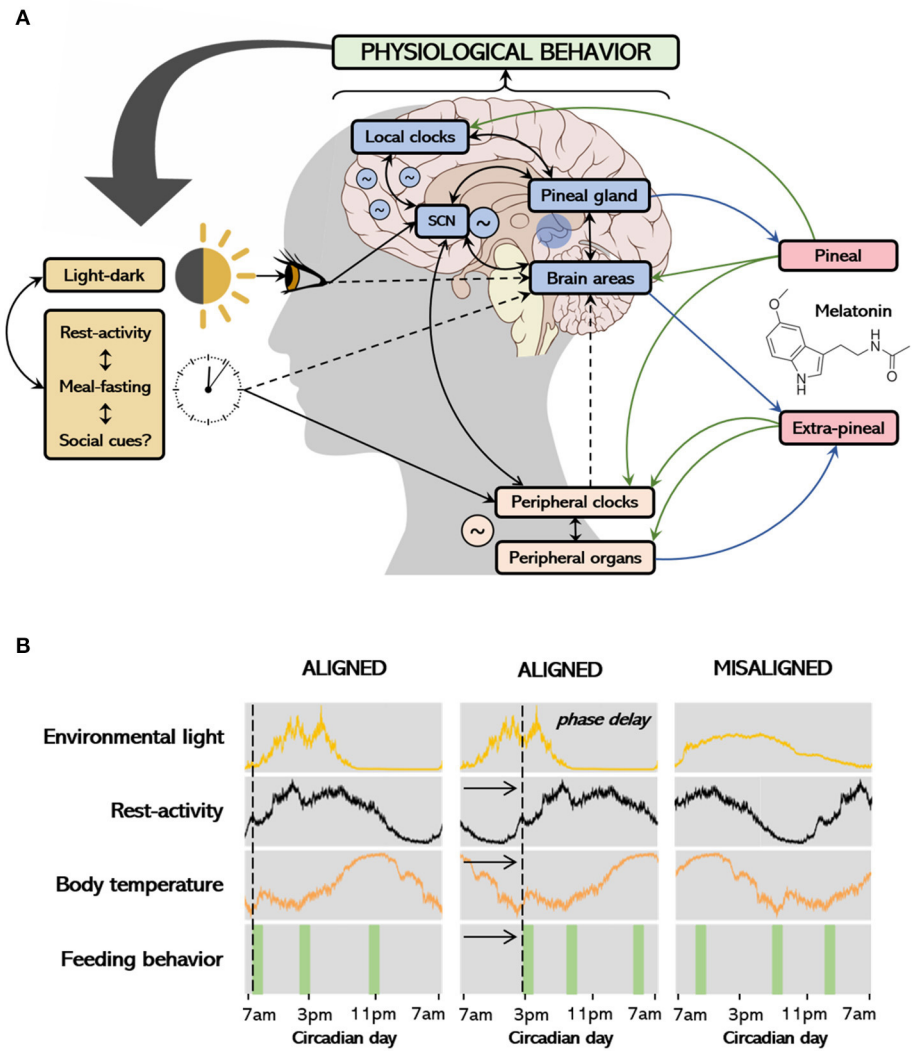

FIGURE 1 | Environmental and internal regulation of biological rhythms, melatonin production and associations with behavior **(A)**; the process of entrainment and alignment **(B)**. The main environmental cue that synchronizes the circadian system is the light-dark cycle. The suprachiasmatic nucleus (SCN) receives photic information and projects to several structures in the body, coordinating peripheral tissues by modulating other oscillators according to light-dark transitions. Other stimuli of importance include feeding schedules, rest-activity rhythm and social cues. Peripheral tissues respond to timing neural and endocrine signals modulated by the SCN, including the release of melatonin to the bloodstream, as well as from feeding. On the other hand, endogenous circadian rhythms also influence daily patterns in behavior/exposure to environmental factors and might also influence melatonin production (see details in text). In **(A)**, black arrows connect the environmental stimuli to the central and peripheral clocks and tissues and show the interconnection among systems. Continuous arrows represent higher levels of evidence and dashed arrows represent lower levels of evidence. Melatonin is produced in the pineal gland, as a result of darkness or on-demand via other pathways (see details in text), acting locally or transducing the dark signal to peripheral tissues (green arrows). Extra-pineal sources of melatonin include the skin, guts, and lungs, which only act locally with no known chronobiotic effect. While the physiological actions of melatonin potentially impact behavior, individual behavior might also determine the timing of light exposure, rest-activity rhythms and feeding behavior, thus regulating melatonin secretion. The first column at **(B)** shows rhythms of activity, temperature, and feeding behavior aligned with the light signal; the second depicts rhythms which are phase delayed. The rhythms at the third column are no longer entrained to the light signal and are not synchronous within themselves, determining a misalignment.

Are Biological Rhythms Related to Depression?

Although the challenges of assessing behavior pose inherent limitations to both experimental and clinical studies, the relationships between depressive-like behavior and biological rhythms have been abundantly documented. Nile grass rats (*Arvicanthis niloticus*), a diurnal rodent, exposed to short photoperiod (5 h light/19 h dark) for 6 weeks presented reduced saccharin preference, higher immobility time in the forced swim test, and no changes on time spent in the light side of the dark/light box in comparison to controls (15). The same species submitted to low light intensity during the daytime (cycles of dim light of 50 lux during 12 h/dark during 12 h) for 4 weeks showed increased immobility and decreased climbing in the

forced swim test; again, no effects were observed in the light/dark test or locomotion in the open field compared to bright light controls. Nile grass rats also show lower dopaminergic and somatostatin neurons in the hypothalamus under both short photoperiods and dim light/dark cycle (16). In nocturnal mice, dim light at night [16 h of light (∼150 lux) and 8 h of darkness (5 lux)] for 4 weeks lead to depression-like behaviors such as increased immobility in the forced swim test and reduced sucrose preference (17). In addition, nocturnal rodents under constant light for 3–4 weeks, a light regimen known to render rats behaviorally arrhythmic (18), exhibited significant higher number of grooming events during the open field test, and diminished sucrose preference throughout the study (19). Under forced desynchrony (light/dark cycles of 22 h), a protocol known to uncouple neuronal oscillators in the suprachiasmatic nucleus (SCN; see **Figure 1**), rats develop into depressive-like phenotypes (i.e., anhedonia, sexual dysfunction, increased immobility in the forced swim test) (3).

Landgraf et al. (4) showed that knocking down *Bmal1* (one of the essential clock genes that participate in the maintenance of the circadian periodicity at the cellular level) in mice suprachiasmatic nucleus (SCN) led to increased escape latencies and number of escape failures in the learned helplessness paradigm, induced higher immobility time in the tail suspension test, and decreased the time spent in the light compartment of the light/dark test. These results indicate that disrupting central SCN rhythms causes helplessness, behavioral despair, and anxiety-like behavior. The experiment supports a causal relationship because environmental light-dark cycles and light input pathways were unchanged. Therefore, results cannot be explained by light affecting depressive-like behavior through other pathways [i.e., without disturbing sleep or circadian rhythmicity, or through pathways unrelated to the SCN (20)]. Unlike in global knockouts in which mice may also present altered behavior related to pleiotropic functions of the clock genes, the model has the advantage of selectively inducing disturbed SCN rhythms while avoiding other neural damages caused by SCN lesions. Moreover, hedonic behavior measured by the sucrose preference test, spatial preference in the open field test, and aversion to eating in a novel environment were unchanged, suggesting that only specific aspects of depressive-like behavior are influenced by the disruption of the central clock (4). Of note, neurotransmitter systems altered in depression show blood levels circadian oscillations (21, 22), though it is unclear whether or how mood states could affect this circadian pattern.

Clinical evidence supports that dysfunctions of the circadian timekeeping system are present in a variety of morbid clinical conditions, such as obesity, diabetes, hypercholesterolemia, cardiovascular diseases, and cancer (23, 24). Mood alterations have also been extensively studied in models of circadian disruption or misalignment (see **Figure 1**). Of relevance to this discussion, depressive symptomatology can assume seasonal variation (i.e., seasonal affective disorder), and is associated with non-respiratory sleep disturbances and distinguishable daily motor activity patterns (i.e., comparing depressed individuals with non-depressed individuals and among depressive subtypes). Diurnal variations in mood have been seen in naturalistic conditions (25) and experimental results suggested mood to be affected by an interaction between circadian phase and duration of prior wakefulness (26). A few initiatives arouse in the past decade aiming to elucidated whether altered patterns of diurnal variations in mood are associated with depressive disorders. These reports show that individuals with depression are more likely to report diurnal variations in mood (27, 28).

Acute or chronic dysfunctions of the circadian timekeeping system (e.g., Eastbound travels across time-zones and rotating night shift workers, respectively) impact the organism with consequences at different biological scales, i.e., cellular, tissue, and systemic level. In the light of the above, circadian misalignment can result from: (1) damage to cerebral structures like the retina, the retino-hypothalamic tract or the SCN; (2) genetic manipulation or variations in *clock* and *clock-controlled* genes; (3) a response to external factors (*zeitgebers*) in situations of rotating night shift work, travel across time zones, lack of light exposure during daytime or the excessive exposure during the night. All these potentially impact the production of pineal melatonin, an important temporal cue of biological rhythms.

MELATONIN: THE NEUROENDOCRINE TIMING SIGNAL

The main environmental cue that synchronizes biological rhythms with the environment is the light-dark cycle. The SCN, a small collection of hypothalamic cells just above the optic chiasm, receives environmental photic information collected by intrinsically photosensitive ganglion cells (ipRGC) in the retina. The ipRGC expresses the photopigment melanopsin, which transduces light wavelengths into neural input through the retino-hypothalamic tract to the SCN. The SCN projects to several structures, including the paraventricular hypothalamic nucleus that communicate with the intermediolateral spinal column (29). The light/dark information reaches the pineal gland through sympathetic postsynaptic fibers of the superior cervical ganglion (30), and melatonin is simultaneously released to the peripheral circulation and to the cerebrospinal fluid (CSF) (31). It is the daily pattern of melatonin secretion that carries information for circadian and seasonal temporal organization (32). Melatonin is also synthesized by the skin, guts, and lungs in a constitutive manner, and on-demand by activated immune-competent cells, such as monocyte-derived and resident macrophages, microglia and lymphocytes (1, 33, 34).

It is currently impossible to monitor the central clock (SCN activity) directly in humans. Since the timing of melatonin secretion is strongly regulated by the SCN, the onset of melatonin secretion under dim light (dim light melatonin onset; DLMO) has been considered a gold standard to assess the central clock's phase and whether it is misaligned in relation to other internal rhythms or environmental cycles. A fair number of studies have investigated the relationship between depressive symptoms and the phase angle difference between DLMO and the timing of other rhythmic functions, including sleep (35, 36). Results are heterogeneous, which may reflect the multifactorial nature of depression and the multiple actions and regulatory systems

related to melatonin. Aside from phase shifts, lower nocturnal melatonin levels are often, though not always, reported in depressed individuals (37–39).

Even though the molecular and mechanistic underpinnings are yet to be unveiled, the way the clock, sleep and behavior co-exist and co-affect each other seems crucial to balance health and disease [see (40) for discussion]. In the context of mood disorders, we still need to identify sub-optimal phase relationships and other altered circadian states that may affect the susceptibility to develop specific depressive-like behaviors. Melatonin—as the important phase marker and *eigen-zeitgeber* it is—is likely to remain a central player in this framework.

MELATONIN PRODUCTION AND PHYSIOLOGICAL ACTIONS

What Is the Pathway of Melatonin Production?

In mammals, the biosynthesis of melatonin starts with the conversion of tryptophan to l-5-hydroxytryptophan, which is converted to serotonin [5-hydroxytryptamine(5-HT)] by the aromatic l-amino acid decarboxylase (AADC). Serotonin is acetylated by the phosphorylated arylalkylamine N-acetyltransferase (P-AANAT), forming N-acetylserotonin (NAS), which is converted to melatonin by N-acetylserotonin O-methyltransferase (ASMT). The melatonin pineal daily rhythm is determined by the conversion of 5HT to NAS under sympathetic control (**Figure 2**). Environmental light modifies the structure of melanopsin in the retinal ganglion cells triggering glutamate excitation at the retino-hypothalamic tract that projects to the SCN. The SCN inhibits the hypothalamic paraventricular nucleus (PVN) via GABAergic projection. In the absence of light, the PVN activates the ganglion cervical nuclei (via the intermediolateral column of the medulla) activating noradrenergic fibers that innervate the pineal gland, ultimately releasing the co-transmitters noradrenaline and ATP (41). Consequently, the production of melatonin depends on the integrity of the brain monoaminergic system (2). Beta-1 adrenergic receptor activation leads to increase in cAMP and activation of protein kinase A, which promotes the phosphorylation of the cyclic AMP regulating element (CREB) and the phosphorylation of AANAT (P-AANAT). Phospho-CREB induces the transcription of the gene that codifies AANAT (the native form of the enzyme), which is immediately degraded by the proteasome. In nocturnal animals both the control of transcription and activation of AANAT plays an important role in melatonin synthesis, while in diurnal animals the transcription of the gene is constitutive (1); thus, nocturnal melatonin surge is delayed in nocturnal rodents when compared to humans. Circulating melatonin levels in the bloodstream accurately reflects pineal synthesis (2). At dark-night the pineal melatonin plasma levels show a 10–20-fold increase in normally entrained individuals. When light is turned on in the middle of the dark night, the pineal melatonin synthesis stops, and melatonin concentration in the blood is abruptly reduced due to liver metabolization (first-pass effect). In the liver, melatonin is metabolized to 6-sulfatoxymelatonin (aMT6s), excreted in the urine, which is well-correlated with plasma melatonin (**Figure 2**) (42).

How Is Melatonin Production Regulated?

The pineal gland is a circumventricular organ that monitors the level of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) in the cerebrospinal fluid (CSF) and peripheral circulation. In pinealocytes, the activation of the NFκB pathway by toll-like receptors and TNFRs inhibits AANAT-gene transcription both in diurnal and nocturnal animals. Cortisol or corticosterone at concentrations compatible with arousal (but not the ones found in models of chronic unpredictable mild stress) potentiate melatonin synthesis, while concentrations compatible with immune suppression blocks it. In immune-competent cells PAMPs and DAMPs promote the synthesis of melatonin by activating the transcription of the gene that codifies AANAT. This process is also dependent on the binding of NFkB dimers to the gene promoter, but it is not an immediate effect. Activation of PAMPs or DAMPs receptors promote the nuclear translocation of the NFkB dimer (p50/p65) that leads to the synthesis of the subunit (cRel) necessary for activating AANAT transcription. The induction of melatonin synthesis in rodents and human macrophages by the transcription factor is a hallmark in inflammatory responses and inflammation (43–46). The fact that stressful conditions increase extra-pineal melatonin synthesis independently of environmental light strongly suggests that the amplitude of daily melatonin rhythm, classically attributed to reduction of nocturnal melatonin synthesis, may also result from an increase of the output from extra-pineal sources (1).

During the mounting of an innate immune response melatonin plays various roles: the reduction of nocturnal melatonin leads to the mobilization of leukocytes from the bone marrow to the blood, and from the blood to the site of lesion; the synthesis of melatonin by macrophages/microglia increases its phagocytic ability and reduces the oxidative stress participating in the recovery phase of the inflammatory response (1). The return of macrophages/microglia/phagocytes counts to baseline requires the return of mediators' levels active at the recovery phase, including melatonin, to basal values. When the recovery from the acute inflammatory response is mediated only by pineal activity return to baseline, without restoration of macrophage melatonin synthesis to baseline, the result is a resilient state (low-inflammatory grade).

Over the past decades, studies reported neuroinflammatory responses in a series of neurodegenerative and psychiatric disorders. Intense research has been undertaken to determine the critical elements of such responses and putative therapies for their suppression. Some reports associate MDD to increased levels of cytokines including TNFα, IL-6, and IL-1β, as well as a reduction in complement C3 (47–49). Increased immune-inflammation, with high oxidative and nitrosative stress leading to changes in neuronal regulating tryptophan catabolites (TRYCATs) and mitochondrial dysfunction, have been documented in the progressive course of MDD (50, 51). In animal models, melatonin treatment significantly abolished

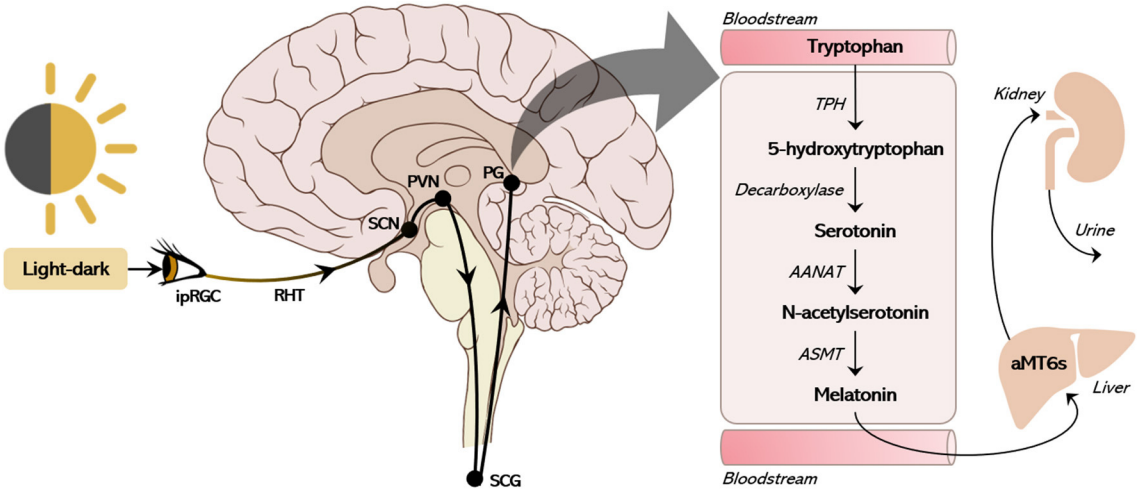

FIGURE 2 | The canonical pathway of pineal melatonin production. The suprachiasmatic nucleus (SCN) receives environmental photic information collected by intrinsically photosensitive ganglion cells (ipRGC) in the retina. The ipRGCs express the photopigment melanopsin, which transduces light wavelengths into neural input through the retinohypothalamic tract (RHT) to the SCN. The SCN constitutively inhibits the hypothalamic paraventricular nucleus (PVN) via GABAergic projection. In the absence of light, the PVN activates the ganglion cervical nuclei (SCG, via the intermediolateral column of the medulla) triggering noradrenergic fibers that innervate the pineal gland (PG), ultimately releasing the co-transmitters noradrenaline and ATP. This sympathetic stimulus triggers the action of arylalkylamine N-acetyltransferase (AANAT) converting serotonin (5TH) into N-acetylserotonin (NAS) within the pinealocyte. With the constitutive action of N-acetylserotonin O-methyltransferase (ASMT), NAS is then converted to melatonin and immediately released into the cerebrospinal fluid and bloodstream. Through first-pass metabolism in the liver, melatonin is converted to 6-sulfatoxymelatonin (aMT6s), which is then excreted in the urine.

the effects of LPS and reduced NF-κB in the cortex and the hippocampus, both effects resulting in an improvement of depressive-like behaviors (52). These results point to the possibility of an "antidepressant effect" of melatonin via the interplay with the immune system. Furthermore, as described above, cortisol functioning and its circadian fluctuation is essential for the adequate melatonin surge. Blunted cortisol rhythms (i.e., lower morning cortisol peak and higher daytime values) have been demonstrated in association with depressive symptoms in humans (49, 53, 54).

How Does Melatonin Exert Its Physiological Actions?

Melatonin effects triggered by a large range of concentrations (pM to mM) are mediated by mechanisms of action with different grades of sensitivity. The effects of exogenous vs. endogenous melatonin, as well as the concentration reached by intracrine/autocrine, paracrine and hormonal sources, depend on the melatonin bioavailability and the mechanisms that mediate each production source. In a concentration range compatible with the nocturnal surge (pM to nM), melatonin orchestrates a plethora of physiological functions by activating GPCRs receptors (MT1 and MT2) localized in plasmatic, nuclei and mitochondrial membranes. At much higher concentrations, melatonin may act as an electron donor, promoting a receptor free non-specific antioxidant response (55). Other non-receptor mediated actions include the regulation of clock genes expression (by directly inhibiting the proteasome), and inhibition of the ubiquitin–proteasome system that ultimately controls protein degradation.

Melatonin receptors MT1 and MT2 couple to Gi/o and beta-arrestins and depending on the context also couple to Gq (55). The second messenger immediate responses result in an increase/decrease of cAMP or increase in intracellular or intramitochondrial free calcium (56). The internalization of the receptors upon stimulation leads to the activation of ERK pathways, which modulate intranuclear responses. MT1/MT2 receptors form heterodimers with pharmacological properties different from MT2 homodimers. The heterodimerization of the MT1 receptor with the orphan receptor GPR50 impairs its interaction with melatonin (57). Thus, another source of variability of melatonin response are the changes in the expression and the dimerization of melatonin receptors induced by pathophysiological and pathological states.

Pineal melatonin exerts a wide range of physiological actions due to its release into the cerebrospinal fluid (CSF) and bloodstream. As a hormone that gains the bloodstream at night in the dark, melatonin is an internal temporal cue that has immediate interactions with its molecular effectors as well as prospective effects, since it primes physiological responses that will take place hours after its peak. The duration, timing, and cyclic-nature of pineal melatonin production, as well as its seasonal variation according to photoperiod changes, contribute to the temporal organization of physiological phenomena into circadian and circannual timescales (58). Finally, transgenerational effects have been described, as maternal melatonin reaches the fetus via placenta and constitutes the only fetal source of intrauterine melatonin. As previously stated, extra-pineal melatonin shows immediate effects in the cells and tissues where it is produced, and the current knowledge

does not support any *eigen-zeitgeber* action (i.e., being an internal temporal cue) or seasonal effect resulting from these sources. A detailed review on the physiological actions of melatonin was composed by Cipolla-Neto and Amaral (59).

MELATONIN AND DEPRESSION: IS THERE A PHYSIOLOGICAL OVERLAP?

How Does Melatonin Relate to the Monoamine and Neurotrophic Hypotheses of Depression?

Since the report of two pharmacological agents in the late 1950s, the pathophysiology of MDD has been linked to a depletion in monoaminergic neurotransmitters, such as noradrenaline, serotonin and dopamine (5, 60). Initial studies showed that severe depressive episodes could be effectively treated with monoamine oxidase inhibitors (MAOi) and tricyclic antidepressants (TCA), which share increased availability of brain catecholamines as a common pathway. The involvement of other monoamines has been studied, with growing evidence to the role of serotonin with the introduction of Selective Serotonin Reuptake Inhibitors (SSRIs) in the 1960s.

Since melatonin production occurs from serotonin after stimulation of adrenoreceptors (see section What Is the Pathway of Melatonin Production? above), it has been hypothesized that a disturbance in melatonin secretion could be present in the acute phase of depressive illness, and possibly be related to its pathophysiology. Moreover, melatonin controls dopamine signaling in the forebrain, hypothalamic and hippocampal areas (61–63). Of note, dopamine is not only the precursor of noradrenaline, but also has been implicated in the circadian regulation of melatonin production (64), via activation of dopamine D4 receptors by noradrenergic signals (65).

Several clinical studies tried to identify alterations in absolute levels of melatonin as a possible maker of depressive states; considering the above detailed physiological regulation of melatonin, this was a misleading proposition, and the evidence is at best controversial. While some earlier studies reported lower nocturnal serum or saliva levels of melatonin in depressed individuals, others reported no differences, or even higher levels compared to controls (39). One study (38) reported that individuals with levels of melatonin in the two lowest quartiles had higher risk of severe symptomatology; nevertheless, the authors described similar levels of nocturnal melatonin in depressed patients and controls. Studies that investigated the nocturnal production of 6-sulfatoxymelatonin (aMT6s) found no difference between depressed individuals and controls before treatment (66, 67), suggesting the absence of a linear relationship between depressive behavior and melatonin levels. One study (37) found lower bedtime levels of plasma melatonin in depressed individuals with melancholic features; others (68, 69) indicated that subgroups of depressed individuals who do not suppress morning cortisol after dexamethasone stimulation test are more likely to present lower nocturnal melatonin. These data indicate that the lack of agreement in findings on melatonin levels in depressed individuals may be a result of (1) the diversity of behavioral and etiological phenomena in depressive states [as previously commented (14)]; (2) methodological heterogeneity, like inconsistency in the methods for melatonin assessment, difficulties in complying with the protocol of melatonin collection, and the distinctions among study designs; and (3) the erroneous premise that (lack of or excessive) melatonin could contribute or counteract (as antidepressant) depression needs to evolve to the understanding that melatonin is an integrative molecule which potentially affects mood through regulation of physiology and behavior in several levels.

Though the immediate mechanisms of action of current antidepressants are well-understood, the delay in clinical outcome conveys the gap of knowledge on the complete pathways that ultimately determine the clinical response. This delayed response shifted theories toward the neuroplastic hypothesis, whereby reduced levels of neurotrophic factors result in atrophic morphological changes, especially at the synaptic level (5). Animal studies that examine the neurobiological changes induced by experimental models that lead to depressive-like behaviors highlighted the relevance of the neuroplasticity phenomena in depression (70). The activation of cellular signaling pathways by antidepressant strategies would eventually elevate the levels of growth factors (e.g., BDNF, VEGF, VGF), promote proliferation on hippocampal progenitor cells, and ultimately restore monoaminergic synapses (70–72).

Melatonin exerts neuroprotective actions by counteracting the NMDA-mediated excitotoxic effects of glutamate, including the impaired BDNF signaling and cell death (73). Exogenous melatonin can differentially modulate glutamate release in CNS structures in rodents (74), suggesting it might correct the imbalance in the glutamatergic system in patients with mood disorders. This body of evidence suggests that melatonin may increase neuroplasticity in the CNS acting on the glutamatergic system.

Can Urinary 6-Sulfatoxymelatonin (aMT6S) Be a Treatment Biomarker?

Biomarkers are easily accessible molecules that discriminate the presence or absence of a disease or predict treatment response. They are based on genomic, proteomic, functional, and structural features that characterize the disorders. "Diagnostic biomarkers" identifiable at any stage of the illness are often measured before treatment. The term "treatment biomarkers" refers either to baseline values that predict how effective treatments may be and guide therapeutic choice, or short-term variation between baseline and an early phase in the course of therapy (75).

A promising group of "treatment biomarkers" for SNRI/SSRI search for consistent changes in monoamines neuronal reuptake inhibition associated with subsequent mood changes (usually after 4–6 weeks). To measure the blockage of neuronal monoamine transporters directly, it would be necessary to assess changes in an immediate molecular product on the postsynaptic level that directly results from the neurotransmitter release (see section What Is the Pathway of Melatonin Production?). We proposed the assessment of 6-sulfatoxymelatonin (aMT6s, **Figure 2**) to detect whether the blockage of noradrenaline

neuronal reuptake on the first day of medication took place. This can be done by using the relationship between the morning aMT6s content 1 day before and 1 day after the installment of the therapy. This procedure is not expected to evaluate the implication of melatonin or the pineal gland in the mechanism of action of the antidepressants, but rather to produce a proxy of the immediate events that follow the consequences of antidepressants at synaptic level. The same protocol could theoretically be used for both SNRIs and SSRIs, as the pinealocyte function depends on the uptake of serotonin, which is mediated by the transporter found in neuronal membranes.

Because the difference in aMT6s urine concentration in the light and dark phases reflects pineal activity, it has been suggested that an increase in the nighttime production of aMT6s after the first dose of antidepressant medication could work as a fingerprint of the integrity of this system; thus, it could predict a later improvement in depressive symptoms (76). A first study to test that hypothesis evaluated the association of a "aMT6-SNRI biomarker" and the improvement of depressive symptoms in MDD patients taking placebo ($n = 12$) or antidepressants ($n = 22$; fluoxetine, duloxetine or *Hypericum perforatum*) for 8 weeks. Both placebo and SNRIs groups showed improvement in depressive symptoms but only the group treated with the drug showed a significant increase in aMT6 urinary excretion in the first urine of the day (77). In patients taking clomipramine, the fraction of aMT6s excreted from 24:00 to 06:00 relative to the total amount excreted in 24 h was significantly higher 24 h after the first dose than at baseline (before treatment) in responders in comparison to non-responders (78). The same pattern was seen in 22 women taking nortriptyline (79), and 20 women taking fluoxetine (80): only responders had a significant increase in aMT6s levels. Altogether these studies suggest that the "aMT6 urinary biomarker" reveals whether the blockage of neuronal uptake was effective on the first day of administration, thus representing that aMT6 reflects the integrity of the monoaminergic systems that are required for antidepressant action. The advantage of this method over those that measure changes in the content of monoamines in leukocytes, platelets, or even plasma is the direct estimation of the increase of neurotransmission efficiency that is the inductor of neuronal plasticity.

Do Exogenous Melatonin and Melatonin Agonists Have Therapeutic and/or Prophylactic Effects on Depression?

In a series of elegant experiments, Nagy et al. (81) showed that de 24-h pattern of the dorsal raphe 5-HT reuptake in C57BL/6J mice under shortened photoperiods may be altered at the transcriptional level by specifically timed melatonin. The data suggest that daily melatonin treatment can induce and sustain receptor 5HT1A mRNA expression throughout the light phase. In the same line, Otsuka et al. (82), using the same mice, showed that daily melatonin treatments 2 h before the end of the light phase can restore the amplitude of the daily rhythm of 5-HT contents in the amygdala. In models with higher face and construct validities, such as the chronic unpredictable mild stress (CUMS), melatonin at high concentrations (10 mg/kg) showed antidepressant-like effects, preventing the CUMS-induced decrease in norepinephrine content and the expression of tyrosine hydroxylase, dopamine-b-hydroxylase and norepinephrine transporter in the adrenal medulla (83), as well as depressive-like behaviors, such as impaired sucrose intake, physical coat deterioration, and decreased grooming (84, 85).

Overall, there is substantial heterogeneity in the body of clinical evidence regarding the prophylactic or therapeutic use of exogenous melatonin or melatonin agonists in depression, complicating a conclusive assessment. Different methodological approaches and small convenience samples precludes robust comparisons among studies, as there is no consensus on dosing and timing. Moreover, it is known that the secretion of pineal melatonin shows a wide interindividual difference as well as significant dependence on the exposure to dark each night (86); thus, therapeutic doses or an optimal serum levels are yet to be determined.

A recent systematic review (87) included eight randomized double-blind controlled trials using exogenous melatonin as an augmentation strategy in major depression, bipolar disorder or seasonal affective disorder in comparison with placebo. The dosage of exogenous melatonin ranged from 0.125 to 10 mg. Among the three studies that evaluated melatonin in the context of depression, one showed that melatonin improved subjective sleep quality but not depressive symptoms [(88), using 5–10 mg slow release melatonin], the second showed no effect [(89), using 6 mg] and the third compared the use of 3 mg slow-release melatonin plus 15 mg buspirone vs. 15 mg buspirone and placebo, showing a significant antidepressant effect of the melatonin combination (90). Of the four studies using melatonin for SAD, one (91) showed significant antidepressant effect (using 0.125 mg twice daily), while the other three did not show significant effects (92–94). Another systematic review (95) pooled results of clinical trials testing the prophylactic or therapeutic effect of melatonin for depression in adults, including comorbid conditions. Among the three studies that tested prophylactic melatonin, one study with older adults with sleep complaints showed lower depressive scores after supplementation with 5 mg melatonin at bedtime. Two other studies were in individuals with irritable bowel syndrome and found no antidepressant effect of 3 mg melatonin. Of the studies testing melatonin as a treatment for depression, one showed a decrease in depressive scores in individuals with Delayed Sleep Phase Syndrome treated with melatonin 5 mg between 19:00 and 21:00; the other six found no significant antidepressant effects. Adverse effects reported in these studies include mild sleepiness, headache, poor sleep, vivid dreams, daytime sleepiness, and fuzzy feeling.

Following the initial evidence on the potential antidepressant effect of melatonin, efforts have been directed toward the development of antidepressants with melatonin agonist activity. Among them, agomelatine (S20098, N-[2-(7-methoxynaphth-1-yl)ethyl]acetamide) first reported in 1992, is by far the best studied. This synthetic molecule has high affinity for MT1 and MT2 receptors, but also present moderate affinity for the serotonin receptor *5HT2C*. There is few clinical evidence of the effect of agomelatine in reducing overall depressive

symptoms: results show agomelatine's higher response and lower remission rate compared to placebo (96, 97) and its similar efficacy compared to other antidepressants (i.e., paroxetine, fluoxetine, sertraline, escitalopram, and venlafaxine) (97, 98). However, most studies did not show superior or additional effects. Agomelatine seems to be better tolerated than other antidepressant agents, as no significant adverse events have been reported; furthermore, it did not show significant discontinuation symptoms. Some studies suggest agomelatine offers benefits for initial insomnia, improvement of sleep quality and efficiency, reduced daytime sleepiness, but no changes in sleep architecture of depressed patients (97). In one study (99), agomelatine was used in difficult-to-treat and refractory patients showing a significant improvement after 12 weeks. Of note, samples are overall composed of mild to moderate cases of depression. In addition, most studies compared agomelatine with low doses of other antidepressants. Finally, the evidence on melatonin or agomelatine efficacy in preventing or treating SAD is highly controversial (100), with no solid support for its prescription.

The knowledge of clinical use of melatonin is growing, with evidence supporting the prescription of exogenous melatonin for a few clinical situations. In the case of adult chronic insomnia, a few studies show modest benefits for sleep onset, sleep latency and total sleep time, although clinical significance is still questionable (101, 102). For this recommendation, typical doses are in the range of 1–5 mg. Melatonin is also currently recommended as an adjuvant treatment of insomnia in children and adolescents, particularly in those with comorbid ADHD or autism (103). In this case, the prescribed dose should be initially of 0.2–0.5 mg, 3–4 h prior to bedtime, with progressive increase up to 3 mg for children or 5 mg for adolescents (104).

Circadian Rhythm Sleep-Wake Disorders are a set of clinical conditions to which melatonin are classically indicated. For sleep disturbances in shift workers, the readjustment to nighttime sleep following a night work shift or the promotion of desired daytime sleep can be reached with doses of no more than 1–3 mg about 30 min prior to the desired sleep onset (105, 106). In the case of jet lag syndrome of eastward trips, exogenous melatonin can promote the adjustment of sleep phase when taken at the desired destination bedtime (107, 108). For individuals with Delayed Sleep-Wake Phase Disorder, 0.5 up to 5 mg melatonin scheduled at ∼1.5–2 h prior to habitual bedtime significantly advanced sleep onset (109–111). Finally, 0.5 mg of melatonin either 1 h prior to a preferred bedtime or at a fixed time can hasten synchronization of individuals with 24-h Sleep-Wake Rhythm Disorder (107).

In conclusion, the evidence supporting the clinical use of melatonin should be analyzed very carefully. A potential therapeutic effect of melatonin for mood disorders can only be expected if compatible with the physiological regulation of melatonin detailed in this review. Pineal melatonin is a neuro-endocrine message of darkness that is physiologically secreted in humans after a few minutes in the dark, peaking shortly after, serving as an internal temporal cue to cells, tissues, and systems. Extra-pineal production of melatonin happens in a constitutive manner, and on-demand production or suppression may also be regulated by levels of glucocorticoids, and different stages of an inflammatory response triggered by immune-competent cells. Hence, the optimization of potential therapeutic uses of melatonin in psychiatric disorders must encompass the specificities of an integrative molecule inextricably linked to entrainment, metabolism, immunity, neurotransmission, and cell homeostasis.

CONCLUSION AND PERSPECTIVES

Localizing phenomena, dissecting underlying mechanisms, and decomposing systems to understand their functioning have been helpful strategies in building knowledge in Biology. The downside of tackling questions in such a manner is that one might overlook how interconnected systems are, and thus lose perspective of the whole. Therein lies the challenge of decomposing complex regulatory structures like the timekeeping and melatonergic systems.

Pineal melatonin is synthesized in the absence of light (typically at night) under sympathetic control. Its production relies on the neurotransmitter norepinephrine and requires serotonin as precursor, monoamines linked to depression. The rhythmic production of pineal melatonin is a message of darkness to the body, which aids in synchronizing oscillations in physiological functions and behaviors. Nevertheless, melatonin may be implicated in the pathophysiology of depressive mood for its chronobiotic effects and the various immunological processes in which it is involved. A growing number of studies highlight the relevance of exploring the potential of melatonin extra-pineal synthesis, as well as the regulation of pineal melatonin production apart from the circadian light stimuli. Therefore, the study of melatonin in the context of depression must acknowledge its regulatory effect via chronobiotic (endocrine), paracrine and autocrine functions. It is crucial to acknowledge that detectable circulating levels of melatonin are a reflection of its amplitude and phase of secretion.

Studies of the pharmacodynamics of exogenous melatonin or melatonin agonists should always anticipate distinct clinical outputs depending on the route of administration, dosage, and timing, as these substances would potentially represent (or interfere with) different physiological actions. The pleiotropic actions through which melatonin might affect mood include the role of melatonin as an internal temporal cue, as a neurohormone in close relation with the monoaminergic system, and its indirect effects on depression via the immune and stress response systems. In this context, the resulting effects of melatonin actions on mood must be understood as a more complex and multifactorial pattern of systemic regulation, rather than an "antidepressant" effect *per se*.

AUTHOR CONTRIBUTIONS

AT and EE reviewed and edited the final manuscript. MH was responsible for funding acquisition. All authors participated in the conceptualization and the writing of the original draft.

FUNDING

The authors wish to acknowledge funding the Research Support Fund at Hospital de Clínicas de Porto Alegre (FIPE-HCPA) MH, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2013/13691-1) RM. The following received fellowships: AT and LP from Higher Education Personnel Improvement Coordination (CAPES); 305331/2012-4 PQ/CNPq to MH; senior fellowship to RM from CNPq.

REFERENCES

1. Markus RP, Fernandes PA, Kinker GS, Cruz-Machado SdS, Marçola M. Immune-pineal axis – acute inflammatory responses coordinate melatonin synthesis by pinealocytes and phagocytes. *Br J Pharmacol.* (2018) 175:3239–50. doi: 10.1111/bph.14083
2. Simonneaux V, Ribelayga C. Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol Rev.* (2003) 55:325–95. doi: 10.1124/pr.55.2.2
3. Ben-Hamo M, Larson TA, Duge LS, Sikkema C, Wilkinson CW, de la Iglesia HO, et al. Circadian forced desynchrony of the master clock leads to phenotypic manifestation of depression in rats. *eNeuro.* (2016) 3. doi: 10.1523/ENEURO.0237-16.2016
4. Landgraf D, Long JE, Proulx CD, Barandas R, Malinow R, Welsh DK. Genetic disruption of circadian rhythms in the suprachiasmatic nucleus causes helplessness, behavioral despair, and anxiety-like behavior in mice. *Biol Psychiatry.* (2016) 80:827–35. doi: 10.1016/j.biopsych.2016.03.1050
5. Hasler G. Pathophysiology of depression: do we have any solid evidence of interest to clinicians? *World Psychiatry.* (2010) 9:155–61. doi: 10.1002/j.2051-5545.2010.tb00298.x
6. Institute of Medicine (US) Committee on Nervous System Disorders in Developing Countries. *Neurological, Psychiatric, and Developmental Disorders: Meeting the Challenge in the Developing World*. Washington, DC: National Academies Press (US) (2001). Available online at: http://www.ncbi.nlm.nih.gov/books/NBK223475/ (accessed February 18, 2021).
7. ICD-11. Available online at: https://icd.who.int/en (accessed December 7, 2020).
8. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington DC: American Psychiatric Association (2013).
9. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* (2016) 388:1545–602. doi: 10.1016/S0140-6736(16)31678-6
10. Mathers C, Fat DM, Boerma JT, World Health Organization eds. *The Global Burden of Disease: 2004 Update*. Geneva, Switzerland: World Health Organization (2008).
11. Fava M. Diagnosis and definition of treatment-resistant depression. *Biol Psychiatry.* (2003) 53:649–59. doi: 10.1016/S0006-3223(03)00231-2
12. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: A STAR*D report. *AJP.* (2006) 163:1905–17. doi: 10.1176/ajp.2006.163.11.1905
13. Kennedy SH, Lam RW, McIntyre RS, Tourjman SV, Bhat V, Blier P, et al. Canadian network for mood and anxiety treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: section 3. Pharmacological treatments. *Can J Psychiatry.* (2016) 61:540–60. doi: 10.1177/0706743716659417
14. Fried EI, Nesse RM. Depression sum-scores don't add up: why analyzing specific depression symptoms is essential. *BMC Med.* (2015) 13:72. doi: 10.1186/s12916-015-0325-4
15. Ashkenazy-Frolinger T, Kronfeld-Schor N, Juetten J, Einat H. It is darkness and not light: depression-like behaviors of diurnal unstriped Nile grass rats maintained under a short photoperiod schedule. *J Neurosci Methods.* (2010) 186:165–70. doi: 10.1016/j.jneumeth.2009.11.013
16. Deats SP, Adidharma W, Yan L. Hypothalamic dopaminergic neurons in an animal model of seasonal affective disorder. *Neurosci Lett.* (2015) 602:17–21. doi: 10.1016/j.neulet.2015.06.038
17. Fonken LK, Nelson RJ. Dim light at night increases depressive-like responses in male C3H/HeNHsd mice. *Behav Brain Res.* (2013) 243:74–8. doi: 10.1016/j.bbr.2012.12.046
18. Ohta H, Yamazaki S, McMahon DG. Constant light desynchronizes mammalian clock neurons. *Nat Neurosci.* (2005) 8:267–9. doi: 10.1038/nn1395
19. Tapia-Osorio A, Salgado-Delgado R, Angeles-Castellanos M, Escobar C. Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat. *Behav Brain Res.* (2013) 252:1–9. doi: 10.1016/j.bbr.2013.05.028
20. LeGates TA, Altimus CM, Wang H, Lee H-K, Yang S, Zhao H, et al. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature.* (2012) 491:594–8. doi: 10.1038/nature11673
21. Hampp G, Ripperger JA, Houben T, Schmutz I, Blex C, Perreau-Lenz S, et al. Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. *Curr Biol.* (2008) 18:678–83. doi: 10.1016/j.cub.2008.04.012
22. Hampp G, Albrecht U. The circadian clock and mood-related behavior. *Commun Integr Biol.* (2008) 1:1–3. doi: 10.4161/cib.1.1.6286
23. Panda S. Circadian physiology of metabolism. *Science.* (2016) 354:1008–15. doi: 10.1126/science.aah4967
24. Sulli G, Lam MTY, Panda S. Interplay between circadian clock and cancer: new Frontiers for cancer treatment. *Trends Cancer.* (2019) 5:475–94. doi: 10.1016/j.trecan.2019.07.002
25. Stone AA, Schwartz JE, Schkade D, Schwarz N, Krueger A, Kahneman D. A population approach to the study of emotion: diurnal rhythms of a working day examined with the day reconstruction method. *Emotion.* (2006) 6:139–49. doi: 10.1037/1528-3542.6.1.139
26. Boivin DB, Czeisler CA, Dijk D-J, Duffy JF, Folkard S, Minors DS, et al. Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. *Arch Gen Psychiatry.* (1997) 54:145–52. doi: 10.1001/archpsyc.1997.01830140055010
27. Oliveira MAB, Epifano K, Mathur S, Carvalho FG, Scop M, Carissimi A, et al. Validation of the English version of the mood rhythm instrument. *BMC Psychol.* (2020) 8:35. doi: 10.1186/s40359-020-00397-2
28. Pilz LK, Carissimi A, Oliveira MAB, Francisco AP, Fabris RC, Medeiros MS, et al. Rhythmicity of mood symptoms in individuals at risk for psychiatric disorders. *Sci Rep.* (2018) 8:11402. doi: 10.1038/s41598-018-29348-z
29. Lazzerini Ospri L, Prusky G, Hattar S. Mood, the circadian system, and melanopsin retinal ganglion cells. *Ann Rev Neurosci.* (2017) 40:539–56. doi: 10.1146/annurev-neuro-072116-031324
30. Reiter RJ. The pineal gland: an intermediary between the environment and the endocrine system. *Psychoneuroendocrinology.* (1983) 8:31–40. doi: 10.1016/0306-4530(83)90039-2
31. Reiter RJ, Tan DX, Kim SJ, Cruz MHC. Delivery of pineal melatonin to the brain and SCN: role of canaliculi, cerebrospinal fluid, tanycytes and Virchow–Robin perivascular spaces. *Brain Struct Funct.* (2014) 219:1873–87. doi: 10.1007/s00429-014-0719-7
32. Pévet P. The internal time-giver role of melatonin. A key for our health. *Rev Neurol.* (2014) 170:646–52. doi: 10.1016/j.neurol.2014.05.008
33. Carvalho-Sousa CE, Pereira EP, Kinker GS, Veras M, Ferreira ZS, Barbosa-Nunes FP, et al. Immune-pineal axis protects rat lungs exposed to polluted air. *J Pineal Res.* (2020) 68:e12636. doi: 10.1111/jpi.12636
34. Fernandes PA, Kinker GS, Navarro BV, Jardim VC, Ribeiro-Paz ED, Córdoba-Moreno MO, et al. Melatonin-Index as a biomarker for predicting the distribution of presymptomatic and asymptomatic SARS-CoV-2 carriers. *Melatonin Res.* (2021) 4:189–205. doi: 10.32794/mr11250090
35. Murray JM, Sletten TL, Magee M, Gordon C, Lovato N, Bartlett DJ, et al. Prevalence of circadian misalignment and its association with

depressive symptoms in delayed sleep phase disorder. *Sleep.* (2017) 40:1–10. doi: 10.1093/sleep/zsw002
36. Robillard R, Carpenter JS, Rogers NL, Fares S, Grierson AB, Hermens DF, et al. Circadian rhythms and psychiatric profiles in young adults with unipolar depressive disorders. *Transl Psychiatry.* (2018) 8:1–8. doi: 10.1038/s41398-018-0255-y
37. Nair NPV, Hariharasubramanian N, Pilapil C. Circadian rhythm of plasma melatonin in endogenous depression. *Prog Neuropsychopharmacol Biol Psychiatry.* (1984) 8:715–8. doi: 10.1016/0278-5846(84)90044-7
38. Sundberg I, Ramklint M, Stridsberg M, Papadopoulos FC, Ekselius L, Cunningham JL. Salivary melatonin in relation to depressive symptom severity in young adults. *PLOS ONE.* (2016) 11:e0152814. doi: 10.1371/journal.pone.0152814
39. Ogłodek EA, Just MJ, Szromek AR, Araszkiewicz A. Melatonin and neurotrophins NT-3, BDNF, NGF in patients with varying levels of depression severity. *Pharmacol Rep.* (2016) 68:945–951. doi: 10.1016/j.pharep.2016.04.003
40. Roenneberg T, Merrow M. The circadian clock and human health. *Curr Biol.* (2016) 26:R432–43. doi: 10.1016/j.cub.2016.04.011
41. Møller M, Baeres FM. The anatomy and innervation of the mammalian pineal gland. *Cell Tissue Res.* (2002) 309:139–50. doi: 10.1007/s00441-002-0580-5
42. Arendt J. Melatonin and human rhythms. *Chronobiol Int.* (2006) 23:21–37. doi: 10.1080/07420520500464361
43. Cruz-Machado S da S, Pinato L, Tamura EK, Carvalho-Sousa CE, Markus RP. Glia-Pinealocyte network: the paracrine modulation of melatonin synthesis by tumor necrosis factor (TNF). *PLoS ONE.* (2012) 7:e40142. doi: 10.1371/journal.pone.0040142
44. Carvalho-Sousa CE, da Silveira Cruz-Machado S, Tamura EK, Fernandes PACM, Pinato L, Muxel SM, et al. Molecular basis for defining the pineal gland and pinealocytes as targets for tumor necrosis factor. *Front Endocrinol.* (2011) 2:10. doi: 10.3389/fendo.2011.00010
45. Cruz-Machado SDS, Carvalho-Sousa CE, Tamura EK, Pinato L, Cecon E, Fernandes PACM, et al. TLR4 and CD14 receptors expressed in rat pineal gland trigger NFKB pathway. *J Pineal Res.* (2010) 49:183–92. doi: 10.1111/j.1600-079X.2010.00785.x
46. Cecon E, Fernandes PA, Pinato L, Ferreira ZS, Markus RP. Daily variation of constitutively activated nuclear factor kappa B (NFKB) in rat pineal gland. *Chronobiol Int.* (2010) 27:52–67. doi: 10.3109/07420521003661615
47. Antonioli M, Rybka J, Carvalho LA. Neuroimmune endocrine effects of antidepressants. *Neuropsychiatr Dis Treat.* (2012) 8:65–83. doi: 10.2147/NDT.S16409
48. Tao H, Chen X, Zhou H, Fu J, Yu Q, Liu Y. Changes of serum melatonin, interleukin-6, homocysteine, and complement C3 and C4 levels in patients with depression. *Front Psychol.* (2020) 11:1271. doi: 10.3389/fpsyg.2020.01271
49. Peacock BN, Scheiderer DJ, Kellermann GH. Biomolecular aspects of depression: a retrospective analysis. *Compreh Psychiatry.* (2017) 73:168–80. doi: 10.1016/j.comppsych.2016.11.002
50. Maes GA M. Oxidative/nitrosative stress and immuno-inflammatory pathways in depression: treatment implications. *Curr Pharm Des.* (2014) 20:3812–47. doi: 10.2174/13816128113196660738
51. Anderson G. Linking the biological underpinnings of depression: role of mitochondria interactions with melatonin, inflammation, sirtuins, tryptophan catabolites, DNA repair and oxidative and nitrosative stress, with consequences for classification and cognition. *Prog Neuropsychopharmacol Biol Psychiatry.* (2018) 80:255–66. doi: 10.1016/j.pnpbp.2017.04.022
52. Ali T, Rahman SU, Hao Q, Li W, Liu Z, Shah FA, et al. Melatonin prevents neuroinflammation and relieves depression by attenuating autophagy impairment through FOXO3a regulation. *J Pineal Res.* (2020) 69:e12667. doi: 10.1111/jpi.12667
53. Adam EK, Quinn ME, Tavernier R, McQuillan MT, Dahlke KA, Gilbert KE. Diurnal cortisol slopes and mental and physical health outcomes: a systematic review and meta-analysis. *Psychoneuroendocrinology.* (2017) 83:25–41. doi: 10.1016/j.psyneuen.2017.05.018
54. Tonon AC, Carissimi A, Schimitt RL, de Lima LS, Pereira FDS, Hidalgo MP. How do stress, sleep quality, and chronotype associate with clinically significant depressive symptoms? A study of young male military recruits in compulsory service. *Braz J Psychiatry.* (2020) 42:54–62. doi: 10.1590/1516-4446-2018-0286
55. Jockers R, Delagrange P, Dubocovich ML, Markus RP, Renault N, Tosini G, et al. Update on melatonin receptors: IUPHAR review 20. *Br J Pharmacol.* (2016) 173:2702–25. doi: 10.1111/bph.13536
56. Suofu Y, Li W, Jean-Alphonse FG, Jia J, Khattar NK, Li J, et al. Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc Natl Acad Sci USA.* (2017) 114:E7997–8006. doi: 10.1073/pnas.1705768114
57. Levoye A, Dam J, Ayoub MA, Guillaume J-L, Couturier C, Delagrange P, et al. The orphan GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. *EMBO J.* (2006) 25:3012–23. doi: 10.1038/sj.emboj.7601193
58. Amaral FG do, Cipolla-Neto J. A brief review about melatonin, a pineal hormone. *Arch Endocrinol Metabol.* (2018) 62:472–9. doi: 10.20945/2359-3997000000066
59. Cipolla-Neto J, Amaral FG do. Melatonin as a hormone: new physiological and clinical insights. *Endocr Rev.* (2018) 39:990–1028. doi: 10.1210/er.2018-00084
60. Mulinari S. Monoamine theories of depression: historical impact on biomedical research. *J History Neurosci.* (2012) 21:366–92. doi: 10.1080/0964704X.2011.623917
61. Alexiuk NAM, Uddin M, Vriend J. Melatonin increases the *in situ* activity of tyrosine hydroxylase in the mediobasal hypothalamus of male syrian hamsters. *Life Sci.* (1996) 59:687–94. doi: 10.1016/0024-3205(96)00350-5
62. Di Chiara G, Morelli M, Consolo S. Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. *Trends Neurosci.* (1994) 17:228–33. doi: 10.1016/0166-2236(94)90005-1
63. Zisapel N, Egozi Y, Laudon M. Inhibition of dopamine release by melatonin: regional distribution in the rat brain. *Brain Res.* (1982) 246:161–3. doi: 10.1016/0006-8993(82)90157-3
64. González S, Moreno-Delgado D, Moreno E, Pérez-Capote K, Franco R, Mallol J, et al. Circadian-related heteromerization of adrenergic and dopamine D4 receptors modulates melatonin synthesis and release in the pineal gland. *PLoS Biol.* (2012) 10:e1001347. doi: 10.1371/journal.pbio.1001347
65. Bailey MJ, Coon SL, Carter DA, Humphries A, Kim J, Shi Q, et al. Night/day changes in pineal expression of >600 genes central role of adrenergic/cAMP signaling. *J Biol Chem.* (2009) 284:7606–22. doi: 10.1074/jbc.M808394200
66. Waterman GS, Ryan ND, Perel JM, Dahl RE, Birmaher B, Williamson DE, et al. Nocturnal urinary excretion of 6-hydroxymelatonin sulfate in prepubertal major depressive disorder. *Biol Psychiatry.* (1992) 31:582–90. doi: 10.1016/0006-3223(92)90244-T
67. Carvalho LA, Gorenstein C, Moreno RA, Markus RP. Melatonin levels in drug-free patients with major depression from the southern hemisphere. *Psychoneuroendocrinology.* (2006) 31:761–8. doi: 10.1016/j.psyneuen.2006.02.010
68. Steiner M, Brown GM, Goldman S. Nocturnal melatonin and cortisol secretion in newly admitted psychiatric inpatients. *Eur Arch Psychiatry Clin Nuerosci.* (1990) 240:21–7. doi: 10.1007/BF02190088
69. Beck-Friis J, Kjellman BF, Aperia B, Undén F, von Rosen D, Ljunggren JG, et al. Serum melatonin in relation to clinical variables in patients with major depressive disorder and a hypothesis of a low melatonin syndrome. *Acta Psychiatr Scand.* (1985) 71:319–30. doi: 10.1111/j.1600-0447.1985.tb02531.x
70. Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature.* (2008) 455:894–902. doi: 10.1038/nature07455
71. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology.* (2008) 33:88–109. doi: 10.1038/sj.npp.1301574
72. Krishnan V, Nestler EJ. Animal models of depression: molecular perspectives. In: Hagan JJ, editor. *Molecular Functional Models in Neuropsychiatry Current Topics in Behavioral Neurosciences.* Berlin, Heidelberg: Springer (2011) p. 121–47.
73. Bavithra S, Sugantha Priya E, Selvakumar K, Krishnamoorthy G, Arunakaran J. Effect of melatonin on glutamate: BDNF signaling in the cerebral cortex of polychlorinated biphenyls (PCBs)-exposed adult male rats. *Neurochem Res.* (2015) 40:1858–69. doi: 10.1007/s11064-015-1677-z

74. Evely KM, Hudson RL, Dubocovich ML, Haj-Dahmane S. Melatonin receptor activation increases glutamatergic synaptic transmission in the rat medial lateral habenula. *Synapse.* (2016) 70:181–6. doi: 10.1002/syn.21892
75. Jentsch MC, Van Buel EM, Bosker FJ, Gladkevich AV, Klein HC, Oude Voshaar RC, et al. Biomarker approaches in major depressive disorder evaluated in the context of current hypotheses. *Biomark Med.* (2015) 9:277–97. doi: 10.2217/bmm.14.114
76. Lane EA, Moss HB. Pharmacokinetics of melatonin in man: first pass hepatic metabolism. *J Clin Endocrinol Metab.* (1985) 61:1214–6. doi: 10.1210/jcem-61-6-1214
77. Carvalho LA, Gorenstein C, Moreno R, Pariante C, Markus RP. Effect of antidepressants on melatonin metabolite in depressed patients. *J Psychopharmacol.* (2009) 23:315–21. doi: 10.1177/0269881108089871
78. Markus RP, Franco DG, Carvalho LA, Gentil V, Gorenstein C. Acute increase in urinary 6-sulfatoximelatonin after clomipramine, as a predictive measure for emotional improvement. *J Psychopharmacol.* (2010) 24:855–60. doi: 10.1177/0269881109102542
79. Hidalgo MPL, Caumo W, Dantas G, Franco DG, Torres IL da S, Pezzi J, et al. 6-Sulfatoxymelatonin as a predictor of clinical outcome in depressive patients. *Hum Psychopharmacol Clin Exp.* (2011) 26:252–7. doi: 10.1002/hup.1204
80. Jury Freitas J, Bertuol Xavier N, Comiran Tonon A, Carissimi A, Timm Pizutti L, Vieira Ilgenfritz CA, et al. 6-Sulfatoxymelatonin predicts treatment response to fluoxetine in major depressive disorder. *Ther Adv Psychopharmacol.* (2019) 9:2045125319881927. doi: 10.1177/2045125319881927
81. Nagy AD, Iwamoto A, Kawai M, Goda R, Matsuo H, Otsuka T, et al. Melatonin adjusts the expression pattern of clock genes in the suprachiasmatic nucleus and induces antidepressant-like effect in a mouse model of seasonal affective disorder. *Chronobiol Int.* (2015) 32:447–57. doi: 10.3109/07420528.2014.992525
82. Otsuka T, Kawai M, Togo Y, Goda R, Kawase T, Matsuo H, et al. Photoperiodic responses of depression-like behavior, the brain serotonergic system, and peripheral metabolism in laboratory mice. *Psychoneuroendocrinology.* (2014) 40:37–47. doi: 10.1016/j.psyneuen.2013.10.013
83. Stefanovic B, Spasojevic N, Jovanovic P, Dronjak S. Melatonin treatment affects changes in adrenal gene expression of catecholamine biosynthesizing enzymes and norepinephrine transporter in the rat model of chronic-stress-induced depression. *Can J Physiol Pharmacol.* (2019) 97:685–90. doi: 10.1139/cjpp-2018-0612
84. Vega-Rivera NM, Ortiz-López L, Granados-Juárez A, Estrada-Camarena EM, Ramírez-Rodríguez GB. Melatonin reverses the depression-associated behaviour and regulates microglia, fractalkine expression and neurogenesis in adult mice exposed to chronic mild stress. *Neuroscience.* (2020) 440:316–36. doi: 10.1016/j.neuroscience.2020.05.014
85. Detanico BC, Piato AL, Freitas JJ, Lhullier FL, Hidalgo MP, Caumo W, et al. Antidepressant-like effects of melatonin in the mouse chronic mild stress model. *Eur J Pharmacol.* (2009) 607:121–5. doi: 10.1016/j.ejphar.2009.02.037
86. Burgess HJ, Fogg LF. Individual differences in the amount and timing of salivary melatonin secretion. *PLoS ONE.* (2008) 3:e3055. doi: 10.1371/journal.pone.0003055
87. Crescenzo FD, Lennox A, Gibson JC, Cordey JH, Stockton S, Cowen PJ, et al. Melatonin as a treatment for mood disorders: a systematic review. *Acta Psychiatr Scand.* (2017) 136:549–58. doi: 10.1111/acps.12755
88. Dolberg OT, Hirschmann S, Grunhaus L. Melatonin for the treatment of sleep disturbances in major depressive disorder. *Am J Psychiatry.* (1998) 155:1119–21. doi: 10.1176/ajp.155.8.1119
89. Serfaty MA, Osborne D, Buszewicz MJ, Blizard R, Raven PW. A randomized double-blind placebo-controlled trial of treatment as usual plus exogenous slow-release melatonin (6 mg) or placebo for sleep disturbance and depressed mood. *Int Clin Psychopharmacol.* (2010) 25:132–42. doi: 10.1097/YIC.0b013e32832c260b
90. Fava M, Targum SD, Nierenberg AA, Bleicher LS, Carter TA, Wedel PC, et al. An exploratory study of combination buspirone and melatonin SR in major depressive disorder (MDD): a possible role for neurogenesis in drug discovery. *J Psychiatr Res.* (2012) 46:1553–63. doi: 10.1016/j.jpsychires.2012.08.013
91. Lewy AJ, Bauer VK, Cutler NL, Sack RL. Melatonin treatment of winter depression: a pilot study. *Psychiatry Res.* (1998) 77:57–61. doi: 10.1016/S0165-1781(97)00128-5
92. Sherer MA, Weingartner H, James SP, Rosenthal NE. Effects of melatonin on performance testing in patients with seasonal affective disorder. *Neurosci Lett.* (1985) 58:277–82. doi: 10.1016/0304-3940(85)90066-7
93. Danilenko KV, Putilov AA. Melatonin treatment of winter depression following total sleep deprivation: waking EEG and mood correlates. *Neuropsychopharmacology.* (2005) 30:1345–52. doi: 10.1038/sj.npp.1300698
94. Lewy AJ, Lefler BJ, Emens JS, Bauer VK. The circadian basis of winter depression. *Proc Natl Acad Sci USA.* (2006) 103:7414–9. doi: 10.1073/pnas.0602425103
95. Hansen MV, Danielsen AK, Hageman I, Rosenberg J, Gögenur I. The therapeutic or prophylactic effect of exogenous melatonin against depression and depressive symptoms: a systematic review and meta-analysis. *Eur Neuropsychopharmacol.* (2014) 24:1719–28. doi: 10.1016/j.euroneuro.2014.08.008
96. Krause M, Gutsmiedl K, Bighelli I, Schneider-Thoma J, Chaimani A, Leucht S. Efficacy and tolerability of pharmacological and non-pharmacological interventions in older patients with major depressive disorder: a systematic review, pairwise and network meta-analysis. *Eur Neuropsychopharmacol.* (2019) 29:1003–22. doi: 10.1016/j.euroneuro.2019.07.130
97. De Berardis D, Marini S, Fornaro M, Srinivasan V, Iasevoli F, Tomasetti C, et al. The melatonergic system in mood and anxiety disorders and the role of agomelatine: implications for clinical practice. *Int J Mol Sci.* (2013) 14:12458–83. doi: 10.3390/ijms140612458
98. Guaiana G, Gupta S, Chiodo D, Davies SJ, Haederle K, Koesters M. Agomelatine versus other antidepressive agents for major depression. *Cochrane Database Syst Rev.* (2013) 12:CD008851. doi: 10.1002/14651858.CD008851.pub2
99. Sparshatt A, Williams RHM, Baldwin DS, Haddad PM, Bazire S, Weston E, et al. A naturalistic evaluation and audit database of agomelatine: clinical outcome at 12 weeks. *Acta Psychiatr Scand.* (2013) 128:203–11. doi: 10.1111/acps.12044
100. Nussbaumer-Streit B, Greenblatt A, Kaminski-Hartenthaler A, Noord MGV, Forneris CA, Morgan LC, et al. Melatonin and agomelatine for preventing seasonal affective disorder. *Cochrane Database Syst Rev.* (2019) 6:CD011271. doi: 10.1002/14651858.CD011271.pub3
101. Brzezinski A, Vangel MG, Wurtman RJ, Norrie G, Zhdanova I, Ben-Shushan A, et al. Effects of exogenous melatonin on sleep: a meta-analysis. *Sleep Med Rev.* (2005) 9:41–50. doi: 10.1016/j.smrv.2004.06.004
102. Low TL, Choo FN, Tan SM. The efficacy of melatonin and melatonin agonists in insomnia - an umbrella review. *J Psychiatr Res.* (2020) 121:10–23. doi: 10.1016/j.jpsychires.2019.10.022
103. Buckley AW, Hirtz D, Oskoui M, Armstrong MJ, Batra A, Bridgemohan C, et al. Practice guideline: treatment for insomnia and disrupted sleep behavior in children and adolescents with autism spectrum disorder: report of the guideline development, dissemination, and implementation subcommittee of the American Academy of Neurology. *Neurology.* (2020) 94:392–404. doi: 10.1212/WNL.0000000000009033
104. Bruni O, Alonso-Alconada D, Besag F, Biran V, Braam W, Cortese S, et al. Current role of melatonin in pediatric neurology: clinical recommendations. *Eur J Paediatr Neurol.* (2015) 19:122–33. doi: 10.1016/j.ejpn.2014.12.007
105. Liira J, Verbeek JH, Costa G, Driscoll TR, Sallinen M, Isotalo LK, et al. Pharmacological interventions for sleepiness and sleep disturbances caused by shift work. *Cochrane Database Syst Rev.* (2014) 8:CD009776. doi: 10.1002/14651858.CD009776.pub2
106. Morgenthaler TI, Lee-Chiong T, Alessi C, Friedman L, Aurora RN, Boehlecke B, et al. Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. An American Academy of Sleep Medicine report. *Sleep.* (2007) 30:1445–59. doi: 10.1093/sleep/30.11.1445
107. Auger RR, Burgess HJ, Emens JS, Deriy LV, Thomas SM, Sharkey KM. Clinical practice guideline for the treatment of intrinsic circadian rhythm sleep-wake disorders: advanced sleep-wake phase disorder (ASWPD), delayed sleep-wake phase disorder (DSWPD), non-24-hour sleep-wake rhythm disorder (N24SWD), and irregular sleep-wake rhythm disorder (ISWRD). An Update for

2015: An American Academy of Sleep Medicine Clinical Practice Guideline. *J Clin Sleep Med.* (2015) 11:1199–236. doi: 10.5664/jcsm.5100

108. Lewy A. Clinical implications of the melatonin phase response curve. *J Clin Endocrinol Metab.* (2010) 95:3158–60. doi: 10.1210/jc.2010-1031
109. Sletten TL, Magee M, Murray JM, Gordon CJ, Lovato N, Kennaway DJ, et al. Efficacy of melatonin with behavioural sleep-wake scheduling for delayed sleep-wake phase disorder: a double-blind, randomised clinical trial. *PLoS Med.* (2018) 15:e1002587. doi: 10.1371/journal.pmed.1002587
110. Mundey K, Benloucif S, Harsanyi K, Dubocovich ML, Zee PC. Phase-dependent treatment of delayed sleep phase syndrome with melatonin. *Sleep.* (2005) 28:1271–8. doi: 10.1093/sleep/28.10.1271
111. Ferracioli-Oda E, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. *PLoS ONE.* (2013) 8:e63773. doi: 10.1371/journal.pone.0063773

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 15 June 2021
doi: 10.3389/fpsyt.2021.626486

The Effect of Adjunctive Mangosteen Pericarp on Cognition in People With Schizophrenia: Secondary Analysis of a Randomized Controlled Trial

Wolfgang Marx[1], David R. Skvarc[1], Mohammadreza Mohebbi[2], Adam J. Walker[1], Alcy Meehan[1], Alyna Turner[1,3], Andrea Baker[4], Seetal Dodd[1,5], Sue M. Cotton[5,6], James Graham Scott[4,7,8], Bianca E. Kavanagh[1], Melanie M. Ashton[1], Ellie Brown[5,6], John J. McGrath[4,9,10], Michael Berk[1] and Olivia May Dean[1,11]*

[1] Deakin University, The Institute for Mental and Physical Health and Clinical Translation (IMPACT), School of Medicine, Geelong, VIC, Australia, [2] Biostatistics Unit, Faculty of Health, Deakin University, Geelong, VIC, Australia, [3] School of Medicine and Public Health, Faculty of Health and Medicine, The University of Newcastle, Newcastle, NSW, Australia, [4] Queensland Center for Mental Health Research, The Park Center for Mental Health, Wacol, QLD, Australia, [5] Center for Youth Mental Health, The University of Melbourne, Parkville, VIC, Australia, [6] Orygen, Parkville, VIC, Australia, [7] QIMR Berghofer Medical Research Institute Mental Health Programme, Herston, QLD, Australia, [8] Metro North Mental Health Service Herston, Herston, QLD, Australia, [9] Queensland Brain Institute, University of Queensland, St Lucia, QLD, Australia, [10] National Center for Register-Based Research, Aarhus University, Aarhus, Denmark, [11] Florey Institute for Neuroscience and Mental Health, University of Melbourne, Parkville, VIC, Australia

OPEN ACCESS

Edited by:
Zhang-Jin Zhang, The University of Hong Kong, China

Reviewed by:
Nenad Naumovski, University of Canberra, Australia
Tomiki Sumiyoshi, National Center of Neurology and Psychiatry, Japan

***Correspondence:**
Wolfgang Marx
wolf.marx@deakin.edu.au

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *06 November 2020*
Accepted: *14 May 2021*
Published: *15 June 2021*

Citation:
Marx W, Skvarc DR, Mohebbi M, Walker AJ, Meehan A, Turner A, Baker A, Dodd S, Cotton SM, Scott JG, Kavanagh BE, Ashton MM, Brown E, McGrath JJ, Berk M and Dean OM (2021) The Effect of Adjunctive Mangosteen Pericarp on Cognition in People With Schizophrenia: Secondary Analysis of a Randomized Controlled Trial. Front. Psychiatry 12:626486. doi: 10.3389/fpsyt.2021.626486

Background: Cognitive impairment is prevalent and often highly burdensome in people with schizophrenia. The aim of this study was to investigate if mangosteen (*Garcinia mangostana* Linn.) pericarp extract may be an effective intervention to improve cognitive performance in this population.

Methods: This was a secondary analysis of a larger randomized placebo-controlled trial that investigated a 24-weeks intervention of mangosteen pericarp extract supplementation in people diagnosed with schizophrenia. A subset of $n = 114$ participants with completed cognitive outcomes at follow up were included in this analysis. Using the Cogstate Brief Battery, the following cognitive outcomes were assessed: psychomotor function, attention, visual learning and memory (visual and working). Subgroup analyses investigated whether baseline clinical parameters (baseline cognitive functioning, illness severity and duration, depressive symptoms) moderated the relationship between mangosteen pericarp extract intervention and change in cognitive outcomes.

Results: There were no significant between-group changes in any cognitive outcomes assessed. Subgroup analysis based on baseline cognition and clinical characteristics did not reveal any significant between-group difference in change.

Conclusions: Mangosteen pericarp extract did not affect cognitive outcomes in people with schizophrenia. Further investigation regarding optimal dosing strategies

for mangosteen interventions and the testing of additional cognitive domains may be warranted.

Trial Registration: ANZCTR.org.au identifier: ACTRN12616000859482, registered 30 June 3 2016.

Keywords: mangosteen, *Mangostana garcinia* Linn., schizophrenia, schizoaffective disorder, psychiatry, mental disorders, cognition

INTRODUCTION

Cognitive impairment is highly prevalent in people living with schizophrenia, with upwards of 80% experiencing considerable cognitive deficits (1). These wide-ranging cognitive impairments negatively influence daily life, with memory and processing speed most affected (2, 3). Prior reviews have demonstrated the negative effect of this cognitive impairment on functional outcomes including career success and independent living (3). These deficits are not explained by prescribed pharmacotherapy, duration of illness, or psychotic symptoms but rather, are core symptoms of schizophrenia (4). There is limited support for conventional pharmacological interventions improving cognition and functioning in schizophrenia. This is highlighted in a large meta-analysis ($n = 93$ trials) that reported a minor pooled treatment effect ($g = 0.10$) for global cognition, with no significant effects for any cognitive subdomain and limited support for any one treatment type (5).

Preliminary clinical and preclinical data suggest that mangosteen (*Garcinia mangostana* Linn.) pericarp contains unique bioactive phytochemicals such as flavonoids and xanthones that may beneficially modulate pathways implicated in schizophrenia-related cognitive impairment, including antioxidant, neuroprotective, anti-inflammatory, and mitochondrial-enhancing properties (6). Mangosteen was found to mitigate cognitive deficits and ameliorate oxidative stress within the hippocampus of Flinders Sensitive Line rats (7), and prevented age-related cognitive impairment and increased BDNF levels in older C57BL/6J (B6) mice (8). Similar results were reported in an animal model of Alzheimer's disease wherein mangosteen mitigated scopolamine-induced memory impairment in Morris water maze and passive avoidance tests as well as mitigated oxidative stress (9).

To date, there are no human intervention studies that have investigated the potential effect of mangosteen on cognitive outcomes in people with schizophrenia or otherwise. Hence, due to the need for novel interventions to manage schizophrenia-related cognitive impairment, coupled with the promising pre-clinical efficacy and mechanistic evidence in support of mangosteen, the aims of this study were to:

1. Determine whether 24 weeks of adjunctive mangosteen pericarp extract supplementation affected change in cognitive functioning from baseline (specifically, psychomotor function, attention, visual learning and visual/working memory) compared to placebo,
2. Investigate whether baseline clinical parameters (baseline cognitive functioning, illness severity and duration, depressive symptoms) moderated the relationship between mangosteen pericarp extract intervention and change in cognitive outcomes.

METHODS

This study is a secondary analysis of a randomized controlled trial (ANZCTR.org.au identifier: ACTRN12616000859482) that completed data collection in February 2019. The full study protocol has been published elsewhere (10). Primary analysis of cognitive outcomes were preregistered and the investigation of subgroup responses was conducted as a *post-hoc* subgroup analysis. In brief, this was a 24-weeks double-blind, placebo-controlled (1:1 treatment allocation ratio) randomized clinical trial that was conducted in two sites in Australia (Geelong, Victoria, and Brisbane, Queensland). The timeframe and dose for this study was informed by a previous pilot study (11). Participants provided written informed consent. Human ethics approval was received from Barwon Health Human Research Ethics Committee (HREC), Geelong, Victoria (reference number 15/26); and Metro South Health Service District HREC, Queensland (reference number HREC/16/QPAH/15). Participating institutions included Deakin University, University of Queensland, Barwon Health and the Metro South Health Service.

Eligibility Criteria

Participants were eligible if they met the following inclusion criteria: aged ≥18 years, diagnosed schizophrenia or schizoaffective disorder using Diagnostic and Statistical Manual of Mental Disorders (fifth edition, DSM-5) diagnostic criteria; scored ≥54 on the PANSS and/or ≥3 on the Clinical Global Impressions severity of illness scale (CGI-S); treatment stable for ≥4 weeks prior to enrolment (if on psychotropic therapy); using effective contraception (if female); able to speak, read, write, and understand the English language; have a current treating physician; and have capacity to consent to the study. Exclusion criteria were the following: known or suspected clinically unstable systemic medical disorder; pregnant or breastfeeding; contraindications or intolerance to mangosteen pericarp or any of the trial preparations; or currently enrolled in another clinical trial.

Intervention

Participants randomly assigned to the intervention received mangosteen pericarp extract capsules (1,000 mg/days, two 500 mg capsules per day, VitalXan, Adelaide, Australia). Further details of the intervention product are described in the protocol

paper (10). Matched placebo tablets were produced to be identical to the intervention in appearance, color and taste. The intervention and placebo were packaged in identical bottles to ensure double-blinding.

Outcomes

Using the CogState Brief Battery, Maruff et al. (12) delivered on identically configured laptop computers at both sites, the following cognitive outcomes were assessed based on participant performance speed (scored using the mean of the log_{10} transformed reaction times for correct responses) and/or accuracy (scored using the arcsine transformation of the square root of the proportion of correct responses):

Psychomotor function was assessed using the speed of performance in the Detection test, which measures processing speed during a simple reaction time design. *Attention* was measured using the speed of performance in the Identification test, which measures attention using a choice reaction time paradigm whereby participants are required to correctly identify the color of flipped cards as quickly as they can. The speed of performance and accuracy scores of the One Card Learning test was used to assess *visual learning and visual memory* using a pattern separation paradigm whereby the participant is asked to identify previously displayed playing cards correctly. *Working memory* was tested using the speed of performance and accuracy scores of the One Back test, which incorporates a n-back paradigm, whereby participants are asked to correctly identify if the current card matches the previously drawn card.

Based on performance on these subtests, two composite outcomes were derived; (i) *Learning-memory* composite, which was derived from the One Back test and One Card Learning test to derive composite accuracy and performance speed outcomes; and (ii) a *Psychomotor composite* score combining performance speed outcomes of the Detection and Identification tests.

To investigate the treatment response based on baseline cognitive function, we defined low baseline cognitive performance as one standard deviation from the mean of the sample due to two considerations. Firstly, a deficit of one standard deviation from the mean performance is characteristic of mild cognitive impairment on the psychomotor composite (13). Second, one standard deviation change is a commonly accepted signifier of cognitive decline over time (14). Treatment response was also assessed according to baseline clinical symptom severity using the Positive and Negative Syndrome Scale (PANSS) total score using cut-offs of >95 (Marked to Severe) (15). Positive ($PANSS_P$) and negative ($PANSS_N$) sub scores (16), duration of illness (years), and depressive symptoms [Montgomery Åsberg Depression Rating Scale, (MADRS)] were also assessed (17).

Statistical Methods

Statistical analyses were conducted using IBM® SPSS® Statistics Version 26.0. *P*-values were set at <0.01 to account for multiple comparisons false discovery rate. Participant characteristics were reported as mean (standard deviation) or as a percentage, as appropriate.

TABLE 1 | Participant demographics.

		Total	Placebo	Intervention
DEMOGRAPHIC				
Gender %male	*n* (%)	80 (70)	39 (72.2)	41 (67.2)
Age	M (SD)	39 (11.77)	39.06 (12.35)	39.05 (11.36)
Country of Birth (Australian Born)	*n* (%)	94 (82)	44 (81.5)	50 (82)
Aboriginal/Torres Strait Islander	*n* (%)	3 (2.6)	3 (5.6)	0 (0)
DIAGNOSIS				
Schizophrenia	*n* (%)	96 (83.5)	44 (81.5)	52 (85.2)
Schizoaffective disorder	*n* (%)	19 (16.5)	10 (18.5)	9 (14.8)
CLINICAL CHARACTERISTICS				
Age of diagnosis	M (SD)	25 (8.11)	25.93 (7.62)	24.48 (8.53)
PANSS total	M (SD)	73 (14.004)	69.13 (12.8)	76.52 (14.21)
MADRS	M (SD)	11 (9.02)	9.94 (7.98)	12.3 (9.79)

MADRS, Montgomery-Asberg Depression Rating Scale; PANSS, Positive and Negative Syndrome Scale.

Following the data analysis approach of the original RCT, a modified intention to treat was implemented and missing data for participants that completed the Cogstate assessment at follow up were imputed using multiple imputation (five imputations) technique with missing at random assumption. All cognitive outcomes were transformed to standardized z scores for analysis. Generalized estimation equation (GEE) approach with identity link assuming Normal distribution for the outcome was implemented for all main and secondary analyses. The GEE model includes nominal time, nominal group allocation and the two-way interaction between time and group allocation. In this setting, the two-way interaction between time and group allocation estimates the between group differential change from baseline to week 24 in the intervention vs. control group. An unstructured covariance pattern was considered to account for within participants autocorrelation in time. Cohen's d of between group differential change were also calculated. Effect sizes > 0.50 are interpreted as large, effect size of 0.50–0.30 as medium, effect size of 0.30–0.10 as small, and those <0.10 as trivial (18).

RESULTS

Of the 145 participants recruited to the original study, 114 participants that completed cognitive assessment at baseline and follow up were included in this analysis. The average age of the sample was 39 years (SD = 11.771), and 70% were male (see **Table 1**). Most participants were Australian born (82%, $n = 94$). Regarding the clinical characteristics of the cohort, most had a diagnosis of schizophrenia (83.5%) with the remainder diagnosed with schizoaffective disorder (16.5%). The average age of formal diagnosis was 25.2 (SD = 8.0) years.

TABLE 2 | Mean (±95% confidence intervals) in cognitive outcomes (standardized Z scores) from baseline to end of treatment.

	Placebo		Intervention			
	Baseline	Follow up	Baseline	Follow up	Between-group change (95% CI)	Cohens D
DETECTION TEST						
Speed of performance	−0.13 (−0.52, 0.27)	0.01 (−0.22, 0.24)	−0.009 (−0.37, 0.35)	0.01 (−0.24, 0.27)	−0.12 (−0.65, 0.42)	0.10
IDENTIFICATION TEST						
Speed of performance	−0.02 (−0.25, 0.21)	0.0004 (−0.23, 0.23)	0.02 (−0.26, 0.29)	−0.02 (−0.27, 0.23)	−0.05 (−0.38, 0.28)	0.05
ONE CARD LEARNING TEST						
Speed of performance	−0.04 (−0.3, 0.22)	−0.0003 (−0.23, 0.23)	0.02 (−0.32, 0.37)	0.003 (−0.25, 0.25)	−0.06 (−0.49, 0.37)	0.05
Accuracy	0.04 (−0.34, 0.43)	0.0003 (−0.22, 0.22)	0.3 (−0.37, 0.97)	0.002 (−0.25, 0.26)	−0.25 (−1.15, 0.64)	0.14
ONE BACK TEST						
Speed of performance	0.0001 (−0.23, 0.23)	0.0004 (−0.23, 0.23)	−0.07 (−0.61, 0.47)	−0.02 (−0.3, 0.25)	0.04 (−0.56, 0.64)	0.03
Accuracy	−0.005 (−0.23, 0.22)	0.00004 (−0.23, 0.23)	−0.06 (−0.36, 0.24)	−0.04 (−0.29, 0.21)	0.02 (−0.41, 0.44)	0.02
LEARNING-MEMORY COMPOSITE						
Speed of performance	−0.04 (−0.4, 0.32)	0.0002 (−0.37, 0.37)	−0.04 (−0.81, 0.73)	−0.03 (−0.44, 0.37)	−0.03 (−0.83, 0.77)	0.11
Accuracy	0.04 (−0.45, 0.53)	0.0003 (−0.4, 0.4)	0.26 (−0.52, 1.03)	−0.03 (−0.41, 0.34)	−0.25 (−1.32, 0.81)	0.01
PSYCHOMOTOR COMPOSITE						
Speed of performance	−0.15 (−0.68, 0.39)	0.01 (−0.39, 0.41)	0.03 (−0.52, 0.58)	0.02 (−0.37, 0.41)	−0.17 (−0.87, 0.54)	0.09

Mangosteen Pericarp Extract Supplementation and Cognitive Outcomes

There was no between-group difference in differential change from baseline to 24 weeks for all cognitive outcomes measured (**Table 2**). Although the mangosteen intervention reported a greater change for all cognitive outcomes compared to the placebo group, the effect sizes were low for all outcomes (Cohens $d < 0.11$).

Similarly, subgroup analyses based on low baseline cognition reported no differences in change between groups (**Table 3**). Further sensitivity analyses based on baseline schizophrenia severity (PANSS total score, negative and positive sub-scores) and duration, as well as depressive symptoms (MADRS), also reported no difference between groups.

DISCUSSION

This is the first study to investigate the effect of mangosteen pericarp extract on cognition in people with schizophrenia. Despite promising preclinical and mechanistic data (6), there was no significant difference in treatment effect compared to placebo, and the results do not support this intervention as an effective therapy for cognitive outcomes in people with schizophrenia. Our findings, together with the finding that mangosteen pericarp extract did not significantly affect between-group differences in change in schizophrenia symptom scores (as measured by the PANSS) (19), weaken the hypothesis that mangosteen pericarp extract has clinical utility for those with schizophrenia.

The results of this trial are also in line with previous nutraceutical interventions for schizophrenia-related cognitive performance (20). A recent review reported that omega-3 fatty acids and taurine failed to improve any measure of cognitive performance (20). N-acetyl cysteine improved some individual cognitive domains, but not global cognition (20). Furthermore, a previous trial that investigated another polyphenol intervention, resveratrol, in people with schizophrenia also reported no significant improvement (21).

The results of this study are in contrast to the extant polyphenol literature in other populations, which has reported improved cognitive outcomes in other populations, including in healthy adults and people with mild cognitive impairment (22, 23). While mangosteen pericarp is rich in polyphenol compounds, the biological properties of the diverse range of polyphenol compounds are not uniform, and so other polyphenol interventions that have demonstrated improvements in other conditions may act on different biological pathways to those polyphenols included in mangosteen pericarp.

While we explored potential baseline factors to identify possible sub-populations that may display greater treatment response, an additional potential explanation for the null findings is that unexplored factors may influence treatment response. In particular, inter-individual differences in the metabolism and pharmacokinetics has been identified for other polyphenol compounds (24). For example, the metabolism of ellagitannins, found in high concentrations in pomegranate husk and juices, is greatly influenced by individual gut microbiota composition (24). The limited studies that have investigated the bioavailability and pharmacokinetics of mangosteen polyphenols also indicate high inter-individual variability with marked variation in the area under the curve (762–4,030 nmol/L × h) of the primary polyphenolic compound, α-mangostin, in serum (25). A related consideration is the need for further investigation of optimal dosing regimens. A previous study reported that roughly 2% of consumed mangosteen polyphenols were absorbed (25), suggesting low bioavailability. Similar low absorption rates have been reported for other polyphenol compounds such as resveratrol and curcumin where novel methods to improve bioavailability have been introduced (26, 27).

These factors speak to the difficulty and complexity of nutraceutical research where the bioavailability and treatment

TABLE 3 | Subgroup analyses of cognitive outcomes (standardized Z scores) from baseline to end of treatment.

	Baseline cognition		Baseline PANSS Total		PANSS positive		PANSS negative		Duration of illness		MADRSS	
	Normal–High	Low	Marked to Severe	Mild to Moderate	High	Low	High	Low	>13 years	<13 years	High	Low
DETECTION TEST												
Speed of performance	−0.04 (−0.6, 0.51)	−0.69 (−1.51, 0.13)	0.43 (−4.01, 4.87)	−0.12 (−0.67, 0.42)	0.24 (−0.29, 0.77)	−0.55 (−1.6, 0.49)	0.11 (−0.46, 0.68)	−0.38 (−1.31, 0.56)	−0.45 (−1.36, 0.46)	0.22 (−0.4, 0.84)	−0.41 (−1.3, 0.48)	0.2 (−0.42, 0.81)
IDENTIFICATION TEST												
Speed of performance	−0.08 (−0.39, 0.24)	0.59 (−0.45, 1.63)	−1.19 (−4.23, 1.85)	0.004 (−0.28, 0.29)	0.03 (−0.46, 0.52)	−0.14 (−0.59, 0.31)	−0.03 (−0.53, 0.48)	−0.09 (−0.49, 0.31)	−0.09 (−0.57, 0.39)	−0.02 (−0.43, 0.4)	−0.11 (−0.57, 0.35)	−0.02 (−0.46, 0.42)
ONE CARD LEARNING TEST												
Speed of performance	−0.19 (−0.6, 0.23)	0.52 (−0.74, 1.77)	−1 (−4.2, 2.2)	−0.0005 (−0.41, 0.41)	0.07 (−0.55, 0.7)	−0.28 (−0.8, 0.24)	−0.04 (−0.6, 0.52)	−0.08 (−0.71, 0.55)	−0.31 (−0.86, 0.24)	0.23 (−0.39, 0.86)	−0.13 (−0.8, 0.53)	−0.01 (−0.56, 0.54)
Accuracy	−0.57 (−3.35, 2.2)	−0.09 (−0.95, 0.77)	−3.91 (−10.43, 2.61)	−0.07 (−0.92, 0.78)	−0.38 (−2.03, 1.28)	−0.1 (−1.15, 0.95)	−0.82 (−1.99, 0.34)	0.45 (−1.31, 2.22)	−0.15 (−1.06, 0.77)	−0.35 (−1.76, 1.05)	−0.44 (−2.53, 1.65)	−0.06 (−1.46, 1.33)
ONE BACK TEST												
Speed of performance	−0.02 (−0.99, 0.95)	0.13 (−0.88, 1.13)	−0.44 (−3.01, 2.13)	0.09 (−0.5, 0.67)	−0.03 (−0.95, 0.88)	0.08 (−0.53, 0.69)	−0.1 (−1.11, 0.91)	0.22 (−0.42, 0.85)	0.2 (−0.68, 1.09)	−0.16 (−0.99, 0.68)	−0.26 (−0.91, 0.39)	0.36 (−0.57, 1.29)
Accuracy	−0.45 (−1.77, 0.88)	0.06 (−0.34, 0.46)	−0.65 (−2.39, 1.09)	0.05 (−0.38, 0.49)	0.04 (−0.59, 0.67)	0.03 (−0.55, 0.6)	0.07 (−0.57, 0.71)	−0.05 (−0.55, 0.46)	0.55 (−0.07, 1.17)	−0.49 (−1.13, 0.15)	−0.13 (−0.6, 0.35)	0.18 (−0.51, 0.88)
LEARNING–MEMORY COMPOSITE												
Speed of performance	−0.02 (−0.99, 0.95)	0.13 (−0.88, 1.13)	−1.41 (−6.54, 3.72)	0.06 (−0.67, 0.79)	0.04 (−1.05, 1.14)	−0.18 (−1.14, 0.77)	−0.14 (−1.46, 1.18)	0.08 (−0.83, 0.98)	−0.13 (−1.2, 0.95)	0.09 (−1.02, 1.2)	−0.39 (−1.35, 0.56)	0.34 (−0.86, 1.55)
Accuracy	−1.41 (−3.78, 0.95)	0.31 (−0.7, 1.31)	−4.74 (−11.9, 2.41)	−0.03 (−1.06, 1.01)	−0.4 (−2.27, 1.48)	−0.07 (−1.41, 1.26)	−0.79 (−2.17, 0.6)	0.12 (−1.44, 1.67)	0.39 (−0.83, 1.61)	−0.95 (−2.56, 0.67)	−0.6 (−2.85, 1.65)	0.12 (−1.44, 1.67)
PSYCHOMOTOR COMPOSITE												
Speed of performance	−0.23 (−0.99, 0.53)	0.1 (−1.24, 1.44)	−0.63 (−7.16, 5.9)	−0.13 (−0.82, 0.55)	0.3 (−0.5, 1.1)	−0.65 (−1.89, 0.58)	0.12 (−0.7, 0.94)	−0.51 (−1.65, 0.64)	−0.53 (−1.67, 0.6)	0.19 (−0.65, 1.04)	−0.53 (−1.62, 0.56)	0.17 (−0.72, 1.07)

Data presented as between-group change (95% CI).

response is potentially modulated by several factors unique to nutraceutical interventions. These factors include the absorption and treatment response of some dietary compounds, including polyphenol compounds, which can be modulated by the food matrix and degree of processing of the nutraceutical formulation. For example, polyphenols derived from apples had a substantially different effect on gene expression depending on the degree of processing (whole apple vs. puree vs. extract) (28). Furthermore, interindividual differences in pathways such as inflammation have also been shown to modulate treatment response to nutraceuticals in psychiatry (29), suggesting that subpopulations may be more amenable to some nutraceutical interventions than others. Related to this is the role of nutrient deficiency and sufficiency in modulating treatment response, whereby nutraceuticals such as vitamin D appear to have a differential treatment effect on depression depending on the baseline serum levels of vitamin D (30). Similarly, due to the presence of some nutraceutical compounds in commonly consumed food items, it is conceivable that some participants may already be consuming higher quantities of nutraceuticals including polyphenols through their habitual diet and that this may in turn, affect individual treatment response. Novel study design features including the measurement of baseline biomarkers, habitual diet, and consideration for the food matrix may improve treatment efficacy in future trials.

Strengths of this study include the rigorous study design, which incorporated double-blinding and placebo control features. The cognitive outcomes of this study were also assessed using a widely-used and validated cognitive battery. Furthermore, adherence, as assessed by pill counts at follow up, was high (94% adherence rate). We acknowledge the following limitations to this analysis. First, while this study was statistically powered based on the primary outcome of the original study (PANSS Total), the subgroup analyses conducted as part of this analysis are likely underpowered. More extensive studies may provide sufficient sample sizes to detect small treatment differences that were not able to be detected in the current analysis. Second, while we included a widely-used and validated tool to measure cognitive outcomes (CogState Brief Battery), this task configuration measures a limited sample of cognitive domains, and so additional cognitive domains may be worthy of future investigation. For example, reasoning/problem solving and social cognition, as recommended by the MATRICS cognitive battery initiative, or the expanded schizophrenia-specific battery developed by CogState (31).

CONCLUSION

Despite promising pre-clinical evidence suggesting a therapeutic effect, this study reports that mangosteen pericarp supplementation did not improve cognitive outcomes in people with schizophrenia. While baseline clinical and cognitive factors did not alter this result, potential inter-individual differences in metabolism require further exploration. Furthermore, further pre-clinical investigation of mangosteen pericarp supplementation may be warranted to identify pharmacologically active compounds and ensure that they are present in the formulation and bioavailable with oral dosage.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by reasonable request to authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human ethics approval was received from Barwon Health Human Research Ethics Committee (HREC), Geelong, Victoria, (reference number 15/26); and Metro South Health Service District HREC, Queensland (reference number HREC/16/QPAH/15). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WM led all phases of this study. DS, AW, and OD informed the analysis. OD supervised all phases of the study. MM supervised the statistical analysis. AT, AB, OD, AW, SD, SC, JS, BK, MA, EB, JM, and MB were all involved in design, data collection, and/or analysis of the original clinical trial. All authors provided input to the development of the manuscript.

FUNDING

The primary study was funded by the Stanley Medical Research Institute (Treatment Trial #14T-007). All authors gratefully acknowledge their support. We wish to thank the participants who took part in the study, the health services including Barwon Health, the West Moreton Hospital and Health Service, the Metro North Mental Health Service, Metro South Mental Health Service, Gold Coast Mental Health Service, the Melbourne Clinic and the Community and Research Network. MB was supported by a NHMRC Senior Principal Research Fellowship 1059660 and 1156072. JM was supported by grant APP1056929 from the John Cade Fellowship from the National Health and Medical Research Council. OD was supported by a NHMRC R.D. Wright Career Development Fellowship (1145634).

REFERENCES

1. McCleery A, Nuechterlein KH. Cognitive impairment in psychotic illness: prevalence, profile of impairment, developmental course, and treatment considerations. *Dial Clin Neurosci.* (2019) 21:239. doi: 10.31887/DCNS.2019.21.3/amccleery
2. Reichenberg A, Harvey PD. Neuropsychological impairments in schizophrenia: integration of performance-based and brain imaging

findings. *Psychol Bull.* (2007) 133:833. doi: 10.1037/0033-2909.133.5.833
3. Green MF, Horan WP, Lee J. Nonsocial and social cognition in schizophrenia: current evidence and future directions. *World Psychiatry.* (2019) 18:146–61. doi: 10.1002/wps.20624
4. Keefe RS. Should cognitive impairment be included in the diagnostic criteria for schizophrenia? *World Psychiatry.* (2008) 7:22. doi: 10.1002/j.2051-5545.2008.tb00142.x
5. Sinkeviciute I, Begemann M, Prikken M, Oranje B, Johnsen E, Lei WU, et al. Efficacy of different types of cognitive enhancers for patients with schizophrenia: a meta-analysis. *NPJ Schizophrenia.* (2018) 4:1–14. doi: 10.1038/s41537-018-0064-6
6. Ashton MM, Dean OM, Walker AJ, Bortolasci CC, Ng CH, Hopwood M, et al. The therapeutic potential of mangosteen pericarp as an adjunctive therapy for bipolar disorder and schizophrenia. *Front Psychiatry.* (2019) 10:115. doi: 10.3389/fpsyt.2019.00115
7. Oberholzer I, Möller M, Holland B, Dean OM, Berk M, Harvey BH. Garcinia mangostana Linn displays antidepressant-like and pro-cognitive effects in a genetic animal model of depression: a bio-behavioral study in the flinders sensitive line rat. *Metab Brain Dis.* (2018) 33:467–80. doi: 10.1007/s11011-017-0144-8
8. Huang H-J, Chen W-L, Hsieh R-H, Hsieh-Li HM. Multifunctional effects of mangosteen pericarp on cognition in C57BL/6J and triple transgenic Alzheimer's mice. *Evid Based Compl Altern Med.* (2014) 2014:813672. doi: 10.1155/2014/813672
9. Sattayasai J, Chaonapan P, Arkaravichie T, Soi-ampornkul R, Junnu S, Charoensilp P, et al. Protective effects of mangosteen extract on H 2 O 2-induced cytotoxicity in SK-N-SH cells and scopolamine-induced memory impairment in mice. *PLoS ONE.* (2013) 8:e85053. doi: 10.1371/journal.pone.0085053
10. Turner A, McGrath JJ, Dean OM, Dodd S, Baker A, Cotton SM, et al. Protocol and rationale: A 24-week double-blind, randomized, placebo controlled trial of the efficacy of adjunctive garcinia mangostana linn (Mangosteen) pericarp for schizophrenia. *Clin Psychopharm Neurosci.* (2019) 17:297. doi: 10.9758/cpn.2019.17.2.297
11. Laupu WK. *The Efficacy of Garcinia mangostana L. (mangosteen) Pericarp as an Adjunctive to Second-Generation Antipsychotics for the Treatment of Schizophrenia: A Double Blind, Randomized, Placebo-Controlled Trial*. Townsville, QLD: James Cook University (2014).
12. Maruff P, Thomas E, Cysique L, Brew B, Collie A, Snyder P, et al. Validity of the CogState brief battery: relationship to standardized tests and sensitivity to cognitive impairment in mild traumatic brain injury, schizophrenia, and AIDS dementia complex. *Arch Clin Neuropsych.* (2009) 24:165–78. doi: 10.1093/arclin/acp010
13. Maruff P, Lim YY, Darby D, Ellis KA, Pietrzak RH, Snyder PJ, et al. Clinical utility of the cogstate brief battery in identifying cognitive impairment in mild cognitive impairment and Alzheimer's disease. *BMC Psych.* (2013) 1:30. doi: 10.1186/2050-7283-1-30
14. Hinton-Bayre AD. Deriving reliable change statistics from test–retest normative data: Comparison of models and mathematical expressions. *Arch Clin Neuropsych.* (2010) 25:244–56. doi: 10.1093/arclin/acq008
15. Leucht S, Kane JM, Kissling W, Hamann J, Etschel E, Engel RR. What does the PANSS mean? *Schizophr Res.* (2005) 79:231–8. doi: 10.1016/j.schres.2005.04.008
16. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull.* (1987) 13:261–76. doi: 10.1093/schbul/13.2.261
17. Montgomery S, Åsberg M. *A New Depression Scale Designed to be Sensitive to Change*. Akkad: Department of Psychiatry, Guy's Hospital (1977).
18. Cohen J. *Statistical Power Analysis for the Behavioral Sciences, 2nd edn*. Hillsdale, NJ: Á/L. Erbaum Press (1988).
19. Turner A, Baker A, Dean OM, Walker AJ, Dodd S, Cotton SM, et al. Adjunctive Garcinia mangostana Linn (mangosteen) pericarp for schizophrenia: a 24-week double-blind, randomised, placebo controlled efficacy trial. *Can J Psychiatry.* (2020) 66:354–66.
20. Firth J, Rosenbaum S, Ward PB, Curtis J, Teasdale SB, Yung AR, et al. Adjunctive nutrients in first-episode psychosis: a systematic review of efficacy, tolerability and neurobiological mechanisms. *Early Interv Psychiatry.* (2018) 12:774–83. doi: 10.1111/eip.12544
21. Zortea K, Franco VC, Guimarães P, Belmonte-de-Abreu PS. Resveratrol supplementation did not improve cognition in patients with schizophrenia: results from a randomized clinical trial. *Frontiers in psychiatry.* (2016) 7:159. doi: 10.3389/fpsyt.2016.00159
22. Travica N, D'Cunha NM, Naumovski N, Kent K, Mellor DD, Firth J, et al. The effect of blueberry interventions on cognitive performance and mood: a systematic review of randomized controlled trials. *Brain Behav Immunity.* (2019) 85:96–105. doi: 10.1016/j.bbi.2019.04.001
23. Desideri G, Kwik-Uribe C, Grassi D, Necozione S, Ghiadoni L, Mastroiacovo D, et al. Benefits in cognitive function, blood pressure, and insulin resistance through cocoa flavanol consumption in elderly subjects with mild cognitive impairment: the Cocoa, Cognition, and Aging (CoCoA) study. *Hypertension.* (2012) 60:794–801. doi: 10.1161/HYPERTENSIONAHA.112.193060
24. Cerdá B, Periago P, Espín JC, Tomás-Barberán FA. Identification of urolithin A as a metabolite produced by human colon microflora from ellagic acid and related compounds. *J Agricult Food Chem.* (2005) 53:5571–6. doi: 10.1021/jf050384i
25. Chitchumroonchokchai C, Riedl KM, Suksumrarn S, Clinton SK, Kinghorn AD, Failla ML. Xanthones in mangosteen juice are absorbed and partially conjugated by healthy adults. *J Nutr.* (2012) 142:675–80. doi: 10.3945/jn.111.156992
26. Marx W, Kelly JT, Marshall S, Cutajar J, Annois B, Pipingas A, et al. Effect of resveratrol supplementation on cognitive performance and mood in adults: a systematic literature review and meta-analysis of randomized controlled trials. *Nutr Rev*. (2018) 2018:nuy010-nuy. doi: 10.1093/nutrit/nuy010
27. Prasad S, Tyagi AK, Aggarwal BB. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice. *Cancer Res Treatment.* (2014) 46:2. doi: 10.4143/crt.2014.46.1.2
28. Monfoulet L-E, Buffière C, Istas G, Dufour C, Le Bourvellec C, Mercier S, et al. Effects of the apple matrix on the postprandial bioavailability of flavan-3-ols and nutrigenomic response of apple polyphenols in minipigs challenged with a high fat meal. *Food Funct.* (2020) 11:5077–90. doi: 10.1039/D0FO00346H
29. Rapaport MH, Nierenberg AA, Schettler PJ, Kinkead B, Cardoos A, Walker R, et al. Inflammation as a predictive biomarker for response to omega-3 fatty acids in major depressive disorder: a proof of concept study. *Mol Psychiatry.* (2016) 21:71–9. doi: 10.1038/mp.2015.22
30. Cheng YC, Huang YC, Huang WL. The effect of vitamin D supplement on negative emotions: A systematic review and meta-analysis. *Depr Anxiety.* (2020) 37:549–64. doi: 10.1002/da.23025
31. Marder SR, Fenton W. Measurement and treatment research to improve cognition in schizophrenia: NIMH MATRICS initiative to support the development of agents for improving cognition in schizophrenia. *Schizophr Res.* (2004) 72:5–9. doi: 10.1016/j.schres.2004.09.010

Conflict of Interest: AT has received grant/research support from the National Health and Medical Research Council (NHMRC), AMP Foundation and Deakin University. OD was a R.D. Wright NHMRC Biomedical Career Development Fellow (APP1145634) and has received grant support from the Brain and Behavior Foundation, Simons Autism Foundation, Stanley Medical Research Institute, Deakin University, Lilly, NHMRC and ASBDD/Servier. She has also received in kind support from BioMedica Nutraceuticals, NutritionCare and Bioceuticals. AW has previously received grant/research support from a Trisno Family Fellowship and Deakin University. SD has received grant support from Stanley Medical Research Institute, NHMRC, Beyond Blue, ARHRF, Simons Foundation, Geelong Medical Research Foundation, Fondation FondaMental, Eli Lilly, Glaxo SmithKline, Organon, Mayne Pharma and Servier. He has received speaker's fees from Eli Lilly, advisory board fees from Eli Lilly and Novartis and conference travel support from Servier. SC was supported by a NHMRC Senior Research Fellowship (APP1136344). SC has received grant support from the NHMRC, the Stanley Medical Research Institute, BeyondBlue, Movember, The University of Melbourne, Australian Catholic University, ARHRF, and Mental Illness Research Fund (Victoria Department of Human Services). JS

was supported by a National Health and Medical Research Council Practitioner Fellowship Grant (APP1105807), has been a speaker for Janssen Cilag, Lundbeck, Servier, and Shire Pharmaceuticals, and served as a consultant to Janssen Cilag, Lundbeck and Roche. BK has received grant/research support from the Australian Postgraduate Research Training Scholarship, Deakin University, Ian Scott Mental Health Ph.D. Scholarship, Australian Rotary Health, and the International Society for the Study of Personality Disorders. MA has received grant/research support from Deakin University, Australasian Society for Bipolar Depressive Disorders, Lundbeck, Australian Rotary Health, Ian Parker Bipolar Research Fund, Cooperative Research Center for Mental Health and PDG Geoff and Betty Betts Award from Rotary Club of Geelong. EB has received grant/research support from the University of Melbourne, the Australian Department of Health, Western Victoria PHN, and the University of the West of England, UK. MB was supported by a NHMRC Senior Principal Research Fellowship (1059660 and 1156072). MB has received Grant/Research Support from the NIH, Cooperative Research Centre, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, Medical Benefits Fund, National Health and Medical Research Council, Medical Research Futures Fund, Beyond Blue, Rotary Health, A2 milk company, Meat and Livestock Board, Woolworths, Avant and the Harry Windsor Foundation, has been a speaker for Abbot, Astra Zeneca, Janssen and Janssen, Lundbeck and Merck and served as a consultant to Allergan, Astra Zeneca, Bioadvantex, Bionomics, Collaborative Medicinal Development, Janssen and Janssen, Lundbeck Merck, Pfizer and Servier—all unrelated to this work. WM was currently funded by an Alfred Deakin Postdoctoral Research Fellowship and a Multiple Sclerosis Research Australia early-career fellowship. WM has previously received funding from the Cancer Council Queensland and university grants/fellowships from La Trobe University, Deakin University, University of Queensland, and Bond University, received industry funding and has attended events funded by Cobram Estate Pty. Ltd, received travel funding from Nutrition Society of Australia, received consultancy funding from Nutrition Research Australia, and has received speakers honoraria from The Cancer Council Queensland and the Princess Alexandra Research Foundation. MM has received Grant/research support from NHMRC, Deakin University School of Medicine, Deakin Biostatistics Unit, Institute for Mental and Physical Health and Clinical Translation, and Medibank Health Research Fund.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer NN declared a past co-authorship with four of the authors WM, OD, MA, and MB to the handling editor.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 04 August 2021
doi: 10.3389/fpsyt.2021.701408

Antidepressant-Like Effects of Chronic Guanosine in the Olfactory Bulbectomy Mouse Model

Roberto Farina Almeida[1], Yasmine Nonose[2], Marcelo Ganzella[3], Samanta Oliveira Loureiro[2], Andréia Rocha[2], Daniele Guilhermano Machado[2], Bruna Bellaver[2], Fernanda Urruth Fontella[2], Douglas T. Leffa[4], Letícia Ferreira Pettenuzzo[2], Gianina Teribele Venturin[5], Samuel Greggio[5], Jaderson Costa da Costa[5], Eduardo R. Zimmer[2,6,7], Elaine Elisabetsky[2] and Diogo O. Souza[2]*

[1] Programa de Pós-Graduação em Ciências Biológicas, Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, Brazil, [2] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [3] Neurobiology Department, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [4] Attention Deficit Hyperactivity Disorder Outpatient Program & Development Psychiatry Program, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [5] Preclinical Imaging Center, Brain Institute (Brains) of Rio Grande do Sul, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil, [6] Graduate Program in Biological Sciences: Pharmacology and Therapeutics, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [7] Departament of Pharmacology, UFRGS, Porto Alegre, Brazil

OPEN ACCESS

Edited by:
Nazzareno Cannella, University of Camerino, Italy

Reviewed by:
Stefania Schiavone, University of Foggia, Italy
Carla Ines Tasca, Federal University of Santa Catarina, Brazil

***Correspondence:**
Roberto Farina Almeida
almeida_rf@yahoo.com.br

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *27 April 2021*
Accepted: *01 July 2021*
Published: *04 August 2021*

Citation:
Almeida RF, Nonose Y, Ganzella M, Loureiro SO, Rocha A, Machado DG, Bellaver B, Fontella FU, Leffa DT, Pettenuzzo LF, Venturin GT, Greggio S, da Costa JC, Zimmer ER, Elisabetsky E and Souza DO (2021) Antidepressant-Like Effects of Chronic Guanosine in the Olfactory Bulbectomy Mouse Model. Front. Psychiatry 12:701408. doi: 10.3389/fpsyt.2021.701408

Major depressive disorder (MDD) leads to pervasive changes in the health of afflicted patients. Despite advances in the understanding of MDD and its treatment, profound innovation is needed to develop fast-onset antidepressants with higher effectiveness. When acutely administered, the endogenous nucleoside guanosine (GUO) shows fast-onset antidepressant-like effects in several mouse models, including the olfactory bulbectomy (OBX) rodent model. OBX is advocated to possess translational value and be suitable to assess the time course of depressive-like behavior in rodents. This study aimed at investigating the long-term behavioral and neurochemical effects of GUO in a mouse model of depression induced by bilateral bulbectomy (OBX). Mice were submitted to OBX and, after 14 days of recovery, received daily (ip) administration of 7.5 mg/kg GUO or 40 mg/kg imipramine (IMI) for 45 days. GUO and IMI reversed the OBX-induced hyperlocomotion and recognition memory impairment, hippocampal BDNF increase, and redox imbalance (ROS, NO, and GSH levels). GUO also mitigated the OBX-induced hippocampal neuroinflammation (IL-1, IL-6, TNF-α, INF-γ, and IL-10). Brain microPET imaging ([^{18}F]FDG) shows that GUO also prevented the OBX-induced increase in hippocampal FDG metabolism. These results provide additional evidence for GUO antidepressant-like effects, associated with beneficial neurochemical outcomes relevant to counteract depression.

Keywords: major depressive disorder, psychopharmacology, purines (source: MeSH), guanosine, purinergic signaling, olfactory bulbectomy

INTRODUCTION

Major depressive disorder (MDD) is a multifactorial disorder characterized by a complex symptomatology, leading to important changes in the mental and social health of afflicted patients (1, 2). Despite its high prevalence (3), there are no validated biomarkers that can be used for a differential diagnosis (4). Current antidepressants are characterized by delayed clinical

response, significant adverse effects, long-term treatment, and, unfortunately, high relapse rates (3, 5–8). Innovation in the field is sorely needed, especially the development of fast acting drugs with improved effectiveness, for which a better understanding of the pathophysiology underlying MDD is a requirement.

The removal of the olfactory bulbs in rodents induces long-term disruption in pathways of the cortical–hippocampal–amygdala circuit, leading to dysfunctional signaling in limbic areas (9, 10). It recapitulates, in rodents, depressive-like behavioral and neurochemical changes observed in MDD patients (9, 11–13). Adding to its face value, the OBX-induced altered behavior can be reversed or attenuated by chronic (and not acute) treatment with the classical antidepressant agents (12, 14). We proposed (15) that OBX in mice provides two-different windows to explore changes in behavior: an early one (up to 4 weeks after surgery), which includes hyperlocomotion, spatial memory deficits, and anhedonia-like behavior, and a latter one (up to 8 weeks after surgery), where only anhedonia-like behavior is no longer observed. This temporal profile adds to the model's face value by replicating the symptom remission documented in a segment of untreated MDD patients (12, 14).

Depressive patients present decreased levels of serum guanosine [GUO, an endogenous nucleoside with neuroprotective properties (16–18)], corroborating the idea that the purinergic signaling is involved in MDD pathophysiology (18–20). Substantial data have demonstrated that systemic or central GUO induces antidepressant-like effects in distinct rodent models with predictive validity [tail suspension test (21), forced swimming test (21, 22), and acute restrain stress (22)]. GUO antidepressant-like effects were also verified with combined subthreshold doses of GUO and ketamine in the novelty-suppressed feeding test (NSF) (23) and the corticosterone-induced depression models (24). Moreover, a single and acute GUO intraperitoneal administration showed fast-onset antidepressant-like activity, comparable to ketamine, in OBX mice (25). Different mechanisms were postulated for GUO antidepressant effects: interaction with NMDA receptors and the mTOR pathway (21, 25), activation of MAPK/ERK and Nrf2/HO-1 pathways, inhibition of GSK-3β (26), attenuation of oxidative stress (22), and facilitation of neuronal plasticity (27).

As no safe fast-onset antidepressant is currently available for long-term use, a better understanding of long-term administration of GUO is warranted. As the OBX model seems to show higher sensitivity, specificity, and reliability than other experimental depression models (9, 14, 28–30), it was the model chosen for this study.

The purpose of this study is to investigate the effects of chronic GUO in the OBX-induced long-lasting changes in behavior (locomotion and cognition) and brain signaling (glutamate transmission, oxidative stress, and neuroinflammation) parameters. Additionally, positron emission micro-tomography (microPET) with [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) was used to investigate brain metabolism.

MATERIALS AND METHODS

Animals

Three cohorts of male C57BL/6 mice (45–50 days, 20–25 g) were obtained from Fundação Estadual de Produção e Pesquisa do Rio Grande do Sul, Porto Alegre, Brazil. Animals were housed five per cage and allocated in a room with controlled temperature (22 ± 1°C), under a 12 h/12 h light/dark cycle, and with *ad libitum* access to food and water. The cages were placed in the experimental room 24 h before behavioral tasks for acclimatization. Behavioral tests were carried out between 13:00 and 17:00 h. The [^{18}F]FDG-microPET was performed between 7:00 and 11 h in the Preclinical Imaging Center at PUC-RS. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the local Ethics Committee (project approval #24577). All efforts were made to minimize suffering and the number of animals used in the experiments.

Drugs

GUO and imipramine (IMI) were purchased from Sigma Chemicals (St. Louis, MO, USA). All drug solutions were freshly prepared (in saline) before administration and intraperitoneally (i.p.) injected (10 ml/kg), as 7.5 mg/kg (25) for GUO and 40 mg/kg for IMI (used as positive control drug) (31, 32). To reduce the influence of the stress from repeated i.p. administration, the behavioral tests and euthanasia were performed 24 h after the last drug administration. To minimize damages from repeated injections, the abdomen quadrant was changed daily.

Bilateral Olfactory Bulbectomy

Bilateral olfactory bulb ablation was performed as previously described (15, 25). Briefly, mice were anaesthetized (i.p.) with a combination of xylazine (6 mg/kg) and ketamine (100 mg/kg) diluted in saline. The animals were fixed in a stereotactic frame (Stoelting Co., USA), the skull was shaven, and a burr hole (circa 2 mm in diameter) was made above the olfactory bulbs, 4 mm rostral to the bregma. Both olfactory bulbs were then disconnected with a surgical micro-scissors and removed by suction with a glass Pasteur pipette. Sham-operated mice were treated in the same way, including piercing of the dura mater, but their olfactory bulbs were left intact.

Treatment Schedule and Behavior

The research designs can be seen in **Figures 1A**, **2A**, **3A** for cohorts 1, 2, and 3, respectively.

In all three cohorts, naïve animals were submitted to the Open Field Task (OFT1) 1 day before surgery. After recovery from surgery (for 14 days), mice were re-submitted to the OFT (OFT2) and a final one (OFT3) after treatments. Daily treatments started immediately after OFT2 and lasted for 45 days, up to 24 h before euthanasia. Behavior or image data collected after the recovery period is considered baseline, and those collected after the treatment are considered follow-up.

From the 80 animals assigned to cohort 1, 12 mice were lost (due to surgical or chronic treatment complications, incomplete bulbectomy, or frontal cortex injury). The following

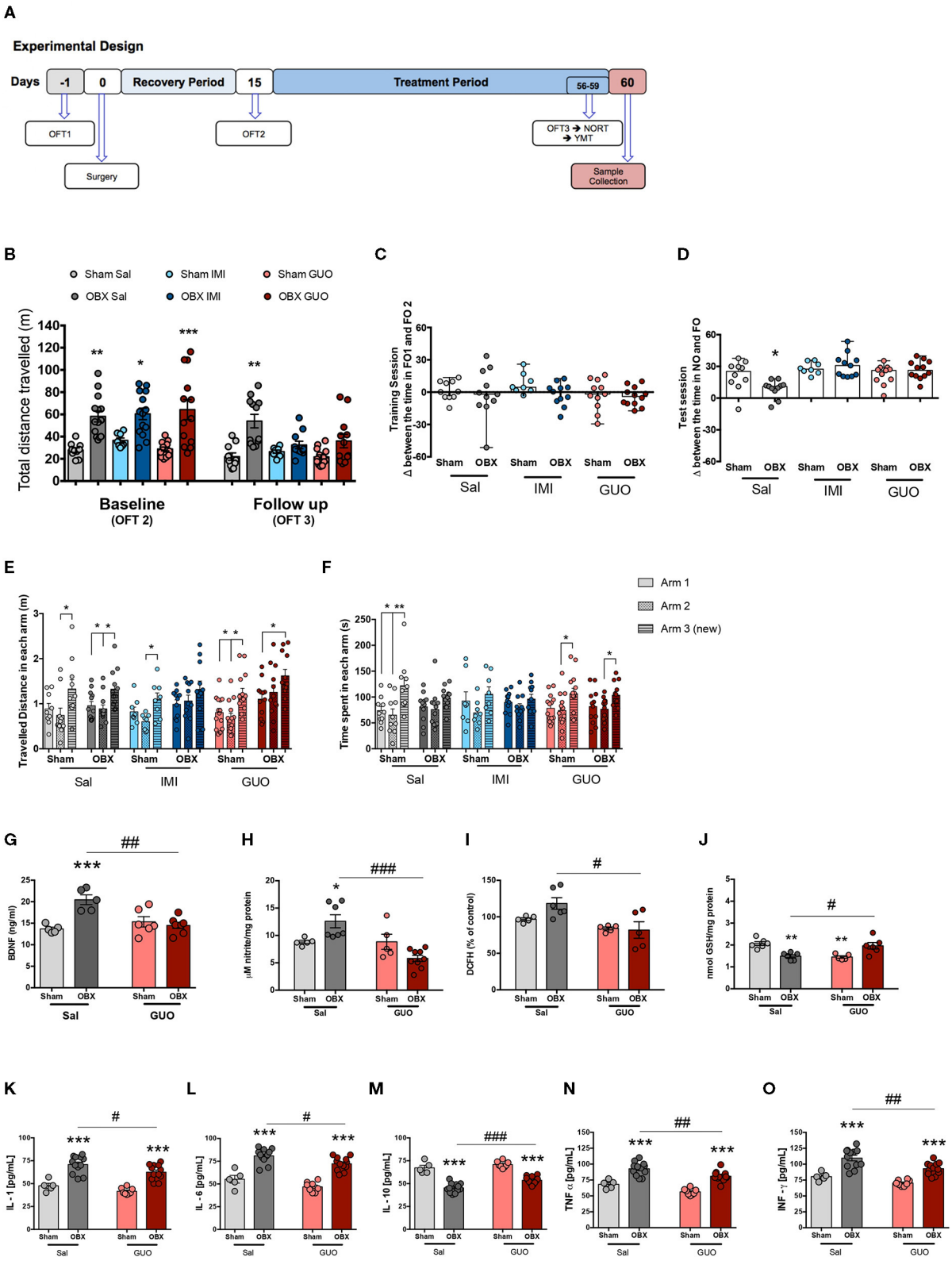

FIGURE 1 | Cohort 1: study design **(A)**. Surgery and treatment effects in locomotor activity in OFT **(B)**: Columns represents mean ± S.E.M. (n = 8–14 mice/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ OBX x respective Sham, two-way ANOVA/Tukey. **(C,D)**: Delta (Δ) between the time spent exploring objects in NORT training (FO1 and FO2) **(C)** and test **(D)** (NO and FO) sessions, respectively. Columns represent median with range ($n = 8$–12). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, two-way

(Continued)

FIGURE 1 | ANOVA/Tukey. **(E,F)**: Total distance travelled **(E)** and time spent in each arm **(F)** in YMT. Columns represent mean ± S.E.M. (n = 8–14). $^{*}p < 0.05$, $^{**}p < 0.01$, one-way ANOVA/Tukey. Hippocampal levels of BDNF **(G)**, NO **(H)**, DCFH **(I)**, GSH **(J)**, IL-1 **(K)**, IL-6 **(L)**, IL-10 **(M)**, TNF-α **(N)**, **(O)** and INF-γ. Columns represent mean ± S.E.M. (n = 5–12). $^{*}p < 0.05$, $^{***}p < 0.001$ OBX x respective sham; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ OBX GUO x Sal; two-way ANOVA/Tukey.

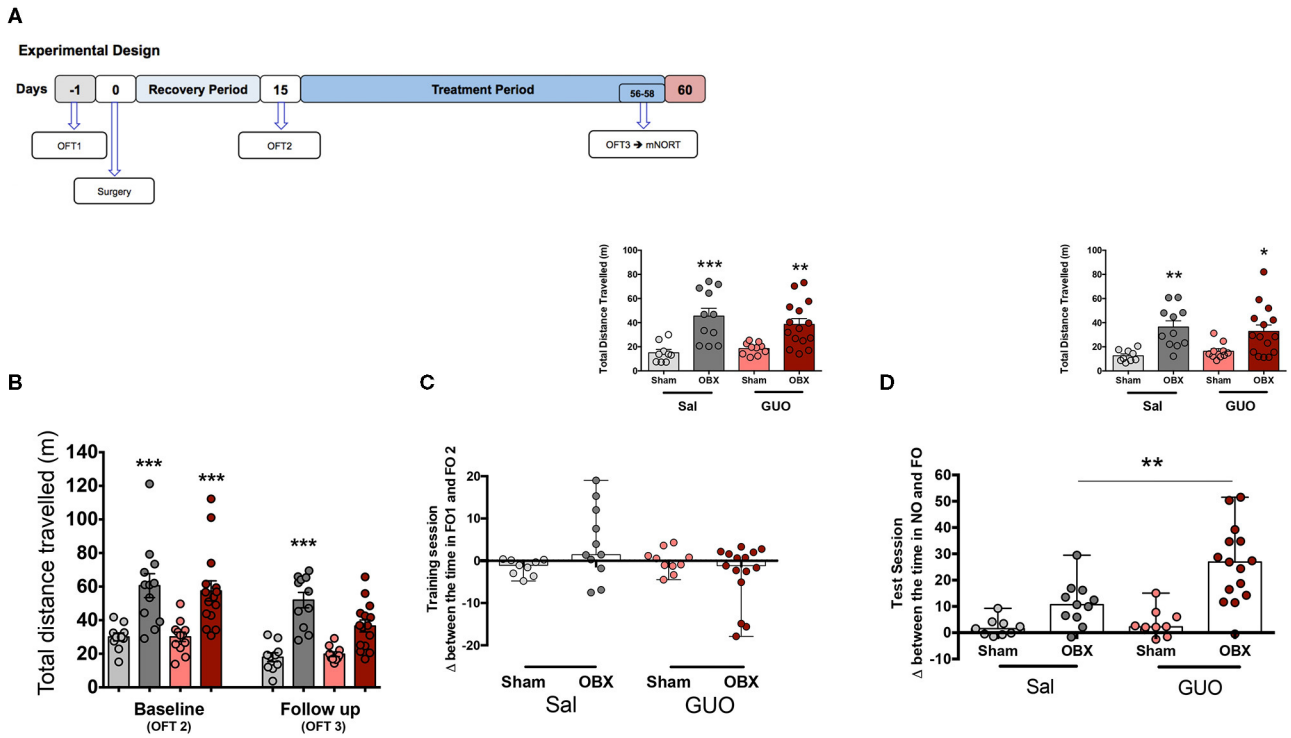

FIGURE 2 | Cohort 2: study design **(A)**. Surgery and GUO treatment effects in locomotor activity in OFT **(B)**. Columns represent mean ± S.E.M. (n = 10–15). $^{*}p < 0.05$, $^{***}p < 0.001$ OBX x Sham, two-way ANOVA/Tukey. Effects of surgery and GUO treatment at mNORT training **(C)** and test **(D)** sessions. Columns represent median with range, and in the inserts, columns represent mean ± S.E.M. (n = 10–15). $^{**}p < 0.001$; total exploratory behavior, two-way ANOVA/Sidak. Inserts represent distance travelled. $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$ two-way ANOVA/Tukey.

experimental groups were subjected to OFT2 (baseline line): Sham Sal (n = 10), OBX Sal (n = 14), Sham IMI (n = 8), OBX IMI (n = 14), Sham GUO (n = 14), and OBX GUO (n = 13). OFT3 (follow-up) was performed 24 h after daily treatment: Sham Sal (n = 10), OBX Sal (n = 12), Sham IMI (n = 8), OBX IMI (n = 11), Sham GUO (n = 14), and OBX GUO (n = 12). OFT3, the novel object recognition test (NORT), and the Y-maze test (YMT) were performed in these very same groups within 24 h from each other.

From the 50 animals assigned to cohort 2 (n = 50), four mice were lost (due to surgical complications, incomplete bulbectomy, or frontal cortex injury). The following groups were subjected to OFT2 (baseline): Sham Sal (n = 10), OBX Sal (n = 12), Sham GUO (n = 12), and OBX GUO (n = 15), while OFT3 (follow-up) was performed for Sham Sal (n = 10), OBX Sal (n = 11), Sham GUO (n = 10), and OBX GUO (n = 15). The modified NORT (mNORT) was performed 24 h after OFT3.

From the 44 mice assigned to the third cohort, 6 mice were lost (due to surgical complications, incomplete bulbectomy, or for frontal cortex injury). OFT2 (baseline) was performed for Sham Sal (n = 8), OBX Sal (n = 11), Sham GUO (n = 8), and OBX GUO (n = 12). OFT3 (follow-up) was performed for Sham Sal (n = 8), OBX Sal (n = 11), Sham GUO (n = 8+), and OBX GUO (n = 12). Twenty-four hours after, the OFT3 mice were scanned for [^{18}F] FDG-microPET imaging.

Locomotor Activity

The OFT was performed as previously detailed (15, 25). Mice were individually placed facing the wall of a gray wooden box (50 × 50 × 50 cm, lighted by 200-lux bulb) and recorded for 10 min by a video-camera (positioned above and at ca. 90° to the square arena) connected to a monitor. The total distance traveled was evaluated using the AnyMaze® software. The apparatus was cleaned with alcohol 70° and dried between trials.

Memory

Novel Object Recognition Task

The novel object recognition task (NORT) was used to evaluate recognition memory (25, 33). NORT was performed at the same OFT apparatus, consisting of an acquisition trial (training

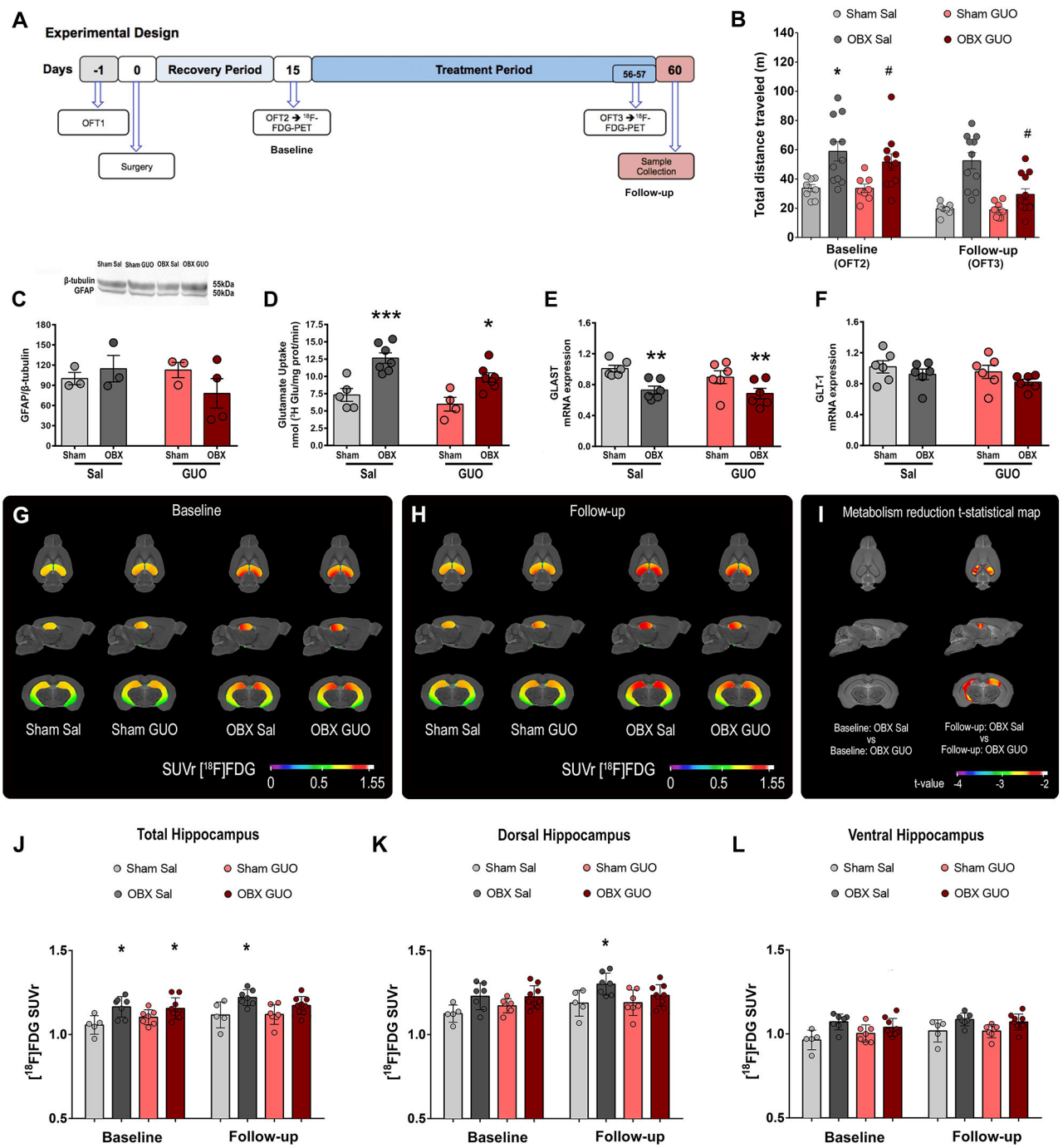

FIGURE 3 | Cohort 3: Study design **(A)**. Effects of surgery and treatment on locomotion at the OFT3 **(B)**. GFAP/β-actin **(C)**, glutamate uptake **(D)**, and mRNA levels of GLAST **(E)** and GLT-1 **(F)** in the hippocampus. Columns represent mean ± S.E.M. (n = 8–11 mice/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ Sham x respective OBX; #$p < 0.05$ OBX GUO x Sal, two-way ANOVA/Tukey. Brain metabolic maps showing the mean SUVr in the hippocampus of groups Sham Sal, Sham GUO, OBX Sal, and OBX GUO in the baseline **(G)** and follow-up **(H)** scans. T-statistical maps showing the statistically significant decrease in metabolism in the hippocampal region on the OBX GUO follow-up group in comparison with the group OBX Sal follow-up **(I)**. VOI means [^{18}F]FDG SUVr values in the total hippocampal region **(J)**, in the dorsal hippocampal region **(K)**, and in the ventral hippocampal region **(L)**. All images are projected into a standard magnetic resonance imaging (MRI) image in axial, sagittal, and coronal planes. Data represented as mean ± S.D. $^{*}p < 0.05$ (multiple comparisons—three-way ANOVA with repeated measures).

session) and a test trial (test session) performed within a 24 h interval. During the training session (10 min), two identical objects were placed in a symmetric position in the center of the apparatus (subjects with training session exploration time inferior to 20 s were excluded from the experiment). The time spent in each object and the total distance traveled were

measured. In the test session (10 min), one of the objects was replaced by a novel object (different shape and material), and again, the time spent exploring each object and distance traveled was measured. Exploration of an object was defined as rearing on or sniffing the object from <1 cm, and/or touching it with the nose. Successful recognition of a previously explored object was reflected by preferential exploration of the novel object in more than 50% of the total time. Experiments were recorded as described in Section **Locomotor Activity**. The total distance in the training and the test sessions were analyzed by AnyMaze® software, while the time spent in each object was analyzed in video records by an experimenter blinded to groups and treatments. After each session, the OFT arena and objects were thoroughly cleaned with 70% ethanol to prevent odor recognition.

Modified Novel Object Recognition Task

The purpose of mNORT was to investigate mice ability to discriminate odor, given the potential recovery of olfactory bulbs. The mNORT was conducted as the NORT except for two main differences: i—the objects (FO1 and FO2) at training and test (FO and NO) sessions were kept in rat cages at the animal facility during the 24 h previous to the experiment; and ii—by the exclusion criteria used in NORT, since no Sham mice explored objects at training session more than 20 s.

Y-Maze Test

A modified version of the Y-maze test was used (34, 35). The apparatus consisted of three identical arms (30 × 8 cm) disposed at 120°, with gray wooden walls of 15 cm height. The test consisted of a sample phase trial and a test phase trial separated by a 30 min trial interval. In the sample phase trial, each mouse was individually placed in the maze with one of the three arms closed and allowed to explore the other two arms freely for 5 min. At the test trial, each animal was placed again in the maze with all three arms opened and allowed to explore freely for 5 min; the arm closed at sample trial was defined as the new arm. The modified Y-maze was used to evaluate short-term recognition memory. Successful recognition was expressed by increasing the time spent or distance traveled in the new arm when compared to arms 1 and 2. Experiments were video-recorded, and the time spent and total distance traveled in all three arms were analyzed by the AnyMaze® software. After each session, the apparatus was thoroughly cleaned with 70% ethanol.

Neurochemistry

Sample Collection

Twenty-four hours after the last behavioral session and drug administration, mice were anesthetized with xylazine (6 mg/kg) and ketamine (100 mg/kg) and decapitated, brains were removed, and the hippocampi were dissected out for immediate analysis or frozen at −80°C for biochemical evaluations.

Redox Homeostase

Reactive Oxygen Species

The hippocampus tissue samples were homogenated in phosphate-KCl (20 mM/140 mM) buffer and centrifuged at 1,000 × g × 5 min at 4°C. An aliquot of the supernatant was used to evaluate 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation (15). DCFH-DA (7 μM) oxidation was determined spectrofluorimetrically. Fluorescence was determined at 488 nm for excitation and 520 nm for emission. A standard curve was carried out using 2′,7′- dichlorofluorescein (DCF). Results are shown as delta of DCFH-DA oxidation between 15 and 30 min of incubation.

Nitrite

NO levels were determined by measuring the amount of nitrite (a stable oxidation product of NO) in hippocampal tissue homogenates, as indicated by the Griess reaction. The Griess reagent was a 1:1 mixture of 1% (w/v) sulphanilamide in 2.5% (w/v) phosphoric acid and 0.1% (w/v) N-(1-napthyl) ethylenediamine dihydrochloride in deionized water. Briefly, the tissue was homogenized in phosphate-KCl (20 mM/140 mM) buffer and centrifuged at 1,000 × g × 5 min at 4°C. The supernatant was deproteinized with 20 μl TCA 25%, centrifuged at 2,000 × g × 10 min at 4°C, and immediately neutralized with 2 M potassium bicarbonate. After this procedure, the Griess reagent was added directly to the neutralized sample and incubated in the dark for 15 min at 22°C (15, 36). Samples were analyzed at 550 nm on a microplate spectrophotometer. Nitrite concentrations were calculated using a standard curve, and the results are expressed as percentages relative to the control conditions.

Glutathione

GSH levels were assessed as previously described (36). The hippocampal tissues were homogenated in a phosphate-KCl (20 mM/140 mM) buffer containing 5 mM EDTA, and protein was precipitated with 1.7% meta-phosphoric acid. The tissue homogenates were centrifuged at 1,000 × g × 5 min at 4°C, and the supernatants were mixed with o-phthaldialdehyde (at a final concentration of 1 mg/ml methanol) and incubated at 22°C for 15 min. Fluorescence was measured using excitation and emission wavelengths of 350 and 420 nm, respectively. A calibration curve was performed with standard GSH solutions. GSH concentrations were calculated as nmol/mg protein.

Neuroinflammation

Hippocampi samples were homogenized in PBS/Tris-HCl/SDS 5% pH 7.4 and centrifuged at 5,000 × g × 10 min at 4°C, and the supernatant was collected (15). Commercial enzyme-linked immunosorbent assay (ELISA) kits for rat IL-1, IL-6, TNF-α, INF-γ, and IL-10 were used according to the manufacturer's instructions (eBIOSCIENCE, San Diego, CA, USA). Briefly, 96-well microplates were incubated with the primary antibody at 4°C overnight, washed, and blocked at room temperature for 1 h. The cytokine standards, calibrators, and samples were added in the plate in triplicate and incubated at room temperature for 2 h. After washing, the secondary antibody conjugated with peroxidase was added and incubated at room temperature for 1 h; the samples were washed and the tetramethylbenzidine chromogen was added. After 15 min, the enzyme reaction was stopped by adding 50 μl phosphoric acid 1 M. The

absorbance was measured at 450 nm. The results are expressed as pg/mg protein.

Glutamate Neurotransmission

Glutamate Uptake by Hippocampi Slices

After dissected out, the hippocampi were immediately cut into transverse slices (300 μm thick) using a Mcllwain Tissue Chopper. Transverse hippocampal slices were immediately immersed in HBSS solution (137 NaCl, 0.63 Na_2HPO_4, 4.17 $NaHCO_3$, 5.36 KCl, 0.44 KH_2PO_4, 1.26 $CaCl_2$, 0.41 $MgSO_4$, 0.49 $MgCl_2$, and 5.5 glucose), pH 7.2, 4°C, and glutamate uptake was performed following an adapted protocol (37). Slices were pre-incubated with HBSS, at 37°C for 15 min, followed by medium change and incubation in the presence of 0.2 μCi/ml L-[$^{3,4-3}$H]glutamate (American Radiolabeled Chemicals, Cat# 0132, Conc. 1 mCi/ml) for 5 min. The incubation was stopped with two ice-cold washes using 1 ml of HBSS, followed by the immediate addition of 200 μl of 0.5 N NaOH, and stored overnight. Na^+-independent uptake was measured using the same protocol, with modifications in the temperature (4°C) and medium composition (choline chloride instead of sodium chloride). Na^+-dependent uptake was defined as the difference between both uptakes. The incorporated radioactivity was measured in a Hidex 300 SL scintillation counter. Results are expressed as nMol of glutamate/protein/minute.

GLAST and GLT-1 Gene Expression

The gene expression of GLAST and GLT-1 was evaluated in hippocampi from each Sal and GUO groups ($n = 6$) by quantitative real-time polymerase chain reaction (RT-PCR). Total RNA was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA) following the instructions from the manufacturer. The purity and concentration of the RNA were determined by spectrophotometry at 260/280 nm ratio. One microgram of total RNA was reverse transcribed using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) in a 20-μl reaction. GLAST (Rn01402419_g1), GLT-1 (Rn00691548_m1), and β-actin (Rn00667869_m1) mRNA levels were quantified using the TaqMan real-time RT-PCR system, using inventory primers and probes purchased from Applied Biosystems (Foster City). Quantitative RT-PCR was performed in duplicate using the Applied Biosystems 7500 fast system. No-template and a no-reverse transcriptase were used in each assay as controls, producing no detectable signal during the 40 cycles of amplification. Therefore, target mRNA levels were normalized to β-actin levels using the $2^{-\Delta\Delta Ct}$ method (38).

Astrocyte Marker

GFAP immunocontent was analyzed by Western blot as previously described (39). Briefly, hippocampal samples from all experimental groups ($n = 6$) were solubilized in ice-cold lysis buffer (4% SDS, 2 mM EDTA, 50 mM Tris-HCl pH 6.8), standardized in sample buffer (62.5 mM Tris-HCl pH 6.8, 2% (*w/v*) SDS, 5% β-mercaptoethanol, 10% (*v/v*) glycerol, 0.002% (*w/v*) bromophenol blue), and boiled at 95°C for 5 min. Samples were separated by SDS-PAGE (35 μg protein/well) and transferred to a nitrocellulose membrane (GE Healthcare). Adequate loading of each sample was confirmed using Ponceau S staining. After blocking with 5% (*w/v*) skim milk overnight, membranes were incubated with primary rabbit antibody one at 4°C (GFAP from Sigma Aldrich, São Paulo/Brazil, 1:3,000 dilution; β-tubulin, 1:2000 dilution), washed, and incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG (NA934V, 1:5,000 dilution, GE Healthcare, UK) secondary antibody for 2 h. Chemiluminescent bands were detected in ImageQuant LAS4000 system (GE Healthcare) using Immobilon Western chemiluminescence kit (#P90720, Millipore) and quantified with ImageQuant TL software (version 8.1, GE Healthcare). The results are expressed in percentage of control levels after normalization using β-tubulin as an internal standard.

Brain Derived Neurotrophic Factor

The hippocampi were homogenized in PBS/Tris-HCl/SDS 5% pH 7.4 and centrifuged at 5,000 × g × 10 min at 4°C. BDNF levels were measured in the supernatants by anti-BDNF sandwich-ELISA, in a plate previously coated with anti-BDNF antibody according to the instructions at ELISA Kit for Brain Derived Neurotrophic Factor (Wuhan USCN Business Co., Ltd, Cat. No. SEA011Ra).

Protein Determination

Protein content was measured using the Pierce BCA® protein kit (Thermo Scientific, Waltham, MA, USA) with bovine serum albumin as standard.

Glucose Metabolism Imaging Procedure

The PET scans were performed using a Triumph™ microPET at the Brain Institute of Rio Grande do Sul [LabPET-4, TriFoil Imaging, Northridge, CA, USA (for technical information, see Bergeron et al. (40)]. Mice from the third cohort were scanned in two points: after recovery (baseline, 15 days post OBX) and after treatments (follow-up, in experimental day 57) (**Figure 3A**).

Animals received an intraperitoneal injection of [^{18}F]FDG (mean ± s.d. = 25 ± 0.5 mCi) after overnight fasting; each mouse was returned to its home cage for a 40 min period of awake uptake of [^{18}F]FDG, immediately followed by a 10 min microPET static acquisition conducted under isoflurane anesthesia (2% at 0.5 L/min oxygen flow). The scan was performed with the animals in a head-first prone position and with the field of view (FOV: 4.6 cm) centered in the animal's head. Throughout these procedures, the animals were kept on a pad heated at 37°C.

Imaging data were reconstructed using the maximum likelihood estimation method (MLEM-3D) algorithm with 20 interactions. Each microPET image was reconstructed with a voxel size of 0.2 × 0.2 × 0.2 mm and spatially normalized into an [^{18}F]FDG template using brain normalization in PMOD v3.8 and the Fuse It Tool (PFUSEIT) (PMOD Technologies, Zurich, Switzerland). Further imaging processing and analysis were carried out using the MINC Tool Kit software (www.bic.mni.mcgill.ca/ServicesSoftware). MicroPET images were manually co-registered to a standard mouse MRI histological template. Activity values were normalized by the region of reference showing the lower standard

deviation among all the images, the lateral septal nucleus (**Supplementary Figure 1A**) and, therefore, are expressed as reference standardized uptake value (SUVr). Mean SUVr of total hippocampus and subregions was extracted using predefined VOI templates (**Supplementary Figures 1B,C**).

Statistics

Data from OFT, NORT, mNORT, and biochemical parameters were compared by two-way ANOVA followed by Tukey's *post hoc*. Data from YMT was analyzed by one-way ANOVA followed by Tukey's *post hoc*. [^{18}F]FDG hippocampal t-statistical maps (voxelwise) were generated comparing groups of interest ($p < 0.05$). Regional values of [^{18}F]FDG data were analysed by three way mixed ANOVA (time × surgery × treatment) followed by Newman–Keuls *post hoc*.

RESULTS

Cohort 1

Results are shown in **Figure 1**. All groups of naïve mice, either assigned to sham or OBX surgeries, and any of the treatment groups (saline, IMI, or GUO) presented comparable performances at OFT1 ($F_{(1,74)} = 0.47$ $p > 0.05$) (data not shown). Data from OFT2 (baseline) show that OBX induced hyperlocomotion ($F_{(1,67)} = 44.99, p < 0.0001$). For OFT3 (follow-up), the two-way ANOVA identified a main effect of OBX ($F_{(2,61)} = 4.34$, $p < 0.05$), and interaction between treatment and OBX ($F_{(1,61)} = 23.69, p < 0.0001$), showing that IMI and GUO reversed the persistent hyperlocomotion of untreated OBX (**Figure 1B**).

As for NORT memory, two-way ANOVA identified OBX as the main effect ($F_{(5,58)} = 10.56$, $p < 0.001$), and interaction between OBX and treatments ($F_{(2,58)} = 4.45, p < 0.05$), showing that IMI and GUO also reversed the OBX-induced NORT memory deficit (**Figures 1C,D**).

Results in Y-maze task indicate that only OBX IMI group present an impaired performance in distance travelled ($F_{(2,30)} = 1.49, p > 0.05$; **Figure 1E**), while GUO (but not IMI) attenuated the OBX-induced Y-maze memory deficit in time spent in the Y-maze new arm ($F_{(2,33)} = 3.31, p < 0.05$; **Figure 1F**).

Two way ANOVA identified OBX as the main effect for hippocampal BDNF ($F_{(1,19)} = 10.07$, $p < 0.05$; 1G) and nitrite levels ($F_{(1,19)} = 12.59$, $p < 0.05$; **Figure 1H**); an interaction between OBX and GUO in BDNF, nitrite, and GSH content was identified, with GUO reversing OBX-induced increased in BDNF ($F_{(1,19)} = 16.50, p < 0.05$; **Figure 1G**) and nitrite ($F_{(1,19)} = 12.76$, $p < 0.05$; **Figure 1H**) levels, and decreased GSH levels ($F_{(1,19)} = 29.52$, $p < 0.05$; **Figure 1J**). OBX per se did not alter DCFH levels; however, in OBX groups GUO decreased DCFH levels in comparison to saline (**Figure 1**).

Two way ANOVA shows that OBX clearly induced neuroinflammation at the hippocampus, and GUO slightly attenuated the increase in IL-1 ($F_{(1,33)} = 66.09$, $p < 0.0001$; **Figure 1K**), IL-6 ($F_{(1,33)} = 94.15, p < 0.0001$; **Figure 1L**), TNF-α ($F_{(1,33)} = 71.66$, $p < 0.0001$; **Figure 1N**), and INF-γ (($F_{(1,33)} = 47.58$, $p < 0.0001$; **Figure 1O**), as well as the decrease in IL-10 ($F_{(1,33)} = 142.1, p < 0.0001$; **Figure 1M**).

Cohort 2

Replicating the findings from cohort 1 in OFT3 (follow-up), OBX-induced hyperlocomotion ($F_{(1,42)} = 52.4$, $p < 0.0001$; **Figure 2B**), the interaction between GUO and OBX ($F_{(1,42)} = 6.06$, $p < 0.05$, **Figure 2B**) shows that GUO reversed the OBX effect on locomotor activity.

Smell is crucial to the performance at NORT. Since olfactory bulbs are known for its neurogenic and proliferative capacity (41–45), and considering that brain circuitry remodeling has been reported after OBX (15), a modified NORT was performed to differentiate the effects of GUO from those of a potential recovery of the sense of smell at follow-up. As expected, at the mNORT, not anosmic sham mice did not reach the exploration criteria, either in training or test session. Corroborating the data from cohort 1, OBX induced memory impairment at NORT test session ($F_{(1,41)} = 27.73, p < 0.0001$; **Figure 1D**), and a positive interaction shows that GUO reversed ($F_{(1,41)} = 4.97$, $p < 0.05$) this deficit. Both not anosmic Sham groups presented decreased locomotion in mNORT training ($F_{(1,41)} = 27.57, p < 0.0001$; insert **Figure 2C**) and test ($F_{(1,41)} = 19.65, p < 0.0001$; insert **Figure 2D**) sessions.

Cohort 3

Replicating cohorts 1 and 2 findings in OFT3 (follow-up), OBX mice present increases in locomotor activity ($F_{(1,35)} = 27.76$ $p < 0.0001$; **Figure 3B**) and the interaction between GUO and OBX ($F_{(1,35)} = 7.360$ $p < 0.05$) confirms GUO reversal in OBX-induced hyperlocomotion.

No differences were identified in GFAP protein expression among groups ($F_{(1,19)} = 2.68, p > 0.05$, **Figure 3C**). OBX induced increased hippocampus glutamate uptake ($F_{(1,19)} = 29.99$, $p < 0.0001$; **Figure 3D**). OBX induced decreased hippocampus GLAST gene expression ($F_{(1,19)} = 27.57, p < 0.0001$; **Figure 3E**), an effect unresponsive to GUO. No differences in GLT-1 gene expression ($F_{(1,19)} = 0.3115$, $p > 0.05$; **Figure 3F**) were found among conditions or treatments.

At baseline, [^{18}F]FDG-microPET showed increased glucose metabolism at the hippocampus of OBX subjects compared to Sham (Hippocampus mean SUVr: Sham Sal = 1.06 ± 0.04, $n = 5$; Sham GUO = 1.08 ± 0.05, $n = 7$; OBX Sal = 1.16 ± 0.05, $n = 9$; OBX GUO = 1.16 ± 0.06, $n = 11$; **Figure 3G**). The mean hippocampal increase in FDG metabolism driven by OBX was up to 8% in comparison to Sham (hippocampus mean percentage of change: OBX Sal = 8.82%; OBX GUO = 7.93%; **Supplementary Figure 1E**). A voxel-wise t-statistical analysis, using the Sham Sal group as control, showed that significant [^{18}F]FDG-microPET differences were found only for OBX groups (OBX Sal $t_{(4)} = 5.76$; $p = 0.006$; OBX GUO $t_{(4)} = 4.25$; $p = 0.013$; Sham GUO $t_{(4)} = 1.21$; $p = 0.292$; **Supplementary Figure 1F**).

At follow-up, [^{18}F]FDG-microPET revealed higher hippocampal glucose metabolism for OBX Sal × Sham Sal, while chronic GUO attenuated the OBX-induced rise in [^{18}F]FDG signal (OBX Sal × OBX GUO) (hippocampus mean SUVr: Sham Sal = 1.10 ± 0.07, $n = 6$; Sham GUO = 1.11 ± 0.05, $n = 8$; OBX Sal = 1.19 ± 0.05, $n = 9$; OBX GUO = 1.16 ± 0.05, $n = 10$; **Figure 3H**). At this point, the hippocampal increase in FDG metabolism in the untreated OBX animals reached a peak of 15% increase (mean of 13.8%) in comparison to Sham

Sal, while the OBX group treated with GUO presented a mean value of 9% (hippocampus mean percentage of change: OBX Sal = 13.85%; OBX GUO = 9.10%; **Supplementary Figure 1G**). A voxel-wise t-statistical analysis, using Sham Sal at follow-up as control, only detected a significant increase in FDG metabolism for the saline treated OBX group (OBX Sal $t_{(5)} = 3.80$; $p = 0.0126$); in the group OBX GUO it increased metabolism was only seen in a small cluster (peak $t_{(7)} = 2.41$; $p = 0.0469$) and no significant difference was seen for the group Sham GUO there (peak $t_{(5)} = 1.65$; $p = 0.1599$; **Supplementary Figure 1H**).

A percentage of change map analysis (data not show) revealed a mean lower hippocampal glucose metabolism for the OBX GUO at follow-up group if compared to the OBX Sal group at the same period (peak $t_{(8)}$ of 3.17; $p = 0.0132$; **Figure 3I**).

A hippocampal mask with 6 VOIs, based on the Allen Mouse Brain Atlas (https://mouse.brain-map.org/), was used to obtain the mean regional [^{18}F]FDG SUVr value of the total hippocampus, of the dorsal hippocampus region, and of the ventral hippocampus region. For the total hippocampus mean SUVr (**Figure 3J**), a three-way ANOVA test—with repeated measures—identified only the effect of OBX [$F_{(1,28)} = 17.75$, $p = 0.0002$], and interaction between OBX and GUO [$F_{(1,20)} = 5.91$, $p = 0.0245$]. The *post hoc* test identified a significant difference for mean total hippocampal SUVr between baseline groups Sham Sal and OBX Sal ($p < 0.05$) and Sham Sal and OBX GUO ($p < 0.05$), and only in Sham Sal and OBX Sal group at follow-up ($p < 0.05$).

At the dorsal region of the hippocampus (**Figure 3K**), the analysis indicated the effect of the OBX [$F_{(1,28)} = 12.9$, $p = 0.0012$], and interaction between OBX and GUO [$F_{(1,20)} = 6.4$, $p = 0.0199$]. Following, a multiple comparisons test identified a significant difference for mean dorsal hippocampal SUVr only comparing the Sham Sal and OBX Sal groups at follow-up ($p < 0.05$).

Finally, at the ventral region of the hippocampus (**Figure 3L**), the three-way ANOVA analysis showed an effect for the OBX [$F_{(1,28)} = 18.32$, $p = 0.0002$] but not for treatment [$F_{(1,20)} = 0.01$; $p = 0.9194$] or time [$F_{(1,28)} = 3.27$, $p = 0.0810$]. No interaction was found.

DISCUSSION

This study shows that 45 day treatment with GUO reversed the hyperlocomotion and attenuated the memory deficit induced by OBX. The biochemical analysis of the hippocampi from those animals shows that treatment with GUO completely reversed the BDNF increase and the redox imbalance, as well as discreetly attenuated the pro-inflammatory status induced by OBX. Additionally, MicroPET imaging analysis indicates that GUO reduced the OBX-induced hippocampal increase in FDG metabolism. The behavioral and neurochemical effects promoted by chronic GUO treatment on the OBX model are similar to those reported for antidepressant agents used clinically (9, 14, 46).

Regarding the antidepressant behavioral potential of GUO, we previously showed that an acute single administration of GUO was already capable to reverse the anhedonic-like behavior, as well as the short-term recognition memory impairment observed after 15 days post OBX surgery (25). Noteworthy, only when chronically administered, as observed in the present study, GUO reversed the hyperlocomotion induced by OBX, whereas acute GUO, as ketamine, administration was ineffective (25). The effects are in line with our proposal that OBX model of depression depicts a time dependent development of depressive-like behaviors (15).

In contrast with the recognition deficit observed in shorter post-surgery periods (25), we show in the present study that 8 weeks after OBX surgery the recognition memory evaluated by NORT is partially recovered. As previously demonstrated for the transient loss of self-care and motivational behavior in OBX mice (15), the same pattern was here verified for recognition memory as assessed by NORT. Nevertheless, the lower discrimination rate of OBX mice at NORT suggests incomplete remission of long-term memory deficits within 2 months of surgery. It has been reported that GUO can promote amnesic effects (25, 47, 48). Amnesic effects were observed when GUO was administered acutely (25) or for a short time (up to 2 weeks) (48), but not after along 6 weeks treatment (49). In the current study, GUO did not induce any memory disturbance; on the contrary, GUO treatment for 8 weeks attenuated the residual memory deficit observed in untreated OBX mice (50). In agreement to our findings, it was recently reported that GUO administered during 26 days induces antidepressant-like effect, with no impairment on learning and memory (51). Taken together, those results suggest that a short-term disturbance in GUO levels can negatively impact learning and memory, but homeostasis is regained after repeated administration cancelling GUO amnesic effect.

In the present study, IMI treatment did not reverse the memory deficit induced by OBX in the YMT task. Similar results were reported for tricyclic antidepressants (52, 53), as well as for chronic IMI treatment on spatial working memory deficits in OBX mice tested 2 weeks after OBX (54). Similar results were found with rats treated with IMI for 10 or 28 days, resulting in impaired delayed in spatial win-shift performance (41). The results suggest that, differently than GUO, treatment with IMI presents a time independent impairment on learning and memory. The discrepancy may be related to the fact that as an endogenous compound GUO metabolism can be adjusted in accordance to GUO level.

Neurogenic and proliferative abilities are recognized for olfactory bulbs (43), with studies showing that after different types of lesions spontaneous recovery occurs over time (42, 44, 45, 55). To test the hypothesis that odor discrimination could be regained over time, we investigated the effects of time and treatment on Sham and OBX mice capability to discriminate odor. By using a modified NORT protocol (mNORT) where objects are smeared with rat odor, it was shown that OBX-induced anosmia was still present at follow-up. As in NORT, chronic GUO improved the OBX-induced residual memory deficit in mNORT. Therefore, the improvement or attenuation of memory impairment induced by OBX was the result of GUO treatment and not a potential recovery of odor discrimination.

Several neuroprotective effects of GUO have been demonstrated, but its exact mechanism of action is still

unclear (17). Nevertheless, *in vivo* and *in vitro* studies evidenced that GUO modulates a broad range of cellular pathways that are closely related to various brain functions (16, 17). Notable, evidences support that MDD physiopathology is also related to alterations in some of those signaling and metabolic pathways (53). Besides neurotransmitter imbalance, altered levels of neurotrophic factors/neurogenesis, altered glial/neuronal biology, impairment in mitochondrial functionality, hypermetabolism in brain specific regions including hippocampus, increased proinflammatory scenario, and nitrosative and oxidative stress are also observed in the patients suffering from MDD, as well as in animals submitted to different models that mimic MDD (12, 56). Therefore, in the present study, we explored some of the neurochemical systems related to depression and previously observed to be modulated by GUO.

BDNF profiles were largely explored in several rodent models of MDD. The literature reveals a tight association between depression phenotype and decreased BDNF content, as well as its reversal by antidepressant when investigated in different stress-induced rodent models of depression (57). However, in the OBX model of depression, the literature suggests distinct hippocampal BDNF modulations (14). Accordingly, an age dependent opposite effect on BDNF level was observed after OBX surgery, as 10 week old mice submitted to OBX presented an up-regulation in BDNF levels (58), while a decrease was observed in older animals (6 months old) (57). Literature supports that BDNF levels decline during normal brain aging, which are often accompanied by mild brain atrophy, reduced neuronal function, and synaptic loss (59). Our result reinforces the data on young mice, as we also observed here an increase on BDNF levels in mice submitted to OBX at 7 weeks old. Considering the critical role of BDNF in synaptic plasticity (60, 61), the increment in BDNF content observed in the current study can be associated with the attenuation of behavioral deficits (NORT and YMT) in untreated OBX mice over time. Given that our data show that GUO prevented the OBX-induced BDNF increment and behavioral deficits, it is conceivable that the neuroprotective effect of GUO diminishes the need to increase BDNF. Alternatively, GUO could have an earlier effect on BDNF levels as it has been shown that GUO stimulates BDNF synthesis or its release inducing neuroplasticity in *in vitro* and *in vivo* experiments (27, 51, 62–66).

Chronic GUO completely reversed the OBX-induced hippocampal redox imbalance. The results corroborate previous data (67–70) indicating that GUO exerts its antioxidant action as a direct radical scavenger, preventing the OBX-induced increase in cellular ROS production, NO levels, and decrease in GSH content. The links among redox imbalance, inflammatory status, and depression are well-documented (71), including in our previous study exploring neurochemical changes in OBX mice model of depression, where we had demonstrated that a hippocampal pro-oxidative status is observed at 2 weeks after OBX, remaining dysregulated at least for more 6 weeks (15). Chronic GUO discreetly attenuated the pro-inflammatory status induced by OBX in mice hippocampi. Chronic GUO did not replicate the inhibitory effect on TNF-α release observed with acute GUO administration (69), or the anti-inflammatory effect obtained by subthreshold doses of GUO plus ketamine in mice submitted to a corticosterone model of depression (72). Overall, results indicate that the potential anti-inflammatory GUO property does not play a significant for the antidepressant effects of chronic GUO.

Numerous evidences pointed to a relevant role for the glutamatergic system in the pathophysiology of depression disorder (73, 74). Accordingly, it has been documented that different brain regions of OBX rodents are more sensitive to release glutamate when exposed to novelty (75–77). A limitation for this study is the absence of data on CSF glutamate content. Nevertheless, we show that OBX induced an increase in hippocampal glutamate uptake, unaffected by chronic GUO. Regarding the main astrocytic glutamate transporters, OBX mice presented decreased GLAST mRNA expression, with no changes in GLT-1, a pattern unchanged by chronic GUO. Considering that OBX increases glutamate release (75–77), it is arguable that increased glutamate uptake is to be expected. In fact, GUO stimulates glutamate uptake *in vitro* and *in vivo* (16, 17), but only in the presence of high glutamate concentration or neurological conditions, respectively (37). As OBX increases glutamate uptake for a long period, it is of interest to investigate, at different brain areas and time points, if GUO minimizes earlier glutamatergic excitotoxicity by increasing glutamate uptake and/or its use as energy substrate during recovery.

Alongside with the increased hippocampal glutamate uptake, OBX also induced increase in hippocampal FDG metabolism. The increase in FDG metabolism is present at baseline (15 days after surgery) and magnified at follow-up (60 days after surgery). The effect is more prominent in the hippocampus dorsal region in comparison to the ventral part. Although previous studies suggested that glucose hypermetabolism might result from increased gliosis (78), we did not identify changes in GFAP astrocytic marker. Further investigation with a broader range of glial markers is necessary to clarify this effect. Stimulation of the glycolytic pathway (mainly in astrocytes) has been associated to increased BDNF signaling (79), increased levels of oxidative and nitrosative radicals (80), enhancement in inflammatory cytokines (81), and/or increment in glutamate release (82). It is therefore arguable that the increment in [^{18}F]FDG signal (83, 84) observed in untreated OBX mice may result from alterations in multiple brain signaling pathways. The increased in glutamate uptake accompained by increased glucose metabolism and BDNF levels is suggestive of a long-lasting plasticity process in the hippocampus of mice subjected to OBX. Forty-five days of GUO treatment prevented the OBX-induced increase in hippocampal FDG metabolism in untreated animals. The lack of increase in FDG metabolism accompanied by attenuated BDNF increase and diminished redox imbalance corroborate the hypothesis that GUO modulates different brain pathways contributing, directly or indirectly, to a better-balanced brain metabolism.

CONCLUSION

Our data clearly show that chronic GUO attenuates the behavioral changes induced by OBX, an effect accompanied by neurochemical changes associated with hippocampal plasticity. The antidepressant effect elicited by GUO seems to be associated with its ability to concurrently prevent OBX-induced BDNF increment and redox imbalance, as well as increase in FDG metabolism.

Adding to previous reports showing that acute GUO acts as a fast-onset antidepressant (25), the present data suggest continued benefits with chronic treatment. This result is of value since data suggest that GUO systemic administration is safe, well-tolerated, and not associated with major side effects (85, 86). Additionally, our data reinforces the role of the purinergic system in MDD physiopathology.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Comissão de Ética no Uso de Animais (CEUA/UFRGS) project number #24577.

AUTHOR CONTRIBUTIONS

RFA was responsible for the design, acquisition, analysis, interpretation, drafting, and approval of the final version of the manuscript. YN, SL, AR, DGM, BB, FF, DL, LP, GV, and SG were responsible for the acquisition of some data displayed in the manuscript. JC was responsible for the *in vivo* microPET scans. EE, MG, EZ, and DS were responsible for the interpretation, drafting, critical revision, and approval of the final version of the manuscript.

FUNDING

This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Instituto Nacional de Ciência e Tecnologia (INCT) para Excitotoxicidade e Neuroproteção, Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and Financiadora de Estudos e Projetos (FINEP) research grant Rede Instituto Brasileiro de Neurociências (IBN-Net), #01.06.0842-00, by Programa de Pós Graduação em Ciências Biológicas at UFOP, and UFOP/PROPP 19/2020 (#23109.000929/2020-88).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.701408/full#supplementary-material

Supplementary Figure 1 | Representative image of the lateral septal nucleus VOI template, used as a reference region for the SUVr normalization **(A)**. Representative images of the total hippocampus **(B)** and subregions, dorsal **(C)** and ventral **(D)** VOI templates. Representative images of the hippocampal positive (metabolism increase) percentage of change between group Sham Sal baseline and groups Sham GUO, OBX Sal, and OBX GUO, in the baseline scan **(E)**. Representative images of the hippocampal positive (metabolism increase) percentage of change between group Sham Sal baseline and groups Sham Sal, Sham GUO, OBX Sal, and OBX GUO, in the follow-up scan **(F)**. T-statistical maps showing the statistically significant increased metabolism in the hippocampal region on groups Sham GUO, OBX Sal, and OBX GUO, in the follow-up scan, in comparison with the group Sham Sal follow-up **(G)**. Representative images of the hippocampal positive (metabolism increase) percentage of change between group Sham Sal follow-up and groups Sham Sal, Sham GUO, OBX Sal, and OBX GUO, in the follow-up scan **(H)**.

REFERENCES

1. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet.* (2012) 380:2163–96. doi: 10.1016/S0140-6736(12)61729-2
2. Schmaal L, Veltman DJ, van Erp TG, Samann PG, Frodl T, et al. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA major depressive disorder working group. *Mol Psychiatry.* (2016) 21: 806–12. doi: 10.1038/mp.2015.69
3. Citrome L, Jain R, Tung A, Landsman-Blumberg PB, Kramer K, Ali S. Prevalence, treatment patterns, and stay characteristics associated with hospitalizations for major depressive disorder. *J Affect Disord.* (2019) 249:378–84. doi: 10.1016/j.jad.2019.01.044
4. Lee MY, Kim EY, Kim SH, Cho KC, Ha K, Kim KP, et al. Discovery of serum protein biomarkers in drug-free patients with major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry.* (2016) 69:60–8. doi: 10.1016/j.pnpbp.2016.04.009
5. Malhi GS, Mann JJ. Depression. *Lancet.* (2018) 392:2299–312. doi: 10.1016/S0140-6736(18)31948-2
6. Trivedi MH, Greer LT. Cognitive dysfunction in unipolar depression: implications for treatment. *J Affect Disord.* (2014) 152–54:19–27. doi: 10.1016/j.jad.2013.09.012
7. Nemeroff CB. The state of our understanding of the pathophysiology and optimal treatment of depression: glass half full or half empty? *Am J Psychiatry.* (2020) 177:671–85. doi: 10.1176/appi.ajp.2020.20060845
8. Paris J. The mistreatment of major depressive disorder. *Can J Psychiatry.* (2014) 59:148–51. doi: 10.1177/070674371405900306
9. Song C, Leonard EB. The olfactory bulbectomised rat as a model of depression. *Neurosci Biobehav Rev.* (2005) 29:627–47. doi: 10.1016/j.neubiorev.2005.03.010
10. Kelly JP, Wrynn AS, Leonard EB. The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther.* (1997) 74:299–316. doi: 10.1016/S0163-7258(97)00004-1
11. Yuan TF, Slotnick MB. Roles of olfactory system dysfunction in depression. *Prog Neuropsychopharmacol Biol Psychiatry.* (2014) 54:26–30. doi: 10.1016/j.pnpbp.2014.05.013

12. Czeh B, Fuchs E, Wiborg O, Simon M. Animal models of major depression and their clinical implications. *Prog Neuropsychopharmacol Biol Psychiatry.* (2015) 64:293–310. doi: 10.1016/j.pnpbp.2015.04.004
13. Mucignat-Caretta C, Bondi M, Caretta A. Time course of alterations after olfactory bulbectomy in mice. *Physiol Behav.* (2006) 89:637–43. doi: 10.1016/j.physbeh.2006.08.003
14. Hendriksen H, Korte SM, Olivier B, Oosting SR. The olfactory bulbectomy model in mice and rat: one story or two tails? *Eur J Pharmacol.* (2015) 753:105–13. doi: 10.1016/j.ejphar.2014.10.033
15. Almeida RF, Ganzella M, Machado DG, Loureiro SO, Leffa D, Quincozes-Santos A, et al. Olfactory bulbectomy in mice triggers transient and long-lasting behavioral impairments and biochemical hippocampal disturbances. *Prog Neuropsychopharmacol Biol Psychiatry.* (2017) 76:1–11. doi: 10.1016/j.pnpbp.2017.02.013
16. Schmidt AP, Lara DR, Souza OD. Proposal of a guanine-based purinergic system in the mammalian central nervous system. *Pharmacol Ther.* (2007) 116:401–16. doi: 10.1016/j.pharmthera.2007.07.004
17. Bettio LE, Gil-Mohapel J, Rodrigues LA. Guanosine and its role in neuropathologies. *Purinergic Signal.* 2016) 12:411–26. doi: 10.1007/s11302-016-9509-4
18. Ali-Sisto T, Tolmunen T, Toffol E, Viinamaki H, Mantyselka P, Valkonen-Korhonen M, et al. Purine metabolism is dysregulated in patients with major depressive disorder. *Psychoneuroendocrinology.* (2016) 70:25–32. doi: 10.1016/j.psyneuen.2016.04.017
19. Ortiz R, Ulrich H, Zarate CA Jr, Machado-Vieira R. Purinergic system dysfunction in mood disorders: a key target for developing improved therapeutics. *Prog Neuropsychopharmacol Biol Psychiatry.* (2015) 57:117–31. doi: 10.1016/j.pnpbp.2014.10.016
20. Kesebir S, Tatlidil Yaylaci E, Suner O, Gultekin KB. Uric acid levels may be a biological marker for the differentiation of unipolar and bipolar disorder: the role of affective temperament. *J Affect Disord.* (2014) 165:131–4. doi: 10.1016/j.jad.2014.04.053
21. Bettio LE, Cunha MP, Budni J, Pazini FL, Oliveira A, Colla AR, Rodrigues LA. Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. *Behav Brain Res.* (2012) 234:137–48. doi: 10.1016/j.bbr.2012.06.021
22. Bettio LE, Freitas AE, Neis VB, Santos DB, Ribeiro CM, Rosa PB, et al. Guanosine prevents behavioral alterations in the forced swimming test and hippocampal oxidative damage induced by acute restraint stress. *Pharmacol Biochem Behav.* (2014) 127:7–14. doi: 10.1016/j.pbb.2014.10.002
23. Camargo A, Pazini FL, Rosa JM, Wolin IAV, Moretti M, Rosa PB, et al. Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway. *J Psychiatr Res.* (2019) 115:103–12. doi: 10.1016/j.jpsychires.2019.05.017
24. Camargo A, Dalmagro AP, Zeni ALB, Rodrigues SAL. Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: possible role of pro-synaptogenic signaling pathway. *J Affect Disord.* (2020) 271:100–8. doi: 10.1016/j.jad.2020.03.186
25. de Almeida RF, Pocharski CB, Rodrigues ALS, Elisabetsky E, Souza OD. Guanosine fast onset antidepressant-like effects in the olfactory bulbectomy mice model. *Sci Rep.* (2020) 10:8429. doi: 10.1038/s41598-020-65300-w
26. Rosa PB, Bettio LEB, Neis VB, Moretti M, Werle I, Leal RB, et al. The antidepressant-like effect of guanosine is dependent on GSK-3beta inhibition and activation of MAPK/ERK and Nrf2/heme oxygenase-1 signaling pathways. *Purinergic Signal.* (2019) 15:491–504. doi: 10.1007/s11302-019-09681-2
27. Bettio LE, Neis VB, Pazini FL, Brocardo PS, Patten AR, Gil-Mohapel J, et al. The antidepressant-like effect of chronic guanosine treatment is associated with increased hippocampal neuronal differentiation. *Eur J Neurosci.* (2016) 43:1006–15. doi: 10.1111/ejn.13172
28. Abelaira HM, Reus GZ, Quevedo J. Animal models as tools to study the pathophysiology of depression. *Braz J Psychiatry.* (2013) 35 (Suppl 2):S112–20. doi: 10.1590/1516-4446-2013-1098
29. Ramaker MJ, Dulawa CS. Identifying fast-onset antidepressants using rodent models. *Mol Psychiatry.* (2017) 22:656–65. doi: 10.1038/mp.2017.36
30. Menard C, Hodes GE, Russo JS. Pathogenesis of depression: insights from human and rodent studies. *Neuroscience.* (2016) 321:138–62. doi: 10.1016/j.neuroscience.2015.05.053
31. Roche M, Shanahan E, Harkin A, Kelly PJ. Trans-species assessment of antidepressant activity in a rodent model of depression. *Pharmacol Rep.* (2008) 60:404–8.
32. Breuer ME, Groenink L, Oosting RS, Westenberg HG, Olivier B. Long-term behavioral changes after cessation of chronic antidepressant treatment in olfactory bulbectomized rats. *Biol Psychiatry.* (2007) 61:990–5. doi: 10.1016/j.biopsych.2006.08.032
33. Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann-Bard P, et al. Object recognition test in mice. *Nat Protoc.* (2013) 8:2531–7. doi: 10.1038/nprot.2013.155
34. de Sousa LP, de Almeida RF, Ribeiro-Gomes FL, de Moura Carvalho LJ, E. Souza TM, de Souza DOG, et al. Long-term effect of uncomplicated Plasmodium berghei ANKA malaria on memory and anxiety-like behaviour in C57BL/6 mice. *Parasit Vectors.* (2018) 11:191. doi: 10.1186/s13071-018-2778-8
35. Jaehne EJ, Corrigan F, Toben C, Jawahar MC, Baune TB. The effect of the antipsychotic drug quetiapine and its metabolite norquetiapine on acute inflammation, memory and anhedonia. *Pharmacol Biochem Behav.* (2015) 135:136–44. doi: 10.1016/j.pbb.2015.05.021
36. Hansel G, Ramos DB, Delgado CA, Souza DG, Almeida RF, Portela LV, et al. The potential therapeutic effect of guanosine after cortical focal ischemia in rats. *PLoS One.* (2014) 9:e90693. doi: 10.1371/journal.pone.0090693
37. Almeida RF, Comasseto DD, Ramos DB, Hansel G, Zimmer ER, Loureiro SO, et al. Guanosine anxiolytic-like effect involves adenosinergic and glutamatergic neurotransmitter systems. *Mol Neurobiol.*(2016) 54:423–36. doi: 10.1007/s12035-015-9660-x
38. Livak KJ, Schmittgen DT. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods.* (2001) 25:402–8. doi: 10.1006/meth.2001.1262
39. Nonose Y, Gewehr PE, Almeida RF, da Silva JS, Bellaver B, Martins LA, et al. Cortical bilateral adaptations in rats submitted to focal cerebral ischemia: emphasis on glial metabolism. *Mol Neurobiol.*(2017) 55:2025–41. doi: 10.1007/s12035-017-0458-x
40. Bergeron M, Cadorette J, Tetrault MA, Beaudoin JF, Leroux JD, Fontaine R, et al. Imaging performance of LabPET APD-based digital PET scanners for pre-clinical research. *Phys Med Biol.* (2014) 59:661–78. doi: 10.1088/0031-9155/59/3/661
41. Fares J, Bou Diab Z, Nabha S, Fares Y. Neurogenesis in the adult hippocampus: history, regulation, prospective roles. *Int J Neurosci.* (2019) 129:598–611. doi: 10.1080/00207454.2018.1545771
42. Marin C, Laxe S, Langdon C, Berenguer J, Lehrer E, Marino-Sanchez F, et al. Olfactory function in an excitotoxic model for secondary neuronal degeneration: role of dopaminergic interneurons. *Neuroscience.* (2017) 364:28–44. doi: 10.1016/j.neuroscience.2017.09.008
43. Marin C, Langdon C, Alobid I, Fuentes M, Bonastre M, Mullol J. Recovery of olfactory function after excitotoxic lesion of the olfactory bulbs is associated with increases in bulbar SIRT1 and SIRT4 expressions. *Mol Neurobiol.* (2019) 56:5643–53. doi: 10.1007/s12035-019-1472-y
44. Marin C, Langdon C, Alobid I, Mullol J. Olfactory dysfunction in traumatic brain injury: the role of neurogenesis. *Curr Allergy Asthma Rep.* (2020) 20:55. doi: 10.1007/s11882-020-00949-x
45. Yuan TF. BDNF signaling during olfactory bulb neurogenesis. *J Neurosci.* (2008) 28:5139–40. doi: 10.1523/JNEUROSCI.1327-08.2008
46. Giuliani P, Buccella S, Ballerini P, Ciccarelli R, D'Alimonte I, Cicchitti S, et al. Guanine-based purines modulate the effect of L-NAME on learning and memory in rats. *Panminerva Med.* (2012) 54 (1 Suppl 4):53–8.
47. Vinade ER, Izquierdo I, Lara DR, Schmidt AP, Souza OD. Oral administration of guanosine impairs inhibitory avoidance performance in rats and mice. *Neurobiol Learn Mem.* (2004) 81:137–43. doi: 10.1016/j.nlm.2003.12.003
48. Vinade ER, Schmidt AP, Frizzo ME, Izquierdo I, Elisabetsky E, Souza OD. Chronically administered guanosine is anticonvulsant, amnesic and anxiolytic in mice. *Brain Res.* (2003) 977:97–102. doi: 10.1016/S0006-8993(03)02769-0

49. Ganzella M, de Oliveira ED, Comassetto DD, Cechetti F, Cereser VH Jr, Moreira JD, et al. Effects of chronic guanosine treatment on hippocampal damage and cognitive impairment of rats submitted to chronic cerebral hypoperfusion. *Neurol Sci.* (2012) 33:985–97. doi: 10.1007/s10072-011-0872-1
50. Nagane A, Baba H, Nakano Y, Maeshima H, Hukatsu M, Ozawa K, et al. Comparative study of cognitive impairment between medicated and medication-free patients with remitted major depression: class-specific influence by tricyclic antidepressants and newer antidepressants. *Psychiatry Res.* (2014) 218:101–5. doi: 10.1016/j.psychres.2014.04.013
51. Piermartiri TCB, Dos Santos B, Barros-Aragao FGQ, Prediger RD, Tasca IC. Guanosine promotes proliferation in neural stem cells from hippocampus and neurogenesis in adult mice. *Mol Neurobiol.* (2020) 57:3814–26. doi: 10.1007/s12035-020-01977-4
52. Mizuki D, Qi Z, Tanaka K, Fujiwara H, Ishikawa T, Higuchi Y, Matsumoto K. Butea superba-induced amelioration of cognitive and emotional deficits in olfactory bulbectomized mice and putative mechanisms underlying its actions. *J Pharmacol Sci.* (2014) 124:457–67. doi: 10.1254/jphs.13252FP
53. Bortolato B, Miskowiak KW, Kohler CA, Maes M, Fernandes BS, Berk M, et al. Cognitive remission: a novel objective for the treatment of major depression? *BMC Med.* (2016) 14:9. doi: 10.1186/s12916-016-0560-3
54. Naudon L, Hotte M, Jay MT. Effects of acute and chronic antidepressant treatments on memory performance: a comparison between paroxetine and imipramine. *Psychopharmacology.* (2007) 191:353–64. doi: 10.1007/s00213-006-0660-4
55. Duman RS, Monteggia ML. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry.* (2006) 59:1116–27. doi: 10.1016/j.biopsych.2006.02.013
56. Kennedy SH, Evans KR, Kruger S, Mayberg HS, Meyer JH, McCann S, et al. Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. *Am J Psychiatry.* (2001) 158:899–905. doi: 10.1176/appi.ajp.158.6.899
57. Antunes MS, Jesse CR, Ruff JR, de Oliveira Espinosa D, Gomes NS, Altvater EET, et al. Hesperidin reverses cognitive and depressive disturbances induced by olfactory bulbectomy in mice by modulating hippocampal neurotrophins and cytokine levels and acetylcholinesterase activity. *Eur J Pharmacol.* (2016) 789:411–20. doi: 10.1016/j.ejphar.2016.07.042
58. Hellweg R, Zueger M, Fink K, Hortnagl H, Gass P. Olfactory bulbectomy in mice leads to increased BDNF levels and decreased serotonin turnover in depression-related brain areas. *Neurobiol Dis.* (2007) 25:1–7. doi: 10.1016/j.nbd.2006.07.017
59. Oh H, Lewis DA, Sibille E. The role of BDNF in age-dependent changes of excitatory and inhibitory synaptic markers in the human prefrontal cortex. *Neuropsychopharmacology.* (2016) 41:3080–91. doi: 10.1038/npp.2016.126
60. Deyama S, Duman SR. Neurotrophic mechanisms underlying the rapid and sustained antidepressant actions of ketamine. *Pharmacol Biochem Behav.* (2020) 188:172837. doi: 10.1016/j.pbb.2019.172837
61. Ciccarelli R, Ballerini P, Sabatino G, Rathbone MP, D'Onofrio M, Caciagli F, et al. Involvement of astrocytes in purine-mediated reparative processes in the brain. *Int J Dev Neurosci.* (2001) 19:395–414. doi: 10.1016/S0736-5748(00)00084-8
62. Bau C, Middlemiss PJ, Hindley S, Jiang S, Ciccarelli R, Caciagli F, et al. Rathbone: guanosine stimulates neurite outgrowth in PC12 cells via activation of heme oxygenase and cyclic GMP. *Purinergic Signal.* (2005) 1:161–72. doi: 10.1007/s11302-005-6214-0
63. Su C, Elfeki N, Ballerini P, D'Alimonte I, Bau C, Ciccarelli R, et al. Guanosine improves motor behavior, reduces apoptosis, and stimulates neurogenesis in rats with parkinsonism. *J Neurosci Res.* (2009) 87:617–25. doi: 10.1002/jnr.21883
64. Rathbone MP, Middlemiss P, Andrew C, Caciagli F, Ciccarelli R, Di Iorio P, et al. The trophic effects of purines and purinergic signaling in pathologic reactions of astrocytes. *Alzheimer Dis Assoc Disord.* (1998) 12 (Suppl 2):S36–45. doi: 10.1097/00002093-199803001-00006
65. Rathbone M, Pilutti L, Caciagli F, Jiang S. Neurotrophic effects of extracellular guanosine. *Nucleosides Nucleotides Nucleic Acids.* (2008) 27:666–72. doi: 10.1080/15257770802143913
66. Rosa PB, Bettio LEB, Neis VB, Moretti M, Kaufmann FN, Tavares MK, et al. Antidepressant-like effect of guanosine involves activation of AMPA receptor and BDNF/TrkB signaling. *Purinergic Signal.*(2021) 17:285–301. doi: 10.1007/s11302-021-09779-6
67. Bellaver B, Rocha AS, Souza DG, Leffa DT, De Bastiani MA, Schu G, et al. Activated peripheral blood mononuclear cell mediators trigger astrocyte reactivity. *Brain Behav Immun.* (2019) 80:879–88. doi: 10.1016/j.bbi.2019.05.041
68. Gudkov SV, Shtarkman IN, Smirnova VS, Chernikov AV, Bruskov IV. Guanosine and inosine as natural antioxidants and radioprotectors for mice exposed to lethal doses of gamma-radiation. *Dokl Biochem Biophys.* (2006) 407:47–50. doi: 10.1134/S1607672906020013
69. D'Alimonte I, Flati V, D'Auro M, Toniato E, Martinotti S, Rathbone MP, et al. Guanosine inhibits CD40 receptor expression and function induced by cytokines and beta amyloid in mouse microglia cells. *J Immunol.* (2007) 178:720–31. doi: 10.4049/jimmunol.178.2.720
70. Jiang S, Bendjelloul F, Ballerini P, D'Alimonte I, Nargi E, Jiang C, et al. Guanosine reduces apoptosis and inflammation associated with restoration of function in rats with acute spinal cord injury. *Purinergic Signal.* (2007) 3:411–21. doi: 10.1007/s11302-007-9079-6
71. Hurley LL, Tizabi Y. Neuroinflammation, neurodegeneration, and depression. *Neurotox Res.* (2013) 23:131–44. doi: 10.1007/s12640-012-9348-1
72. Camargo A, Dalmagro AP, Rosa JM, Zeni ALB, Kaster MP, Tasca CI, et al. Subthreshold doses of guanosine plus ketamine elicit antidepressant-like effect in a mouse model of depression induced by corticosterone: Role of GR/NF-kappaB/IDO-1 signaling. *Neurochem Int.* (2020) 139:104797. doi: 10.1016/j.neuint.2020.104797
73. Almeida RF, Thomazi AP, Godinho GF, Saute JA, Wofchuk ST, Souza DO, et al. Effects of depressive-like behavior of rats on brain glutamate uptake. *Neurochem Res.* (2010) 35:1164–71. doi: 10.1007/s11064-010-0169-4
74. Murrough JW, Abdallah CG, Mathew JS. Targeting glutamate signalling in depression: progress and prospects. *Nat Rev Drug Discov.* (2017) 16:472–86. doi: 10.1038/nrd.2017.16
75. Ho YJ, Chang YC, Liu TM, Tai MY, Wong CS, Tsai FY. Striatal glutamate release during novelty exposure-induced hyperactivity in olfactory bulbectomized rats. *Neurosci Lett.* (2000) 287:117–20. doi: 10.1016/S0304-3940(00)01152-6
76. Jimenez-Sanchez L, Castane A, Perez-Caballero L, Grifoll-Escoda M, Lopez-Gil X, Campa L, et al. Activation of AMPA receptors mediates the antidepressant action of deep brain stimulation of the infralimbic prefrontal cortex. *Cereb Cortex.* (2016) 26:2778–89. doi: 10.1093/cercor/bhv133
77. Ruda-Kucerova J, Amchova P, Havlickova T, Jerabek P, Babinska Z, Kacer P, et al. Reward related neurotransmitter changes in a model of depression: an in vivo microdialysis study. *World J Biol Psychiatry.* (2015) 16:521–35. doi: 10.3109/15622975.2015.1077991
78. Barron AM, Tokunaga M, Zhang MR, Ji B, Suhara T, Higuchi M. Assessment of neuroinflammation in a mouse model of obesity and beta-amyloidosis using PET. *J Neuroinflammation.* (2016) 13:221. doi: 10.1186/s12974-016-0700-x
79. Burkhalter J, Fiumelli H, Allaman I, Chatton JY, Martin LJ. Brain-derived neurotrophic factor stimulates energy metabolism in developing cortical neurons. *J Neurosci.* (2003) 23:8212–20. doi: 10.1523/JNEUROSCI.23-23-08212.2003
80. Tilleux S, Hermans E. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. *J Neurosci Res.* (2007) 85:2059–70. doi: 10.1002/jnr.21325
81. Backes H, Walberer M, Ladwig A, Rueger MA, Neumaier B, Endepols H, et al. Glucose consumption of inflammatory cells masks metabolic deficits in the brain. *Neuroimage.* (2016) 128:54–62. doi: 10.1016/j.neuroimage.2015.12.044
82. Hertz L, Chen Y. Integration between glycolysis and glutamate-glutamine cycle flux may explain preferential glycolytic increase during brain activation, requiring glutamate. *Front Integr Neurosci.* (2017) 11:18. doi: 10.3389/fnint.2017.00018
83. Zimmer ER, Parent MJ, Souza DG, Leuzy A, Lecrux C, Kim HI, et al. [(18)F]FDG PET signal is driven by astroglial glutamate transport. *Nat Neurosci.* (2017) 20:393–5. doi: 10.1038/nn.4492

84. Nortley R, Attwell D. Control of brain energy supply by astrocytes. *Curr Opin Neurobiol.* (2017) 47:80–5. doi: 10.1016/j.conb.2017.09.012
85. Molz S, Dal-Cim T, Budni J, Martín-de-Saavedra M, Egea J, Romero A, et al. Neuroprotective effect of guanosine against glutamate-induced cell death in rat hippocampal slices is mediated by the phosphatidylinositol-3 kinase/Akt/glycogen synthase kinase 3β pathway activation and inducible nitric oxide synthase inhibition. *J Neurosci Res.* (2011) 89:1400–8. doi: 10.1002/jnr.22681
86. Tasca CI, Lanznaster D, Oliveira KA, Fernández-Dueñas V, Ciruela F. Neuromodulatory effects of guanine-based purines in health and disease. *Front Cell Neurosci.* (2018) 12:376. doi: 10.3389/fncel.2018.00376

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 19 January 2022
doi: 10.3389/fpsyt.2021.596017

The Cognitive-Enhancing Effects of *Dendrobium nobile* Lindl Extract in Sleep Deprivation-Induced Amnesic Mice

Ning Jiang[1,2†], *Yu-jiao Li*[1†], *Meng-di Wang*[1], *Hong Huang*[2], *Shanguang Chen*[3], *Yinghui Li*[3], *Lina Qu*[3], *Fengzhong Wang*[4], *Xinmin Liu*[1,2*] and *Qiong Wang*[1,4*]

[1] *Sino-Portugal TCM International Cooperation Center, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, China,* [2] *Research Center for Pharmacology and Toxicology, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China,* [3] *National Key Laboratory of Human Factors Engineering/State Key Laboratory of Space Medicine Fundamentals and Application, Chinese Astronaut Research and Training Center, Beijing, China,* [4] *Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China*

OPEN ACCESS

Edited by:
Zhang-Jin Zhang,
The University of Hong Kong, Hong Kong SAR, China

Reviewed by:
Elizabeth Mary Williamson, University of Reading, United Kingdom
Biao Li, Chinese Academy of Medical Sciences and Peking Union Medical College, China

***Correspondence:**
Qiong Wang
luyiwangqiong@163.com;
wqimplad@126.com
Xinmin Liu
liuxinmin@hotmail.com

[†] *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *02 September 2020*
Accepted: *27 October 2021*
Published: *19 January 2022*

Citation:
Jiang N, Li Y-j, Wang M-d, Huang H, Chen S, Li Y, Qu L, Wang F, Liu X and Wang Q (2022) The Cognitive-Enhancing Effects of Dendrobium nobile Lindl Extract in Sleep Deprivation-Induced Amnesic Mice. Front. Psychiatry 12:596017. doi: 10.3389/fpsyt.2021.596017

Chronic sleep deprivation (SD) causes neurological and neurodegenerative dysfunction including learning and memory deficit. The orchid *Dendrobium nobile* Lindl (DNL), is widely used as a Yin tonic and medicinal food throughout Asia, and has many reported pharmacological effects. This study focused on the cognitive-enhancing effects of DNL in sleep deprivation-induced amnesia in mice and its biochemical mechanisms. Our results showed that the mice displayed significant cognitive deficits after 2-week SD while treatment with the extract of DNL prevented these impairments. In the novel object recognition and object location recognition tasks, a significant increase in the discrimination index was observed in DNL-treated (200 and 400 mg/kg) mice. In the MWM test, DNL (200 and 400 mg/kg) treatment shorten the prolongation of latency and increased the crossing numbers compared with SD mice. The biochemical analysis of brain tissue showed a decrease in NE, dismutase (T-SOD) and catalase (CAT) activity and an increase in 5-HT and malondialdehyde (MDA) concentration after the treatment with DNL in mice. Our findings indicated that DNL exerted a positive effect in preventing and improving cognitive impairment induced by SD, which may be mediated via the regulation of neurotransmitters and alleviation of oxidative stress.

Keywords: ***Dendrobium nobile*** **Lindl, sleep deprivation, learning and memory, neurotransmitters (5-HT and NE), oxidative stress markers 3**

INTRODUCTION

Chronic sleep deprivation is a common problem, and it has been estimated that 50–70 million adults in the United States have sleep or wakefulness disorder (1). Sleep loss contributes to neurological and neurodegenerative disorders including memory deficits which are increasingly regarded as a major public health and safety issue (2) and financial and social burden. Cognitive enhancers including as modafinil and donepezil, and stimulants such as caffeine and nicotine have been reported to prevent the memory impairment induced by chronic sleep deprivation (SD), but many have undesirable side-effects and cause habituation (3). Safer and more effective treatments for memory impairment induced by SD are thus under investigation.

Dendrobium nobile Lindl (DNL) is a precious traditional Chinese medicine, it's an epiphytic orchid distributed throughout tropical and subtropical Asia (N. E India, China, Malaysia, Japan) which is used a tonic and medicinal food (**Figure 1**). The main chemical components of DNL are alkaloids, polysaccharides, amino acids, phenols, volatile oils, etc. The DNL has a wide range of pharmacological effects (4, 5). Recently researches focused on its neuroprotective and cognitive-enhancing effects (6, 7), and its effect on improving nerve cell damage (8). It was reported that the alkaloids and polysaccharide in DNL have obvious protective effects on LPS-induced learning and memory impairment in rats (9). DNL also has displayed ameliorative effects on memory impairment induced by lipopolysaccharide and $A\beta_{25-35}$ in rats, but its activity against memory impairment induced by sleep deprivation has not been reported (10, 11). The present study was designed to investigate the effect of DNL extract on learning and memory in a sleep deprivation model and its underlying mechanisms, to explore its potential application in treating SD-induced impairment.

FIGURE 1 | The picture of DNL.

TABLE 1 | Chemical profile of *Dendrobium nobile* extract (DNL).

Number	Peak area	Compound	Molecular formula
1	2805.415137	-	-
2	100197.7715	Ficusal-4-O-β- d -glucopyranoside	C24H28O11
3	144967.1248	-	-
4	22285.69431	dendroside G	C21H34O10
5	11267.58756	dendrobin A	C16H18O4
6	14866.99716	dendronobiloside A	C27H48O12
7	686.8800255	dendronobiloside C	C27H44O12
8	33576.83579	-	-
9	258.3121648	-	-
10	75152.12667	Citrusin C	C16H22O7
11	87059.25145	Trans-methyl cinnamate-2-O-beta-D-glucoside	C16H20O8
12	108334.4155	-	-
13	23859.5398	Zhepiresinol	C14H16O6
14	21912.22788	-	-

MATERIALS AND METHODS

Drugs

Donepezil hydrochloride were purchased from Eisai (Ibaraki, Japan). Norepinephrine (NE) from the National institutes for Food and Drug Control (Beijing, China); dopamine (DA), 5-hydroxytryptamine (5-HT), 5-HIAA (5-hydroxyindole acetic acid), GABA (Gamma amino acid butyric acid) and DOPAC (Dihydroxy- phenyl acetic acid) from Sigma-Aldrich Co. (St. Louis, MO, USA) and Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) commercial kit were from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). Air-dried stems of DNL were purchased from Luzhou in SiChuan province and further authenticated by professor Bengang Zhang (Institute of Medicinal Plant Development, Beijing, China) according to their macroscopic characteristics. The air-dried stems of DNL were crushed and soaked in 80% acidic ethanol for 1 night. The extract was re-extracted three times with boiling acidic ethanol. After filtration, the solution was concentrated by vacuum-rotary evaporation at 60°C and then freeze-drying 12 h, The extraction value was 14%.

Analysis

DNL was characterized chromatographically under the following conditions: Waters Acquity UPLC HSS T3 (1.8 um, 2.1 × 100 mm); flow rate: 0.3 ml/min, column temperature: 30°C; mobile phase A, 0.1% formic acid aqueous solution; phase B acetonitrile as a gradient elution. The chemical profile is shown in **Table 1**.

Animals and Treatments

72 ICR male mice (20–22 g) (Institute of the Chinese Academy of Medical Science Center, Beijing, China) were housed under standard conditions for 3 days prior to testing to adapt to the new environment. All experiments were conducted according to the "Principles of Laboratory Animal Care" (NIH publication No.86-23 1996) and P.R. China legislation. The protocols were approved by the committee for the Care and Use of Laboratory Animals of IMPLAD, CAMS & PUMC, China (NO. 20161028), all experiments adhered to standard biosecurity and institutional safety procedures.

Animals were divided into 6 groups: the control group, the SD model group, the DNL-treated groups (100, 200, and 400 mg/kg) and the donepezil group (3 mg/kg). The doses of DNL and Donezepil were based on our previous study (12). DNL and donepezil were administrated orally once a day to mice for 14 days before SD until the end of experiment. The control group and the SD model group were given the same volume of distilled

Abbreviations: SD, sleep deprivation; DI, discrimination index; DNL, Dendrobium nobile Lindl; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MWM, Morris water maze; NOR, novel object recognition; OLR, object location recognition.

water, and except for the control group, other mice were exposed to SD for 14 days.

After habituation for 3 days, all except the control group were subjected to SD from 8 a.m. to 11 a.m. each day. Then all animals were moved back to the housing room. The process lasted for 2 days in order that the mice could acclimatize. Following the induction period, all test groups were exposed to SD for 14 days. After a 2-week SD exposure, behavioral changes tests were conducted as follows: open field test (OFT), novel object recognition task (NOR), object location recognition task (OLR), and Morris water maze test (MWM). Animals were sacrificed after the behavior tests and blood and brain tissue were taken for analysis (**Figure 2**).

The SD Mice Model

The SD procedure was performed as our previously studies in our lab (3, 13, 14). Briefly, except the mice in the control group, other mice were placed in Sleep Deprivation Apparatus (developed by the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, and the Chinese Astronaut Center, patent No. 201210356645.X) for 2 weeks continuously. Before encountered to the SD procedure, the mice receipt 3 days' adaptation (3 h per day, during 12:00 p.m.−15:00 p.m.). The speed of SD apparatus was 60 s per rotation and there was a 2 min pause between two rotations. After finishing the adaptation, the mice were put into the SD apparatus and suffered for 14 consecutive days' SD modeling, then the behavioral tests were tested in mice. The mice were free to have water and food during the SD experiments.

Behavioral Tests

Open-Field Test

The open field test was conducted on day 15. The apparatus consists of four metal tanks (diameter 30 cm, height 40 cm) with a video camera fixed at the top (15). For each experiment, four mice were place in the center of each tank and their locomotor activities in 10 min were detected. The total distance was recorded as the index of the locomotive activity in mice.

Novel Object Recognition Task

This task consists of three phases: habituation, familiarization, and test. In the habituation phase, animals were placed individually in a square metal box (black, 60 × 40 × 80cm) with no objects in it, for 10 min in three consecutive days. On day 4, in the familiarization phase, two identical objects (A1 and A2) were placed on opposite sides of the box, and the animal was placed in the box for 5 min to explore the two objects. After 20 min in the home cage, the test was performed and the mice placed in the same metal box and presented with two objects, the old familiar A1, and a new object B, to replace object A2 for 5 min. The discrimination index (DI) was calculated as the percentage of time spent exploring the novel object over the total time spent exploring the two objects (16).

Object Location Recognition Task

The Object Location Recognition (OLR) task is an accepted method for testing spatial location memory. The protocol was similiar as the novel object recognition test. Instead of replacing one of the original identical objects (A1 and A2), A1 or A2 was moved to a new location (17). The discrimination index (DI) was calculated as before (18).

Morris Water Maze Test

The Morris Water Maze test was performed on the 21st day of SD to further investigate the effects of DNL on spatial memory in mice. The water maze is a circular pool (100 cm in diameter, 40 cm in high), with the water (23–25°C) made opaque with black ink. An "invisible" platform (metal, black, 6 cm in diameter, 15 cm in high) was placed 1.5 cm below the water surface, providing the only outlet channel. The protocol was described in our previous studies with minor modifications (19–21).

Escape Acquisition

The mice were subjected to three trials per day for 5 days. At the beginning of the test, the mice were trained to remember the platform by being placed on it for 10 s, before being released into the water, facing the wall, at a different starting point each time, and allowed to swim for a maximum of 90 s in each trial. If they failed to escape within 90 s, the mice were gently guided to the platform and allowed to stay there again to learn, and the escape latency was recorded as 90 s.

Probe Trial

After the escape acquisition test, each animal was subjected to the probe trial, in which the platform was absent. The mice were released from the opposite quadrant where the platform had been located and allowed to swim and explore the pool for 90 s. Swimming distance in the target quadrant and the number of target crossings were recorded as measures for spatial memory.

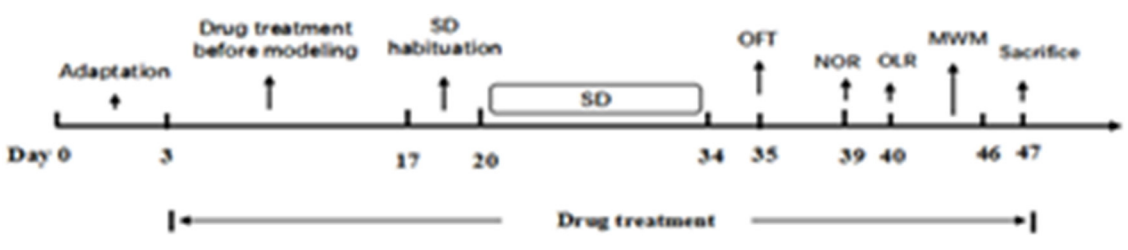

FIGURE 2 | The experimental procedure.

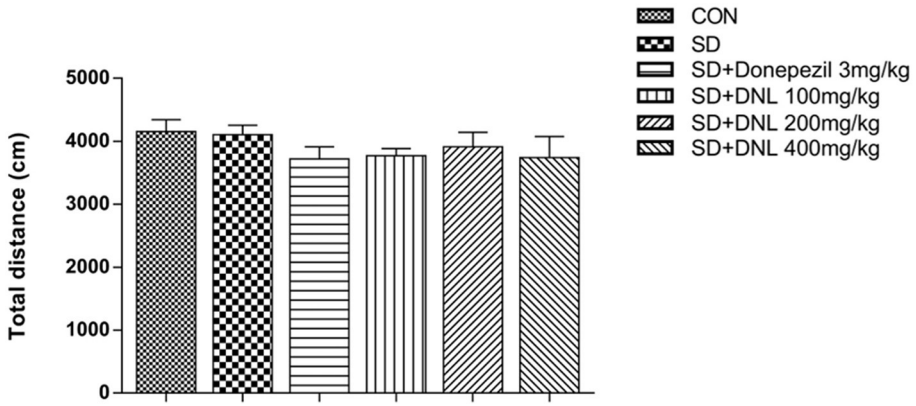

FIGURE 3 | The effect of DNL on the locomotor activities in the open field test after SD for 2 weeks in mice. Values are mean ± SEM, $n = 10$–12.

Biochemical Analysis

The levels of neurotransmitters were determined in the brain tissues in rats described as below:

Brain Tissue Extraction

After the behavioral tests, mice were sacrificed by decapitation. The brains were removed and placed on ice, and the hippocampus and cerebral cortex tissues were dissected out rapidly, weighed, and stored at −80°C.

Measurement of Neurotransmitter Levels in the Hippocampus

Hippocampus tissues were homogenized and mixed with acetonitrile containing the internal standard (5 μg/mL, 3, 4-dihydroxybenzylamine, DHBA). After centrifuging at 4°C (20,000 rpm, 30 min), the supernatant was collected, and neurotransmitter levels measured by liquid chromatography-tandem mass spectrometry. Chromatographic separation was performed on a TSK Gel amide 80 column (2.0 mm × 15 cm, 3 μm); column temperature 35°C; mobile phase system acetonitrile-ammonium formate solution (15 mmol·mL-1, pH = 5.5) (40: 60), flow rate 0.4 mL·min^{-1}; operated under the multiple reaction monitoring (MRM) mode using electrospray ionization (ESI) in the positive ion mode, at m/z 177.0→ 160.0 (5-HT), 170.0152.0 (NE) and 140.0→ 123.0 (DHBA as internal standard). Neurotransmitter concentrations were quantified using peak area ratios vs. internal standard.

Detection of Brain T-SOD, CAT Activities, and MDA Levels

The hippocampus and cerebral cortex were each homogenized in 10 volumes of cold saline. After centrifuging at 4°C (2,500 rpm, 10 min), the supernatants were collected. The total protein content of sample was estimated with the Pierce BCA Assay kit, using bovine serum albumin as the standard (22). T-AOC and CAT activity and MDA levels were determined according to the manufacturer's protocols (Jiancheng Institute of Biotechnology, Nanjing, China).

Data and Statistical Analyses

Statistical analysis was carried out using SPSS 17.0 software (Chicago, IL, USA). All data are expressed as mean ± SEM. Data recorded from the acquisition trials of the MWM among the groups over a period of 5 days were analyzed by using repeated-measure two-way ANOVA. Other data were analyzed by one-way ANOVA followed by multiple *post hoc* comparisons using the LSD test. Statistical significance was defined as $p < 0.05$ or $p < 0.01$.

RESULTS

Effects of DNL in the Open-Field Test

The total distance is used to indicate the locomotive activity after 2-week SD. **Figure 3** showed there was no significant difference between the six groups.

Effects of DNL in the NOR Task

After 2-week SD, in the familiar phase, the mice spent comparable time on exploring the two similar objects, with no preference between positions of the objects. During the test phase, the DI significantly decreased in the SD model group in comparison with the control group. Compared with the SD group, the DI of the three DNL-treated groups showed a significant decrease ($p < 0.05$, **Figure 4**).

Effects of DNL in the OLR Task

Be similiar with the NOR, mice in the OLR task showed no difference in preference in the familiarization phase. In the test phase, the control mice spent significantly longer exploring on the object in the new location compared with the SD mice. DNL treatment (200 and 400 mg/kg) significantly increased DI compared to the SD group ($p < 0.01$; **Figure 5**), however the donepezil group (3 mg/kg) showed no significant difference compared to the SD group ($p > 0.05$).

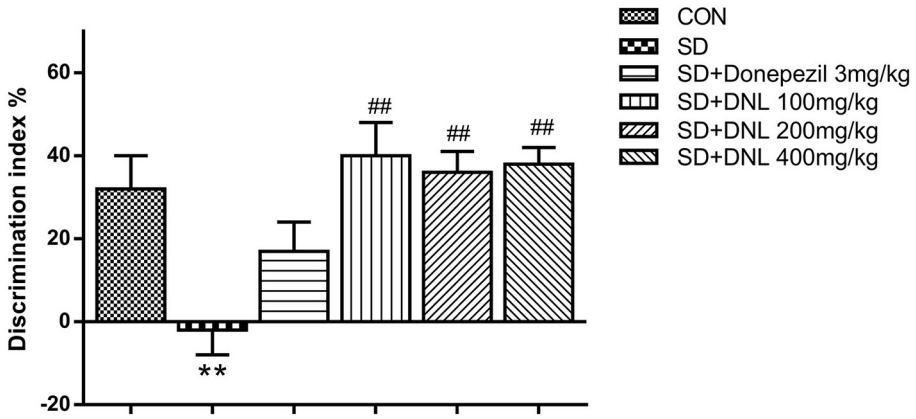

FIGURE 4 | The effect of DNL on the discrimination index (DI) during the testing session in the NOR task after SD for 2 weeks in mice. Values are mean ± SEM, n = 10–12. $^{**}p < 0.01$ vs. the Con group; $^{\#\#}p < 0.01$ vs. the SD group.

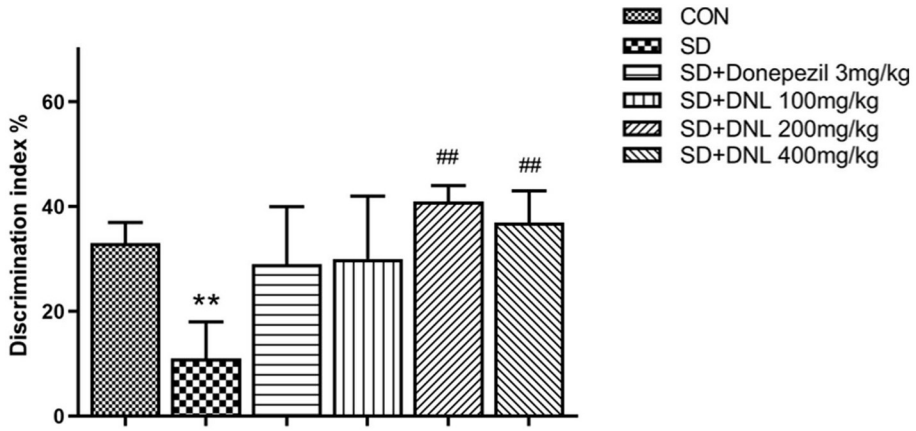

FIGURE 5 | The effect of DNL on the discrimination index (DI) during the testing session in the OLR task after SD for 2 weeks in mice. Values are mean ± SEM, n = 10–12. $^{**}p < 0.01$ vs. the Con group; $^{\#\#}p < 0.01$ vs. the SD group.

Effects of DNL in the MWM Test

The Morris water maze task was conducted over 6 days, ending with the probe test on the last day. As shown in **Figures 6A–C**, in the escape acquisition phase, the escape latency and the swimming distance in SD mice was higher than the control group from the second day ($p < 0.05$; $p < 0.01$). The swimming speed in the SD group was decreased from day 3 ($p < 0.05$). DNL at a dose of 200 mg/kg attenuated the spatial learning deficits, as observed by a reduction of the escape latency and swimming distance from day 3 to day 5 ($p < 0.01$; $p < 0.05$). Similarly, the high-dose group (400 mg/kg) on days 4 and 5 reduced escape latency compared to the SD mice. Donepezil (3 mg/kg) reduced the escape latency and the swimming distance and increased the swimming speed on the 5th day ($p < 0.01$; $p < 0.05$).

In the probing phase (**Figures 6D–G**), SD rats showed a decreased percentage in swimming distance and swimming duration in the target quadrant ($p < 0.05$) compared to the control rats, whereas DNL (200, 400 mg/kg) mitigated the trend. The swimming speed in the target quadrant was no different in all groups. The number of SD rats crossing through the platform where had previously been located was significantly reduced compared with the control rats ($p < 0.01$). With the treatment of DNL (200, 400 mg/kg), the crossing number was increased compared with the SD group ($p < 0.05$).

Effects of DNL on 5-HT and NE in the Hippocampus

The neurotransmitter content of the hippocampus after 44 days of DNL treatment is shown in **Figure 7**. After SD induction, hippocampus level of 5-HT in SD mice were increased, while the concentration of NE was decreased compared to the control group ($p < 0.01$). Treatment with DNL (100, 200, and 400 mg/kg) decreased 5-HT level while elevating NE level in the hippocampus in SD rats ($p < 0.05, p < 0.01, p < 0.05$). No marked difference in 5-HT and NE levels detected in donepezil-treated SD mice.

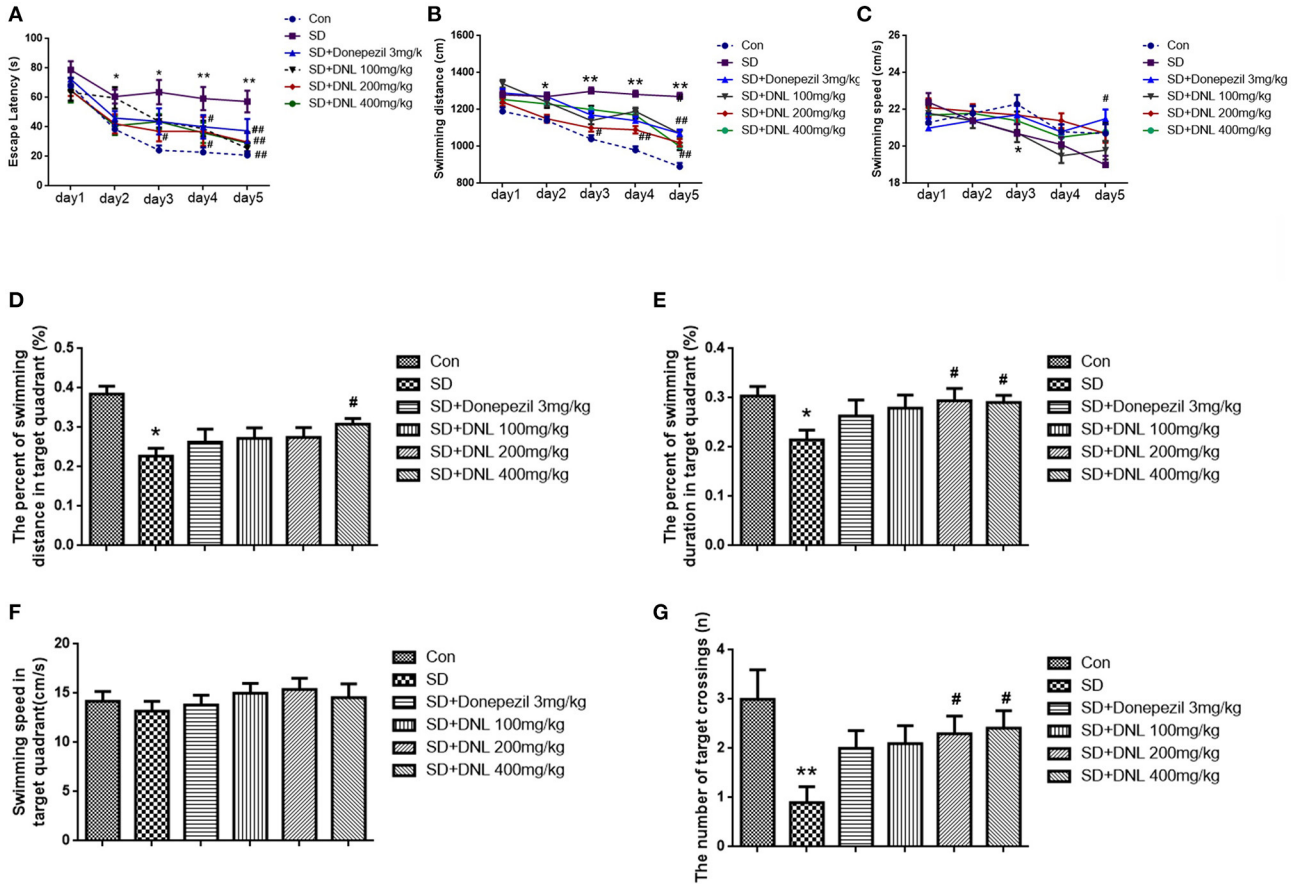

FIGURE 6 | The effect of DNL on the Escape Latency **(A)**; Swimming distance **(B)**; Swimming speed **(C)**; The percent of swimming distance in target quadrant **(D)**; The percent of swimming duration in target quadrant **(E)**; Swimming speed in target quadrant **(F)**; The number of target crossings **(G)** in the MWM test after SD 2 weeks in mice. Values are mean ± SEM, $n = 10$–12. $^{*}p < 0.05$ vs. the Con group, $^{**}p < 0.01$ vs. the Con group; $^{\#}p < 0.05$ vs. the SD group, $^{\#\#}p < 0.01$ vs. the SD group.

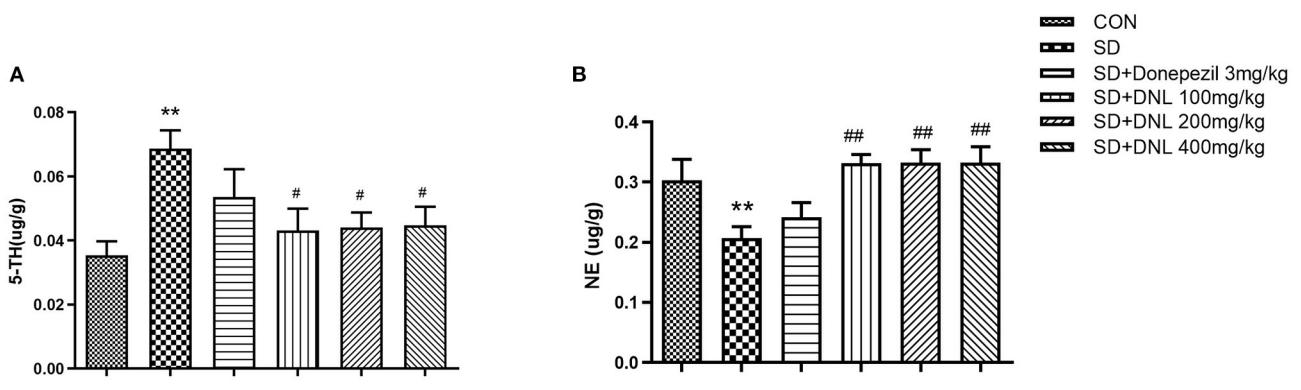

FIGURE 7 | The effect of DNL on 5-hydroxytyptamine (5-HT) **(A)** and norepinephrine (NE) **(B)** levels in the hippocampus after SD 2 weeks in mice. Values are mean ± SEM, $n = 8$. $^{**}p < 0.01$ vs. the Con group; $^{\#}p < 0.05$ vs. the SD group, $^{\#\#}p < 0.01$ vs. the SD group.

Effects of DNL on Oxidative Stress Markers in Hippocampus and Cerebral Cortex

The T-SOD and CAT activities were significantly decreased, while MDA levels was markedly increased in both hippocampus and cerebral cortex in the SD group compared with the control group ($p < 0.05$) (**Figure 8**). Compared with the SD mice, brain T-SOD activities in all the DNL-treated groups were increased ($p < 0.05$, $p < 0.01$). DNL (400 mg/kg) treated mice showed a significant

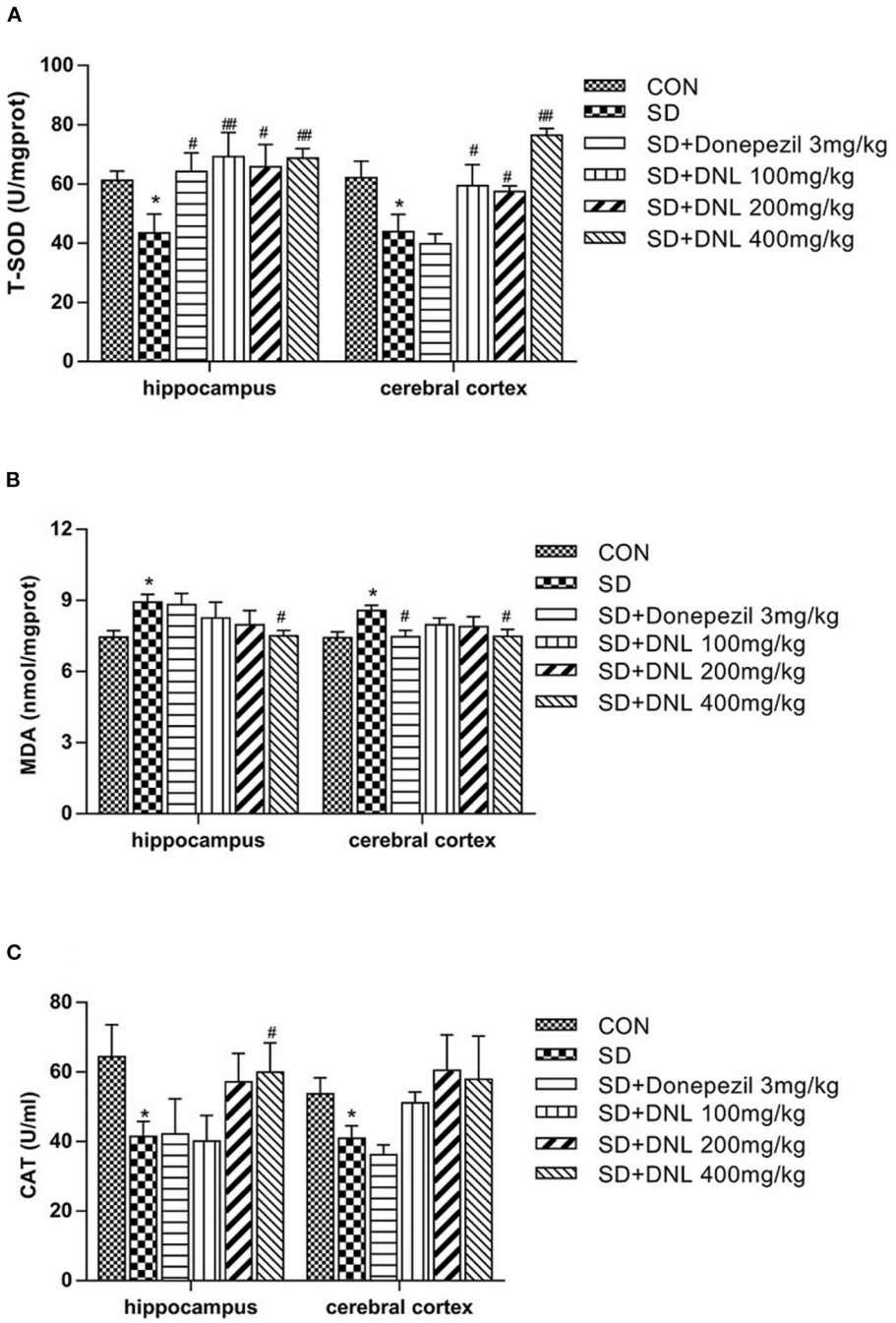

FIGURE 8 | The effect of DNL on the activities of Total Superoxide Dismutase (T-SOD) **(A)**; Catalase (CAT) **(B)** and Malonaldehyde (MDA) **(C)** in the hippocampus and cerebral cortex of mice after SD for 2 weeks. Values are mean ± SEM, $n = 8$. $^{*}p < 0.05$ vs. the Con group; $^{\#}p < 0.05$ vs. the SD group, $^{\#\#}p < 0.01$ vs. the SD group.

decrease in the level of MDA in both brain regions compared to the SD group ($p < 0.05$). Hippocampus CAT activities of DNL (400 mg/kg) group were increased compared to the SD mice ($p < 0.05$). Donepezil (3 mg/kg) treatment significantly increased hippocampus T-SOD activities and reduced cerebral cortex MDA level in the SD mice ($p < 0.05$).

DISCUSSION

Sleep Deprivation Could Induce Cognitive Impairments

Sleeping plays a key role on human's health, especially for the brain health. Adequate sleep is basically imperative for memory

consolidation (23). Accumulating evidence suggests that sleep deprivation produces could induce memory deficits, and in animals these can be assessed by using behavioral tests, including avoidance tasks (24), object recognition tasks and the Morris water maze task (25). Sleep deprivation as cognitive challenge may therefore provide a promising preclinical model of memory impairment and a useful tool to study cognition enhancing drugs (26). Previous studies exhibited that sleep deprivation could induce serious cognitive dysfunction (27, 28). In our experiment, we found SD 2 weeks in mice could induced obviously cognitive associated behavioral impairments, such as spatial and non-spatial learning and memory deficits. These results are consistent with previous studies.

DNL Has No Sensorimotor Effect in the Open-Field Test in the SD Mice

Open-field test is mainly used to test the locomotor activities of animals in a relatively closed environment during a certain period of time, and widely used in animal experiment (29). The results of this study showed that after 2-week of SD, there was no significant difference between any two groups, which showed DNL had no influence on the locomotor activity in mice. This result indicated that the effect of DNL was originally mnemonic, rather than the sensorimotor effect.

DNL Could Ameliorate Short-Term Memory in the ORT in the SD Mice

The object recognition task (ORT) is based on the principle of preference for novel objects in rodents and can assess recognition memory in the spontaneous state (30). The ORT takes advantage of the nature of preference for "novel" objects in rodents in the spontaneous state (31). During the experiment, the animals are in accordance with the characteristics, the location, the order of appearance, and the background of objects to distinguish the different "identity" objects, achieving the transition through detection of simple non-spatial memory to complex spatial, temporal and episodic memory (32). Based on the ORT, the object location recognition (OLR) and the novel object recognition (NOR) were established. The former was used to assess short-term, spatial memory, while the latter was designed to evaluate short-term, non-spatial memory. In agreement with the previous studies (33, 34), our results showed that SD damaged the normal performance of mice in ORT tasks. However, compared with the SD model group, the treatment of DNL effectively enhanced DI in both OLR and NOR tasks. For the first time, we have demonstrated that DNL administration could significantly ameliorate impaired short-term memory in SD mice.

DNL Could Ameliorate Long-Term and Spatial Memory in the Morris Water Maze in the SD Mice

Morris water maze test was used to assess effects of DNL on the long-term, spatial learning and reference memory. In this task, two main parameters are essential for assessing the spatial learning and memory ability in mice. The first is the escape latency in the acquisition trial, which means that the mice must learn the accurate position of the platform and develop suitable swimming approaches to reach the same from the randomly chosen starting point within 90 s. The second is the virtual-platform crossing numbers when the platform was absent in probe trial, which is a key indicator assessed reference memory (35, 36). In our study, compared with the SD group, the treatment of DNL effectively shorten escape latencies after 2 days' training to find the hidden platform in the escape acquisition phase, which showed DNL has a strong effect on ameliorating the reference memory impairment in the SD mice. In the probe trial, we found DNL could effectively increase the number of target crossings in the SD mice. These results indicating that DNL treatment remarkably improved the long-term, spatial memory in the SD mice.

DNL Could Modulate Memory Deficit via 5-HT and NE in the Hippocampus of SD Mice

Monoamine neurotransmitters play an important role in the learning and memory. 5-HT mediates processes involved in central nervous system fatigue, helping to control sleep (37, 38). NE plays a major role in the maintenance of REM sleep (39), and levels of NE in the brain have been shown to be closely related to memory. In the present study, chronical sleep deprivation induced an obvious elevation of hippocampus 5-HT levels and a marked decline of hippocampus NE levels in mice, which was consistent with the previous study (40, 41). DNL treatment at all doses significantly reversed the elevation of 5-HT and the decrease of NE in SD mice, indicating that the cognitive-enhancing effects of DNL might be at least in part due to modulation of these two major monoamine neurotransmitters.

DNL Could Repair Oxidative Damage in the Hippocampus and Cortex of SD Mice

Oxidative stress plays a prominent role in the pathogenesis of several cognitive impairment processes. Oxidative stress occurs in brain tissues whenever there is increased generation of reactive oxygen species (ROS) and impaired antioxidant defense systems. Brain tissue in particular is more susceptible to the deleterious effects of ROS because of its high rate of oxygen consumption and reduced antioxidant defense systems (19). Excessive ROS production may result in oxidative damage to proteins, lipids, and DNA and eventually in apoptosis or cell death, but ROS levels are balanced by the action of antioxidant enzymes, including CAT, SOD, and glutathione oxide. Sleeping deprivation could weaken the free radical scavenging enzyme system and cause system imbalance, which further aggravates brain damage which ultimately results in learning and memory disorders (42, 43). The changed hippocampal synaptic protein GAP-43, SYP, PSD-95 levels, serum corticosterone levels, and neuroinflammation were found in sleeping deprivation related memory impairment mice (44). Studies have confirmed the abnormal alterations in MDA,

SOD, and CAT activities associated with memory impairment after sleep deprivation (45, 46). Similar changes were exhibited in our study: a decrease of SOD and CAT activities and an increase of MDA level in hippocampus and cerebral cortex of SD mice. Treatment with DNL enhanced antioxidant defense in the brain by increasing SOD and CAT levels and reducing MDA concentration induced by SD, contributing to the cognitive enhancement effect of DNL.

CONCLUSION

The present study shows for the first time the cognitive-enhancing effect of DNL in a chronic stress model induced by SD in mice. The improvement effect of DNL in SD mice may be partially due to regulate the neurotransmitters and mitigate oxidative stress levels in the brain. Our results also indicate that, as a traditional medicinal herbal plant, DNL may be used as a potential candidate agent in preventing and treating cognition impairment induced by stress.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Care and Use of Laboratory Animals of IMPLAD, CAMS & PUMC, China (No. 20161028).

AUTHOR CONTRIBUTIONS

NJ: for doing animal experiments and process all experimental data. Y-jL: assist in animal experiments and process the data. M-dW: provide some experimental equipment help. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Science & Technology Department of Sichuan Province (2019YFH0023), the opening foundation of the State Key Laboratory of Space Medicine Fundamentals and Application, Chinese Astronaut Research and Training Center (SMFA18K06), the National Key Research and Development Program of China (2016YFE0131800), Office of Sciences & Technology and Talent Work of Luzhou (2018LZXNY-ZK32), and the High - end Talents Recruitment Program (Liu Xinmin group) of Luzhou Municipal People's Government.

REFERENCES

1. Strand LB, Carnethon M, Biggs ML, Djoussé L, Kaplan RC, Siscovick DS, et al. Sleep disturbances and glucose metabolism in older adults: the cardiovascular health study. *Diabetes Care.* (2015) 38:2050–8. doi: 10.2337/dc15-0137
2. William DSK. Effects of sleep deprivation on cognition. *Prog Brain Res.* (2010) 185:105–29. doi: 10.1016/B978-0-444-53702-7.00007-5
3. Lu C, Shi Z, Dong LM, Lv JW, Xu P, Li YH, et al. Exploring the effect of ginsenoside Rh1 in a sleep deprivation-induced mouse memory impairment model. *Phytother Res.* (2017) 31:763–70. doi: 10.1002/ptr.5797
4. Huang J, Huang NQ, Zhang MH, Nie J, Xu YY, Wu Q, et al. Dendrobium alkaloids decrease Aβ by regulating α- and β-secretases in hippocampal neurons of SD rats. *PeerJ.* (2019) 6:e7627. doi: 10.7717/peerj.7627
5. Ma C, Meng CW, Zhou QM, Peng C, Liu F, Zhang JW, et al. New sesquiterpenoids from the stems of *Dendrobium nobile* and their neuroprotective activities. *Fitoterapia.* (2019) 138:104351. doi: 10.1016/j.fitote.2019.104351
6. Zheng XK, Cao XW, Feng WS, Kuang HX. Current status in studies of *Dendrobium nobile* Lindl. *Chinese N Drugs J.* (2005) 7:4.
7. Chen JW, Ma H, Huang XN, Gong QH, Wu Q, Shi JS. Improvement of *Dendrobium nobile* Lindl. alkaloids on cognitive deficit in rats induced by lipopolysaccharides. *Chin J Pharmacol Toxicol.* (2008) 22:6. doi: 10.3867/j.issn.1000-3002.2008.06.002
8. Li SY, Zhou JX, Xu SF, Li J, Liu J, Lu YF, et al. Induction of Nrf2 pathway by *Dendrobium nobile* Lindl. *alkaloids protects against carbon tetrachloride induced acute liver injury. Biomed Pharmacother.* (2019) 117:109073. doi: 10.1016/j.biopha.2019.109073
9. Bang YH. ChemInform abstract: phenanthrenes from *Dendrobium nobile* and their inhibition of the LPS-Induced production of nitric oxide in macrophage RAW 264.7 cells. *ChemInform.* (2010) 41. doi: 10.1002/chin.201045217
10. Nie J, Tian Y, Zhang Y, Lu YL, Li LS, Shi JS. Dendrobium alkaloids prevent $A\beta_{25-35}$-induced neuronal and synaptic loss via promoting neurotrophic factors expression in mice. *PeerJ.* (2016) 4:e2739. doi: 10.7717/peerj.2739
11. Yang S, Gong QH, Wu Q, Li F, Lu YF, Shi JS. Alkaloids enriched extract from *Dendrobium nobile* Lindl. *attenuates tau protein hyperphosphorylation and apoptosis induced by lipopolysaccharide in rat brain. Phytomedicine.* (2014) 21:712–6. doi: 10.1016/j.phymed.2013.10.026
12. Jiang N, Fan LX, Yang YJ, Liu XM, Lin HY, Gao L, et al. Antidepressant effects of the extract of *Dendrobium nobile* Lindl on chronic unpredictable mild stress-induced depressive mice. *Sheng Li Xue Bao.* (2017) 69:159–66. doi: 10.13294/j.aps.2017.0006
13. Lu C, Lv JW, Jiang N, Wang HX, Huang H, et al. Protective effects of Genistein on the cognitive deficits induced by chronic sleep deprivation. *Phytother Res.* (2020) 34:846–58. doi: 10.1002/ptr.6567
14. Feng L, Wu HW, Song GQ, Lu C, Li YH, Qu LN, et al. Chronical sleep interruption-induced cognitive decline assessed by a metabolomics method. *Behav Brain Res.* (2016) 302:60–8. doi: 10.1016/j.bbr.2015.12.039
15. Li LS, Lu YL, Nie J, Xu YY, Zhang W, Yang WJ, et al. *Dendrobium nobile* Lindl alkaloid, a novel autophagy inducer, protects against axonal degeneration induced by Aβ25-35 in hippocampus neurons in vitro. *CNS Neurosci Ther.* (2017) 23:329–40. doi: 10.1111/cns.12678
16. Zhao FK, Wu Q, Lu YF, Yang WJ, Yao XD, Zhang MS, et al. The extraction and the improvement of determination method for total alkaloids in *Dendrobium nobile* Lindl. *Jo Zunyi Med Univer.* (2015) 38:5. doi: 10.14169/j.cnki.zunyixuebao.2015.0127
17. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, its modifications. *Cogn Process.* (2012) 13:93–110. doi: 10.1007/s10339-011-0430-z
18. Denninger JK, Smith BM, Kirby ED. Novel object recognition and object location behavioral testing in mice on a budget. *J Vis Exp.* (2018) 141:10.3791/58593. doi: 10.3791/58593
19. Lu C, Lv JW, Dong LM, Jiang N, Wang Y, Fan B. The protective effect of 20(S)-protopanaxadiol (PPD) against chronic sleep deprivation (CSD) -

induced memory impairments in mice. *Brain Res Bull.* (2018) 137:249–56. doi: 10.1016/j.brainresbull.2017.12.012
20. Wang Q, Sun LH, Jia W, Liu XM, Dang HX, Mai WL, et al. Comparison of ginsenosides Rg1 and Rb1 for their effects on improving scopolamine-induced learning and memory impairment in mice. *Phytother Res PTR.* (2010) 24:1748–54. doi: 10.1002/ptr.3130
21. Wang Q, Zhang YL, Li YH, Chen SG, Gao JH, Chen YX, et al. The memory enhancement effect of Kai Xin San on cognitive deficit induced by simulated weightlessness in rats. *J Ethnopharmacol.* (2016) 187:9–16. doi: 10.1016/j.jep.2016.03.070
22. Zhou BZ, Li MX, Cao XY, Zhang QL, Liu YT, Ma Q, et al. Phenylethanoid glycosides of Pedicularis muscicola Maxim ameliorate high altitude-induced memory impairment. *Physiol Behav.* (2016) 157:39–46. doi: 10.1016/j.physbeh.2016.01.037
23. Heckman PRA, Roig Kuhn F, Meerlo P, Havekes R. A brief period of sleep deprivation negatively impacts the acquisition, consolidation, and retrieval of object-location memories. *Neurobiol Learn Mem.* (2020) 175:107326. doi: 10.1016/j.nlm.2020.107326
24. Akbarabadi A, Niknamfar S, Vousooghi N, Sadat SMS, Toolee H, Zarrindast MR. Effect of rat parental morphine exposure on passive avoidance memory and morphine conditioned place preference in male offspring. *Physiol Behav.* (2017) 184:143–9. doi: 10.1016/j.physbeh.2017.11.024
25. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods.* (1984) 11:47–60. doi: 10.1016/0165-0270(84)90007-4
26. Li YF, Li F, Gong QH, Wu Q, Shi JS. Inhibitory effects of Dendrobium alkaloids on memory impairment induced by lipopolysaccharide in rats. *Planta Med.* (2011) 77:117–21. doi: 10.1055/s-0030-1250235
27. Xie G, Huang X, Li H, Wang P, Huang P. Caffeine-related effects on cognitive performance: roles of apoptosis in rat hippocampus following sleep deprivation. *Biochem Biophys Res Commun.* (2020) 534:632–8. doi: 10.1016/j.bbrc.2020.11.029
28. Zhang K, Lian NQ, Ding R, Guo CL, Dong X, Li YY. Sleep deprivation aggravates cognitive impairment by the alteration of hippocampal neuronal activity and the density of dendritic spine in isoflurane-exposed mice. *Front Behav Neurosci.* (2020) 14:589176. doi: 10.3389/fnbeh.2020.589176
29. Paulus MP, Dulawa SC, Ralph RJ, Geyer MA. Behavioral organization is independent of locomotor activity in 129 and C57 mouse strain. *Brain Res.* (1999) 835:27–36. doi: 10.1016/S0006-8993(99)01137-3
30. Fernandes SL, Patti CL, Zanin KA, Fernandes HA, Tufik S, Andersen ML, et al. Sleep deprivation impairs emotional memory retrieval in mice: influence of sex. *Prog Neuropsychopharmacol Biol Psychiatry.* (2012) 38:216–22. doi: 10.1016/j.pnpbp.2012.03.014
31. Sutcliffe JS, Marshall KM, Neill JC. Influence of gender on working and spatial memory in the novel object recognition task in the rat. *Behav Brain Res.* (2006) 177:117–25. doi: 10.1016/j.bbr.2006.10.029
32. Pitsikas N, Rigamonti AE, Cella SG, Muller EE. The non-NMDA receptor antagonist NBQX does not affect rats performance in the object recognition task. *Pharmacol Res.* (2002) 45:43–6. doi: 10.1006/phrs.2001.0898
33. Antonio FB, Millón C, Gago B, Laura GD, Noelia CC, Coveñas R. Galanin (1-15)-fluoxetine interaction in the novel object recognition test. Involvement of 5-HT1A receptors in the prefrontal cortex of the rats. *Neuropharmacology.* (2019) 155:104–12. doi: 10.1016/j.neuropharm.2019.05.023
34. Nikolaos P. The role of nitric oxide in the object recognition memory. *Behav Brain Res.* (2015) 285:200–7. doi: 10.1016/j.bbr.2014.06.008
35. Ahmed A, Zeng GR, Jiang DJ, Lin HY, Azhar M, Ahsana DF, et al. Time-dependent impairments in learning and memory in Streptozotocin-induced hyperglycemic rats. *Metab Brain Dis.* (2019) 34:1431–46. doi: 10.1007/s11011-019-00448-7
36. Mai WL, Wang Q, Liu XM, Li YH, Chen SG, Wang LW, et al. Significant Parameters in evaluation of spacetaneous activity in mice. *Acta Lab Anim Sci Sinica.* (2008) 3:172–5
37. Kwon KJ, Lee EJ, Kim MK, Jeon SJ, Choi YY, Shin CY, et al. The potential role of melatonin on sleep deprivation-induced cognitive impairments: implication of FMRP on cognitive function. *Neuroscience.* (2015) 301:403–14. doi: 10.1016/j.neuroscience.2015.05.079
38. Zhang YL, Wang Q, Chen HL, Liu XM, Lv K, Wang TM, et al. Involvement of cholinergic dysfunction and oxidative damage in the effects of simulated weightlessness on learning and memory in rats. *Biomed Res Int.* (2018) 2018:2547532. doi: 10.1155/2018/2547532
39. Miyazaki S, Uchida S, Mukai J, Nishihara K. Clonidine effects on all-night human sleep: Opposite action of low- and medium-dose clonidine on human NREM–REM sleep proportion. *Psychiatry Clin Neurosci.* (2004) 58:138–44. doi: 10.1111/j.1440-1819.2003.01207.x
40. Colavito V, Fabene PF, Grassi ZG, Pifferi F, Lamberty Y, Bentivoglio M, et al. Experimental sleep deprivation as a tool to test memory deficits in rodents. *Front Syst Neurosci.* (2013) 7:106. doi: 10.3389/fnsys.2013.00106
41. Zhu L, Zhang R, Li TL. Effects of Acanthopanax on learning and memory and monoamine neurotransmitters in hippocampus of sleep deprived rats. *Chin J Traditional Med Formulae.* (2012) 18:4. doi: 10.1016/J.BRAINRESBULL.2021.06.013
42. Silva RH, Abílio VC, Takatsu AL, Kameda SR, Grassl C, Chehin AB, et al. Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. *Neuropharmacology.* (2004) 46:895–903. doi: 10.1016/j.neuropharm.2003.11.032
43. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav Brain Res.* (1988) 31:47–59. doi: 10.1016/0166-4328(88)90157-X
44. Farajdokht F, Vatandoust SM, Hosseini L, Fekri K, Rahigh Aghsan S, Majdi A, et al. Sericin protects against acute sleep deprivation-induced memory impairment via enhancement of hippocampal synaptic protein levels and inhibition of oxidative stress and neuroinflammation in mice. *Brain Res Bull.* (2021) 174:203–11. doi: 10.1016/j.brainresbull.2021.06.013
45. Alzoubi KH, Khabour OF, Rashid BA, Damaj IM, Salah HA. The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: the role of oxidative stress. *Behav Brain Res.* (2012) 226:205–10. doi: 10.1016/j.bbr.2011.09.017
46. Villafuerte G, Miguel PA, Rodríguez EM, Machado S, Manjarrez E, Arias CO. Sleep deprivation and oxidative stress in animal models: a systematic review. *Oxid Med Cell Longev.* (2015) 2015:234952. doi: 10.1155/2015/234952

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership