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Original article

Alteration of Depressive-like Behaviors by *Psilocybe cubensis* Alkaloid Extract in Mice: the Role of Glutamate Pathway

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Abstract

Background and objectives: Considering the increasing prevalence of depression, many studies are launched to investigate new antidepressant treatments. The present research has shown how psilocybin as an active compound of *Psilocybe cubensis* (Earle) Singer extract (PCE) can change the parameters related to depression and anxiety in animal models. Both serotonin (5-hydroxytryptamine: 5-HT) and glutamate modulate depressive-like behaviors and, therefore, we examined the possible interaction of psilocybin as 5-HT_1 agonist with glutamate receptor N-methyl-D-aspartate (NMDA). **Methods**: *Psilocybe cubensis* extract of this mushroom was prepared by ethyl acetate. NMRI mice involved in all experiments and were treated with the vehicle, extract, or standard drug intraperitoneally. Open field (OFT), forced swimming (FST) and tail suspension tests (TST) were applied to measure the intended parameters. OFT was performed to verify the applied doses for measuring the following antidepressant activity. **Results**: PCE at the doses of 100 mg/kg significantly changed the locomotion, time spent in center and velocity of the animals in OFT. While treatment of the animals with PCE 10 and 40 mg/kg or ketamine 1 mg/kg did not alter the locomotor activity, co-administration of these subeffective amounts significantly reduced the immobility time in both FST and TST. **Conclusion**: These effects may indicate possible implication of psilocybin with NMDA receptor which consequently produces the antidepressant effects.

Keywords: anxiety; depression; NMDA receptor; *Psilocybe cubensis*; psilocybin

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Introduction

Major depressive disorder (MDD) is a health concern in all countries which impairs the quality of life. Lifetime prevalence of MDD is between 11.1-14.6% [1]. The main issues with the current depression therapies are insufficient efficacy in attenuation of the symptoms and the relapse after stopping the psycho- or pharmacotherapy. Due to the insufficient efficacy of the currently used agents for depression management, recently some prohibited substances have been used as new antidepressant tools [2,3]. Psilocybin and psilocin, psychoactive compounds of *Psilocybe* mushroom, are potent 5-hydroxytryptamine$_{1A, 2A, 2C}$ receptor agonists. These serotonin receptors play a pivotal role in the pathogenesis of depression in dentate gyrus (DG) of hippocampus [4]. Long-lasting deposition of serotonin in subventricular and the subgranular zones of DG enhances neurogenesis mediated by different subtypes of 5-HT receptor [5]. While acute administration of 5-HT_1 agonists produces neurogensis in DG, chronic stimulation has negative effects on those regions of the brain. Interestingly, 5-$HT_{2A/2C}$ mimetics such as 2,5-

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dimethoxy-4-iodoamphetamine (DOI) shows inverse effects. Both acute and chronic activation of 5-HT_2 receptor diminished the neural proliferation in DG [6]. Either acute or chronic alteration of serotonin by selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine have shown antidepressant effects in animal tests. They decrease immobility time in both forced swimming test (FST) [7], tail suspension test (TST) [8], and increase of exploratory activity in the open field test (OFT) [9]. However, the effects of some selective 5-$HT_{1A,2A}$ agonist psilocybin remained unclear. These effect patterns on depressive-like parameters have been reported by glutamate receptor modulators. Sanacora et al. reviewed the involvement of N-methyl-D-aspartate (NMDA) receptor in animal models of depression [10]. The considerable evidences in this review supported that a decrease in function or expression of NMDA receptor with the known antidepressants are consequences of depression treatment. Moreover, recently, clinical trials have implicated the long lasting effects of a single administration of 5-$HT_{1A,\ 2A}$ agonists as well as NMDA antagonists on depression [11-13]. These experiences in animal models of depressive-like behaviors can be attributed to human. Also, there are several case reports about the efficacy of psilocybin and psilocin in the treatment of obsessive–compulsive disorder (OCD) [14], and also some clinical trials are in progress for OCD and MDD in the end stage patient with cancer (available at: www.clinicaltrials.gov).

In this study, the OFT, TST, and FST related parameters were assessed to measure the effective doses of *P. cubensis* extract which did not produce hallucination. Consequently, a pharmacological interaction was applied by a NMDA antagonist ketamine to clarify the potential implication of NMDA receptor.

Material and Methods

Ethical considerations

The tested animals were treated in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23) and Shahid Beheshti university guideline for animal use. Animal Ethics Committee of Shahid Beheshti University of Medical Sciences approved all procedures performed in the studies (IR.SBMU.PHNM.1396.887).

Chemicals

Fluoxetine powder was purchased from Abidi Pharmaceutical Co. (Tehran, Iran). Ketamine injectable solution (Alfasan, Netherlands) was applied in this work.

Cultivation of mushroom

Production of mycelium of *P. cubensis* was done according to a previously well-described method [15]. Briefly, parts of the inner tissue of mushroom were cut out and used for inoculation into Petri dish containing potato dextrose agar supplemented with 0.5% cyclohexamide (Sigma Aldrich, Germany) and 0.05% chloramphenicol (Merck Darmstadt, Germany). All dishes were incubated at 28 °C for 5 days. After appearance of the radial, the mycelia on the agar surface and the stock cultures were transferred to the potato dextrose broth and shook well for 10-12 days at 32 °C. Afterwards, mycelia material was collected from the broth medium by a sterile syringe. To get a cap of mushroom, wheat grains were utilized as grain substrate for planting spawn. Mycelium was added to autoclaved wheat grains at a rate of 10% (V/W) and incubated at 25 °C for two weeks in full darkness. Then, spawns obtained were inoculated with wheat straw as a substrate at the ratio of 20% (W/W). Spawning was carried out by mixing spawn with the substrate and placing in polyethylene bags. The bags were tied and incubated at 25 °C and 85% humidity to allow the fruiting bodies to emerge [16].

Animals

Male NMRI mice (Pasteur Institute, Tehran, Iran) weighing 20-25 g were used. The animals were housed under standard conditions (Each experimental group consisted of 8 animals, temperature: 23±2°C, 12h light-dark cycle, and free access to food and water). All behavioral observations were conducted between 10:00 and 14:00.

Identification of *P. cubensis*

In order to confirm the genus and the specie, 5 g of the growing mushroom was applied for DNA extraction by promega Wizard Genomic DNA Purification Kit (Promega, Italy) according to the manufacturer's protocol. Amplification of nSLU gene was done using primers from the 5 'portion of the nuclear large ribosomal subunit of rRNA

(nSLU rRNA) F(5′--TGAGAAGAAGCGAC3′) R(5′--TACTACCACCAAGATCT-3′) [17].

Sequencing analysis was performed on purified amplification products. The obtained sequences were compared to the sequences present in gene bank database (www.ncbi.nlm.nih.gov).

Extraction of alkaloids

Alkaloid extraction was performed as described by Sarwar et al. [18]; 100 mL of 10 % acetic acid was poured to 10 g of mushroom, sonicated for 10 min; 100 mL of deionized water was poured and the mixture was ground finely. The supernatant was neutralized by adding sodium bicarbonate following transferring the mixture into a test tube and centrifugation for 3 min. Equal amount of chloroform was added to extract the solution. Then, the chloroform layer was collected and dried under vacuum. Finally, the dried chloroform extract was stored at -20 ºC.

GC and GC-Mass analysis

The alkaloids content was analyzed on a Young Lin instrument with a DB-5 capillary column, 30 m×0.25 mm i.d. and FID detector.

Carrier gas: He, flow: 0.8 mL/min, linear velocity: 30 cm/sec. The temperature of injection: 290 °C. The volume of injection: 1.0 µL. The mode of injection: Split (1:50). The program of temperature: 50 °C for 1 min, increasing at 3 °C /min to 240 °C, then increasing at 15 °C/min to 300 °C, sustained at 300 °C for 3 min. FID (290 °C): The flow of H2 and air: 50 and 400 ml/min, respectively.

GC-MS analysis was carried out by an Agilent (6890/5973 N) and DB-5 capillary column, 30 m×0.25 mm i.d. Carrier gas: He, linear velocity: 32.4 cm/sec, flow: 0.8 ml/min. The temperature of injection: 290 °C. The volume of injection: 1.0 µL. The mode of injection: split (1:10). The program of temperature: 50 °C, for 5 min, increasing at 3 °C/min to 240 °C, then increasing at 15 °C/min to 300 °C, sustained at 300 °C for 3 min. The temperature of MS interface: 290 °C, The mode of MS: EI, The voltage of ionization: 70 eV; The range of mass: 40-500 u; The speed of scan: 3.18 scans/sec; interval: 0.50 s (2 Hz). Data handling was operated using a Chem. Station (Agilent).

Identification of the components

Psilocin was recognized by comparison of its retention index, which was considered by using the retention times of *n*-alkanes (C8-C28) with the similar chromatographic situations, along with the fragmentation models of the mass spectra with the literature and the published mass spectra or WILEY library [19]. The measure of the detected compounds was estimated based on GC peak areas.

Animal grouping and drug administration

Eight groups of mice were conducted to OFT: NaCl 0.9% as the control group, fluoxetine 20 mg/kg as the standard drug, PCE (10, 40 and 100 mg/kg) and ketamine (1, 5 and 10 mg/kg). Both FST and TST were designed with seven experimental groups including the control, fluoxetine 20 mg/kg) subeffective doses of PCE (10 and 40 mg/kg) and ketamine (1 mg/kg).

Each group consisted of eight mice. All injections were performed thirty minutes before each test intraperitoneally. NaCl 0.9% was used as the vehicle for PCE and fluoxetine-HCl.

Open field test (OFT)

The OFT was used to elucidate the effects of treatments on motor function and anxiety-like behaviors [20]. The apparatus consisted of a box (square arena 50×50 and 30 cm height) made by white opaque Plexiglas and dimly illuminated. The floor of the box was divided into 16 equal squares (12.5×12.5 cm). The test was initiated by gently placing the mouse over the central square (25×25 cm), and its behaviors were continuously recorded by a video camera placed over the apparatus for 5 min and analyzed by Ethovision software version 8 (Noldus, Netherlands). The horizontal activity parameters (distance moved and velocity) were comprised. The surface of the apparatus was carefully cleaned with alcohol after every test.

Forced swimming test (FST)

An open glass cylinders (diameter: 10 cm, height: 25 cm), containing 19 cm depth of water (23±1 °C) was used during FST [21]. Mice were placed in an open glass cylinder individually and forced to swim. The prolonged immobility times considered when the animal remained floating motionless in water which presented the depressive-like symptoms. The immobility time was scored from the 2 to 6 min of the test (4 min).

Tail suspension test (TST)

The tail suspension test was based on the method of Steru et al. [22]. Briefly, the mice were suspended 5 cm above the floor by means of an adhesive tape placed about 1 cm from tip of tail. The total duration of immobility (s) was recorded during the last 4 min of a single 6-min test session. Mice were considered immobile when they were completely motionless. The experimental procedure was carried out at minimal noise in a dark room.

Statistical analysis

The statistical analysis was performed using ANOVA followed by Tukey's multiple comparison when appropriate. Differences between groups were considered statistically significant at a probability level of 0.05.

Results and Discussion

Alkaloids extraction was analyzed by capillary GC and GC-MS, and the main compound was determined via areas under the peaks. Psilocin (11.5%) was identified as the main component in the alkaloid extract (figure 1).

One-way ANOVA showed a significant difference in total distance moved between the applied group in open field [$F_{(7,56)}$ = 16.06, p<0.001]. While administration of PCE 100 mg/kg and 5 and 10 mg/kg ketamine reduced the total distance moved compared to the control (p<0.001).

Ketamine 1 mg/kg and PCE 10 and 40 mg/kg showed no significant decrease in the total distance moved by mice (p>0.05, figure 2a).

One-way ANOVA showed a significant difference in velocity of mice between the groups [$F_{(7,56)}$ = 17.79, p<0.001]. Ketamine at doses of 10 and 5 mg/kg and PCE 100 mg/kg reduced the velocity of the mice in OFT, but administration of 1 mg/kg ketamine did not cause any change (*p*>0.05). Similarly, administration of 10 or 40 mg/kg PCE showed no significant alteration in the velocity of the mice (p>0.05 for both, figure 2b).

The doses which produced no alteration in exploratory behaviors were applied in FST and TST. Then, doses of the extract above 40 mg were excluded from the experiment. A significant difference between the immobility times in FST was showed by ANOVA [$F_{(6,49)}$ = 20.99, p<0.001]. While fluoxetine 20 mg/kg reduced the immobility time (p<0.05), administration of 10 and 40 mg/kg PCE did not change the immobility time significantly in FST (p>0.05). Also, ketamine 1 mg/kg failed to reduce the immobility time, but co-administration of ketamine 1 mg/kg and 10 or 40 mg/kg PCE significantly decreased that time compared to the control (p<0.001, figure 3). A significant difference between the immobility times in FST was showed by ANOVA [$F_{(6,49)}$ = 20.1, p<0.001]. Significant reductions in immobility time were observed in fluoxetine 20 mg/kg group (p<0.001).

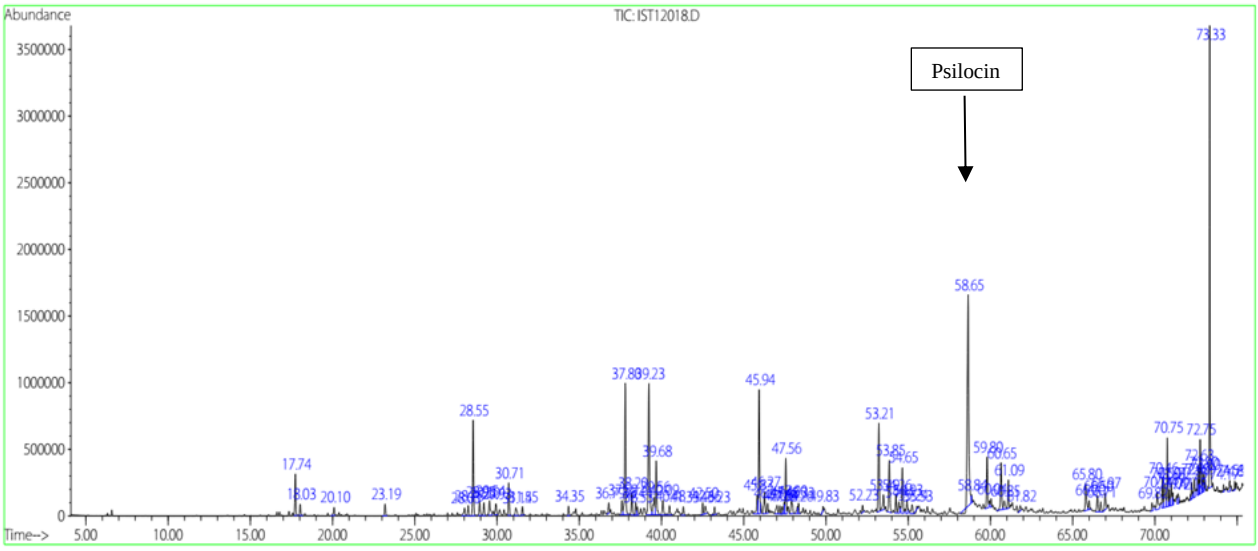

Figure 1. GC chromatogram of *Psilocybe cubensis* alkaloid extract; psilocin appeared at 58 min

No significant difference was indicated by administration of 10 and 40 mg/kg PCE and ketamine 1 mg/kg in the immobility time in TST ($p>0.05$ for both). However, co-treatment of mice by ketamine 1 mg/kg and 10 or 40 mg/kg PCE significantly decreased that time ($p<0.001$, figure 4).The present study investigated the effects of alkaloid extract of *P. cubensis* on depressive-like behaviors of NMRI mice. OFT which draws an indication for animal's emotional status was applied as a reliable assessment test to evaluate the locomotor activity behaviors in rodents. Rodents prefer to spend more time in the peripheral zone and anxiolytics promote this behavior.

Reduction in OFT recorded parameters by PCE 100 mg/kg was comparable to fluoxetine 20 mg/kg a SSRIs prescribed as a first line treatment for depression. However, this parameter did not differ by PCE at concentrations of 10 and 40 mg/kg in our experiment. Thus, the above mentioned doses were considered as subeffective doses.

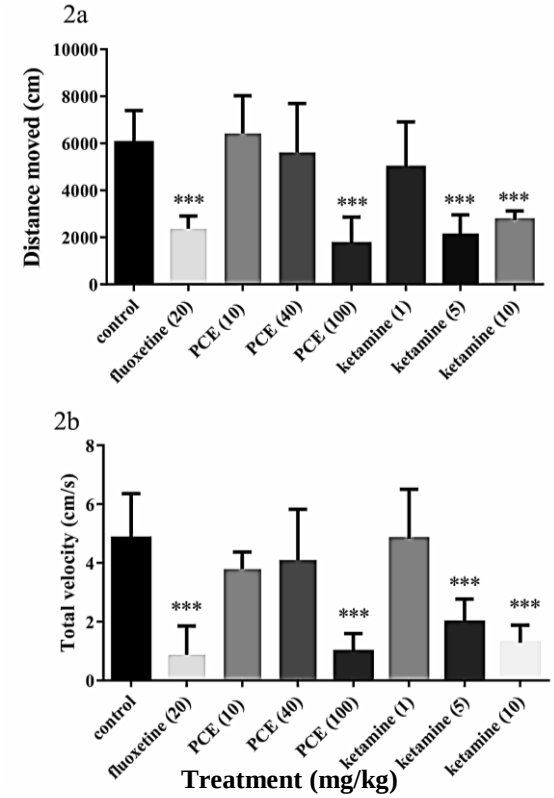

Figure 2. Effects of different doses of NMDA receptor antagonists and PCE on the locomotion in OFT. Locomotor activities of mice in OFT after administration of PCE and ketamine: PCE 10 and 40 mg/kg and ketamine 1 mg/kg did not chang ethe total distance moved (**2a**) and velocity (**2b**) ($p>0.05$, n = 8). Values are mean±SEM.

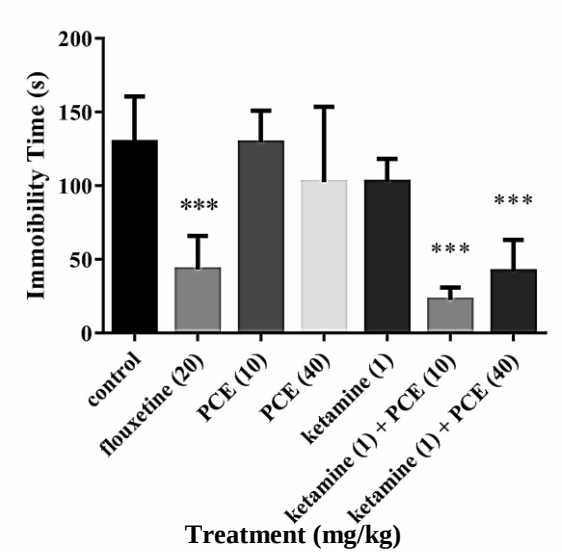

Figure 3. Effects of subeffective doses of ketamine, PCE and ketamine+PCE on the immobility time in FST. Administration of PCE 10, 40 or ketamine 1 mg/kg had no effects of FST while the co-treatment of ketamine and PCE significantly reduced the immobility time (***$p<0.001$, n = 8). Values have been expressed as the mean ± SEM.

In the TST, immobility state reveals despairs which can be minimized by administration of the number of therapeutics for depression. Also, in the FST, behavioral despaired animals were enforced to swim in a water tank. This inescapable state simulates the depressive behaviors in human [23].In this regards, these subeffective doses and not 100 mg/kg, which have no locomotor inhibitory activity were chosen for the subsequent experiments. Moreover, according to the results, PCE 10 or 40 mg/kg did not reverse the depressive-like behaviors in the FST and TST. In this study, the low dose of the ketamine (1 mg/kg); a NMDA antagonist which did not show any sedative action or suppression of locomotion was selected based on previous investigation [24]. Ketamine 1 mg/kg and PCE 10 and 40 mg did not show any sedative action or suppression of locomotion. Then, ketamine or PCE at the applied doses displayed no false negative responses in the depression measurement tests (FST and TST). Our findings indicated that co-injection of subeffective doses of NMDA receptor antagonists and PCE possessed significant antidepressant effects in FST and TST. The major bioactive alkaloid of PCE is psilocin, which is known as a serotonergic system agonist. Serotonin, by activating different receptor

subtypes, regulates membrane excitability in the central nervous system in a complex manner [25]. The beneficial role of fluoxetine in the recorded parameters of FST seems be related to increased availability of reuptake inhibition of neurotransmitter serotonin (5HT) at the synapse.

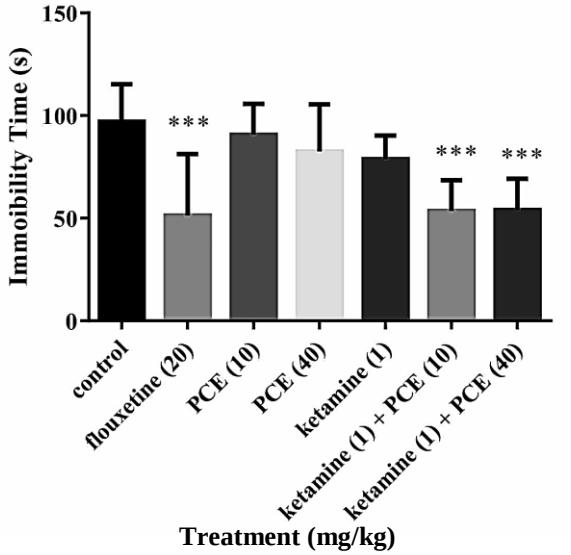

Figure 4. Effects of subeffective doses of ketamine, PCE and ketamine+PCE on the immobility time in TST. Administration of PCE 10, 40 or ketamine 1 mg/kg had no effects of TST. Co-administration of ketamine 1 mg/kg and PCE 10 and 40 mg/kg significantly decreased the immobility time in mice (***$p<0.001$, $n = 8$). Values have been expressed as the mean ± SEM.

The interaction of serotonin receptors with various channel receptors in the neural excitability has been proven. NMDA glutamate receptor has been implicated in the function of cognitive behaviors, depression and anxiety [26]. NMDA receptors blockage, improves depression and anxiety behaviors in both animal and human studies [13,27]. However, co-injection of these two subeffective doses significantly reduced the immobility time in FST and TST, indicating the effect of PCE on specific subunit(s) of the receptor. There are two NR2A and NR2B subtypes of NMDA receptor that have different roles in the exhibition of the receptor functions [28]. It has been shown that NR2B subunit antagonists (e.g. CP-101606) displays antidepressant properties, while anxiolytic activities are related to NR2A subtype [29]. In our study, the significant reduction in immobility time in FST and TST with no changes in OFT variables could be the outcomes of the action on NR2B subunit of NMDA receptor which is responsible for antidepressant properties.

Moreover, both serotonin and glutamate level were regulated presynaptically by 5-HT_{1A} receptors. The 5-HT_{1A} receptors are widely distributed in the brain, and found on serotonergic nerves as an autoreceptor [30]. 5-HT_{1A} receptor reduces serotonin and glutamate levels in the prefrontal cortex. Then, a shift of serotonin-glutamate balance may be occurred in the regions of the brain involved in anxiety and depression related behavior [31]. It seems that the additive/synergistic effects of 5-HT_{1A} agonist psilocin and NMDA antagonist ketamine, which were observed in this study, can be considered as the outputs of that imbalance.

In addition $5HT_{2C}$ receptor which has a pivotal role in anxiety behaviors is one of the targets of psilocin [32]. Our data exhibited no antianxiety effect of psilocin at the mentioned doses, enlightening the low affinity of psilocin at some regions of brain such as raphe nucleus which is involved in anxiety [33].

Overall, this study demonstrated that antidepressant effects of a combination of low doses of ketamine+psilocybin was comparable with the standard treatment fluoxetine. Interaction of psilocybin/psilocin, agonists of specific subtype's of serotonin receptors, with NMDA receptor influenced the antidepressant behaviors in rodents. Hence, a single treatment by psilocybin/psilocin showed antidepressant effects, which at least partially mediated by interaction with NMDA receptors. Thus, psilocybin at subeffective doses could exert an antidepressant effects in the presence of ketamine.

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Author contributions

In the present work the mushrooms were cultured by Elaheh Mahmoudi. Extraction of the alkaloids was made by Reza Hajiaghaee and Mehrdad Faizi. Ali Razmi carried out the behavioral tests as well as data managements and writing the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Bromet E, Andrade LH, Hwang I, Sampson N A, Alonso J, de Girolamo G, de Graaf R, Demyttenaere K, Hu C, Iwata N, Karam AN, Kaur J, Kostyuchenko S, Lepine JP, Levinson D, Matschinger H, Mora ME, Browne MO, Posada-Villa J, Viana MC, Williams DR, Kessler RC. Cross-national epidemiology of DSM-IV major depressive episode. *BMC Med.* 2011; 9(90): 1-16.

[2] Nutt DJ, King LA, Nichols DE. Effects of Schedule I drug laws on neuroscience research and treatment innovation. *Nat Rev Neurosci.* 2013; 14(8): 577-585.

[3] Young SN. Single treatments that have lasting effects: some thoughts on the antidepressant effects of ketamine and botulinum toxin and the anxiolytic effect of psilocybin. *J Psychiatry Neurosci.* 2013; 38(2): 78-83.

[4] Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology.* 1999; 38(8): 1083-1152.

[5] Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci.* 2000; 20(24): 9104-9110.

[6] Klempin F, Babu H, de Pietri Tonelli D, Alarcon E, Fabel K, Kempermann G. Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. *Front Mol Neurosci.* 2010; 3(14): 1-11.

[7] Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, Kavalali ET, Monteggia LM. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature.* 2011; 475(7354): 91-95.

[8] Mantovani M, Pertile R, Calixto JB, Santos AR, Rodrigues AL. Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. *Neurosci Lett.* 2003; 343(1): 1-4.

[9] Plaznik A, Palejko W, Nazar M, Jessa M. Effects of antagonists at the NMDA receptor complex in two models of anxiety. *Eur Neuropsychopharmacol.* 1994; 4(4): 503-512.

[10] 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(5): 426-437.

[11] Diazgranados N, Ibrahim L, Brutsche NE, Newberg A, Kronstein P, Khalife S, Kammerer WA, Quezado Z, Luckenbaugh DA, Salvadore G, Machado-Vieira R, Manji HK, Zarate CA. A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Arch Gen Psychiatry.* 2010; 67(8): 793-802.

[12] Zarate CA, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry.* 2006; 63(8): 856-864.

[13] Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry.* 2000; 47(4): 351-354.

[14] Delgado PL, Moreno FA. Hallucinogens, serotonin and obsessive-compulsive disorder. *J Psychoactive Drugs.* 1998; 30(4): 359-366.

[15] Nicholas LG, Ogamé K. Psilocybin mushroom handbook: easy indoor & outdoor cultivation. 1st ed. Canada: Quick American Archives, 2006.

[16] Catalfomo P, Tyler V. The production of psilocybin in submerged culture by *Psilocybe cubensis. Lloydia.* 1964; 27(1): 53-63.

[17] Nugent KG, Saville BJ. Forensic analysis of hallucinogenic fungi: a DNA-based approach. *Forensic Sci Int.* 2004; 140(2-3): 147-157.

[18] Sarwar M, McDonald J. A rapid extraction and GC/MS methodology for the identification of psilocyn in mushroom/chocolate concoctions. *Microgram J.* 2003; 1(3-4): 177-183.

[19] Adams RP. Identification of essential oil components by gas chromatography-mass spectroscopy. Carol Stream: Allured Publishing Corp, 1995.

[20] Kulesskaya N, Voikar V. Assessment of mouse anxiety-like behavior in the light-dark

box and open-field arena: role of equipment and procedure. *Physiol Behav*. 2014; 133: 30-38.
[21] Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther*. 1977; 229(2): 327-336.
[22] Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)*. 1985; 85(3): 367-370.
[23] Willner P, Mitchell PJ. The validity of animal models of predisposition to depression. *Behav Pharmacol*. 2002; 13(3): 169-188.
[24] Haj-Mirzaian A, Amiri S, Kordjazy N, Rahimi-Balaei M, Haj-Mirzaian A, Marzban H, Aminzadeh A, Dehpour AR, Mehr SE. Blockade of NMDA receptors reverses the depressant, but not anxiogenic effect of adolescence social isolation in mice. *Eur J Pharmacol*. 2015; 750: 160-166.
[25] Andrade R. Regulation of membrane excitability in the central nervous system by serotonin receptor subtypes. *Ann N Y Acad Sci*. 1998; 861: 190-203.
[26] Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: diversity, development and disease. *Curr Opin Neurobiol*. 2001; 11(3): 327-335.
[27] Trullas R, Skolnick P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur J Pharmacol*. 1990; 185(1): 1-10.
[28] Preskorn SH, Baker B, Kolluri S, Menniti F S, Krams M, Landen JW. An innovative design to establish proof of concept of the antidepressant effects of the NR2B subunit selective N-methyl-D-aspartate antagonist, CP-101,606, in patients with treatment-refractory major depressive disorder. *J Clin Psychopharmacol*. 2008; 28(6): 631-637.
[29] Dere E, Topic B, De Souza Silva MA, Fink H, Buddenberg T, Huston JP. NMDA-receptor antagonism via dextromethorphan and ifenprodil modulates graded anxiety test performance of C57BL/6 mice. *Behav Pharmacol*. 2003; 14(3): 245-249.
[30] Zhong P, Yuen EY, Yan Z. Modulation of neuronal excitability by serotonin-NMDA interactions in prefrontal cortex. *Mol Cell Neurosci*. 2008; 38(2): 290-299.
[31] Martin-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F. Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J Neurosci*. 2001; 21(24): 9856-9866.
[32] Passie T, Seifert J, Schneider U, Emrich H M. The pharmacology of psilocybin. *Addict Biol*. 2002; 7(4): 357-364.
[33] Aghajanian GK, Gallager DW. Raphe origin of serotonergic nerves terminating in the cerebral ventricles. *Brain Res*. 1975; 88(2): 221-231.

Abbreviations

FST: Forced Swimming Test, TST: Tail Suspension Test, OFT: Open Field Test, NMDA: N-methyl-D-aspartate, PCE: *Psilocybe cubensis* extract, MDD: Major depressive disorder, 5HT: 5-hydroxytryptamine