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Research Article

Assessment of the 3-substituted thietane-1,1-dioxide derivative antidepressant effect using rat model of depression induced by reserpine

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Abstract

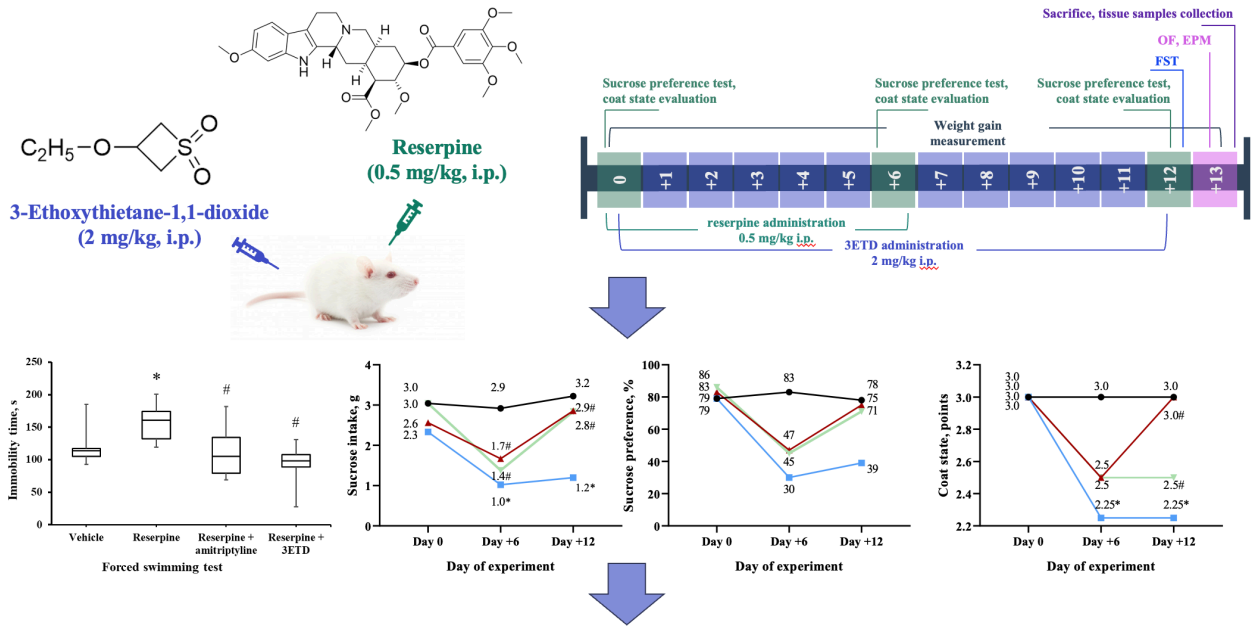
Introduction: 3-ethoxythietane-1,1-dioxide (3ETD) is a novel molecule that has demonstrated significant antidepressant properties when administered intraperitoneally (i.p.) to mice once or repeatedly. **The aim of the study** was to evaluate the antidepressant effect of 3ETD in a model of depressive-like state in rats induced by seven-fold administration of reserpine.

Materials and Methods: We conducted two sets of experiments in white outbred male rats. In Experiment 1, reserpine (0.5 mg/kg) was administered intraperitoneally daily from Day 0 to Day +6; 3ETD (2 mg/kg) and the reference drug amitriptyline (10 mg/kg) were administered i.p. daily from Day 0 to Day +11. The sucrose intake and preference (Days 0, +6, +12), the coat state (Days 0, +6, +12), the behavior of the rats in the forced swimming test (FST, +12 day), the open field test (OF, Day +13) and the elevated plus maze test (EPM, Day +13), as well as the body weight (daily) were assessed. On Day +13, the animals were sacrificed and the weight coefficients of their internal organs (liver, spleen, thymus, adrenal glands) were measured and serum chemistry was performed. Brain-derived neurotrophic factor (BDNF) level in the hippocampus was quantified by enzyme-linked immunosorbent assay (ELISA). In Experiment 2, reserpine, amitriptyline, and 3 ETD were administered similarly to Experiment 1, and on Day +13, rats were euthanized and brain samples were collected for immunohistochemical determination of glial fibrillary acidic protein (GFAP) and anti-apoptotic B-cell lymphoma-2 protein (Bcl-2) in the hippocampus.

Results and Discussion: Seven-fold administration of reserpine significantly increased immobility time (IT) in FST, caused anhedonia and coat state deterioration, decreased motor activity in OF and EPM, decreased body weight, increased the adrenal glands and spleen weight coefficients, increased GFAP level and decreased Bcl-2 level in the hippocampus. 3ETD (2 mg/kg) significantly reduced IT FST by 39%, increased sucrose preference to the level of the control group and sucrose consumption by 137%, eliminated self-care deficit, prevented weight loss in rats by the end of the experiment (Experiment 1); significantly reduced GFAP level (CA1-CA4, dentate gyrus) and increased Bcl-2 level (CA1, dentate gyrus) in the hippocampus compared with the reserpine group (Experiment 2).

Conclusion: 3ETD eliminated depressive-like symptoms in rats induced by seven-fold administration of reserpine.

Graphical abstract



3ETD eliminated depressive-like symptoms (despair behavior, anhedonia, coat state deterioration) in rats induced by seven-fold administration of reserpine

Keywords

thietane, antidepressive agents, rats, reserpine, depression

Introduction

According to the Institute for Health Metrics and Evaluation (IHME) for 2021, 19.8 million people in the Russian Federation suffer from mental disorders, which are socially significant diseases, with depressive disorders being the most common (6.8 million people). Despite the fact that a number of original antidepressants have been developed and introduced into clinical practice over the past 30 years, the prevalence and burden of depressive disorders have not decreased since 1990 (Herrman et al. 2022), and the COVID-19 pandemic has sharply exacerbated the existing upward trend (Santomauro et al. 2021). The demand for antidepressants, which are the main treatment option for depressive disorders (Akhapkin et al. 2021), will continue to grow, and their global market will expand from US$ 13 billion (almost 50% of the psychotropic drugs market share) in 2020 to US$ 20 billion by 2031. Therefore, the search for new antidepressants is relevant.

The results of our previous studies confirm that 3-substituted thietane-1,1-dioxides are a promising class for the development of new psychotropic drugs. Molecules with antidepressant activity have already been found within the class (Klen et al. 2017), in particular, low-toxic 3-ethoxythietane-1,1-dioxide (3ETD), which exhibits pronounced antidepressant properties after single (Khaliullin et al. 2020) and repeated (Nikitina and Gaisina 2022) intraperitoneal (i.p.) administration to male mice in a wide range of doses (Gaisina and Nikitina 2020), and is characterized by an atypical mechanism of action (Nikitina et al. 2022). In the present study, we investigated the antidepressant effect of 3ETD in a model of reserpine-induced depression in rats.

Materials and Methods

Experimental animals

The experiments were performed on white outbred male rats weighing 280-350 g, kept in a 12-h light regime (08:00-20:00) with free access to water and food (pellets for laboratory animals, GOST R 50258-92, GROUP-SPETSKOM LLC, Russia). All animal procedures were carried out in accordance with the requirements of *The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes* (ETS No. 123, 1986) and *The Rules of Good Laboratory Practice of the Eurasian Economic Union in the Field of Drugs* (Decision No. 81 of the Council of the Eurasian Economic Commission dated November 3, 2016 "On Approval of Rules of Good Laboratory Practice of the Eurasian Economic Union in the Sphere of Circulation of Medicines"). The study was approved by the Expert Council on Biomedical Ethics in Theoretical Disciplines of Bashkir State Medical University (BSMU, minutes No. 9, 2020).

Drugs and treatment

3-Ethoxythietane-1,1-dioxide (3ETD) was synthesized at the Department of Pharmaceutical, Analytical and Toxicological Chemistry of BSMU (Head of the Department – Elena E. Klen, Doctor of Pharmaceutical Sciences). 3ETD (2 mg/kg) and reserpine (0.5 mg/kg; substance, Sigma Aldrich, USA) were suspended with Tween-80 (Panreac Quimica S.A.U., Spain), diluted in saline and administered i.p. in accordance with the design of the experiment (see "Experimental design") at the rate of 0.4 ml/200 g of animal body weight. The reference drug amitriptyline (Amitriptyline, solution for intravenous and intramuscular administration 10 mg/ml, Federal State Unitary Enterprise "Moscow Endocrine Plant", Russia) was diluted in saline and administered i.p. at a dose of 10 mg/kg similarly to 3ETD. The control group received an equivalent volume of saline i.p.

Experimental design

A depressive-like state in rats was induced by 7-fold administration of reserpine at low doses according to Antkiewicz-Michaluk et al. (2015) and Park et al. (2018).

Two sets of experiments were conducted according to the same design (Fig. 1). There were 4 experimental groups in both experiments: Group 1 (control) received saline, i.p.; Group 2 (reserpine) – reserpine (0.5 mg/kg), i.p.; Group 3 (amitriptyline) – reserpine (0.5 mg/kg) and amitriptyline (10 mg/kg), i.p.; Group 4 (3ETD) – reserpine (0.5 mg/kg) and 3ETD (2 mg/kg), i.p.

In Experiment 1, reserpine was administered daily for 7 days (from Day 0 to Day +6), and amitriptyline and 3ETD were administered daily for 12 days (from Day 0 to Day +11). On Day +12, forced swimming test (FST) (Porsolt 1979) was performed; on Day +13, open field (OF) and elevated plus maze (EPM) tests (Sestakova et al. 2014) were conducted. On Days 0, +6, and +12, anhedonia (sucrose intake and preference) and coat state deterioration were assessed. Additionally, the weight gain of the animals was measured daily. At the end of the experiment (Day +13), the animals were euthanized and the mass of their internal organs (liver, spleen, thymus, adrenal glands) was assessed. Brain samples were collected to determine the level of brain-derived neurotrophic factor (BDNF) in hippocampus by enzyme-linked immunosorbent assay (ELISA); blood

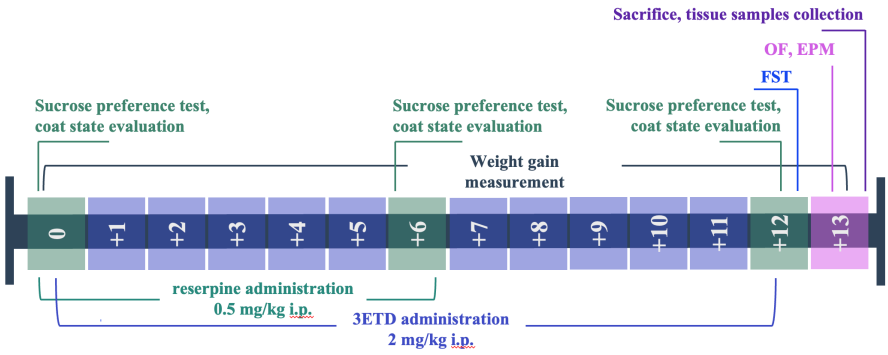


Figure 1. Design of the reserpine-induced depression model. ***Note:*** FST – forced swimming test, OP – open field test, EPM – elevated plus maze test, i.p. – intraperitoneally.

samples were also collected for biochemical analysis (alanine aminotransferase (ALT), and aspartate aminotransferase (AST), cholesterol, lactate dehydrogenase (LDH), urea, creatinine, alkaline phosphatase, bilirubin, total protein, triglycerides, glucose and albumin concentrations were assessed).

In Experiment 2, reserpine was similarly administered daily for 7 days (from Day 0 to Day +6), and amitriptyline and 3ETD were administered daily for 12 days (from Day 0 to Day +11). On Day +13, rats were euthanized, and brains samples were collected for histological and immunohistochemical (IHC) analysis.

Behavioral tests

The behavior of animals was assessed in FST (Day +12), OF and EPM (Day +13). The experiments were recorded on camera for 5 min, and the video recordings were analyzed using the RealTimer (RPC OpenScience Ltd, Russia) software. In FST, the immobility time (IT) was recorded (s). In EPM, the time spent in center / closed arms / open arms of the maze (s), the number of entries into closed / open arms of the maze, closed arms returns and head dippings over the sides of the open arms were calculated. In OF, the number of behavioral patterns "movement", "movement in place", "vertical rearing", "supported rearing", "hole peeking", "sniffing", "grooming", "sitting" were recorded; the indicators "emotional anxiety" (EA – the sum of the patterns "movement in place", "vertical rearing", "supported rearing") and "orientation-exploratory activity" (OEA – the sum of the patterns "sniffing", "movement", "hole peeking") were calculated.

Evaluation of anhedonia and coat state deterioration

Anhedonia was assessed on Days 0, +6, and +12 by intake and preference for 1% sucrose solution. Each experimental group received 2 drinking bottles with plain water and 2 drinking bottles with 1% sucrose solution. The sucrose solution was prepared 12 h prior to the test (white crystalline refined sugar, GOST 33222-2015). The amount of liquid (water/sucrose) consumed during 2 h was measured in g, and then sucrose preference (SP) was calculated according to Strekalova et al. (2011) as a percentage of the consumed sucrose solution (g) to the total amount of liquid drunk (g). SP less than 65% was defined as anhedonia in animals.

Coat state was evaluated on Days 0, +6, and +12, using a scale from 3 to 0 in 0.5 point decrements (3 points – a fur is clean and well-cared, 0 points – a fur is dirty, with bald patches) (Costa-Ferreira et al. 2019).

Evaluation of weight gain and internal organs mass

Throughout the experiment, the animals' body weight was recorded daily. On Day +13, the animals were sacrificed, the weight of their internal organs (liver, spleen, thymus and adrenal glands) was recorded, and the internal organ weight coefficients were calculated as a percentage of the organ weight (g) to the body weight (g).

Determination of the brain-derived neurotrophic factor level by enzyme-linked immunosorbent assay

After the behavioral tests, the rats were anesthetized, decapitated, and their brains were removed, weighed, and washed in ice-cold phosphate-buffered saline. The hippocampus was dissected on ice, weighed, frozen in liquid nitrogen, and stored at -80˚C. Before analysis, the samples were ground in liquid nitrogen and homogenized in lysis buffer (1 mL of buffer per 50 mg of the wet tissue) with the protease inhibitor (phenylmethylsulfonyl fluoride, 0.2 g per sample). The homogenates were centrifuged at +4˚C and 10,000g for 5 min; then the supernatants were collected and stored at -20˚C. The analysis was performed using an ELISA kit for BDNF (SEA011Ra, Cloud-Clone Corp., USA) according to the manufacturer's protocol using enzyme immunoassay analyzer Uniplan AIFR-01 (CJSC Pikon, Russia).

Histological and immunohistochemical analysis of rat hippocampus

Paraffin sections (6 μm, LEICA 4RM 2145 microtome, Germany) were stained with Nissl, and then morphometric analysis of the hippocampus was performed. The structures of the hippocampal region and the cytoarchitectonic fields of the hippocampus were defined according to the rat brain atlas (Paxinos and Watson 2006). The diameter and area of cells (in μm^2) in the hippocampal fields CA1, CA2, CA3, CA4 and dentate gyrus (DG) were measured using a 3DHISTECH PANNORAMIC 250 Flash scanning microscope (3DHISTECH Ltd, Hungary), and 3DHISTECH image analysis software.

The level of the astrocytic marker – glial fibrillary acidic protein (GFAP), and the anti-apoptotic B-cell lymphoma-2 protein (Bcl-2) were determined using IHC staining. Mouse monoclonal antibodies (Santa Cruz Biotechnology, CA) and a universal secondary detection system (Novocastra TM, UK) were used. Paraffin sections (6 μm) were stained according to the manufacturer's protocol using a Bond-Max histostainer (Leica, Germany), counterstained with hematoxylin and embedded in balsam. The total area of immunopositive cells relative to the total area of the studied hippocampal region (%) was calculated at 400x magnification in fields CA1, CA2, CA3, CA4 and DG of the hippocampal formation using ImageJ software (v 1.53).

Statistical analysis

Statistical data processing was performed using the Statistica 13.3 software package (TIBCO Software Inc., USA), and graphs were plotted using GraphPad Prism 8.0.1 software (GraphPad Software, USA). The pattern of distribution was assessed using Shapiro-Wilk test, all parameters being differed from a normal distribution. The median (Me), interquartile range, minimum and maximum were calculated. Comparative statistics included the Kruskal-Wallis (multiple comparison) and Mann-Whitney (pairwise comparison) tests for independent variables, the Friedman (multiple comparison) and Wilcoxon (pairwise comparison) tests for dependent variables (White 2019). The results were considered statistically significant at a p-level<0.05.

Results

Effect of 3ETD on the behavior of rats with reserpine-induced depression in the forced swimming, open field, and elevated plus maze tests

In FST (Day +12), reserpine significantly increased IT by 41% (p=0.010) compared to the control group, and 3ETD and amitriptyline reversed the effect of reserpine, reducing IT by 39% (p=0.001) and 35% (p=0.017), respectively, compared to the reserpine group (Fig. 2A).

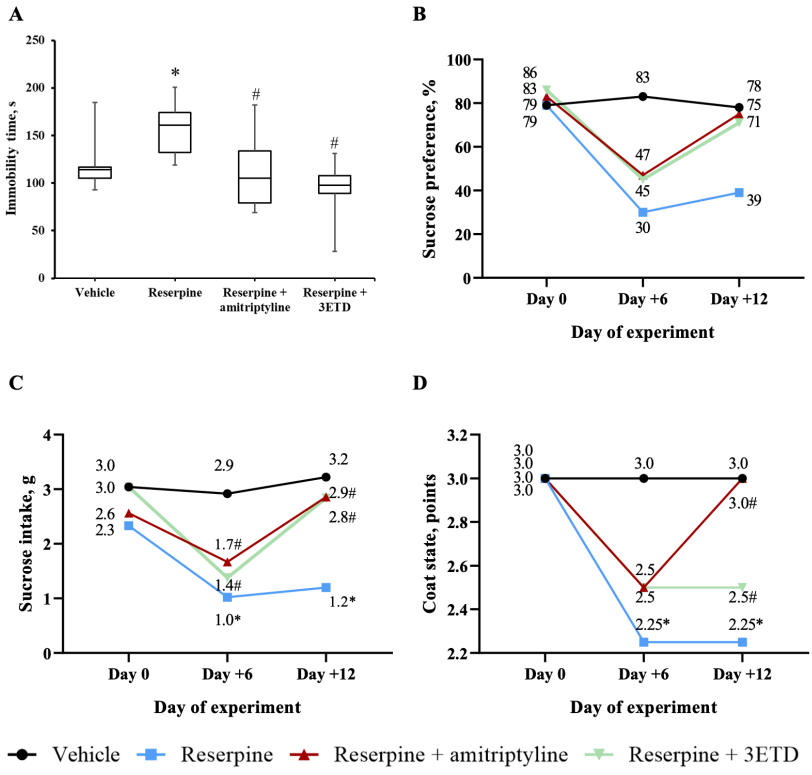


Figure 2. Effect of 3-ethoxythietane-1,1-dioxide (3ETD) on immobility time in the forced swimming test (**A**), sucrose preference (**B**), sucrose intake (**C**) and coat state (**D**) in rats with reserpine-induced depression. ***Note:*** graphs show group medians (A-D), interquartile range (A), minimum and maximum (A); * – p<0.05 for the Mann-Whitney test compared with the control group; # – p<0.05 for the Mann-Whitney test compared with the reserpine group.

Seven-fold administration of reserpine significantly decreased the motor activity of rats in OF and EPM (Day +13). In OF, reserpine significantly reduced the number of all assessed indicators compared to the control group (in particular, "movement" – by 98%, OEA – by 82%, $p<0.05$), and in EPM, the animals did not enter any of the arms of the maze, remaining in the central zone throughout the entire recording period (Me=300 s). 3ETD and amitriptyline did not have a significant effect on the behavior of rats with reserpine-induced depression – the values of all indicators were comparable with the reserpine group.

Effect of 3ETD on the development of anhedonia and coat state deterioration in rats with reserpine-induced depression

Reserpine induced anhedonia in rats, decreasing sucrose preference to 30% on Day +6 and to 39% on Day +12 (Fig. 2B). Sucrose intake decreased compared to the control group (by 65% on Day +6, by 63% on Day +12, $p<0.001$) and that on Day 0 (by 49-56%, $p=0.005$, Fig. 2C). 3ETD and amitriptyline eliminated reserpine-induced anhedonia, increasing sucrose preference to the level of the control group on Day +12 (Fig. 2B), sucrose consumption – on Days +6 and +12 (on Day +6 – by 36% and 64%, respectively, on Day +12 – by 137% and 138%, respectively, $p<0.05$, Fig. 2C).

In the reserpine group, a coat state deterioration was observed on Days +6 and +12: the coat state decreased to 2.25 points, both compared to the control group ($p=0.001$) and to the base level of Day 0 ($p=0.012$ and $p=0.008$, respectively). 3ETD and amitriptyline counteracted the effect of reserpine on Day +12, improving the coat state to the level of that in the control group ($p<0.05$ compared to the reserpine group); no statistically significant difference was found between the 3ETD and amitriptyline groups (Fig. 2D).

Effect of 3ETD on weight gain and internal organ mass in rats with reserpine-induced depression

Reserpine reduced the body weight of rats by 35 g compared to the control group (Day +13) and by 39 g compared to Day 0 ($p<0.05$). Amitriptyline did not influence the effect of reserpine, while 3ETD prevented reserpine-induced weight loss – the weight of rats on Days +3, +4 and +12 and +13 – with the control group (Fig. 3).

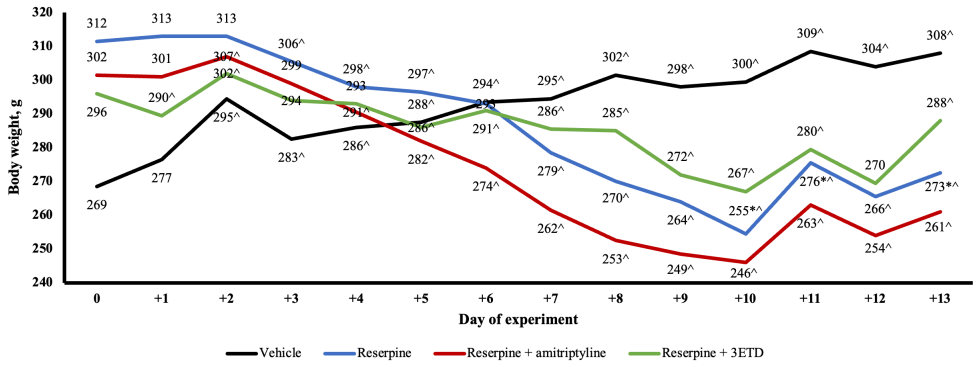


Figure 3. Effect of 3ETD on body weight of rats with reserpine-induced depression. ***Note:*** graphs show group medians; * – $p<0.05$ for the Mann-Whitney test compared with the control group; # – $p<0.05$ for the Mann-Whitney test compared with the reserpine group; ^ – $p<0.05$ for the Wilcoxon test compared with the base level of Day 0.

Reserpine increased the weight coefficients of the spleen and adrenal glands (by 37% and 69%, respectively, compared with the control group, $p<0.05$), 3ETD and amitriptyline had no effect compared with the reserpine group.

Effect of 3ETD on serum biochemical markers and hippocampal brain-derived neurotrophic factor levels in rats with reserpine-induced depression

Reserpine decreased the levels of ALT and AST (both by 31%, $p<0.01$), triglycerides (by 13%, $p=0.041$) and albumin (by 12%, $p=0.045$), increased the content of urea (by 26%, $p=0.007$) in the blood serum of rats compared with the control group. Reserpine also insignificantly reduced bilirubin levels by 21% and did not change the level of glucose.

3ETD decreased the level of urea (by 31%, p=0.037) and increased the concentration of triglycerides (by 18%, p=0.041) compared with the reserpine group, and amitriptyline decreased the concentration of bilirubin, exacerbating the effect of reserpine (by 5%, p=0.018 compared with the reserpine group). Both 3ETD and amitriptyline increased glucose levels by 11% and 15% compared with the reserpine group (p=0.003 and p=0.002, respectively).

Reserpine had no effect on BDNF levels in the rat hippocampus. BDNF levels in the 3ETD and amitriptyline groups did not differ significantly from the reserpine group.

Effect of 3ETD on morphological characteristics and level of apoptosis regulators in the hippocampus of rats with reserpine-induced depression

Reserpine caused prominent morphological changes in the hippocampus compared with the control group: it decreased the pyramidal neuron density in the CA1, CA2, CA3, CA4 fields, and pycnomorphic neurons and neurons with wrinkled perikaryon and reduced basophilia were found in field CA1. In DG, numerous astrocytes were visualized on the surface of basket cells, and neurons had light cytoplasm. In the CA1 and CA3 fields, a decrease in the diameter and area of cells was noted (by 10 and 22% in CA1, by 19 and 39% in CA3, respectively, p<0.05 compared to the control group), and in the CA4 field – their increase (by 27% and 60%, respectively, p<0.05) (Table 1).

Table 1. Effect of 3ETD on morphometric parameters of the hippocampus of rats with reserpine-induced depression

Indicator	Vehicle	Reserpine	Reserpine + amitriptyline	Reserpine + 3ETD
Dentate gyrus				
Cell diameter, μm	**8.0** (7.3-8.8) n=11	**8.4** (7.5-9.1) n=11	**8.3** (7.7-9.0) n=11	**7.7** (7.4-8.6) n=11
Cell area, μm²	**50.4** (41.8-61.0) n=11	**55.7** (44.6-64.5) n=11	**53.7** (46.5-63.6) n=11	**46.8** (43.0-58.5) n=11
CA1				
Cell diameter, μm	**13.9** (12.3-14.5) n=11	**12.5*** (11.3-14.2) n=11	**11.6#** (11.0-12.5) n=11	**11.3#** (10.5-11.8) n=11
Cell area, μm²	**152.6** (118.4-164.1) n=11	**123.5*** (100.5-158.0) n=11	**104.9#** (94.4-122.1) n=11	**99.4#** (86.8-109.0) n=11
CA2				
Cell diameter, μm	**12.3** (11.7-13.4) n=11	**12.1** (11.4-12.7) n=11	**11.6** (10.6-12.3) n=11	**13.2** (11.5-14.2) n=11
Cell area, μm²	**118.0** (107.2-141.7) n=11	**115.1** (101.4-127.2) n=11	**104.9** (87.6-119.1) n=11	**135.9** (103.3-158.9) n=11
CA3				
Cell diameter, μm	**14.5** (13.0-15.1) n=11	**11.4*** (10.8-12.3) n=11	**12.5#** (11.9-13.6) n=11	**8.7#** (8.2-9.4) n=11
Cell area, μm²	**165.0** (132.9-178.9) n=11	**101.4*** (92.3-118.1) n=11	**123.4#** (111.2-144.7) n=11	**60.1#** (53.3-69.9) n=11
CA4				
Cell diameter, μm	**9.9** (9.2-11.1) n=11	**12.5*** (11.1-12.9) n=11	**11.9#** (10.9-12.8) n=11	**11.5#** (10.3-13.0) n=11
Cell area, μm²	**76.7** (65.8-96.3) n=11	**122.9*** (96.2-131.1) n=11	**110.5#** (93.5-127.6) n=11	**104.5#** (83.9-132.9) n=11

Note: the table shows group medians, interquartile range and the number of observations (n) in each group; * – p<0.05 for the Mann-Whitney test compared with the control group, # – p<0.05 for the Mann-Whitney test compared with the reserpine group.

Almost the same histological alterations were observed in the group treated with amitriptyline. The pyramidal layer of the CA2 field was formed by densely located cells with moderately chromophobic cytoplasm and dust-like Nissl bodies; astrocytes and oligodendrocytes were visualized on their surface and in the intercellular space; neurons of the CA3 and CA4 pyramidal layer were characterized by cytoplasmatic chromophobia.

In the 3ETD group, the pyramidal cells in the CA1 and CA2 fields were located more densely compared to the reserpine and amitriptyline groups, and the neurohistological picture was similar to the control group. In the CA3 and CA4 fields, a loose arrangement of cells was noted; neurons had light chromophilic cytoplasm.

3ETD and amitriptyline decreased the diameter and area of neurons compared to both the reserpine group and the control group ($p<0.05$) in the CA1 and CA4 fields. In the CA3 field, 3ETD also decreased morphometric parameters compared to the reserpine group, while amitriptyline, on the contrary, increased them ($p<0.05$) (Table 1).

The presence of reactive astrocytes in the rat hippocampus was determined based on the relative density of GFAP-positive cells. In the reserpine group, a more than 2-fold increase in the number of GFAP-positive cells was found in all areas of the hippocampus compared with the control group ($p<0.05$). 3ETD reduced GFAP staining density in all hippocampal fields compared with the reserpine group by 9% (CA2) – to 77% (DG) ($p<0.05$ for all fields), and, in CA4 and DG, the value was comparable to the control group. Amitriptyline also reduced the density of GFAP-positive cells in all hippocampal fields by 43% (CA2) – to 73% (CA4) ($p<0.05$ for all fields) compared with the reserpine group, and, in the fields CA1, CA3 and CA4, the value was comparable to the control group. The effect of amitriptyline was more pronounced than the effect of 3ETD in the fields CA1, CA2 and CA3 (1.5, 1.6 and 1.2 times, respectively, $p<0.05$), and was inferior to it in DG (1.7 times, $p<0.05$). The results of the analysis of the relative density of GFAP-positive cells in the hippocampus are shown in Table 2 and Figure 4.

Reserpine reduced the level of the anti-apoptotic marker Bcl-2 by 1.5-2 times compared with the control group ($p<0.05$) in all hippocampal fields. 3ETD counteracted the effect of reserpine in CA1 and DG, increasing the density of Bcl-2-positive cells by 24% and 55% ($p<0.05$), respectively, compared with the reserpine group, and in DG – to the level of the control group (Table 3, Figure 5).

Discussion

The reserpine-induced depression model is based on the monoamine theory of depression (Czéh et al. 2016) and is a basic method for studying the antidepressant activity of novel compounds (Habriev 2005). Reserpine, being an inhibitor of vesicular monoamine transporter 2 (VMAT2), causes monoamine depletion in neuronal terminals and leads to the development of a depressive-like symptoms in animals, such as locomotor disturbances (Antkiewicz-Michaluk et al. 2015), anhedonia (Czéh et al. 2016), and decreased exploratory behavior. This model is commonly used by researchers due to its simplicity and reproducibility, despite questionable construct and predictive validity (Czéh et al. 2016). Our study design included 7-fold i.p. administration of low-dose reserpine (0.5 mg/kg) from Day 0 to Day +6 and 12-fold i.p. administration of 3ETD and amitriptyline from Day 0 to Day +11.

Analysis of rat behavior showed that 3ETD and amitriptyline corrected the depressogenic effect of reserpine in FST (Day +12), reducing IT to the level of the control group, but did not eliminate the suppression of motor activity in OF and EPM (Day +13) and caused a tendency to worsen it (insignificantly decreased EA and OEA in OF). It is known that the behavior of rats in OF is largely determined by dopaminergic neurotransmission: for example, stimulation of D1 and/or D2 dopamine receptors increases the motor activity of animals in OF, and stimulation of D3 receptors decreases it (Zamudio et al. 2005). Therefore, it can be assumed that the enhancement of reserpine effects under the influence of 3ETD is probably associated with a decrease in dopaminergic neurotransmission combined with dopamine depletion in neuronal terminals. No statistically significant differences were found between the 3ETD and amitriptyline groups; however, it should be noted that the inhibition of motor activity in animals in the group of 3ETD was more pronounced than in the group of amitriptyline. Thus, in EPM, the animals of the 3ETD group remained in the central zone throughout the recording time and did not enter any of the arms of the maze, while in the group of amitriptyline, the rats were able to move to one of the arms. In OF, the EA and OEA values were higher in the amitriptyline group. These data confirm the differences between 3ETD and amitriptyline in the

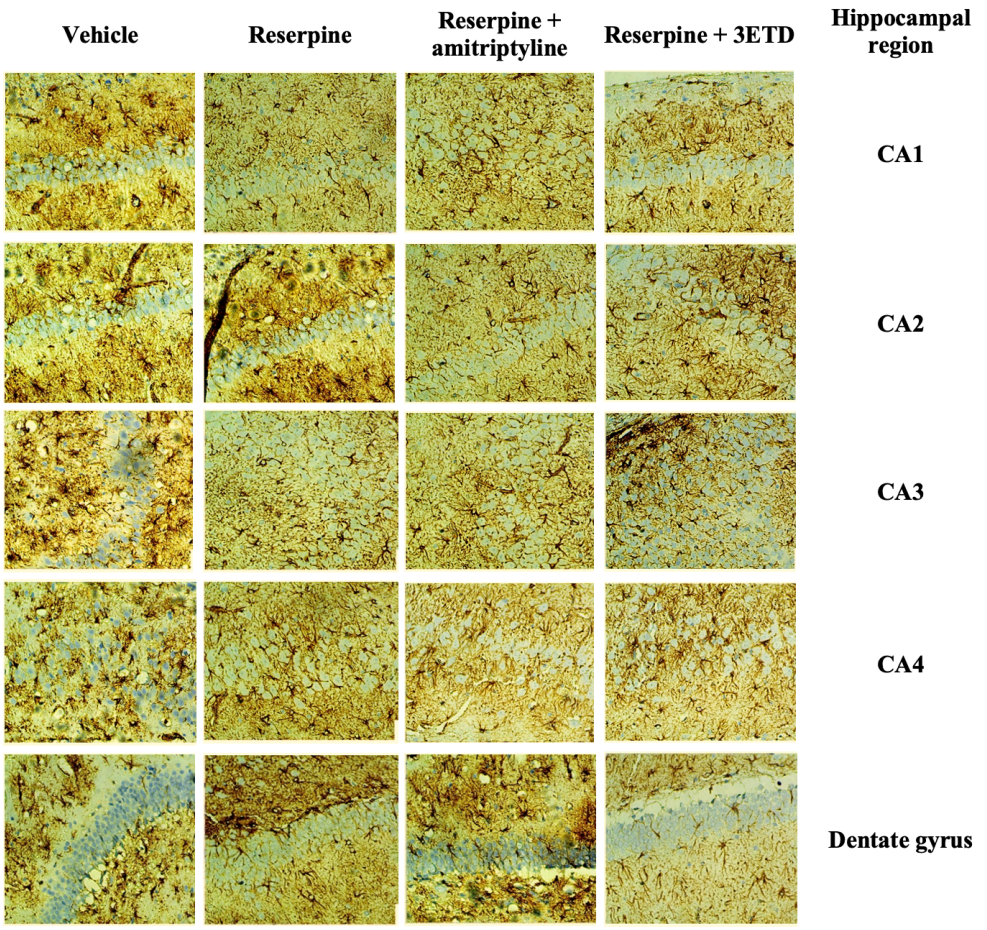


Figure 4. Effect of 3ETD on immunolocalization of GFAP in the hippocampal regions CA1, CA2, CA3, CA4 and dentate gyrus in rats with reserpine-induced depression (magnification x400x).

Table 2. Effect of 3ETD on the relative density of GFAP-positive cells (in %) in the hippocampus of rats with reserpine-induced depression

Hippocampal region	Vehicle	Reserpine	Reserpine + amitriptyline	Reserpine + 3ETD
Dentate gyrus	**3.52** (3.02-6.32) n=11	**16.74*** (15.51-18.51) n=11	**6.48#** (5.64-7.85) n=11	**3.9#** (3.36-5.32) n=11
CA1	**5.95** (4.67-6.79) n=11	**14.71*** (11.89-15.95) n=11	**5.57#** (4.68-7.92) n=11	**8.3#** (7.62-10.81) n=11
CA2	**5.77** (4.46-6.45) n=11	**12.7*** (11.16-13.68) n=11	**7.29#** (5.82-7.41) n=11	**11.51#** (7.45-12.22) n=11
CA3	**5.78** (5.44-6.65) n=11	**17.98*** (14.21-21.01) n=11	**7.76#** (5.63-8.74) n=11	**8.9#** (8.73-10.73) n=11
CA4	**7.25** (6.78-8.28) n=11	**23.5*** (21.77-24.94) n=11	**6.32#** (4.95-7.78) n=11	**8.12#** (6.07-11.23) n=11

Note: the table shows group medians, interquartile range and the number of observations (n) in each group; * – $p<0.05$ for the Mann-Whitney test compared with the control group, # – $p<0.05$ for the Mann-Whitney test compared with the reserpine group.

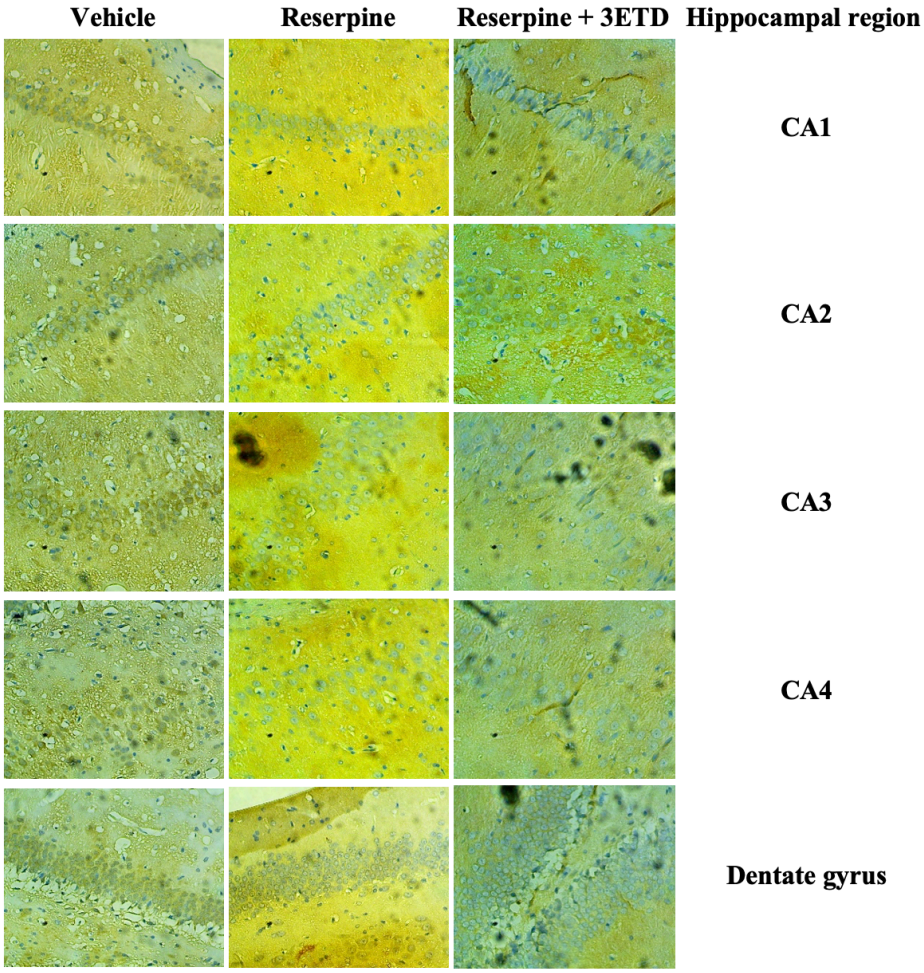


Figure 5. Effect of 3ETD on immunolocalization of Bcl-2 in the hippocampal regions CA1, CA2, CA3, CA4 and dentate gyrus in rats with reserpine-induced depression (magnification x400x).

Table 3. Effect of 3ETD on the relative density of Bcl-2-positive cells (in %) in the hippocampus of rats with reserpine-induced depression

Hippocampal region	Vehicle	Reserpine	Reserpine + 3ETD
Dentate gyrus	**5.9** (5.12-7.86) n=13	**3.5*** (2.64-4.17) n=13	**5.41#** (4.64-6.75) n=13
CA1	**8.49** (6.58-11.19) n=13	**3.92*** (2.35-4.76) n=13	**4.87#** (4.29-5.54) n=13
CA2	**8.7** (5.63-10.8) n=13	**4.66*** (3.18-5.9) n=13	**5.77** (4.46-6.45) n=13
CA3	**8.61** (6.49-11.33) n=13	**5.67*** (4.62-6.43) n=13	**5.67** (4.52-6.45) n=13
CA4	**8.37** (7.46-9.38) n=13	**5.76*** (4.7-7.41) n=13	**4.42** (4.22-5.03) n=13

Note: the table shows group medians, interquartile range and the number of observations (n) in each group; * – $p<0.05$ for the Mann-Whitney test compared with the control group, # – $p<0.05$ for the Mann-Whitney test compared with the reserpine group.

mechanism of action, which is consistent with the results of neuropharmacological interaction tests (Nikitina and Gaisina 2022; Nikitina et al. 2022) and may be associated with increased dopaminergic neurotransmission.

One of the key depressive symptoms is anhedonia, which is equivalent to a deficit in the capacity to feel pleasure in humans (Petković and Chaudhury 2022). Decreased intake and/or preference for palatable solutions is an overall validated behavioural measure of hedonic deficit (Strekalova et al. 2011); therefore, preference and consumption of a 1% sucrose solution were evaluated on Days 0, +6, and +12; the criterion for anhedonia in rats was a sucrose preference level <65% (Strekalova et al. 2011). 3ETD and amitriptyline increased sucrose intake and eliminated anhedonia on Day +12, which, along with the correction of despair behavior in FST, indicates their ability to counteract the reserpine depressogenic effect.

Coat state deterioration has been described as a reliable and well-validated index of depressed-like state (Costa-Ferreira et al. 2019), which is presumably equivalent to feelings of worthlessness or excessive or inappropriate guilt in humans (Czéh et al. 2016). 3ETD and amitriptyline eliminated the self-grooming deficit in animals by the end of the experiment (the fur condition of animals in these groups was comparable to the control group), indicating their antidepressant activity.

Depression-like symptoms in animals also include changes in body weight (Czéh et al. 2016). Reserpine reduced rat body weight both compared to the control group (by 35 g) and to the base level of Day 0 (by 39 g), which is consistent with the depressive-like state development. Amitriptyline did not counteract the effect of reserpine. The weight of animals in the 3ETD group was comparable with the reserpine group, but unlike amitriptyline, 3ETD prevented reserpine-induced weight loss over time compared with the base level of Day 0. This may be due to the antidepressant effect of 3ETD and indicates differences in 3ETD and amitriptyline mechanisms of action.

Additionally, the weight coefficients of internal organs and biochemical parameters of blood serum were assessed. Reserpine increased the weight coefficients of the adrenal glands and spleen compared to the control group, which is natural, since the development of depressive states is associated with hypothalamic-pituitary-adrenal dysregulation (in particular, an enlargement of the adrenal glands) (Packard et al. 2016; Dean and Keshavan 2017) and alterations within the peripheral immune system (Ménard et al. 2016). 3ETD, like amitriptyline, did not prevent the development of hypertrophy of the spleen and adrenal glands. At the same time, the weight coefficient of the liver in both groups receiving compounds was higher than in the reserpine group, but did not differ significantly from the control group.

In the reserpine group, there was a decrease in the levels of ALT, AST, triglycerides and albumin, a slight decrease in LDH level, and an increase in the content of urea in the blood serum compared to the control group, which may be a manifestation of the early phase of toxic injury of the liver (impaired hepatic synthetic function) and kidneys (decreased glomerular filtration). In the amitriptyline and 3ETD groups, a decrease in the level of ALT, AST, LDH and bilirubin was also noted, which may indicate an impairment of the synthetic ability of the liver, but no nephrotoxic effect was observed – the level of urea in these groups was comparable with the control group. At the same time, 3ETD and amitriptyline increased glucose and triglycerides levels compared with the reserpine group, probably interfering with its anorexigenic effect.

It is known that the development of depressive states, including in animals, is associated with impaired neurotrophic support, and antidepressants, regardless of their mechanism of action, are able to increase the level of neurotrophins (Dean and Keshavan 2017). Therefore, the level of BDNF was determined in the hippocampus of rats with reserpine-induced depression. We assumed that long-term administration of reserpine would result in a decrease in BDNF expression, and that antidepressants would counteract reserpine effect, as has been shown in several studies (Park et al. 2018; El-Marasy et al. 2021; Zaazaa et al. 2022). However, BDNF expression in reserpine-treated rats did not change significantly compared to the control group. Since the development of a depressive-like state in rats was confirmed by despair behavior, anhedonia, self-grooming deficit and weight loss, it was concluded that the lack of changes in BDNF expression may be due to both the design of the model and differences in the sensitivity of rodent strains to the effects of depressogenic factors (Duclot and Kabbaj 2015).

It is known that VMAT2 performs a neuroprotective function by accumulating monoamines in synaptic vesicles and thereby preventing their spontaneous oxidation in the cytosol; consequently, blockade of this protein causes neurotoxic effects (Antkiewicz-Michaluk et al. 2015). Therefore, the ability of 3ETD to exert a neuroprotective effect was

additionally investigated. For this purpose, the level of the astrocytic apoptosis marker GFAP and the anti-apoptotic marker Bcl-2 were determined in the rat hippocampus using IHC staining. In the reserpine group, an increase in the number of GFAP-positive cells and a decrease in the number of Bcl-2-positive cells were found in all hippocampal fields compared with the control group. 3ETD administration reduced apoptosis in the hippocampus: the density of GFAP staining decreased (in CA1-CA4, DG), and the density of Bcl-2 staining increased (in CA1, DG) compared with the reserpine group. Amitriptyline caused a similar effect, increasing the density of Bcl-2-positive cells (in CA1, CA3 and DG) and decreasing the density of GFAP-positive cells (in all hippocampal fields). The results obtained may indicate that 3ETD exhibited neuroprotective properties in the reserpine-induced depression model.

Conclusion

Administration of reserpine (0.5 mg/kg) for 7 days resulted in the development of a depressive-like state in rats, manifested by increased IT FST, anhedonia, self-grooming deficit, decreased motor activity in OF and EPM, weight loss, increased adrenal and spleen weight coefficients, as well as increased GFAP expression and decreased Bcl-2 expression in the hippocampus.

3ETD (2 mg/kg) and amitriptyline (10 mg/kg) counteracted the reserpine-induced symptoms in rats, decreasing IT FST, eliminating anhedonia and self-grooming deficit, as well as decreasing GFAP levels and increasing Bcl-2 levels in the hippocampus compared with the reserpine group. 3ETD also prevented reserpine-induced weight loss in animals. The obtained results indicate the prospects of further preclinical study of 3ETD and the possibility of developing a candidate drug for the treatment of depressive disorders with neuroprotective activity based on it.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The study was approved by the Expert Council on Biomedical Ethics in Theoretical Disciplines of Bashkir State Medical University (BSMU, minutes No. 9, 2020).

Data availability

All of the data that support the findings of this study are available in the main text.

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