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ORIGINAL ARTICLE

Combined actions of blueberry extract and lithium on neurochemical changes observed in an experimental model of mania: exploiting possible synergistic effects

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Abstract

Bipolar disorder is a psychiatric disease characterized by recurrent episodes of mania and depression. Blueberries contain bioactive compounds with important pharmacological effects such as neuroprotective and antioxidant actions. The aim of this study was to investigate the effects of blueberry extract and/or lithium on oxidative stress, and acetylcholinesterase (AChE) and Na^+, K^+-ATPase activity in an experimental ketamine-induced model of mania. Male Wistar rats were pretreated with vehicle, blueberry extract (200 mg/kg), and/or lithium (45 mg/kg or 22.5 mg/kg twice daily) for 14 days. Between the 8th and 14th days, the animals also received an injection of ketamine (25 mg/kg) or vehicle. On the 15th day the animals received a single injection of ketamine; after 30 min, the locomotor activity was evaluated in an open field test. Ketamine administration induced an increase in locomotor activity. In the cerebral cortex, hippocampus and striatum, ketamine also induced an increase in reactive oxygen species, lipid peroxidation and nitrite levels, as well a decrease in antioxidant enzyme activity. Pretreatment with blueberry extract or lithium was able to prevent this change. Ketamine increased the AChE and Na^+, K^+-ATPase activity in brain structures, while the blueberry extract partially prevented these alterations. In addition, our results showed that the neuroprotective effect was not potentiated when lithium and blueberry extract treatment were given together. In conclusion, our findings suggest that blueberry extract has a neuroprotective effect against an experimental model of mania. However, more studies should be performed to evaluate its effects as an adjuvant therapy.

Keywords Bipolar disorder · Hyperlocomotion · Anthocyanins · Oxidative stress · Acetylcholinesterase · Na^+, K^+-ATPase

Introduction

Bipolar disorder (BD) is one of the most debilitating mental illnesses, characterized by recurrent episodes of elevated mood, mania, and depression, interspersed with periods of euthymia (Phillips and Kupfer 2013). This disorder is associated with a significant mortality and morbidity, with high rates of suicide and medical comorbidities (Sagar and Pattanayak 2017) and is among the leading causes of disability worldwide (Walker et al. 2015). In manic episodes, people undergo from exaggerated self-esteem, less need to sleep, is easily distracted, hyperactivity and has increased risk behavior (Phillips and Kupfer 2013).

The pathophysiology of this disorder is complex and multifactorial, and its mechanisms remain unclear. However, studies have demonstrated that mitochondrial dysfunction, disturbances in neurotransmitters systems, alterations in membrane transport,

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and oxidative stress seem to play important roles in the pathogenesis of BD (Sigitova et al. 2016).

Oxidative stress is characterized as an imbalance between antioxidant and pro-oxidant processes, resulting in the increase in reactive species levels which have potential to cause cellular damage (Adam-Vizi and Chinopoulos 2006). Biomarkers of oxidative stress, such as an increase in lipid peroxidation and decrease in antioxidant defenses, have been described in BD patients (Mansur et al. 2016; Chowdhury et al. 2017), indicating that free radicals are involved in neurochemical changes in this psychiatric disorder.

Brain oxidative stress damage also may alter the structure and function of crucial enzymes such as acetylcholinesterase (AChE) and Na^+, K^+-ATPase (Dobrota et al. 1999). AChE is the main enzyme involved in the degradation of the neurotransmitter acetylcholine, while the Na^+, K^+-ATPase is responsible for electrochemical gradients across the neuronal membranes by regulating the entry of K^+ and exit of Na^+ from cells (Erecinska and Silver 1994; Zugno et al. 2009; Arnaiz and Ordieres 2014). As a consequence of their key physiological roles, the activity of these enzymes has been studied in different pathological and experimental conditions (Cho 1995; Zugno et al. 2009; Carvalho et al. 2012; Gutierres et al. 2014). Thus, molecules capable of modulating the Na^+, K^+-ATPase and AChE enzymes increase the possibility to develop new therapeutic strategies for reducing the severity and complications of BD.

Lithium is one of the most widely used drugs in the treatment of BD. Studies have demonstrated that this drug has neuroprotective effects by significantly reducing the severity and frequency of mania, and preventing bipolar depression and suicide (Moreno et al. 2005; Fornaro et al. 2016). However, side effects and low therapeutic index are well-recognized limitations for lithium monotherapy in BD.

In recent years, considerable attention has been directed towards the identification of natural compounds that may be used for human health promotion and disease prevention. Blueberry (*Vaccinium* spp.) has gained worldwide interest as it contains various bioactive compounds (Wu et al. 2017) that may have potent biological activities such as antioxidant, anti-inflammatory and neuroprotective actions (Gutierres et al. 2014; Debom et al. 2016). Thus, the consumption of diets enriched with polyphenols may present new perspectives for the prevention or treatment of neuropsychiatric diseases (Gazal et al. 2014; Debom et al. 2016).

Considering that BD is one of the most debilitating psychiatric diseases and that its therapy has many adverse effects, this study aims to evaluate the potential therapeutic of blueberry extract on AChE and Na^+, K^+-ATPase activities and oxidative stress parameters in brain regions of rats submitted to ketamine-induced model of mania. In addition, we also evaluated the effects of the joint blueberry extract and lithium treatment.

Material and methods

Chemicals

Acetylthiocholine iodide (AcSCh), Coomassie Brilliant Blue G, Trizma Base, thiobarbituric acid, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), dichloro-dihydro-fluorescein diacetate (DCFH-DA), ethylenediaminetetraacetic acid (EDTA), epinephrine and ouabain octahydrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid and hydrogen peroxide were purchased from Synth® (Brazil). All other reagents used in the experiments were of analytical grade and the highest purity.

Blueberry extract preparation and phytochemical determination

Extraction

Fruits of *Vaccinium virgatum* were harvested at Embrapa Clima Temperado (Brazilian Agricultural Research Corporation) Pelotas/RS, Brazil (31°40′47″S and 52°26′24″W). After collection, the fruit were stored at −20 °C until the extraction process. The extract was prepared according to Bordignon et al. (2009). Briefly, 30 g of unprocessed blueberries were weighed and sonicated for 30 min at 25 °C in 90 mL 70:30 *v*/v ethanol-water (pH 1.0). The extracts were filtered, the ethanol removed under reduced pressure, and then lyophilized until dry. These procedures were performed in triplicate and sheltered from light.

LC/MS and anthocyanin identification

Anthocyanins were individually identified using liquid chromatography (Acquity-UPLC™) coupled to a photodiode array detector (PDA) and a high-resolution mass spectrometer (Xevo® G2 QTof model – WATERS®) equipped with an electrospray ionization source (ESI) operating in positive mode. The chromatographic separation was performed using a Synergi™ Polar-RP column (Phenomenex® - id 4 μm, 150 × 2.0 mm) at 40 °C and the injection volume was set at 5 μL. The elution was performed using an aqueous phase consisting of 2% formic acid (solvent A) and acetonitrile containing 1% formic acid (solvent B). A linear gradient according to the following conditions was used: 0–10 min, 5–12% B; 10–29 min, 12–18% B; 29–33 min, 18% B; 33–34 min, 5% B with a constant flow of 0.4 mL/min. The detection was performed at 520 nm, and the range of spectral scanning in the visible region ranged from 450 to 600 nm (PDA). Mass scanning ranged from *m/z* 200 to 1500 with a scan time of 0.5 s. MS/MS analyses were performed using a collision energy ramp (10–30 eV) with argon as the collision gas. A capillary voltage of 1.0 kV, source block temperature of 120 °C,

desolvation temperature of 600 °C; nebulizer nitrogen flow rate of 80 L/h, desolvation nitrogen gas flow of 800 L/h, and cone voltage of 40 V were used controlled by MassLynx v.4.1 software for data acquisition and processing. All samples were analyzed in triplicate.

Animals

Adult male Wistar rats (60 days, 250–300 g) were obtained from the Central Animal House of Federal University of Pelotas, Pelotas, RS, Brazil. The animals were housed in standard cages at an ambient temperature of 23 ± 1 °C, with 12 h light/dark cycles and free access to water and food. The Committee of Ethics and Animal Experimentation of the Federal University of Pelotas, Brazil, under protocol number CEEA 9085–2016, approved all animal procedures. The use of animals was in accordance with the Brazilian Guidelines for the Care and Use of Animals in Scientific Research Activities (DBCA), which is in agreement with the National Council of Control of Animal Experimentation (CONCEA).

Animal model of mania and prevention protocol with blueberry extract and/or lithium

Sixty animals were divided into six groups ($n = 10$): I (vehicle, saline), II (ketamine, 25 mg/kg), III (ketamine 25 mg/kg + lithium 45 mg/kg), IV (ketamine 25 mg/kg + blueberry extract 200 mg/kg), V (ketamine 25 mg/kg + lithium 45 mg/kg + blueberry extract 200 mg/kg), and VI (ketamine 25 mg/kg + lithium 22.5 mg/kg + blueberry extract 200 mg/kg). The animals in groups IV, V, and VI received the blueberry extract orally, while the animals of III, V, and VI received lithium (twice a day). The animals in groups I and II received the same volume of saline solution. From the 8th to the 14th day, the animals in groups II, III, IV, V, and VI also received ketamine treatment, while group I received vehicle intraperitoneally. On the 15th day of treatment, the animals received a single injection of ketamine or saline. Thirty minutes later, the locomotor activity was assessed in an open-field apparatus (Fig. 1). The blueberry extract, lithium, and ketamine dosages and treatment times were based on previous studies described in the literature (Ghedim et al. 2012; Gazal et al. 2014; Debom et al. 2016).

Open-field test

Locomotor behavior was evaluated using an open-field apparatus (Gazal et al. 2014; Debom et al. 2016). The apparatus consisted of a wooden box measuring 72 × 72 × 33 cm (width × length × height), with the floor divided into 16 equal squares (18 × 18 cm). The number of quadrants crossed over a period of 5 min was the parameter used to evaluate locomotor activity. The apparatus was cleaned with 40% ethanol and dried after each individual animal session.

Brain tissue preparation

After the open field test, the animals were euthanized by decapitation and the brain was collected and the cerebral cortex, striatum, and hippocampus were dissected. The brain structures were homogenized in specific buffer and centrifuged. The supernatant was used for all biochemical determinations.

Acetylcholinesterase (AChE) activity assay

AChE activity was determined as previously described by Ellman et al. (1961). The reaction system, composed of 10 mM DTNB, 100 mM phosphate buffer (pH 7.5), and 15 μL homogenate was incubated for 2 min at 27 °C. After this, 8 mM AcSCh was added to the reaction. The absorbance was read using a spectrophotometer at 412 nm for 2 min at 25 °C. AChE activity was expressed as μmol AcSCh/h/mg of protein.

Na^+, K^+-ATPase activity assay

Na^+, K^+-ATPase activity was measured as previously described by Carvalho et al. (2012). The reaction mixture for the Na^+, K^+-ATPase activity assay contained 6 mM $MgCl_2$, 50 mM NaCl, 5 mM KCl, 0.1 mM EDTA, and 30 mM Tris-HCl, pH 7.4. After 10 min pre-incubation at 37 °C, the reaction was initiated by the addition of ATP to a final concentration of 3 mM and incubated for 30 min. Control experiments were carried with the addition of 1 mM ouabain. Released inorganic phosphate (Pi) was measured using the malachite green method (Chan et al. 1986). Na^+, K^+-ATPase activity was expressed as pg Pi/mg protein/min.

Brain oxidative stress assays

Oxygen reactive species (ROS) assay

The oxidation of DCFH-DA to fluorescent 2′,7′-dichlorofluorescein (DCF) was measured to detect intracellular reactive oxygen species. DCF fluorescence intensity was recorded using excitation at 488 nm and emission at 525 nm 30 min after the addition of DCFH-DA to the medium. ROS formation was determined according to Ali et al. (1992) with some modifications and was expressed as μmol DCF/mg of protein.

Nitrite levels assay

Nitrite levels were assessed as per Stuehr and Nathan (1989). The supernatants were used in a colorimetric reaction with Griess reagent, where 50 μl sulphanilamide in 5% phosphoric acid was added to 50 μl supernatant. After 10 min, samples were mixed with 100 μl N-(1-naphthyl) ethylenediamine dihydrochloride and incubated for 10 min in the dark. The

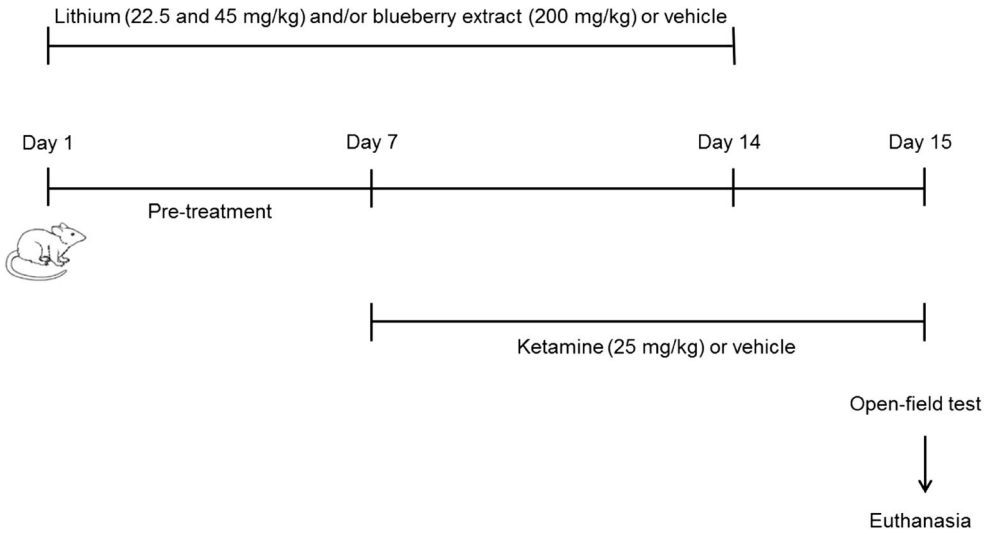

Fig. 1 Animal model of mania induced by ketamine and prevention protocol with blueberry and/or lithium treatment

absorbance was measured at 540 nm and the amount of nitrite is expressed as μmol nitrite/mg of protein.

Thiobarbituric acid reactive substances (TBARS) quantification

Lipid peroxidation was quantified using TBARS, according to Esterbauer and Cheeseman's (1990) method. First, homogenates were mixed with 15% trichloroacetic acid and 0.67% thiobarbituric acid. This mixture was heated for 30 min at 95 °C and then cooled for 10 min. TBARS was determined by the absorbance at 535 nm. Results are expressed as nmol TBARS/mg of protein.

Total sulfhydryl content (SH content) quantification

This assay, performed as described by Aksenov and Markesbery (2001), was based on the reduction of DTNB by thiols, which in turn become oxidized (disulfide), generating a yellow derivative (TNB) whose absorption is measured in the spectrophotometer at 412 nm. Homogenates were added to PBS buffer, pH 7.4, containing EDTA. The reaction was started by the addition of DTNB. Results were expressed as nmol TNB/mg of protein.

Superoxide dismutase (SOD) activity

This assay is based on the inhibition of the autoxidation of adrenaline in a spectrophotometer at 480 nm. The intermediate reaction produces superoxide, which is scavenged by SOD. One SOD unit was defined as the enzyme amount to cause 50% inhibition of adrenaline autoxidation. Total SOD activity was measured using the method described by Misra and Fridovich (1972) and the specific activity of SOD is expressed as units/mg of protein.

Catalase (CAT) activity

CAT activity was determined using the method described by Aebi (1984). The reduction in the amount of H_2O_2 was monitored for 180 s using a spectrophotometer at 240 nm at 37 °C. One unit of the enzyme is defined as the mount required to consume one nmol of H_2O_2 per minute and the specific activity is expressed as units/mg of protein.

Glutathione S-transferase (GST) activity assay

GST activity was determined according to the method of Habig et al. (1974), which uses 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The activity is expressed as μmol GS-DNB min/mg of protein.

Protein determination

For SOD, CAT, and GST activity, and TBARS and total sulfhydryl content levels, the protein levels were measured using the method detailed by Lowry et al. (1951) using bovine serum albumin as the standard. For Na^+, K^+-ATPase and AChE activity, and ROS and nitrite levels, the protein levels were measured using the Coomassie blue method according to Bradford (1976), with bovine serum albumin as the standard.

Lithium content analysis

The lithium concentration in the serum was performed using the Agilent 4200 Microwave Induced Plasma Optical Emission Spectrometry (MIP OES) equipped with a double pass cyclonic glass spray chamber (Agilent Technologies, Melbourne, Australia). The nitrogen used was extracted from atmospheric air by means of a nitrogen generator (4107 Nitrogen Generator – Agilent Technologies, Melbourne, Australia). Torch alignment and spectral line were

performed using a 5% (*v*/v) HNO_3 solution and optimized automatically by the instrument with MP Expert Software (1.5.1.0 version, Agilent Technologies, Melbourne, Australia). For lithium determination, the nebulizer gas flow (0.75 L/min), plasma observation position (0) and the wavelength (460.289 nm) were used.

Statistical analysis

Data were analyzed using analysis of variance (one-way ANOVA), followed by Tukey's multiple comparison test. $P \leq 0.05$ was considered to represent a significant difference in the analysis. All data are expressed as the mean ± S.E.M.

Results

Anthocyanin identification in blueberry extract

Anthocyanin identification was based on the fragmentation pattern and exact mass. Five aglycone fragments were identified: cyanidin, peonidin, delphinidin, petunidin, and malvidin. The aglycone fragmentation pattern and exact mass identified fifteen monoglycosylated anthocyanins (Table 1).

Open-field test

Ketamine treatment induced hyperlocomotion in rats ($P < 0.05$), as represented by an increase in the number of crossings in the open field test. Pretreatment with lithium-alone (45 mg/kg) ($P < 0.001$), blueberry extract-alone (200 mg/kg) ($P < 0.01$), and lithium (45 mg/kg) plus blueberry extract (200 mg/kg) ($P < 0.01$) prevented this increase. Co-treatment with lithium (22.5 mg/kg) plus blueberry extract (200 mg/kg) was not able to prevent this behavioral change ($P > 0.05$) (Fig. 2).

Acetylcholinesterase (AChE) activity in the cerebral cortex, hippocampus, and striatum

In the cerebral cortex, no change in AChE activity was observed in the animals treated only with ketamine; however, an increase in enzyme activity was observed in animals treated with both lithium and blueberry extract ($P < 0.01$; Fig. 3). In the hippocampus and striatum, an increase in AChE activity was observed in the ketamine group ($P < 0.05$). Both lithium ($P < 0.05$) and blueberry extract ($P < 0.05$) treatments were able to prevent this alteration (Fig. 3). Lithium and blueberry extract co-treatment was not effective in preventing the changes in AChE activity induced by ketamine administration in the hippocampus and striatum of rats (Fig. 3).

Na^+, K^+-ATPase activity in the cerebral cortex, hippocampus, and striatum

In the cerebral cortex, our results show an increase in Na^+, K^+-ATPase activity in the ketamine group ($P < 0.05$); treatment with lithium-alone ($P < 0.01$) and blueberry extract-alone ($P < 0.01$) were able to prevent this alteration (Fig. 4). In the hippocampus, an increase in Na^+, K^+-ATPase activity was observed in the ketamine group ($P < 0.05$); only lithium (22.5 mg/kg) and blueberry extract (200 mg/kg) co-treatment was able to prevent this ketamine-induced change ($P < 0.001$). In the striatum, an increase in Na^+, K^+-ATPase activity in the ketamine group ($P < 0.05$) was observed; treatment with blueberry extract-alone ($P < 0.05$), and lithium (45 and 22.5 mg/kg) plus blueberry extract (200 mg/kg) ($P < 0.05$) were able to prevent this alteration (Fig. 4).

Oxidative stress parameters in the cerebral cortex

Fig. 5 shows that ketamine caused an increase in ROS levels ($P < 0.05$); pretreatment with lithium-alone ($P < 0.001$) and blueberry extract-alone ($P < 0.01$) were able to prevent this increase. The administration of both treatments together was not effective in preventing the increase in ROS levels induced by ketamine (Fig. 5). Treatment with lithium-alone ($P < 0.05$), blueberry extract-alone ($P < 0.01$), and lithium (45 mg/kg) plus blueberry extract ($P < 0.05$) prevented the increase in the TBARS levels induced by ketamine administration ($P < 0.05$). In all experimental groups evaluated, no significant changes were observed in nitrite levels in the cerebral cortex.

Pretreatment with lithium-alone ($P < 0.01$), blueberry extract-alone ($P < 0.05$), and lithium and blueberry extract together ($P < 0.05$) prevented the decrease in the total sulfhydryl content induced by ketamine. Ketamine also caused a decrease in the SOD activity ($P < 0.05$), while lithium-alone ($P < 0.05$) and blueberry extract-alone ($P < 0.05$) pretreatment were effective in preventing this alteration. Similar results were observed in the catalase activity in the ketamine group ($P < 0.05$); again, this change was prevented by pretreatment with lithium-alone ($P < 0.05$), blueberry extract-alone ($P < 0.05$), and lithium (45 mg/kg or 22.5 mg/kg) and blueberry extract together ($P < 0.01$).

Oxidative stress parameters in the hippocampus

In the hippocampus, ketamine administration also caused an increase in ROS levels ($P < 0.05$). Only pretreatment with blueberry extract-alone was able to prevent this alteration ($P < 0.01$). In the hippocampus, ketamine increased the TBARS levels; pretreatment with lithium-alone ($P < 0.05$), blueberry extract-alone ($P < 0.01$) and lithium

Table 1 Anthocyanin identification of blueberry extract

Anthocyanin	λ_{max} (nm)	Molecular formula	$[M]^+$ *m/z* (Error - ppm)	Main fragments *m/z*
Delphinidin-*O*-galactoside	507	$C_{21}H_{21}O_{12}^+$	465.1060 (5.8)	303
Delphinidin-*O*-glucoside	507	$C_{21}H_{21}O_{12}^+$	465.1060 (5.8)	303
Cyanidin-*O*-galactoside	515	$C_{21}H_{21}O_{11}^+$	449.1065 (−4.2)	287
Delphinidin-*O*-pentoside	505	$C_{20}H_{19}O_{11}^+$	435.0892 (3.0)	303
Cyanidin-*O*-glucoside	515	$C_{21}H_{21}O_{11}^+$	449.1065 (−4.2)	287
Petunidin-*O*-galactoside	522	$C_{22}H_{23}O_{12}^+$	479.1161 (−6.1)	317
Petunidin-*O*-glucoside	522	$C_{22}H_{23}O_{12}^+$	479.1161 (1.1)	317
Cyanidin-*O*-pentoside	517	$C_{20}H_{19}O_{10}^+$	419.0931 (−4.7)	287
Peonidin-*O*-galactoside	517	$C_{22}H_{23}O_{11}^+$	463.1215 (−5.4)	301
Petunidin-*O*-pentoside	517	$C_{21}H_{21}O_{11}^+$	449.1087 (−0.1)	317
Peonidin-*O*-glucoside	517	$C_{22}H_{23}O_{11}^+$	463.1215 (−5.4)	301
Malvidin-*O*-galactoside	530	$C_{23}H_{25}O_{12}^+$	493.1326 (−4.1)	331
Malvidin-*O*-glucoside	530	$C_{23}H_{25}O_{12}^+$	493.1326 (−4.1)	331
Peonidin-*O*-pentoside	515	$C_{21}H_{21}O_{10}^+$	433.1101 (−7.9)	301
Malvidin-*O*-pentoside	522	$C_{22}H_{23}O_{11}^+$	463.1207 (−7.1)	331

(45 mg/kg) plus blueberry extract (200 mg/kg) ($P < 0.01$) were able to prevent this alteration (Fig. 6). In relation to antioxidant enzymes, our results show that ketamine induced a decrease in SOD and CAT activity in the hippocampus when compared with the control group ($P < 0.05$). It is important to note that all treatments evaluated in this study could prevent the alterations in CAT activity, while only the lithium-alone ($P < 0.001$), blueberry extract-alone ($P < 0.001$) and lithium (45 mg/kg) plus blueberry extract ($P < 0.001$) pretreatment were able to restore SOD activity when compared with the ketamine group (Fig. 6). No changes were observed in the nitrite levels or total sulfhydryl content in the hippocampus (Fig. 6).

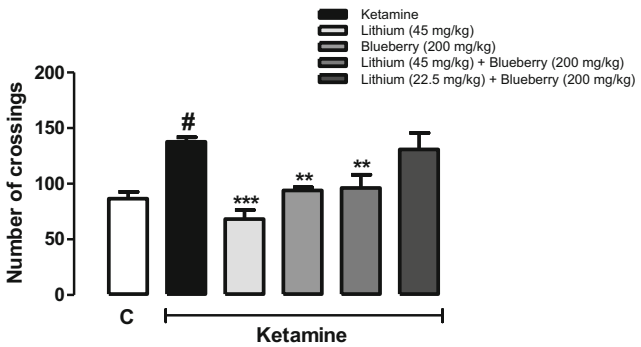

Fig. 2 Effect of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg, p.o.) plus blueberry extract (200 mg/kg, p.o.) pretreatment on ketamine-induced hyperactivity in an open-field test in rats. Data are expressed as mean ± S.E.M. (#) Denotes $P < 0.05$ when compared with the vehicle/saline group. (**) $P < 0.01$ and (***) $P < 0.001$ when compared with the vehicle/ketamine group (One-way ANOVA followed by Tukey test, $n = 7–10$)

Oxidative stress parameters in the striatum

In the striatum, ketamine induced an increase in ROS ($P < 0.05$) and nitrite ($P < 0.05$) levels when compared with the control group (Fig. 7). Pretreatment with lithium-alone, blueberry extract-alone, and both together prevented these alterations to similar levels ($P < 0.05$) (Fig. 7). Lithium-alone ($P < 0.05$) and blueberry extract-alone ($P < 0.05$) pretreatment also prevented the increase in TBARS levels induced by ketamine ($P < 0.05$). In all experimental groups evaluated, no changes were observed in the total sulfhydryl content in the striatum. Ketamine administration did not alter the SOD or CAT activity in the rat striatum ($P > 0.05$), however, our results showed a decrease in the SOD activity in the groups treated with lithium (45 mg/kg or 22.5 mg/kg) and blueberry extract together ($P < 0.05$). In addition, an increase in the CAT activity was observed only in the striatum of animals treated with lithium (45 mg/kg) plus blueberry extract ($P < 0.001$) (Fig. 7).

Glutathione S-transferase (GST) activity in the cerebral cortex, hippocampus, and striatum

Fig. 8 shows that ketamine causes an increase in the activity of the GST enzyme in cerebral cortex ($P < 0.05$). Pretreatment with lithium, blueberry extract, and both together were able to prevent these alterations to similar extents ($P < 0.05$) (Fig. 8). In the hippocampus, an increase in GST activity was observed only in the animals pretreated with lithium (22.5 mg/kg) plus blueberry extract when compared with the other groups ($P < 0.05$). No changes were observed in GST activity in the striatum ($P > 0.05$) (Fig. 8).

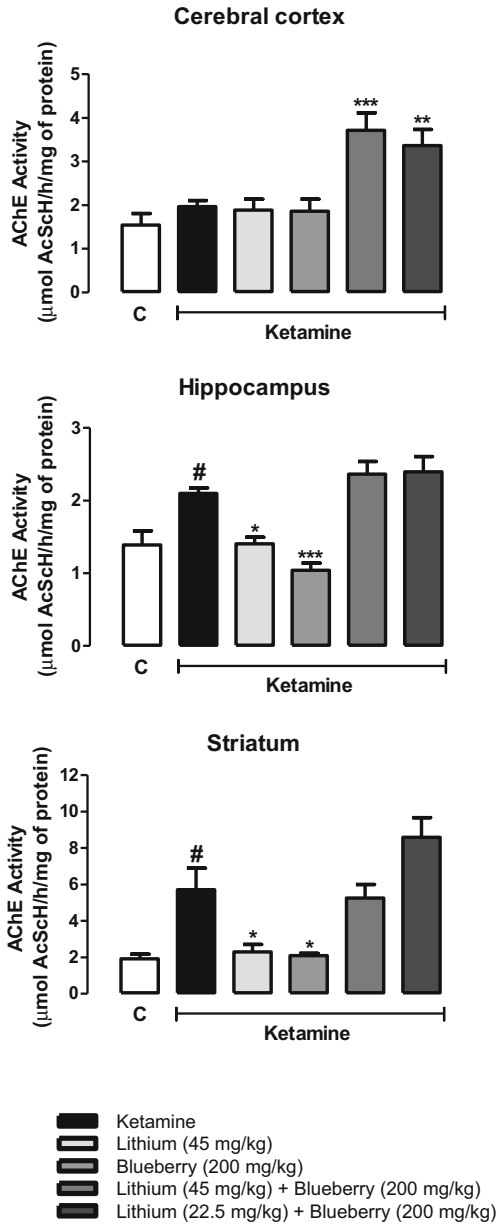

Fig. 3 Effect of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg, p.o.) plus blueberry extract (200 mg/kg, p.o.) pretreatment on acetylcholinesterase (AChE) activity in the cerebral cortex, hippocampus, and striatum of animals in a ketamine-induced model of manic-like behavior. Data are expressed as mean ± S.E.M. (#) Denotes $P < 0.05$ when compared with the vehicle/saline group. (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ when compared with the vehicle/ketamine group (One-way ANOVA followed by Tukey test, $n = 7$–10)

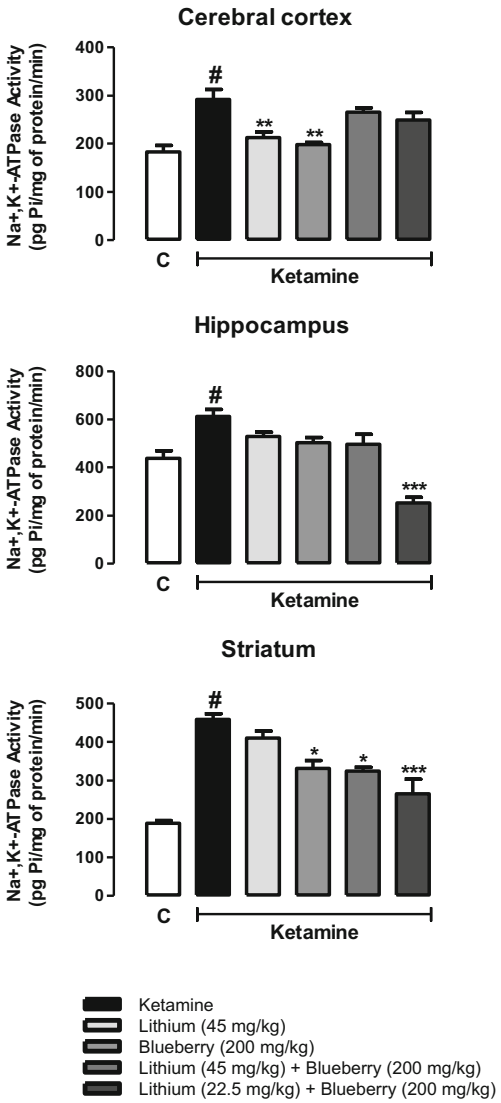

Fig. 4 Effect of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg, p.o.) plus blueberry extract (200 mg/kg, p.o.) pretreatment on Na^+, K^+-ATPase activity in the cerebral cortex, hippocampus, and striatum of animals in a ketamine-induced model of manic-like behavior. Data are expressed as mean ± S.E.M. (#) Denotes $P < 0.05$ when compared with the vehicle/saline group. (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ when compared with the vehicle/ketamine group (One-way ANOVA followed by Tukey test, $n = 5$–6)

Lithium concentration in serum

Fig. 9 shows an increase in serum lithium concentration of animals treated with lithium alone (45 mg/kg) and lithium (45 mg/kg) plus blueberry extract (200 mg/kg) when compared to the vehicle/saline group ($P < 0.001$). In the co-treatment with lithium (22.5 mg/kg) plus blueberry extract (200 mg/kg) lower concentrations were observed when compared with lithium alone (45 mg/kg) and lithium (45 mg/kg) plus blueberry extract (200 mg/kg) ($P < 0.05$).

Discussion

In the present study we evaluated the therapeutic potential of blueberry extract, as well as its effects when administered together with lithium, on neurochemical and behavior changes induced by an animal model of mania.

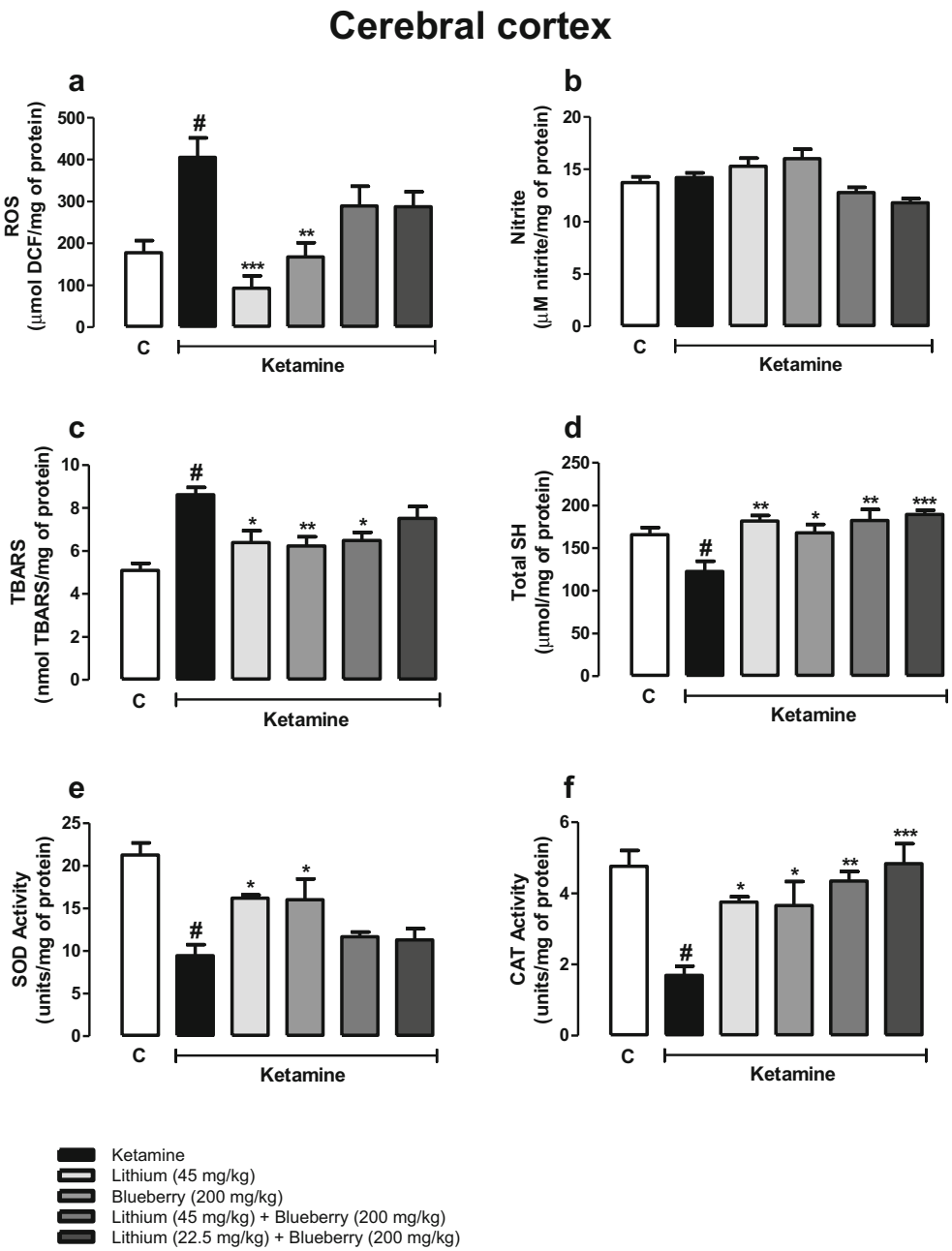

Fig. 5 Effect of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg, p.o.) plus blueberry extract (200 mg/kg, p.o.) pretreatment on levels of ROS, nitrite, TBARS, total thiol content, and superoxide dismutase and catalase activity in the cerebral cortex of animals in a ketamine-induced model of manic-like behavior. Data are expressed as mean ± S.E.M. ($^{\#}$) Denotes $P < 0.05$ when compared with the vehicle/saline group. (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ when compared with the vehicle/ketamine group (One-way ANOVA followed by Tukey test, $n = 5–6$)

The animal model of mania induced by ketamine is an experimental model used for the elucidation of neurological dysfunctions in manic episodes. Ketamine is a non-competitive N-methyl-D aspartate (NMDA) receptor antagonist capable of affecting a variety of receptors and cellular processes (Kapczinski and Quevedo 2016). Ketamine at subanesthetic doses alters mitochondrial respiratory chain activity in various brain structures (De Oliveira et al. 2011) and induces neurotoxicity in primary neuronal culture (Wang et al. 2008). Here, our results showed that ketamine induces hyperactivity in rats, similar to behavior observed in individuals with BD during a manic episode (Nestler and Hyman 2010; Dickerson et al. 2012). These findings are consistent with previous studies that also demonstrated that blueberry extract or lithium treatment were capable of preventing hyperlocomotion induced by ketamine (Debom et al. 2016).

Regarding the brain enzymes, ketamine induced an increase in AChE activity in the hippocampus and striatum. These findings are in conformity with previous studies that showed changes in the cholinergic system in BD (Sigitova et al. 2016). Cholinergic signaling is regulated by AChE, an enzyme that rapidly hydrolyses ACh (Sperling et al. 2008). ACh is involved in motor function, cognition, and attention, and an increase in AChE activity could reduce Ach levels, contributing to cholinergic signaling disruption in the brain. In fact, Cummings (2000) demonstrated that the reduction of

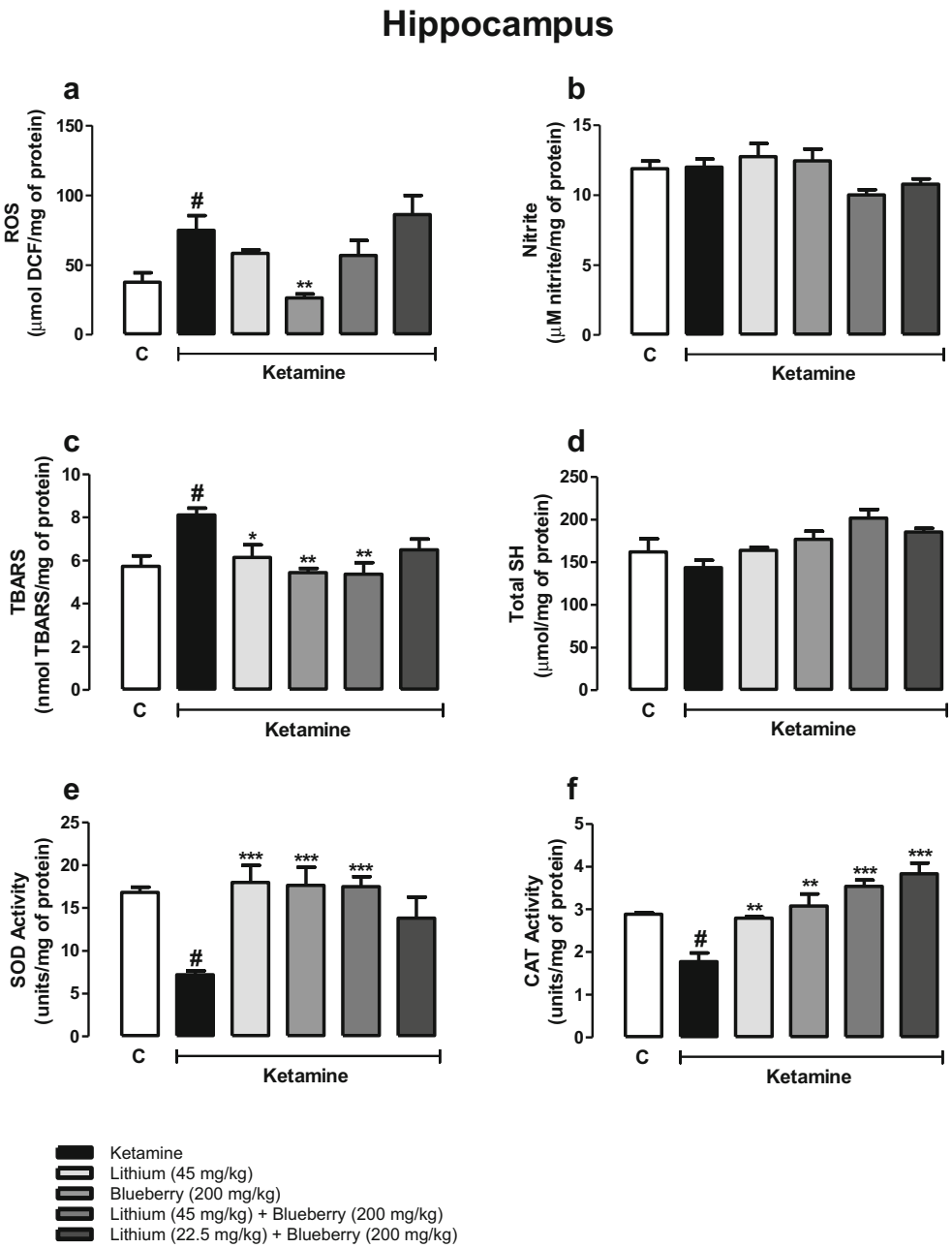

Fig. 6 Effect of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg) plus blueberry extract (200 mg/kg) pretreatment on levels of ROS, nitrite, TBARS, total thiol content, and superoxide dismutase and catalase activity in the hippocampus of animals in a ketamine-induced model of manic-like behavior. Data are expressed as mean ± S.E.M. (#) Denotes $P < 0.05$ when compared with the vehicle/saline group. (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ when compared with the vehicle/ketamine group (One-way ANOVA followed by Tukey test, $n = 5–6$)

ACh levels is associated with the manifestation of psychiatric diseases, particularly psychosis. In addition, the use of AChE inhibitors has been associated with a reduction of neuropsychiatric symptoms (Digby et al. 2012; Obermayer et al. 2017).

Treatment with blueberry extract or lithium was capable of preventing the alterations in AChE activity. Previous studies have shown that lithium improved memory and decreased AChE activity in the brain, and that these effects may be associated with the inhibition of GSK-3β (Jing et al. 2012). Blueberry extract has also been described to improve memory and decrease AChE activity in experimental models (Papandreou et al. 2011). Thus, our findings provide additional mechanisms by which bioactive compounds in blueberry extract could have neuroprotective effects in BD.

Ketamine administration also induced an increase in Na^+, K^+-ATPase activity in all evaluated brain structures. It has been established that dysfunction in Na^+, K^+-ATPase activity alters neuronal excitability leading to brain damage. Ketamine can activate glutamatergic neurotransmission by activating AMPA or kainate receptors in dopaminergic neurons, increasing the release of dopamine in the synaptic cleft (Tan et al. 2012; Duan et al. 2013). Dopamine is involved in the regulation of several proteins, including the Na^+, K^+-ATPase (Yang et al. 2007). Thus, the increase in Na^+, K^+-ATPase

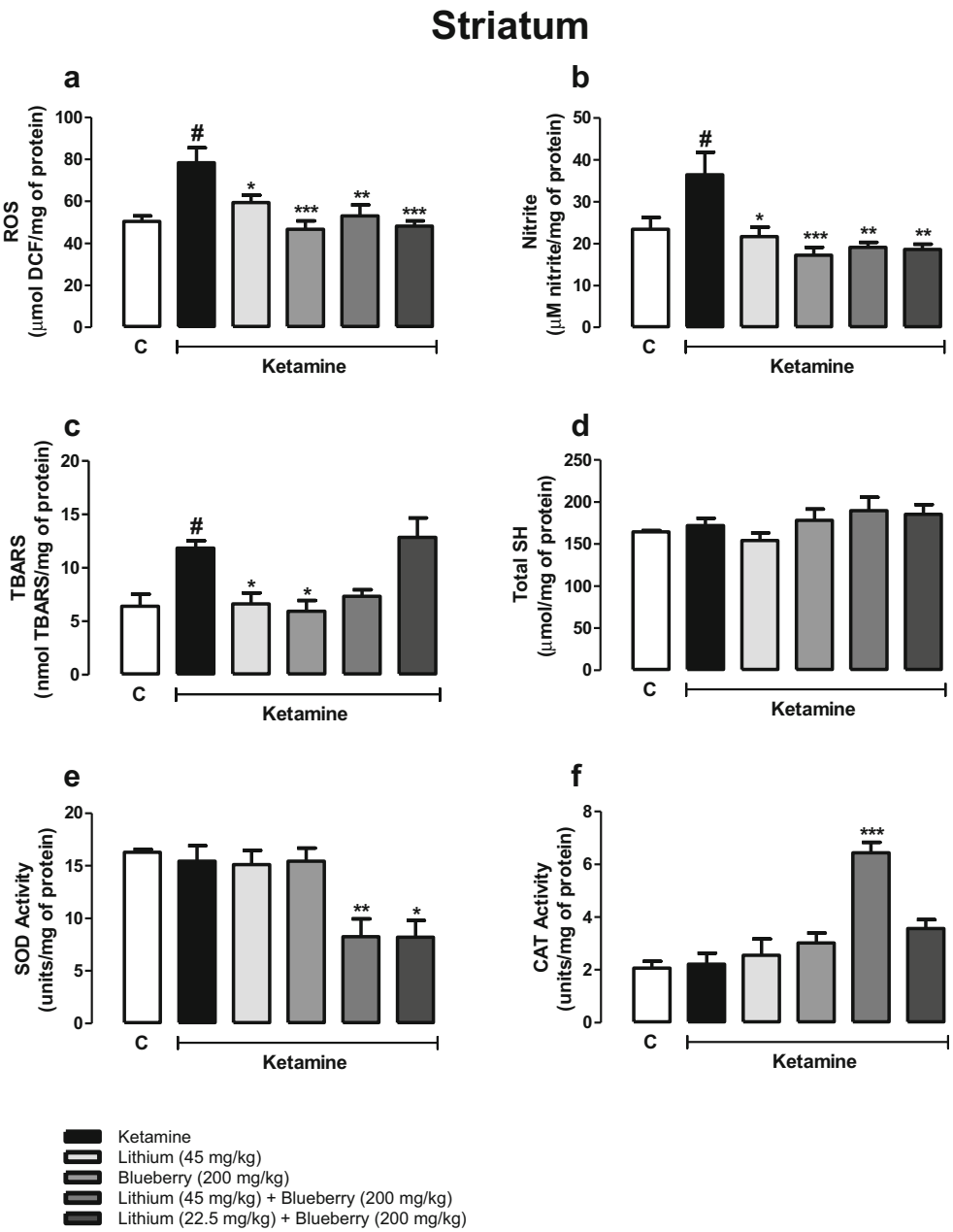

Fig. 7 Effect of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg) plus blueberry extract (200 mg/kg) pretreatment on levels of ROS, nitrite, TBARS, total thiol content, and superoxide dismutase and catalase activity in the striatum of animals in a ketamine-induced model of manic-like behavior. Data are expressed as mean ± S.E.M. (#) Denotes $P < 0.05$ when compared with the vehicle/saline group. (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ when compared with the vehicle/ketamine group (One-way ANOVA followed by Tukey test, $n = 5–6$)

activity could be explained, at least in part, by the activation of dopamine D2 receptors (Yamaguchi et al. 1996).

Interestingly, lithium treatment was able to prevent the change in Na^+, K^+-ATPase activity only in the cerebral cortex. Corroborating our study, Cho (1995) also showed that lithium decreased Na^+, K^+-ATPase activity in rat brain synaptosomes. Blueberry extract also prevented the increase in Na^+, K^+-ATPase activity in the cerebral cortex and striatum. Data from the literature have shown that anthocyanins are responsible for many beneficial properties of blueberries, such as their antioxidant (Debom et al. 2016; Oliveira et al. 2017) and anti-inflammatory (Smeriglio et al. 2016) activities. Neuroprotective actions of anthocyanins in experimental models, such as those for Alzheimer's disease (Gutierres et al. 2014), Huntington disease (Kreilaus et al. 2016), and depression (Nabavi et al. 2017) have also been described. In addition, experimental evidence has suggested that cyanidin-3-*O*-glucoside protects cortical neurons against glutamate excitotoxicity (Bhuiyan et al. 2011). Of particular importance, extract rich in anthocyanins inhibited hippocampal cell death (Ahn et al. 2011) and reverted alterations in Na^+, K^+-ATPase activity in the cortex cerebral and hippocampus in models of memory deficits (Gutierres et al. 2014).

Ketamine administration increased ROS and TBARS levels and decreased the activity of the antioxidant enzymes, SOD and CAT, in the cerebral cortex and hippocampus. These findings are in accordance with other studies using this animal model of

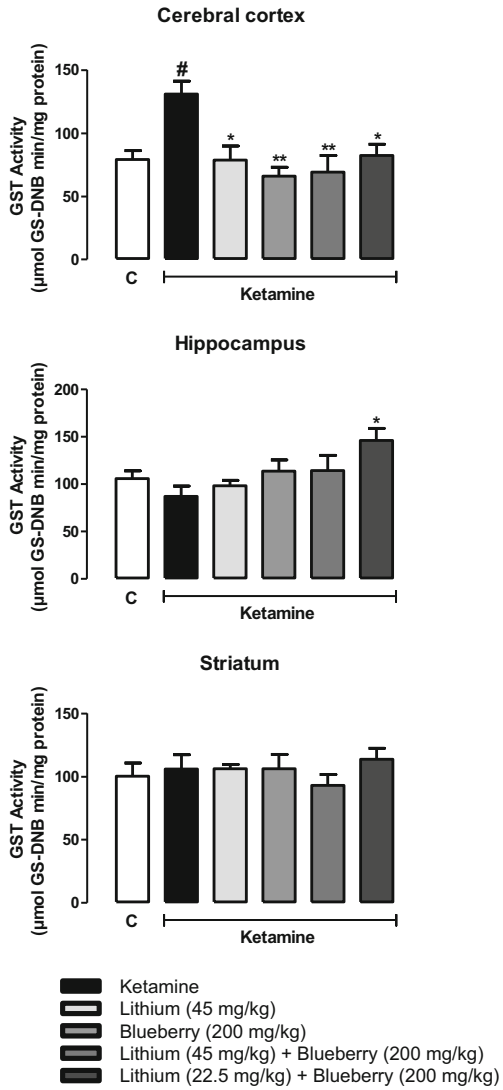

Fig. 8 Effect of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg) plus blueberry extract (200 mg/kg) pretreatment on glutathione S-transferase activity in the cerebral cortex, hippocampus, and striatum of animals in a ketamine-induced model of manic-like behavior. Data are expressed as mean ± S.E.M. ($^{\#}$) Denotes $P < 0.05$ when compared with the vehicle/saline group. (*) $P < 0.05$ and (**) $P < 0.01$ when compared with the vehicle/ketamine group (One-way ANOVA followed by Tukey test, $n = 5$–6)

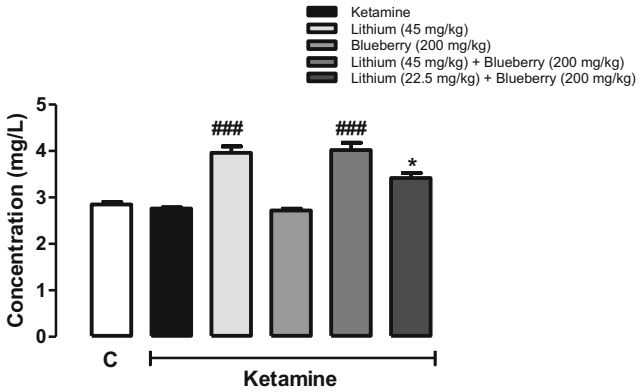

Fig. 9 Lithium concentration (mg/L) after administration of lithium (45 mg/kg, p.o.), blueberry extract (200 mg/kg, p.o.), and lithium (45 or 22.5 mg/kg) plus blueberry extract (200 mg/kg) in serum of animals in a ketamine-induced model of manic-like behavior. Data are expressed as mean ± S.E.M. ($^{\#\#\#}$). Denotes $P < 0.001$ when compared with the vehicle/saline group. (*) $P < 0.05$ when compared with the lithium/ketamine and lithium (45 mg/kg) plus blueberry (One-way ANOVA followed by Tukey test)

mania (Gazal et al. 2014; Debom et al. 2016). Zuo et al. (2007) also demonstrated that subanesthetic doses of ketamine led to the transient generation of hydroxyl radicals in mice. An increase in the TBARS level and alterations in antioxidant enzymes in the serum, plasma, and red blood cells has also been described in patients with BD (Machado-Vieira et al. 2007; Andreazza et al. 2008; Kapczinski and Quevedo 2016).

SOD is the enzyme responsible for converting the superoxide anion radical ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2), while CAT metabolizes H_2O_2 into water and molecular oxygen (Halliwell 2012). The decrease in activity of these antioxidant enzymes in the cerebral cortex and hippocampus may result in an accumulation of $O_2^{\cdot-}$ and H_2O_2 radicals (Pisoschi and Pop 2015), which may be associated with an increase in ROS and TBARS levels, and a decrease in the total thiol content seen in this study. Overproduction of ROS and dysfunctions in antioxidant system can have deleterious effects on signal transduction and cell resilience, mainly by inducing lipid peroxidation in the membranes and damage to proteins (Valko et al. 2007). Thus, it is plausible to suggest that oxidative stress could also contribute to the alterations in AChE and Na^+, K^+-ATPase activity seen in this study.

It is important to note that ketamine also induced an increase in the nitrite levels in the striatum. Nitrite is a metabolite of nitric oxide (NO), an important messenger involved with the regulation of mitochondrial function in the brain (Riobó et al. 2001). Yanik et al. (2004) showed an increase in the plasma nitrite levels in patients with BD. In addition, Venâncio et al. (2015) suggested that ketamine interferes with the activity of complex I, resulting in an increase in mitochondrial nitric oxide synthase (mitNOS) activity and elevated levels of NO. Furthermore, NO contributes to oxidative stress, because it can react with $O_2^{\cdot-}$ and lead to the formation of peroxynitrite (ONOO-), a powerful oxidant (Adam-Vizi 2005).

The antioxidant effect of blueberry extract may be associated with anthocyanins. It has already been shown that they can cross the blood-brain barrier (Andres-Lacueva et al. 2005) and act as a scavenger of free radicals (Del Bo et al. 2015). Among the main mechanisms involved in the antioxidant activity of anthocyanins is the ability to donate hydrogen atoms to free radicals, as well as the chelation of metallic ions (Han et al. 2015). In relation to antioxidant effects of lithium, evidence has suggested that this drug may increase the levels of the bcl-2 protein in the brain (Chen et al. 1999; Manji et al. 2000). Bcl-2 has been associated with mitochondrial activity,

as well as with the antioxidant effects of lithium (Machado-Vieira and Soares 2007).

In the present study, we also found that GST activity was increased in the cerebral cortex of the rats used in the mania model. In another study, Chan et al. (2008) demonstrated that the administration of ketamine also increased the activity of hepatic GST. Although we cannot state the exact mechanisms by which this increase occurred, a study in bipolar patients also demonstrated that the GST activity is enhanced (Andreazza et al. 2009), suggesting that the alteration in this enzyme's activity can be considered a compensatory mechanism.

In relation to our findings, the effects of lithium or blueberry in hyperlocomotion and brain oxidative stress on animal model of mania are in agreement with previous studies from our research group (Debom et al. 2016). Although lithium has a range of adverse effects, this drug is the standard pharmacological treatment of patients with BD (McKnight et al. 2012). Thus, the results obtained in this study suggest that the bioactive compounds in the blueberry extract could have the same therapeutic molecular targets as lithium.

Data from the literature have led to new perspectives on natural compounds, as well as the synergistic potential of numerous substances in the treatment of diseases (Hemaiswarya et al. 2008; Choi et al. 2012). The combination of selective therapy could enhance the beneficial effects, decrease doses compared to when the same drugs are administered individually, and reduce the side effects caused by drug monotherapy (Hemaiswarya et al. 2008). However, in our study, we found that the preventive effects in a model of mania was decreased when lithium and blueberry extract treatment were provided together. Considering that blueberry extract has many bioactive compounds with pharmacological effects, potential interactions with conventional drugs are possible.

The mechanism involved in this interaction between blueberry extract and lithium is complex and is important to consider that among the determinants for this difference in responses is the capacity for metal chelation, absorption, bioavailability, plasma concentrations, and a slow and continuous release of phenolic compounds from the gut into the bloodstream (Fernandes et al. 2014; Ferrars et al. 2014; Kamiloglu et al. 2015; Marín et al. 2015).

Unfortunately we cannot exactly determine the reason of the lack of effects when blueberry extract was administered in combination with lithium, however some findings obtained suggest that this seems not to be related with absorption. The same behavioral and brain parameters results (oxidative stress, AChE and Na^+, K^+-ATPase) were obtained when lithium and blueberry extract were administered concomitantly or when lithium and blueberry extract were administered in the animals with an interval of the six hours (data not shown). Additional experiments realized demonstrated also that lithium concentration in the animals that received only lithium (45 mg/kg twice a day) and animals that received lithium (45 mg/kg twice a day) plus blueberry extract (200 mg/kg) were similar (Fig. 9). So, it is possible that there is no prejudice in lithium absorption. However, a limitation of this research was the analytical method inability to detect, differently, free and complexed lithium in plasma. Besides, due to the inherent molecular complexity of natural matrix, other limitation is the difficult to know the absorption, distribution and pharmacological action mechanisms of the substances.

In conclusion, our findings demonstrate that blueberry extract prevents ketamine-induced alterations in AChE and Na^+, K^+-ATPase activity and oxidative stress in the brain in an experimental model of mania. Blueberry extract is a natural and inexpensive therapeutic means to support a healthy brain in psychiatric disorders. However, more studies are necessary to evaluate the potential interactions with lithium and its use as an adjuvant therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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