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ORIGINAL ARTICLE

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Lithium ameliorates sleep deprivation-induced mania-like behavior, hypothalamic-pituitary-adrenal (HPA) axis alterations, oxidative stress and elevations of cytokine concentrations in the brain and serum of mice

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Correction added on 17 July 2017, after first online publication: A new coauthor and affiliation added.

Objectives: The goal of the present study was to investigate the effects of lithium administration on behavior, oxidative stress parameters and cytokine levels in the periphery and brain of mice subjected to an animal model of mania induced by paradoxical sleep deprivation (PSD).

Methods: Male C57 mice were treated with saline or lithium for 7 days. The sleep deprivation protocol started on the 5th day during for the last 36 hours of the treatment period. Immediately after the sleep deprivation protocol, animals locomotor activity was evaluated and serum and brain samples was extracted to evaluation of corticosterone and adrenocorticotropic hormone circulating levels, oxidative stress parameters and citokynes levels.

Results: The results showed that PSD induced hyperactivity in mice, which is considered a mania-like behavior. PSD increased lipid peroxidation and oxidative damage to DNA, as well as causing alterations to antioxidant enzymes in the frontal cortex, hippocampus and serum of mice. In addition, PSD increased the levels of cytokines in the brains of mice. Treatment with lithium prevented the mania-like behavior, oxidative damage and cytokine alterations induced by PSD.

Conclusions: Improving our understanding of oxidative damage in biomolecules, antioxidant mechanisms and the inflammatory system – alterations presented in the animal models of mania – is important in helping us to improve our knowledge concerning

the pathophysiology of BD, and the mechanisms of action employed by mood stabilizers.

KEYWORDS

animal model of mania, bipolar disorder, cytokine levels, lithium, oxidative stress

1 | INTRODUCTION

Bipolar disorder (BD) is a common, complex, and severe mood disorder with progressive social and cognitive function disturbances.[1,2] BD is characterized by the presence of mania or hypomania, and episodes of depression. Mania or hypomania is characterized by persistent increases in energy, accompanied by an elevated and expansive or irritable mood. Mania and hypomania are differentiated by the severity, duration, and number of symptoms, with hypomania presenting with less severe symptoms than mania. The depressive episodes are characterized by a profound loss of motivation and interest.[3] The gold standard used in the treatment of BD is lithium (Li), which is a mood stabilizer approved by the Food and Drug Administration (FDA).[4] Previous studies have shown that Li is effective in acute manic episodes, and reduces the risk of manic and depressive relapses.[2,5]

Despite its importance, little is known about the precise neurobiological underpinnings of BD. However, a considerable number of studies have reported the involvement of glucocorticoids, oxidative stress and inflammatory cytokines in the pathophysiology of BD.[6-11] Muneer[12] has suggested that an interaction occurs in the pathophysiology of BD between the hypothalamic-pituitary-adrenal (HPA) axis, inflammatory mediators and oxidative stress, which deregulate hormonal, metabolic, and circadian homeostasis. Indeed, oxidative stress leads to cortisol resistance by decreasing the movement of the glucocorticoid receptor from the cytosol to the nucleus.[12] In turn, HPA axis deregulation by oxidative stress induces an inflammatory response which increases the levels of cytokines.[12-14] On the other hand, the increase of glucocorticoids released from the adrenal gland in response to stress-induced activation of the HPA axis can induce oxidative stress, with this becoming a continuous cycle.[15]

In situations in which the generation of reactive oxygen species (ROS) exceeds the capacity of antioxidant defense, oxidative stress may induce direct damage to cellular proteins, DNA and lipids, thus impairing neuronal function.[16,17] The role of oxidative stress in the physiopathology of BD has been investigated in several studies, which have consistently reported changes in antioxidant enzymes, lipid peroxidation, and protein/DNA oxidation, in both the blood and brains of bipolar patients.[7,19-22] A previous *postmortem* study found an increase of lipid peroxidation in an analysis of lipid hydroperoxides (LPHs), 8-isoprostane (8-ISO) and 4-hydroxy-2-nonenal (4-HNE) in the frontal cortex of bipolar patients.[18] In addition, a higher number of manic episodes was correlated with higher levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is a marker of DNA oxidative damage.[7] Alterations in antioxidant enzymes are also observed in bipolar patients. Gawryluk and colleagues[19] demonstrated glutathione reductase (GR) and glutathione peroxidase (GPx) alterations in the frontal cortex of bipolar patients.

The inflammatory system is part of the non-specific immune response, which is activated in response to harmful stimuli such as pathogens, damaged cells, or irritants.[23] Some clinical and preclinical studies have suggested that inflammatory processes in the periphery and the brain are involved in the pathophysiology of BD.[24,25] Previous studies demonstrated that serum levels of the cytokines interleukin (IL)-4, IL-1β, IL-10 and tumor necrosis factor alpha (TNF-α) are elevated in subjects with BD when compared to healthy controls.[26-29]

Paradoxical sleep deprivation (PSD) in mice has been considered a good animal model of mania because it induces some aspects of a manic episode, such as hyperactivity, hypersexuality and aggressive behavior.[30,31] PSD is not able to induce BD in mice, but does induce a mania-like behavior. Some studies have demonstrated that the mania-like behaviors induced by PSD are reversed by Li.[32,33] In addition, circadian rhythms and genes involved in the molecular clock have long been implicated in BD.[34]

The goal of the present study was to investigate the effects of Li administration on behavior, oxidative stress parameters and cytokine levels in the serum, frontal cortex and hippocampus of mice subjected to the animal model of mania induced by PSD. In the same context, corticosterone and adrenocorticotropic hormone (ACTH) levels were evaluated in the serum of mice.

2 | MATERIALS AND METHODS

2.1 | Animals

In the present study, male C57 mice were used and grouped five per cage. Mice were exposed to a 12-hour light/dark cycle with unrestricted access to water and food. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Brazilian Society for Neuroscience and Behavior (SBNeC) guidelines. This study was approved by the local ethics committee (*Comissão de Ética no Uso de Animais da Universidade do Extremo Sul Catarinense*) under protocol 007/2016-1.

2.2 | Treatments

The mice were treated over a period of 7 days with saline solution (SAL; NaCl 0.09%, 1 mL/kg, 1 injection per day, intraperitoneally [i.p.])

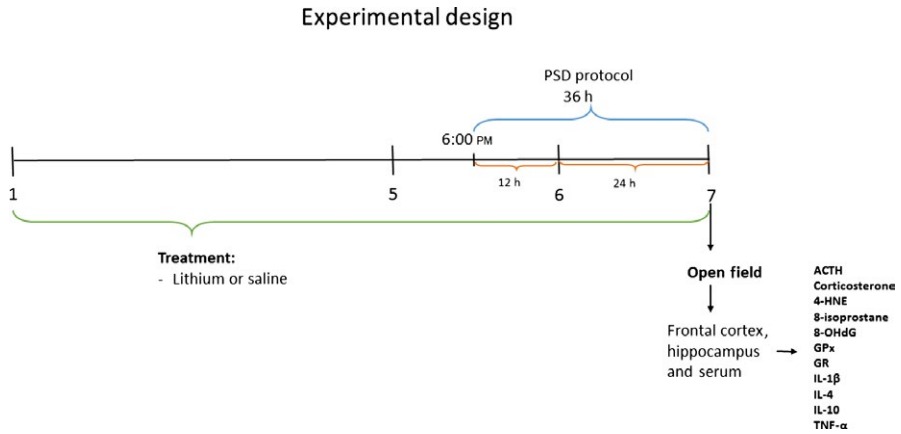

FIGURE 1 Experimental design. On the first day of the experiment, treatment with lithium or saline was started. On the 5th day, the PSD protocol was started at 6 PM. After 36 h, the animals were subjected to an open field test, and then the brain was dissected to obtain the frontal cortex and hippocampus, and the serum was collected for future evaluations. ACTH, adrenocorticotropic hormone; 4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; GPx, glutathione peroxidase; GR, glutathione reductase; IL, interleukin; TNF-α, tumor necrosis factor alpha [Colour figure can be viewed at wileyonlinelibrary.com]

or Li (47.3 mg/kg, 1 mL/kg, 1 injection per day, i.p.). These doses and the treatment schedule used were based on a previous study undertaken by our research group.[24]

All Li-treated animals had Li plasma levels between 0.6 and 1.2 mEq/L, as recommended in the treatment of BD patients.[24]

2.3 | Paradoxical sleep deprivation (PSD) protocol

The PSD protocol was started on the 5th day of the treatment, at 6:00 PM (see Figure 1). For drug administration during the PSD protocol, the mice were removed from the platform and replaced immediately after the injection. The mice were placed 5 per cage (38×31×17 cm), each cage containing 12 platforms (3.5 cm diameter). In the same box, we placed a volume of water 1 inch deep, obligating the animals to stay on the platforms. They could, however, freely move from one platform to another.[34,35] Thus, when animals entered the paradoxical phase of sleep, due to muscle atonia, they were awoken by falling into the water. Food and water were available ad libitum. The present study adopted the period of 36 hours of PSD, since this period of PSD increased the level of locomotor activity, which is considered a mania-like behavior, of animals used in previous studies.[35] The mice in the control group were exposed to the same conditions, except there was no water in the bottom of the box.

2.4 | Experimental groups

The mice were randomly distributed in the four groups (n=10 per group) that are listed below: (1) control+SAL; (2) control+Li; (3) PSD+SAL; (4) PSD+Li.

2.5 | Open field test

The open field test (OFT) was used to evaluate the locomotor activity of the animals. In order to perform the OFT, an apparatus consisting of a 45×60 cm white plywood arena, surrounded by 50-cm-high wooden walls and containing a frontal glass wall, was used. The bottom of the OFT arena was divided into nine equal portions (15×20 cm each) with black lines. The mice were carefully put into the left rear quadrant, and then left to explore the arena for a period of 5 minutes. The locomotor activity (number of horizontal line crossings) of each mouse during the 5 minutes was then recorded.

2.6 | Samples

2.6.1 | Serum samples

Immediately after the behavioral test, the animals were killed by decapitation and individual peripheral blood samples were collected in a microtube, in the morning between 8:00 AM and 12:00 AM, for subsequent analyses. Serum was obtained by centrifugation at 3000*g* for 5 minutes and then kept frozen at −70°C until the experiment.

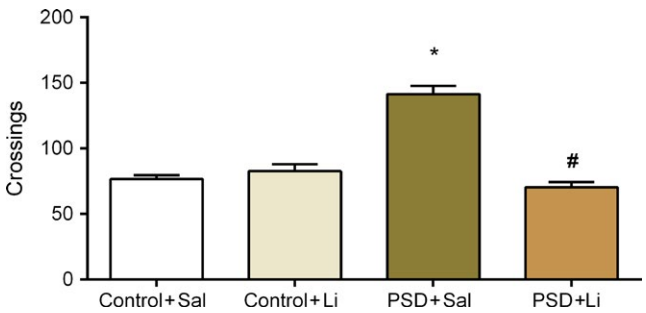

FIGURE 2 Effects of paradoxical sleep deprivation (PSD) on the number of crossings in animals subjected to the PSD-induced animal model (n=10 per group). Data were analyzed by two-way analysis of variance followed by the Duncan test when *F* was significant. Values are expressed as mean±SD. *$P<.05$ compared to the Control+Sal group. #$P<.05$ compared to the ouabain group. Li, lithium; Sal, saline [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 Data from two-away ANOVA

Effects	Behavioral parameter	*F* (1.16)	*P*
PSD	Crossing	29.74	<.001
Li	Crossing	46.05	<.001
Li×PSD	Crossing	64.56	<.001
Effects	**Circulating corticosterone and ACTH**	***F* (1.16)**	***P***
PSD	ACTH	38.81	<.001
	Corticosterone	31.12	<.001
Li	ACTH	37.86	<.001
	Corticosterone	33.43	<.001
Li×PSD	ACTH	80.89	<.001
	Corticosterone	33.31	<.001

		Frontal cortex		Hippocampus		Serum	
Effects	**Parameters**	***F*(1.16)**	***P***	***F*(1.16)**	***P***	***F*(1.16)**	***P***
Oxidative stress							
PSD	HNE	69.86	<.001	29.09	<.001	18.42	<.001
	8-ISO	53.37	<.001	26.60	<.001	34.67	<.001
	8-OHdG	29.33	<.001	16.89	<.001	9.79	.006
Li	HNE	40.57	<.001	30.19	<.001	23.37	<.001
	8-ISO	31.65	<.001	60.45	<.001	25.89	<.001
	8-OHdG	46.64	<.001	19.54	<.001	24.20	<.001
Li×PSD	HNE	47.56	<.001	20.61	<.001	17.00	<.001
	8-ISO	49.31	<.001	29.95	<.001	29.07	<.001
	8-OHdG	35.55	<.001	19.06	<.001	16.18	<.001
Antioxidant enzymes							
PSD	GPx	16.14	<.001	12.05	.003	0.50	.49
	GR	8.13	.11	35.92	<.001	0.56	.467
Li	GPx	47.10	<.001	18.13	<.001	0.02	.88
	GR	21.51	<.001	39.11	<.001	1.13	.303
Li×PSD	GPx	26.54	<.001	13.94	.0018	0.01	.90
	GR	13.48	.002	24.58	<.001	0.04	.838
Cytokine levels							
PSD	IL-1β	120.98	<.001	95.12	<.001	4.22	.056
	IL-4	130.31	<.001	5.93	.027	0.54	.471
	IL-10	146.66	<.001	27.92	<.001	2.22	.156
	TNF-α	76.21	<.001	10.38	.005	0.26	.616
Li	IL-1β	27.13	<.001	78.80	<.001	0.73	.40
	IL-4	8.53	.0099	7.72	.013	0.03	.859
	IL-10	46.26	<.001	8.21	.011	0.048	.829
	TNF-α	80.98	<.001	17.83	<.001	0.50	.49
Li×PSD	IL-1β	29.83	<.001	96.77	<.001	0.95	.34
	IL-4	4.31	.054	0.47	.5	3.82	.068
	IL-10	26.79	<.001	3.45	.081	0.291	.597
	TNF-α	49.36	<.001	21.31	<.001	1.70	.211

Values of *F* and *P* for behavioral parameters, ACTH levels, corticosterone levels, oxidative stress parameters, antioxidant enzymes and cytokine levels. 4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ACTH, adrenocorticotropic hormone; GPx, glutathione peroxidase; GR, glutathione reductase; IL, interleukin; Li, lithium; PSD, paradoxical sleep deprivation; TNF-α, tumor necrosis factor alpha.

2.6.2 | Brain samples

The mice were killed by decapitation immediately after the OFT. The frontal cortex and hippocampus from the mouse brains were then dissected, rapidly frozen, and stored at −70°C until assayed. The samples taken from the frontal cortex and hippocampus of the mice were homogenized in KCl KH_2PO_4 (12 mM KCl and 0.038 mM KH_2PO_4, pH=7.4).

2.7 | Corticosterone and ACTH circulating levels

Corticosterone levels were determined using enzyme immunoassay (EIA) kits (from Diagnostic Products Corporation, Los Angeles, CA, USA). Serum concentrations of ACTH were determined using commercially available radioimmunoassay kits (from Diagnostic Products Corporation) for animals.

2.8 | Evaluation of oxidative stress parameters in the mouse brains

2.8.1 | Measures of lipid peroxidation

Two separate markers of lipid peroxidation, 4-HNE (Cell Biolabs, Inc., San Diego, CA, USA; STA-338) and 8-ISO (Cayman Chemical, Paulinia, Brazil; Item No. 516351), were analyzed following the manufacturers' instructions. 4-HNE protein adducts to lysine, histidine, or cysteine were quantified by standard sandwich enzyme-linked immunosorbent assay (ELISA) using an EIA. 8-ISO was quantified using ACE™ competitive EIAs with 8-ISO-acetylcholinesterase conjugate as a tracer and 8-ISO-specific rabbit anti-serum (Cayman Chemical; Item No. 500431). As 8-ISO and the tracer compete for limited anti-serum binding, the color intensity caused by tracer binding was inversely proportional to the amount of 8-ISO.

2.8.2 | Nuclear extraction from frontal cortex and hippocampus

The obtained samples were flash-frozen and stored at −80°C until nuclear proteins were extracted. Tissue samples were subjected to a nuclear extraction protocol with a commercial Nuclear Extraction Kit (Chemicon, Temecula, CA, USA; Item No. 2900). Briefly, samples were homogenized in cytoplasmic lysis buffer containing dithiothreitol (DTT) and protease inhibitors. The suspension was kept on ice for 15 minutes and was later centrifuged at 250***g*** for 5 minutes at 4°C. The supernatant was discarded, and the pellet was resuspended in two volumes of cold cytoplasmic lysis buffer. The suspension was homogenized using a small-gauge needle syringe and centrifuged at 8000***g*** for 20 minutes at 4°C. The resulting pellet contained the nuclear portion of the cell lysate. The pellet was resuspended in a nuclear extraction buffer containing DTT and protease inhibitors, and the suspension was homogenized with a small-gauge needle syringe. The resulting sample was kept in slow agitation for 30–60 minutes in an orbital shaker at 4°C. Later, the nuclear suspension was centrifuged at 16 000***g*** for 5 minutes at 4°C, and the nuclear extract-containing supernatant was transferred to a new tube and stored at −80°C until further analysis.

2.8.3 | 8-OHdG analysis

8-OHdG is produced by oxidative damage of DNA by reactive oxygen and nitrogen species and serves as an established marker of oxidative stress. An 8-hydroxy-20-deoxy guanosine assay kit purchased from Cell Biolabs (STA-320) was used. It is a competitive assay that can be used for the quantification of 8-OHdG in serum and nuclear extraction from the frontal cortex and hippocampus. It recognizes both free and DNA-incorporated 8-OHdG. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (ache) conjugate (8-OHdG tracer) for a limited amount of 8-OHdG monoclonal antibody. All procedures were carried out in accordance with the manufacturer's instructions.

2.9 | Activity of antioxidant enzymes

2.9.1 | Glutathione peroxidase (GPx)

GPx activity was measured using the assay kit from Cayman Chemical. Oxidized glutathione is produced via the reduction of hydrogen peroxide by GPx, and is recycled into its reduced state by GR and oxidized nicotinamide adenine dinucleotide phosphate (NADP+). The oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP+ is followed by a decrease in the absorbance of light at 340 nM. One unit of GPx is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP+ per minute at 25°C.

2.9.2 | Glutathione redutase (GR)

GR activity was measured using the assay kit from Cayman Chemical. Using this kit, it was possible to measure the rate of oxidation of NADPH to NADP+, which is followed by a decrease in absorbance at 340 nM. One unit of GR is defined as the amount of enzyme that causes the oxidation of 1.0 nmol of NADPH to NADP+ per minute at 25°C.

2.9.3 | Assessment of IL-1, IL-4, IL-10, and TNF-α levels

The hippocampus and frontal cortex were homogenized in phosphate-bufferid saline extraction solution containing aprotinin (100 mg of tissue per 1 mL). The concentrations of cytokines were determined for the serum, hippocampus, striatum and frontal cortex using commercially available ELISAs, following the instructions supplied by the manufacturer (DuoSet kits; R&D Systems, Minneapolis, MN, USA). The results are shown in pg/100 mg of tissue for the hippocampus and frontal cortex. The results are shown in pg/mL of sample for the serum.

TABLE 2 Data from two-away ANOVA

Behavioral test (crossing)		
Group	**Mean±SD**	
Control+Sal	76.6±6.76757	
Control+Li	82.6±11.80254	
PSD+Sal	141.4±14.02854*	
PSD+Li	70.2±8.92749#	
	ACTH	**Corticosterone**
Group	**Mean±SD**	**Mean±SD**
Circulating corticosterone and ACTH		
Control+Sal	8.38±1.398928	10.5±1.64469
Control+Li	11.34±2.514558	10.48±1.90184
PSD+Sal	24.24±3.537372*	32.86±8.03449*
PSD+Li	8.46±0.95551#	10.1±2.59519#

Oxidative stress parameters			
	Frontal cortex	**Hippocampus**	**Serum**
Group	**Mean±SD**	**Mean±SD**	**Mean±SD**
HNE levels			
Control+Sal	0.00205±0.000723	0.003125±0.000726	0.003655±0.00071
Control+Li	0.002205±0.000486	0.002763±0.001006	0.003341±0.000839
PSD+Sal	0.006535±0.000839*	0.006893±0.001000*	0.007364±0.001569*
PSD+Li	0.002635±0.000515#	0.003087±0.000582#	0.003416±0.000467#
8-ISO levels			
Control+Sal	11.5138±1.320112	15.041±1.586463	14.4598±3.09785
Control+Li	12.5892±1.606498	12.4114±2.902931	14.8968±2.710521
PSD+Sal	22.55068±2.010965*	27.1804±3.076175*	30.6932±4.12929*
PSD+Li	12.8074±1.87088#	12.051±2.382667#	15.611±2.724967#
8-OHdG levels			
Control+Sal	2.38388±0.727886	2.452±0.837902	2.8648±1.127493
Control+Li	2.0978±0.848764	2.4324±0.844751	2.4804±0.804611
PSD+Sal	6.1382±0.742536*	5.4846±0.798465*	5.9268±1.012993*
PSD+Li	1.9174±0.612942#	2.3408±0.712302#	2.0982±0.849576#
GPx activity			
Control+Sal	0.04512±0.005614	0.047076±0.007096	0.04846±0.008583
Control+Li	0.0395±0.006003	0.04492±0.008549	0.0471±0.011301
PSD+Sal	0.075226±0.006658*	0.076758±0.01264*	0.044376±0.008439
PSD+Li	0.035776±0.010185#	0.04384±0.007492#	0.044236±0.014604
GR activity			
Control+Sal	0.033097±0.019177	0.025174±0.007829	0.039892±0.010927
Control+Li	0.02786±0.009096	0.021128±0.007743	0.043986±0.007558
PSD+Sal	0.068434±0.007296*	0.05936±0.005645*	0.04247±0.010156
PSD+Li	0.02342±0.009121#	0.02436±0.006466#	0.048548±0.013365

(Continues)

2.9.4 | Protein determination

All biochemical measures were normalized to the protein content with bovine albumin as standard.[36]

2.10 | Statistical analysis

All data are presented as mean±SEM. Differences among experimental groups were determined by two-way ANOVA followed

TABLE 2 (Continued)

Cytokine levels			
	Frontal cortex	Hippocampus	Serum
Group	Mean±SD	Mean±SD	Mean±SD
IL-1β			
Control+Sal	17181.72±3258.71	14713.79±1310.09	1377.8±80.6331
Control+Li	18075.45±1835.08	16055.52±1169.22	1386.4±80.1299
PSD+Sal	75299.39±15090.55*	42096.04±5187.23*	1601.8±297.1375
PSD+Li	37626.54±2756.75*,#	15937.78±3014.04#	1466.4±90.5058
IL-4			
Control+Sal	16288±2279.46	12861.8±506.907	1694.2±63.2669
Control+Li	14363.6±1274.94	14539.7±1498.903	1762.2±100.7135
PSD+Sal	46969.6±6912.57*	14264±1483.972	1797.4±61.6141
PSD+Li	35605.05±6988.31*,#	17049.2±2863.076*	1715.6±106.9921
IL-10			
Control+Sal	17049.2±2863.08	23131.2±7624.78	1456.6±134.5597
Control+Li	13352.8±1196.11	17306.8±5195.09	1442.6±129.8299
PSD+Sal	56375.6±8125.97*	64450±16649.18*	1498.2±41.6617
PSD+Li	29125.81±5276.56*,#	37121.18±17520.68*,#	1531.4±40.3708
TNF-α			
Control+Sal	17557±1124.693	15078.28±1310.798	1647.8±63.03729
Control+Li	13253.96±1028.966	16446.44±2371.597	1628.8±82.06217
PSD+Sal	51913.2±3375.176*	42319.92±6385.643*	1622.6±68.60612
PSD+Li	16970.34±2303.146#	11599.8±434.447#	1686.6±70.00214

Mean and standard deviation (SD) are shown.
*P<.05 compared to the Control+Sal group.
#P<.05 compared to the ouabain group.
4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ACTH, adrenocorticotropic hormone; GPx, glutathione peroxidase; GR, glutathione reductase; IL, interleukin; Li, lithium; PSD, paradoxical sleep deprivation; Sal, saline; TNF-α, tumor necrosis factor alpha.

by Tukey's post hoc test. P<.05 was considered statistically significant.

3 | RESULTS

3.1 | Behavioral test

The PSD protocol increased crossings (locomotion), whereas the locomotor activity was decreased with Li administration. Li in control mice not subjected to PSD did not alter behavioral measures, indicating that the effects of mood stabilizer on PSD-subjected mice were not associated with sedation (Figure 2). Table 1 presents the results of the two-way ANOVA, and means and standard deviations are given in Table 2.

3.2 | Corticosterone and ACTH circulating levels

Figure 3A shows that ACTH and corticosterone levels in the serum of the mice were increased in the PSD group, and the Li treatment prevented these PSD-induced HPA-axis alterations. Table 1 presents the results of the two-way ANOVA, and means and standard deviations are given in Table 2.

3.3 | Oxidative stress parameters in the mouse brains and serum

Figures 4A and B show oxidative damage to lipids determined by assessing the levels of HNE and 8-ISO in samples of the frontal cortex, hippocampus and serum of the mice used in the study. PSD induced a marked increase of HNE and 8-ISO levels in the frontal cortex, hippocampus and serum. The administration of Li prevented PSD-induced increases of HNE and 8-ISO levels in all samples analyzed. Table 1 presents the results of the two-way ANOVA, and means and standard deviations are given in Table 2.

Figure 4C shows oxidative damage to DNA determined by assessing the amount of 8-OHdG in the samples of the frontal cortex, hippocampus and serum of the mice used in the study. OHdG was also significantly different between groups, with significant increases in PSD-subjected mice compared with controls. The administration of Li prevented the PSD-induced increase of OHdG levels in all samples

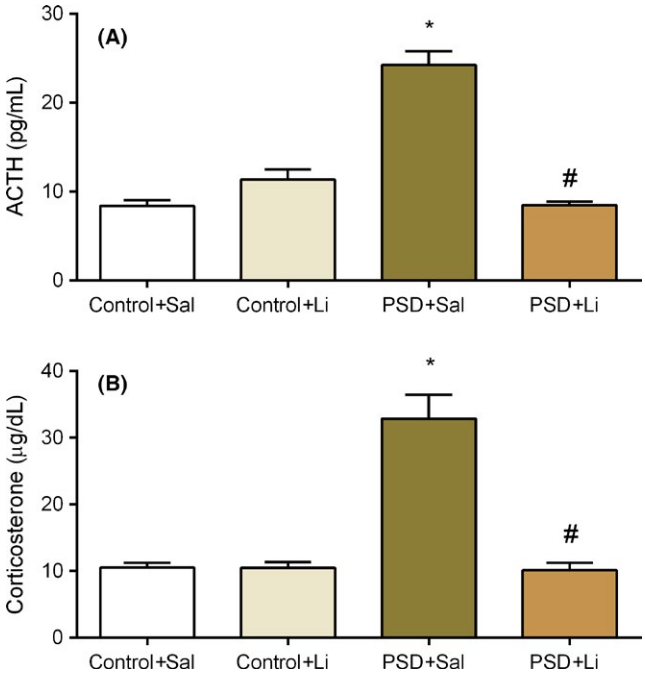

FIGURE 3 Effects of administration of lithium (Li) on the levels of adrenocorticotropic hormone (ACTH) (A) and corticosterone (B) in the serum of animals subjected to the paradoxical sleep deprivation (PSD)-induced animal model (n=8 per group). Data were analyzed by two-way analysis of variance followed by the Duncan test when *F* was significant. Values are expressed as mean±SD. **P*<.05 compared to the Control+Sal group. #*P*<.05 compared to the ouabain group. Sal, saline [Colour figure can be viewed at wileyonlinelibrary.com]

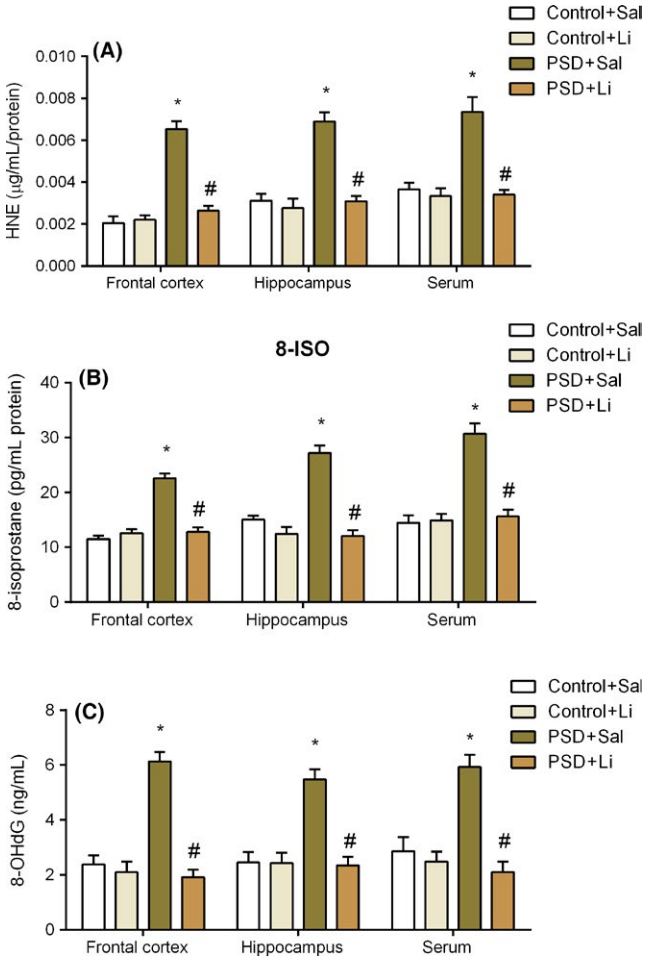

FIGURE 4 Effects of administration of lithium (Li) on the levels of 4-hydroxy-2-nonenal (HNE) (A), 8-isoprostane (8-ISO) (B) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) (C) in the frontal cortex, hippocampus and serum of animals subjected to the paradoxical sleep deprivation (PSD)-induced animal model (n=8 per group). Data were analyzed by two-way analysis of variance followed by the Duncan test when *F* was significant. Values are expressed as mean±SD. **P*<.05 compared to the Control+Sal group. #*P*<.05 compared to the ouabain group. Sal, saline [Colour figure can be viewed at wileyonlinelibrary.com]

analyzed. Table 1 presents the results of the two-way ANOVA, and means and standard deviations are given in Table 2.

3.4 | Activity of antioxidant enzymes in the mouse brains and serum

The GPx and GR activities in the hippocampus, frontal cortex and serum of the mice are shown in Figures 5A and B, respectively. The PSD protocol increased GPx activity in the hippocampus and frontal cortex of the mice, but not in the serum. The administration of Li prevented PSD-induced increases of GPX and GR levels in all brain samples analyzed. Table 1 presents the results of the two-way ANOVA, and means and standard deviations are given in Table 2.

3.5 | IL-1β, IL-4, IL-10, and TNF-α levels in the mouse brains and serum

Figure 6 shows the IL-1β (A), IL-4 (B), IL-10 (C), and TNF-α (D) levels in the frontal cortex, hippocampus and serum of the mice. The PSD protocol increased the IL-1β levels in the frontal cortex and hippocampus; however, Li treatment reversed the IL-1β alteration in the hippocampus. The Li pretreatment significantly diminished the PSD- induced IL-1β increase in the frontal cortex, although it was not able to return the IL-1β concentration to control levels (Figure 6A). PSD increased the IL-4 levels in the frontal cortex, but not in the hippocampus. Li pretreatment significantly diminished the IL-4 increase induced by PSD, although it was not able to return the IL-4 concentration to control levels (Figure 6B). In addition, PSD increased the IL-10 levels in the frontal cortex and hippocampus (Figure 6C), whereas Li treatment reduced this PSD-induced increase. Figure 6D shows that PSD increased the IL-10 levels in the frontal cortex and hippocampus; however, Li treatment was able to prevent these cytokine alterations. No significant alterations in cytokine levels were observed in the serum. Table 1 presents the results of the two-way ANOVA, and means and standard deviations are given in Table 2.

4 | DISCUSSION

The present results demonstrate that PSD, an environmental model of mania, induced mania-like behavior, and treatment with Li reversed

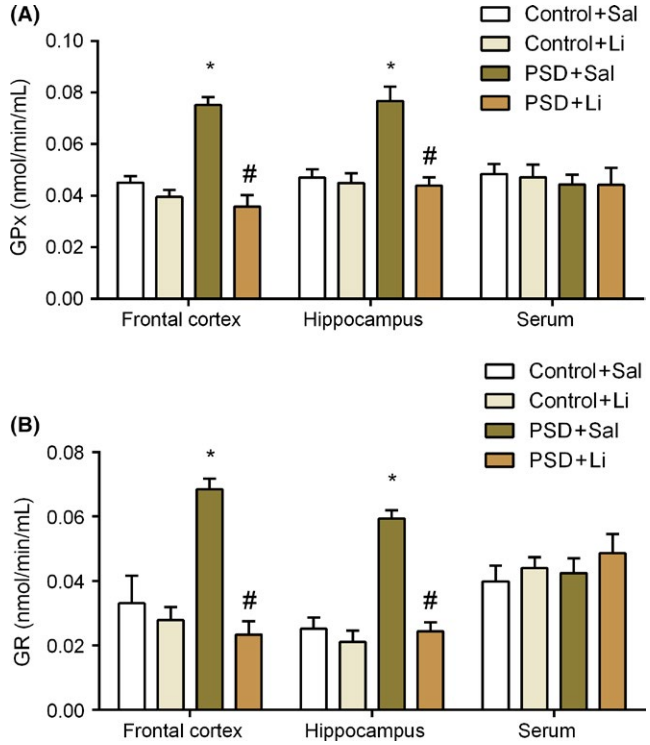

FIGURE 5 Effects of administration of lithium (Li) on the levels of glutathione peroxidase (GPx) (A) and glutathione reductase (GR) (B) in the frontal cortex, hippocampus and serum of animals subjected to the paradoxical sleep deprivation (PSD)-induced animal model (n=8 per group). Data were analyzed by two-way analysis of variance followed by the Duncan test when *F* was significant. Values are expressed as mean±SD. **P*<.05 compared to the Control+Sal group. #*P*<.05 compared to the ouabain group. Sal, saline [Colour figure can be viewed at wileyonlinelibrary.com]

this effect. Likewise, a previous study using the animal model of mania induced by PSD showed the antimanic proprieties of Li.[35,37] These results suggested that alterations in circadian rhythm can lead to mania-like behavior, which represents manic episodes in bipolar patients.[34] In fact, the manic episodes are characterized by a marked decrease in the need for sleep. Therefore, PSD may be considered an important tool for the elucidation of the physiopathology and etiology of BD.[38]

In the present study, the mania-like behavior induced by PSD was accompanied by increases in the circulating levels of corticosterone and ACTH, which indicates that this model of PSD activates the HPA axis. In agreement with the present findings, a recent study demonstrated an elevated corticosterone concentration in the serum of PSD rats when compared to controls.[39] In addition, and corroborating the findings of the present study, several studies have demonstrated that the levels of corticosterone are increased after PSD.[40-43] Suchecki and colleagues [40] showed that PSD induced using different methods increased ACTH and corticosterone secretion. Indeed, insomnia is associated with increases of ACTH and cortisol secretion, and activation of the HPA axis or administration of glucocorticoids can lead to arousal and sleeplessness.[44,45]

It has been reported that there is HPA axis dysregulation in bipolar patients.[46] A previous clinical study demonstrated increases in cortisol secretion in bipolar patients, suggesting HPA axis hyperactivity in this disorder.[47] Kim and colleagues [48] found that ACTH-treated animals exhibited increased locomotor activity. In the same study, Kim and colleagues [48] suggested that ACTH administration in rats could induce a putative animal model of mania, with face validity, by mimicking the manic episodes of BD. Therefore, the mania-like behavior induced by PSD in the present study can be explained, at least in part, by an increase of ACTH levels also induced by PSD.

It is important to note that, in the present study, administration of Li prevented the increases of ACTH and corticosterone levels in the serum of mice subjected to PSD. On the other hand, previous studies have demonstrated that Li administration increased the levels of ACTH and corticosterone in the plasma of rats.[49-53] Wood and colleagues [54] demonstrated that 36 days of Li treatment did not significantly alter corticosterone levels. The effects of Li on the HPA axis are therefore somewhat controversial. This discrepancy can be explained by the fact that the methodologies and durations of the Li treatments used in the studies were different in each case. It is important to emphasize that, in the present study, Li alone did not alter the levels of corticosterone or ACTH, its effects being limited to alterations within the HPA axis, which were induced by PDS. It is well described in the literature that Li acts on the mechanisms of circadian rhythms.[55,56] A previous preclinical study demonstrated that Li delays biochemical circadian rhythms in rats. In that study, the authors found that the levels of prolactin, corticosterone, and aldosterone in plasma showed delays in their circadian rhythms in the Li-treated rats.[57] Therefore, it can be suggested that Li indirectly regulates the levels of corticosterone and ACTH in PSD-subjected rats, acting on the mechanisms controlling circadian rhythms.

Some studies have demonstrated that glucocorticoids released from the adrenal gland in response to stress-induced activation of the HPA axis can induce oxidative stress.[15] In the present study, it was demonstrated that PSD increased oxidative damage to lipids (4-HNE and 8-ISO) and DNA (8-OHdG) in the frontal cortex, hippocampus and serum. Previous studies found that PSD increased lipid oxidative damage parameters, such as lipid hydroperide, 4-HNE and malondialdehyde levels in the frontal cortex and hippocampus of mice.[58,59] In agreement with the present study, Vollert and colleagues [60] also demonstrated that an increase in serum corticosterone was accompanied by increases in lipid peroxidation markers (8-ISO and malondialdehyde) in the hippocampus, cortex and amygdala of rats subjected to PSD. There is also evidence linking the redox state and circadian rhythm.[61,62] Wang and colleagues [61] showed alterations to the redox systems in mutant mice that had undergone deletion or disruption of their CLOCK genes. The circadian rhythm has a natural oscillation in the redox state which is controlled by a biological clock. This in turn triggers the production of antioxidant enzymes and ROS depending on the time of day.[61] Thus, it can be suggested that PSD, which interrupts the circadian rhythm oscillation, may be deregulating the redox state, thus generating oxidative stress.

Oxidative damage to lipids and DNA in bipolar patients has also been observed in both the brain and serum.[7,18,63] 8-ISO and 4-HNE were found to be increased in the postmortem prefrontal cortex from

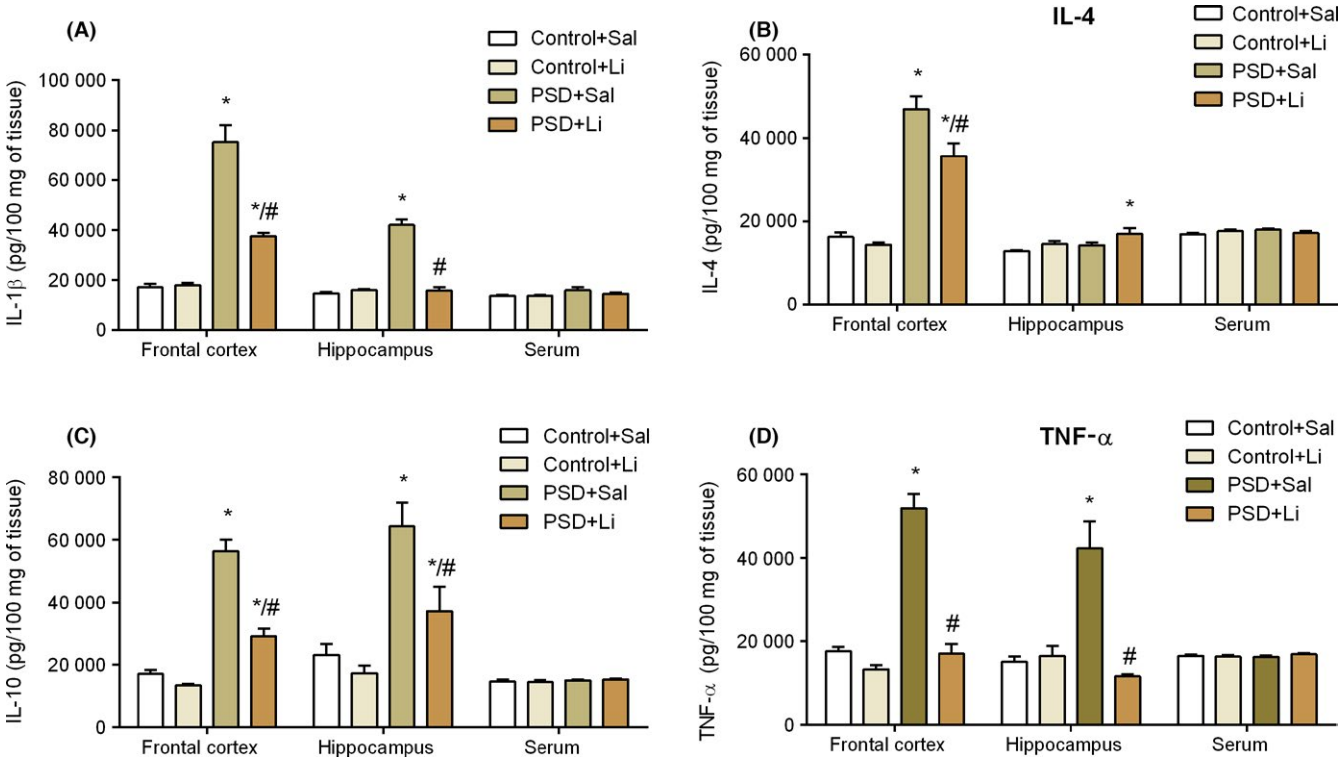

FIGURE 6 Effects of administration of lithium (Li) on the levels of interleukin (IL)-1β (A), IL-4 (B), IL-10 (C) and tumor necrosis factor alpha (TNF-α) (D) in the frontal cortex, hippocampus and serum of animals subjected to the paradoxical sleep deprivation (PSD)-induced animal model (n=8 per group). Data were analyzed by two-way analysis of variance followed by the Duncan test when *F* was significant. Values are expressed as mean±SD. **P*<.05 compared to the Control+Sal group. #*P*<.05 compared to the ouabain group. Sal, saline [Colour figure can be viewed at wileyonlinelibrary.com]

patients with BD when compared with controls.[18] In addition, clinical research has also demonstrated elevated levels of lipid peroxidation in the serum of bipolar patients.[63] Interestingly, Versace and colleagues [63] demonstrated a relationship between white matter damage and peripheral lipid peroxidation in bipolar patients. Additionally, a previous study demonstrated that the levels of DNA 8-OHdG were higher in BD patients when compared to healthy controls.[7] Together, these studies suggest the role of lipid peroxidation and DNA oxidation in BD. Given that PSD also induces an increase in the markers of lipid peroxidation and DNA oxidation, PSD-induced mania-like behavior may provide a useful animal model in which to test the hypothesis of the involvement of oxidative stress in BD.

Herein, Li was able to prevent lipid peroxidation and DNA oxidation in the brain and serum of mice subjected to PSD. Previous preclinical studies using other animal models of mania have demonstrated the antioxidant effects of Li. Valvassori and colleagues [64] found that Li modulated antioxidant enzymes and prevented ouabain-induced mania-like behavior and oxidative damage in the brains of rats that were subjected to this model of mania. Li also reversed the oxidative damage in the brains of rats subjected to the animal model of mania induced by amphetamine.[65] It was also found that, in an animal model of sepsis, treatments with Li at doses of 25 and 50 mg/kg decreased the levels of 8-ISO.[66] Together with the present results, these studies suggest that Li has antioxidant properties, protecting the brain against oxidative brain damage.

In the present study, the levels of GPx and GR were increased in the frontal cortex and hippocampus of rats after PSD, and Li prevented these enzyme alterations. In agreement with our data, previous studies have demonstrated that PSD induces GPx and GR alterations in the brains of mice.[59] Another study examined glutathiolation in brain slices taken at several points across the circadian cycle. It was observed that there was a peak in glutathiolation in the early night, indicating an oxidized state of glutathione, while glutathiolation was lower at midday, indicating a relatively reduced state.[61] Thus, it can be hypothesized that PSD, which interrupts the oscillation of the circadian rhythm, may be deregulating the glutathiolation cycle and, consequently, altering glutathione activity.

It is important to note that Li prevented the PSD-induced increase in GPx and GR levels in the frontal cortex and hippocampus of mice. Brocardo and colleagues [67] demonstrated that Li modulates GPx and GR, protecting the brain against oxidative damage induced by ouabain in an animal model of mania. In addition, a previous study showed that Li modulates other antioxidant enzymes, such as superoxide dismutase and catalase, and prevents ouabain-induced oxidative damage in the frontal cortex and hippocampus of rats subjected to the model of mania induced by ouabain.[68] In a preclinical study using the animal model of mania induced by methylphenidate, it was demonstrated that the rats with mania-like behavior showed decreases in their levels of both glutathione and GPx activity, and these alterations were reversed by Li treatment.[69] Together, these data suggest that Li can modulate antioxidant enzymes, protecting the brain against oxidative damage.

As discussed above, the antioxidant properties of Li have been already described in the literature; however, the present study demonstrated that the neuroprotector effect of Li can be HPA axis-modulated. In fact, it is know that ACTH and cortisol release can lead to an oxidative stress state,[15] and these alterations could play a pivotal role in PSD-induced behavioral changes.[40,44] As Li was able to reduce ACTH and cortisol release induced by PSD, it is possible that, in the present study, the effects of Li on oxidative stress may have been mediated, at least in part, by ACTH and cortisol modulation.

It is known that oxidative stress is an important etiologic factor in the pathogenesis of chronic inflammatory diseases such as atherosclerosis, metabolic disorders, and cancer.[70-72] There is an increasing amount of data showing that sleep loss is associated with HPA axis alterations, activation of inflammatory parameters and increases in oxidative stress parameters in humans and in animal models.[39,59,73,74] Indeed, the present study showed that HPA axis alterations and oxidative stress were accompanied by increased levels of IL-1β, IL-4, IL-10 and TNF-α in the brains of mice subjected to PSD. In agreement with our study, Ashley and colleagues [39] demonstrated that PSD mice exhibited increases in IL-1β and TNF-α pro-inflammatory gene expression in the hypothalamus, hippocampus and frontal cortex. In a previous study, using the animal model of mania induced by amphetamine, it was demonstrated that IL-4, IL-6, IL-10 and TNF-α were increased in the brains of rats with mania-like behavior.[24] Some clinical studies have demonstrated that the progressive neuropathological processes in BD are linked to alterations in inflammatory cytokines.[25,75] A clinical study demonstrated that changes observed in the sleep of patients with BD are also related to the elevation of IL-6.[74] Therefore, together, these studies suggest that sleep deprivation and mania-like behaviors are associated with inflammatory system alteration.

It is important to note that Li reversed the alterations to cytokines induced by PSD. A previous study from our laboratory demonstrated that Li reversed the alterations to cytokines in the frontal cortex and striatum of rats subjected to the animal model of mania induced by amphetamine.[24] Interestingly, Li showed anti-inflammatory effects in an animal model of sepsis.[66] The anti-inflammatory effects of Li, such as the suppression of cyclooxygenase-2 expression, inhibition of IL-1β and TNF-α production, and enhancement of IL-2 and IL-10 synthesis, have been well described in the literature.[76-78] A clinical study demonstrated that cytokine concentrations in patients in remission treated with Li were no different from those of healthy subjects, suggesting that Li maintenance brings the cytokine status to a level similar to that in healthy control subjects.[79] As demonstrated previously, Li prevents oxidative damage to lipids and DNA in rats subjected to PSD. Because PSD-induced ROS overproduction can lead to inflammation, Li could act as an antioxidant and indirectly modulate cytokine levels.

In conclusion, the present data demonstrated that PSD induced HPA axis alterations, and increases in the levels of cytokines and oxidative stress parameters. Therefore, it can be suggested that circadian rhythm alterations observed in BD may be related to alterations in the HPA axis, activation of the inflammatory system and oxidative stress; all these effects were also observed in the animal model induced by PSD. In addition, Li could reverse the neurochemical alterations induced by PSD, suggesting that this mood stabilizer could act on the mechanisms controlling circadian rhythms, and protect the brain and serum against HPA axis alteration and increases in the levels of oxidative stress and cytokines.

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DISCLOSURE

The authors declares that there is no conflict of interest regarding this article.

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