Antidepressant-like action of the bark ethanolic extract from Tabebuia avellanedae in the olfactory bulbectomized mice

ARTICLE *in* JOURNAL OF ETHNOPHARMACOLOGY · DECEMBER 2012

Impact Factor: 3 · DOI: 10.1016/j.jep.2012.11.040 · Source: PubMed

Journal of Ethnopharmacology 145 (2013) 737–745

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Antidepressant-like action of the bark ethanolic extract from *Tabebuia avellanedae* in the olfactory bulbectomized mice

Andiara E. Freitas [a], Daniele G. Machado [a], Josiane Budni [a], Vivian B. Neis [a], Grasiela O. Balen [a], Mark W. Lopes [a], Luiz F. de Souza [a], Patricia O. Veronezi [b], Melina Heller [b], Gustavo A. Micke [b], Moacir G. Pizzolatti [b], Alcir L. Dafre [a], Rodrigo B. Leal [a], Ana Lúcia S. Rodrigues [a,*]

[a] Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina, Campus Universitário—Trindade, 88040-900 Florianópolis-SC, Brazil
[b] Department of Chemistry, Center of Physical and Mathematical Sciences, Universidade Federal de Santa Catarina, Campus Universitário—Trindade, 88040-900 Florianópolis-SC, Brazil

ARTICLE INFO

Article history:
Received 5 October 2012
Received in revised form
14 November 2012
Accepted 18 November 2012
Available online 10 December 2012

Keywords:
Antidepressant
Olfactory bulbectomy
Open-field
Splash test
Tabebuia avellanedae
Tail suspension test
GSK-3β
ERK1/2
CREB
BDNF

ABSTRACT

Ethnopharmacological relevance: *Tabebuia avellanedae* Lorentz ex Griseb is a plant employed in tropical America folk medicine for the treatment of several diseases, including depressive disorders.
Aim of the study: To investigate the ability of *Tabebuia avellanedae* ethanolic extract (EET) administered chronically to cause an antidepressant-like effect in the tail suspension test (TST), a predictive test of antidepressant activity, and to reverse behavioral (hyperactivity, anhedonic-like behavior and increased immobility time in the TST) and biochemical changes induced by olfactory bulbectomy (OB), a model of depression, in mice.
Materials and methods: Mice were submitted to OB to induce depressive-related behaviors, which were evaluated in the open-field test (hyperactivity), splash test (loss of motivational and self-care behavior indicative of an anhedonic-like behavior) and TST (increased immobility time). Phosphorylation levels of Akt, GSK-3β, ERK1/2 and CREB, as well as BDNF immunocontent, were evaluated in the hippocampus of bulbectomized mice or sham-operated mice treated for 14 days by p.o. route with EET or vehicle.
Results: EET (10 and 30 mg/kg) given 14 days by p.o route to mice reduced the immobility time in the TST without altering locomotor activity, an indicative of an antidepressant-like effect. EET *per se* increased both CREB (Ser^{133}) and GSK-3β (Ser^{9}) phosphorylation (at doses of 10–30 and 30 mg/kg, respectively) in sham-operated mice. OB caused hyperactivity, loss of motivational and self-care behavior, increased immobility time in the TST and an increase in CREB and ERK1 phosphorylation, as well as BDNF immunocontent. EET abolished all these OB-induced alterations except the increment of CREB phosphorylation. Akt (Ser^{473}) and ERK2 phosphorylation levels were not altered in any group.
Conclusions: EET ability to abolish the behavioral changes induced by OB was accompanied by modulation of ERK1 and BDNF signaling pathways, being a promising target of EET. Results indicate that this plant could constitute an attractive strategy for the management of depressive disorders, once more validating the traditional use of this plant.

1. Introduction

Depression is a chronic, recurring and potentially life-threatening illness that affects up to 17% of the population across the globe (Kessler et al., 2005). It is projected from the World Health Organization to be the second cause of morbidity and mortality worldwide by 2020 (Murray and Lopez, 1997). Depressive symptoms include somatic and cognitive alterations such as: depressed mood, anhedonia (loss of interest or pleasure in almost all activities), irritability, feelings of hopelessness, worthlessness or guilt, decreased ability to concentrate and think, decreased or increased appetite, weight loss or weight gain, insomnia or hypersomnia, psychomotor retardation or agitation, fatigue, low energy and recurrent thoughts of death and suicide. Although the current pharmacotherapy of depression includes a battery of drugs, many are inconsistently effective and exert undesirable side effects (Morilak and Frazer, 2007). Therefore, considerable

Abbreviations: ANOVA, Analysis of variance; BDNF, Brain-derived-neurotrophic factor; CREB, Cyclic-AMP responsive-element binding protein; ERK, Extracellular signal-regulated kinase; FST, The forced swimming test; GSK-3β, Glycogen synthase kinase-3β; MAPK, Mitogen-activated protein kinase; OB, Olfactory bulbectomy; PI-3K, Phosphatidylinositol 3′-kinase; TST, Tail suspension test

* Corresponding author. Tel.: +55 48 3721 5043; fax: +55 48 3721 9672.
E-mail addresses: analucia@mbox1.ufsc.br, alsrodri@gmail.com (A.L.S. Rodrigues).

0378-8741/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2012.11.040

efforts are invested in the development of alternative therapeutic approaches for the management of depressive disorders.

Tabebuia avellanedae Lorentz ex Griseb (Bignoniaceae) is a tree native to tropical rain forests in the northeast of Brazil commonly known as "pau d'arco" or "ipê-roxo". There are several ethnopharmacological uses for the barks of this plant. It is called "divine tree" by indigenous peoples of South America, because according to them, it is one of the most effective, economical and versatile remedies against a multitude of acute and chronic diseases (Lüebeck, 1999). Colombians use the bark infusion as stimulant of central nervous system (Jones, 1995); Bahamians commonly use the bark decoction to prepare an energizing tonic for strength (Jones, 1995), and Brazilians use this plant to treat malaria, cancer, fever, stomach disorders, bacterial and fungal infections and to relief of a variety of mental and emotional states such as anxiety, poor memory, irritability and depression (Lüebeck, 1999; Gómez Castellanos et al., 2009). Indeed, it was demonstrated that *Tabebuia avellanedae* ethanolic extract (EET) has a protective action against gastric lesions in rats (Twardowschy et al., 2008; Pereira et al., 2012) and a recent study by Freitas et al. (2010) showed that the acute administration of EET exerts an antidepressant-like effect in the tail suspension test (TST), a behavioral test used to assess the efficacy of antidepressant compounds (Steru et al., 1985). The antidepressant-like action of EET was shown to be mediated by an activation of the monoaminergic systems. Furthermore, EET produced a synergistic antidepressant-like effect when combined with conventional antidepressants in the TST (Freitas et al., 2010). However, there is no study reporting the ability of *Tabebuia avellanedae* to abolish depressive-like behavior induced by a model of depression that mimics several symptoms observed in depressed patients.

The olfactory bulbectomy (OB) is a well-established animal model of depression capable of detecting antidepressant activity almost exclusively following chronic treatment (Leonard and Tuite, 1981). OB has been proposed as an animal model of depression in terms of construct validity, since it induces alterations in behavior, and in the endocrine, immune and neurotransmitter systems that reproduces many of those seen in patients with depression (Kelly et al., 1997; Leonard and Tuite, 1981). Noteworthy, the OB model standardized by our group in female mice reproduces depressive-like behaviors (Freitas et al., 2012; Machado et al., 2012a,b) and activates hippocampal cell signaling pathways implicated in synaptic plasticity, namely extracellular signal-regulated kinase (ERK) 1, cyclic-AMP responsive-element binding protein (CREB), and brain-derived-neurotrophic factor (BDNF), without altering phosphatidylinositol 3′-kinase (PI-3K)-Akt and glycogen synthase kinase-3β (GSK-3β) phosphorylation (Freitas et al., 2012).

Considering the information mentioned above, the aims of this study were to examine the ability of the repeated (14 days) p.o. administration of EET to alter hippocampal Akt, GSK-3β, ERK1/2 and CREB phosphorylation and BDNF immunocontent and to reverse behavioral and biochemical changes induced by OB.

2. Methods and materials

2.1. Plant material and preparation of EET

Tabebuia avellanedae barks were provided by Chamel Indústria e Comércio de Produtos Naturais Ltda (Campo Largo, Brazil), lot 4753. The identification was performed by the botanist Elide Pereira dos Santos and a voucher specimen has been deposited at the Herbarium of the Department of Botany at the Universidade Federal do Paraná (UFPR), Brazil. Dried and powdered barks (5 kg) were extracted three times by maceration with 95% ethanol for 7 days at room temperature. The combined ethanolic extract was filtered, the solvent evaporated under reduced pressure (40–50 °C) and lyophilized to give a red-brown solid (919.2 g; 18.4% yield), as described previously (Pereira et al., 2012).

2.2. Phytochemical analyses of EET

The analyses of EET was performed in a capillary electrophoresis system (CE) (HP3DCE, Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector set a 200 nm. The measurements were conducted at 25 °C in an uncoated fused-silica capillary (48.5 cm × 50 μm I.D. × 375 μm O.D.) obtained from Polymicro (Phoenix, AZ, USA). In the first conditioning, the capillary was washed for 30 min with sodium hydroxide 1.0 M followed by deionized water for 30 min. Between runs the capillary was rinsed for 5 min with running electrolyte (sodium tetraborate 20 mmol L^{-1} and methanol 10%, pH 9.0). Standard solutions and samples were introduced from the inlet capillary extremity and injected hydrodynamically at 50 mbar (50 mbar = 4996.2 Pa) for 6 s. The applied separation voltage was 30 kV, positive polarity in the injection side. Caffeic acid (100 mg/L) was utilized as internal standard and detection at 330 nm. Data acquisition and treatment were performed with HP Chemstation software. Sample preparation: 0.5299 g of EET were solubilized into 10 mL of methanol:water 50% (v/v). As reported previously (Pereira et al., 2012), the following compounds were identified by the electropherogram of a sample of EET: *p*-hydroxybenzoic acid, anisic acid, veratric acid and caffeic acid.

2.3. Animals

Female Swiss mice (50–55 days, 35–40 g) were maintained at constant room temperature (20–22 °C) with free access to water and food, under a 12:12 h light:dark cycle (lights on at 07:00 h). The cages were placed in the experimental room 24 h before the test for acclimatization. All manipulations were carried out between 9:00 and 17:00 h, with each animal used only once. The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the local Ethics Committee. All efforts were made to minimize animal suffering and the number of animals used in the experiments.

2.4. Surgical procedure

After a 2-week acclimatization period, bilateral OB was performed by suction method described previously by Leonard and Tuite (1981) and standardized in our laboratory (Freitas et al., 2012; Machado et al., 2012a,b). Animals were randomly divided into two groups: one group underwent OB and the other underwent sham operations. Briefly, the mice were anesthetized with a combination of xylazine (6 mg/kg, i.p.) and ketamine (100 mg/kg, i.p.) diluted in saline. An incision was made in the skin overlying the skull, and, after exposure of the skull, holes were drilled on both sides of the mid-line. Then the olfactory bulbs were bilaterally aspired by blunt hypodermic needle (with for 1.0–1.2 cm long and with a rounded tip of 0.80–1.2 mm of diameter) attached to a 10-ml syringe that was used to create suction. Care was taken to avoid damaging the frontal cortex. To stop the bleeding, the holes were filled with swabs and covered with dental cement. All surgical procedure was carried out employing alcohol 70% to eliminate contaminations. Sham-operated animals underwent all of the same surgical procedures, but the olfactory bulbs were left intact. The mice were allowed to recover under a warming lamp to help with body temperature maintenance. Each animal was given 14 days to recover from the

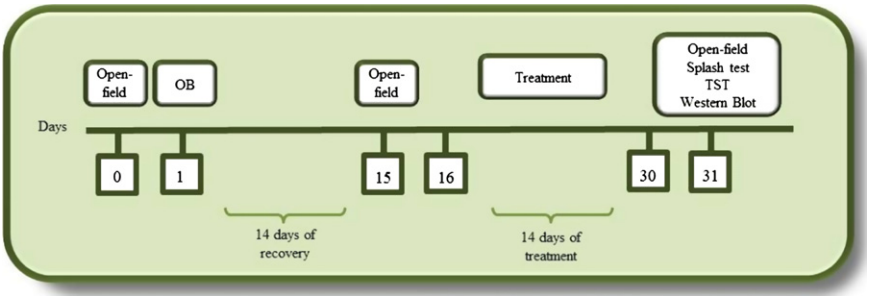

Fig. 1. Diagram of the experimental schedule. The effect of OB on locomotor activity was assessed in the open-field paradigm in 3 time periods: pre-surgically, after 2 weeks of surgery, and 2 weeks post-treatment. Prior to the start of the treatment protocols, the animals were given 14 days to recover from their surgeries. EET doses of 10 and 30 mg/kg (p.o.) was administered once daily for 14 days. Twenty-four hours after the last administration, the mice were submitted to the open-field, splash test 1 h later, and after 1 h to the tail suspension test. Immediately after the conclusion of the behavioral tests, hippocampi were rapidly dissected and prepared to western blot assay.

surgery prior to undergoing any further treatment (Freitas et al., 2012).

2.5. Drugs and treatment

EET (10–100 mg/kg) was dissolved in distilled water with 5% Tween 80 and was administered by oral route (p.o.) by gavage. The dissolution of EET was freshly done from the lyophilized power immediately before its administration. A control group received distilled water with 5% Tween 80 as vehicle. All the animals were fasted for 120 min before the oral treatment.

In the experiments designed to study the antidepressant-like effect of the repeated treatment (for 14 days) of EET, the immobility time in the TST and the locomotor activity in the open-field were assessed in independent groups of mice 24 h after the last daily administration of EET (10–100 mg/kg, p.o.). This experiment was carried out in order to choose the doses of EET to be used in order to investigate its ability to abolish the depressive-like behavior induced by OB.

As shown in Fig. 1, two weeks after surgery, EET (10–30 mg/kg, p.o.) was administered once daily for 14 days. Animals were assigned to the following groups: (a) sham/vehicle, (b) sham/extract 10 mg/kg, (c) sham/extract 30 mg/kg, (d) OB/vehicle, (e) OB/extract 10 mg/kg and (f) OB/extract 30 mg/kg. Number of mice per group was 9–12.

2.6. Open-field test

The OB model of depression reproduces in preclinical assays the psychomotor agitation consistent with that is observed in agitated depression (Zueger et al., 2005). To assess the effects of OB on locomotor activity, mice were evaluated in the open-field paradigm as previously described (Freitas et al., 2012). The test was consecutively performed in 3 time periods: pre-surgically, 2 weeks after surgery, and post-treatment (after 2 weeks of fluoxetine or water p.o. treatment). The number of squares crossed with all paws (crossings) was counted in a 6 min session. The apparatus was cleaned with a solution of 10% ethanol between tests to remove any traces of each animal.

2.7. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was registered during a 6-min period (Machado et al., 2009; Freitas et al., 2010).

2.8. Splash test

The splash test was carried out 24 h after the last repeated drug administration as described by Isingrini et al. (2010), with minor modifications as performed previously (Freitas et al., 2012; Machado et al., 2012a,b). The test consists of squirting a 10% sucrose solution on the dorsal coat of a mouse placed individually in clear Plexiglas boxes ($9 \times 7 \times 11$ cm). Because of its viscosity, the sucrose solution dirties the mouse fur and animals initiate grooming behavior. After applying sucrose solution, the latency to grooming and the grooming time were manually recorded for a period of 5 minutes as an index of self-care and motivational behavior. The apparatus were cleaned with a solution of 10% ethanol between tests to remove any traces of each animal.

2.9. Western blot

After 2 weeks of treatment, and 24 h after the last administration of extract or vehicle by oral rote, mice were decapitated. Brains were removed and the lesion was estimated macroscopically immediately after brain removal; all brains with incomplete surgery or cortex damage were discarded from the experiment. The hippocampus was rapidly dissected and placed in cold saline solution. Western blot analysis was performed as previously described (Leal et al., 2002; Cordova et al., 2004; Freitas et al., 2012). Briefly, hippocampal tissue were mechanically homogenized in 400 μl of Tris-base 50 mM pH 7.0, EDTA 1 mM, sodium fluoride 100 mM, PMSF 0.1 mM, sodium vanadate 2 mM, Triton X-100 1%, glycerol 10%, and then incubated for 30 min in ice. Lysates were centrifuged ($10{,}000 \times g$ for 10 min, at 4 °C) to eliminate cellular debris, and supernatants diluted 1/1 (v/v) in Tris-base 100 mM pH 6.8, EDTA 4 mM, SDS 8%, glycerol 16%. Protein content was estimated by the method previously described by Peterson (1977) and concentration calculated by a standard curve with bovine serum albumin. To compare signals obtained, the same amount of protein (70 μg per lane) for each sample was electrophoresed in 10% SDS-PAGE minigels (after addition of bromophenol blue 0.2% and β-mercaptoethanol 8%) and transferred to nitro-cellulose or polyvinylidene fluoride membranes. To verify the efficiency of the transfer process, the gels were stained with Coomassie blue (Coomassie blue R-250 0.1%, methanol 50%, acetic acid 7%) and membranes with Ponceau 0.5% in acetic acid 1%.

After this process, blots were incubated in a blocking solution (5% non-fat dry milk in Tris buffer saline solution, TBS) for 1 h at room temperature and then probed at 4 °C with anti-phospho-Akt (Sigma Chemical Co., 1:1000), anti-phospho-CREB (Ser^{133}) (Cell Signaling, 1:1000), anti-phospho-GSK-3β (Cell Signaling, 1:1000), anti-phospho-ERK1/2 (Cell Signaling, 1:2000), anti-ERK1/2 (Sigma Chemical, 1:40000), anti-Akt (Sigma Chemical, 1:1000), anti-GSK-3β

(Cell Signaling, 1:1000), anti-CREB (Cell Signaling, 1:1000) and anti-BDNF (Millipore, 1:1000), all in TBS containing 0.05% Tween 20 (TBS-T). Next, the membranes were incubated with anti-rabbit horse radish peroxidase (HRP)-conjugated secondary antibody (1:5000) for 1 h and the immunoreactive bands were developed by chemiluminescence (LumiGLO®, Cell Signaling, Beverly, MA, USA). All blocking and incubation steps were followed by three washes (5 min) of the membranes with TBS-T. In order to detect phosphorylated and total forms of ERK1/2, Akt and CREB in the same membrane, the immunocomplexes were stripped as previously described (Posser et al., 2007). Briefly, membranes were washed once with deionized water (5 min), followed by incubation with NaOH 0.2 M (5 min), washing with deionized water (5 min) and with TBST (10 min). The membranes that had been stripped of immunocomplexes were subsequently blocked and treated according to the same protocol as the one described above. In order to ascertain the same protein load for each experimental group, the expression level of a house keeping protein, β-actin, was evaluated using a mouse anti-β-actin antibody (Santa Cruz, 1:1000) and anti-mouse HRP-conjugated (Millipore 1:4000) secondary antibody.

The optical density (O.D.) of the bands was quantified using Scion Image software®. The phosphorylation levels of Akt, CREB, GSK-3β and ERK1/2 were determined as ratios of O.D. of the phosphorylated band to the O.D. of total bands. The immunocontent of BDNF was determined making the relationship between the O.D of BDNF band and the O.D of the β actin band.

2.10. Statistical analysis

Comparisons between experimental and control groups were performed by one-way (dose-response curve of EET in the TST) or two-way ANOVA (experiments dealing with the ability of EET to abolish OB induced alterations) followed by Duncan's multiple range test, when appropriate. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of the repeated administration of EET on the immobility time in the TST and locomotor activity in the open-field test

The results depicted in Fig. 2a shows that EET given by oral route for 14 days decreased the immobility time in the TST, a behavioral profile characteristic of an antidepressant-like effect. One-way ANOVA revealed a significant effect of the extract [$F(3,29)=4.42$, $P<0.01$]. Post hoc analysis indicated a significant decrease in the immobility time elicited by EET at doses of 10 and 30 mg/kg. Fig. 2b shows that the administration of EET for 14 days (dose range 10–100 mg/kg, p.o.) produced no effect in the locomotor activity assessed in the open-field test [$F(3,30)=0.10$, $P=0.96$].

3.2. Effect of the repeated treatment with EET on hyperactivity induced by OB

The results presented in Fig. 3 shows that the repeated administration of EET (10 and 30 mg/kg, p.o.) abolished the hyperactivity induced by OB in the open-field test. A two-way ANOVA revealed significant differences for OB [$F(1,52)=68.87$, $P<0.01$], EET treatment [$F(2,52)=10.74$, $P<0.01$] and OB × EET treatment interaction [$F(2,52)=4.49$, $P=0<0.1$]. Post hoc analyses indicated that EET (10 and 30 mg/kg, p.o.) treatment for 14 days prevented the hyperactivity caused by OB.

3.3. Effect of EET administration (14 days) on anhedonic-like behavior induced by OB

The results depicted in Fig. 4a shows that the increase in latency to grooming, an indicative of loss of self-care and motivational behavior, produced by OB was significantly blocked by EET (10 and 30 mg/kg, p.o.) treatment. The two-way ANOVA revealed significant differences for OB [$F(1,51)=7.99$, $P<0.01$], EET treatment [$F(2,51)=4.04$, $P<0.05$] and OB × EET treatment interaction [$F(2,51)=4.52$, $P<0.01$]. Also, Fig. 4b shows that the reduced grooming time caused by OB, another parameter that indicates a loss of self-care and motivational behavior, was significantly abolished by the extract. A two-way ANOVA revealed significant differences for OB [$F(1,54)=24.02$, $P<0.01$], EET treatment [$F(2,54)=3.99$, $P<0.05$] and OB × EET treatment interaction [$F(2,54)=15.93$, $P<0.01$].

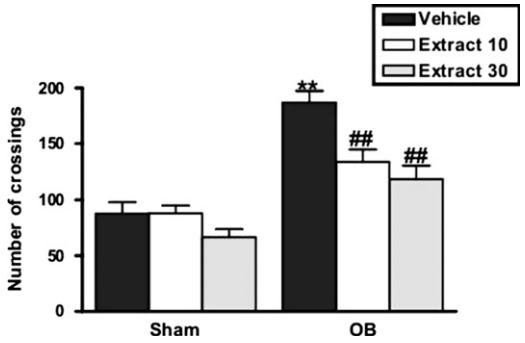

Fig. 3. Effect of the repeated (14 days) treatment of bulbectomized mice with EET (10–30 mg/kg, p.o.) in the locomotor activity in the open-field test. Each column represents the mean+S.E.M. of 9–12 animals. Statistical analysis was performed by two-way ANOVA, followed by the Duncan's test. $^{**}P<0.01$ as compared with the control group (Sham-vehicle); $^{\#\#}P<0.01$ as compared with the OB-vehicle group.

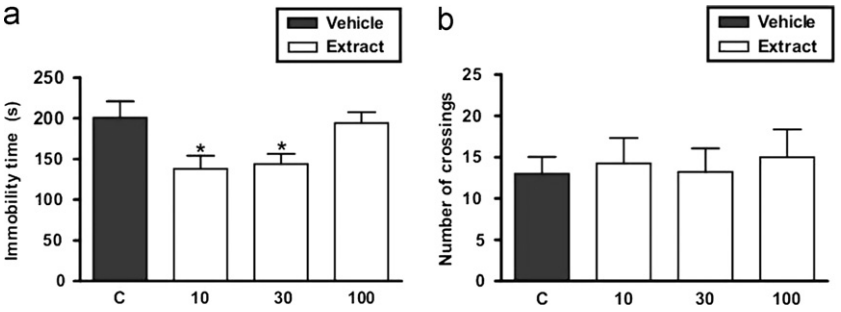

Fig. 2. Effect of repeated (14 days) administration of EET (dose range 10–100 mg/kg, p.o.) in the TST (panel a) and open-field test (panel b). Each column represents the mean+S.E.M. ($n=7$–9). Statistical analysis was performed by one-way ANOVA, followed by Duncan's test. $^{*}P<0.05$ as compared with the vehicle-treated group (C).

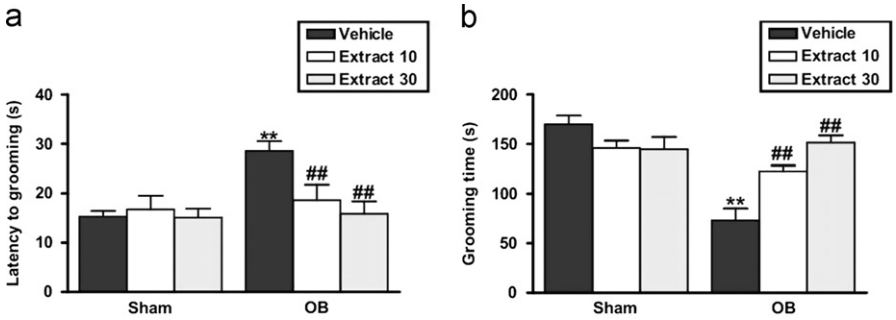

Fig. 4. Effect of the repeated (14 days) treatment of bulbectomized mice with EET (10–30 mg/kg, p.o.) in the latency to grooming (panel a) and grooming time (panel b) in the splash test. Each column represents the mean+S.E.M. (n=9–10). Statistical analysis was performed by two-way ANOVA, followed by the Duncan's test. $^{**}P<0.01$ as compared with the control group (Sham-vehicle); $\#\#P<0.01$ as compared with the OB-vehicle group.

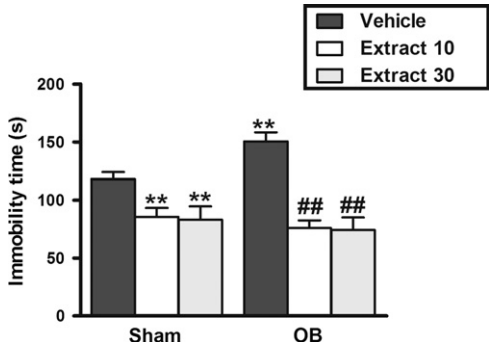

Fig. 5. Effect of the repeated (14 days) treatment of bulbectomized mice with EET (10–30 mg/kg, p.o.) in the immobility-time in the TST. Each column represents the mean+S.E.M. of 9–12 animals. Statistical analysis was performed by two-way ANOVA, followed by the Duncan's test. $^{**}P<0.01$ as compared with the control group (Sham-vehicle); $\#\#P<0.01$ as compared with the OB-vehicle group.

3.4. Effect of EET administration (14 days) on depressive-like behavior induced by OB

Fig. 5 illustrates that the increase in the immobility time produced by OB was significantly abolished by EET (10 and 30 mg/kg, p.o.) treatment during 14 days. The two-way ANOVA revealed a significant main effect of EET treatment [$F(2,43)$= 27.52, $P<0.01$], and OB × EET treatment interaction [$F(2,43)$= 4.06, $P<0.05$], but did not show a significant main effect of OB [$F(1,43)$=0.44, P=0.51].

3.5. Evaluation of Akt, GSK-3β, ERK1/2 and CREB phosphorylation and BDNF immunocontent by Western blot assay

Akt is a serine/threonine kinase activated by phosphorylation at Ser^{473} residue (Yang et al., 2002). Western blot analysis from hippocampal tissue homogenates showed that neither the OB procedure nor the repeated treatment with EET altered Akt phosphorylation in hippocampus of mice (Fig. 6B). The two-way ANOVA revealed no differences for OB [$F(1,18)$=0.31, P=0.58], EET treatment [$F(2,18)$=0.14, P=0.86] and OB × EET treatment interaction [$F(2,18)$=0.28, P=0.76]. Fig. 6D shows that the treatment of Sham or OB-mice with EET (30 mg/kg, p.o.) caused a significant increase in GSK-3β phosphorylation (Ser^{9}) as compared with Sham-operated group or OB-vehicle group, respectively. The two-way ANOVA revealed a significant main effect of EET treatment [$F(2,18)$=7.52, $P<0.01$], but did not show a significant effect of OB [$F(1,18)$=3.35, P=0.08] and OB × EET treatment interaction [$F(2,18)$=0.002, P=0.99].

The results depicted in Fig. 7B illustrates that the increase in ERK1 phosphorylation produced by OB was significantly abolished by EET (10 and 30 mg/kg, p.o.) treatment during 14 days. The two-way ANOVA revealed a significant main effect of OB [$F(1,18)$=5.44, $P<0.05$], and OB × EET treatment interaction [$F(2,18)$=4.56, $P<$ 0.05], but did not show a significant main effect of EET treatment [$F(2,18)$=0.94, P=0.40]. Fig. 7C illustrates that ERK2 phosphorylation was not altered in any experimental condition. The two-way ANOVA revealed no differences for OB [$F(1,18)$=2.25, P=0.15], EET treatment [$F(2,18)$=0.81, P=0.46], and OB × EET treatment interaction [$F(2,18)$=1.23, P=0.32].

Finally, the effect of the repeated treatment of OB-mice with EET on CREB phosphorylation and BDNF immunocontent were verified by western blot assay. Fig. 8B shows that EET (10 and 30 mg/kg, p.o.) administration and OB caused a significant increase in CREB phosphorylation (Ser^{133}). The two-way ANOVA revealed a significant main effect for OB [$F(1,14)$=6.78, $P<0.05$] and EET treatment [$F(2,14)$=3.50, $P<0.05$], but not for OB × EET treatment interaction [$F(2,14)$=2.59, P=0.11]. Fig. 8D shows that the repeated treatment of OB-mice with EET at doses of 10 and 30 mg/kg (p.o.) was able to prevent the BDNF immunocontent augmentation caused by olfactory bulbs ablation. The two-way ANOVA revealed a significant main effect for OB [$F(1,18)$=7.68, $P<0.01$] and OB × EET treatment interaction [$F(2,18)$=3.93, $P<0.05$], but not for EET treatment [$F(2,18)$=0.54, P=0.59].

4. Discussion

This study supports pharmacological and biochemical evidence for the antidepressant-like effect of the EET administered chronically by oral route to mice. The phytochemical analyses of the EET identified the following compounds: *p*-hydroxybenzoic acid, anisic acid, veratric acid and caffeic acid, as reported previously (Pereira et al., 2012). Data from literature have shown that *p*-hydroxybenzoic acid has antimicrobial properties (Sánchez-Maldonado et al., 2011), anisic acid is an anti-inflammatory compound (Singh et al., 2006), veratric acid is antihypertensive and antioxidant (Saravanakumar and Raja, 2011) and caffeic acid is an antioxidant compound (Simić et al., 2007) that has antidepressant effects (Takeda et al., 2002, 2003, 2006; Dzitoyeva et al., 2008). Thus, it is likely that caffeic acid alone or in combination with the other compounds identified in EET contributes to the antidepressant action of *Tabebuia avellanedae*.

In the present work, we demonstrated that EET administered for 14 days produced a significant antidepressant-like effect in the TST, a commonly used behavioral test that predict the efficacy of antidepressant treatment (Steru et al., 1985; Bourin et al., 2005;). Additionally, a consistent antidepressant-like activity of EET in a well-established animal model of depression, the olfactory bulbectomy model (Leonard and Tuite, 1981), was shown. Noteworthy, this

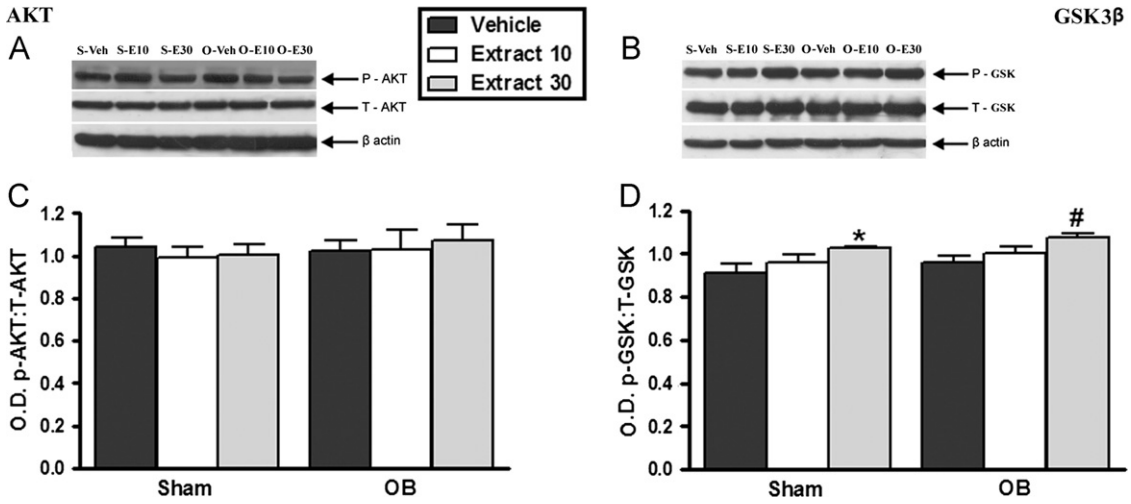

Fig. 6. Effect of repeated (14 days) treatment of bulbectomized mice with EET (10–30 mg/kg, p.o.) on Akt (panels A and B) and GSK-3β (panels C and D) phosphorylation. Panels A and C show a representative western blot of the phosphorylation and total content of Akt and GSK-3β. Quantitative analyses are illustrated in panels B and D. The data are expressed as ratio of optic density (O.D.) between phosporylated (P-Akt, P-GSK-3β) and total (T-Akt, T-GSK-3β) forms of Akt and GSK-3β. Each column represents the mean+S.E.M. of 4 independent experiments. Statistical analysis was performed by two-way ANOVA, followed by the Duncan's test. $^{*}P < 0.05$ as compared with the control group (Sham-vehicle); # $P < 0.05$ as compared with the OB-vehicle group. Abbreviations: sham/vehicle (S-Ct), sham/extract 10 mg/kg (S-E10), sham/extract 30 mg/kg (S-E30), OB/vehicle (B-Ct), OB/extract 10 mg/kg (B-E10) and OB/extract 30 mg/kg (B-E30) groups.

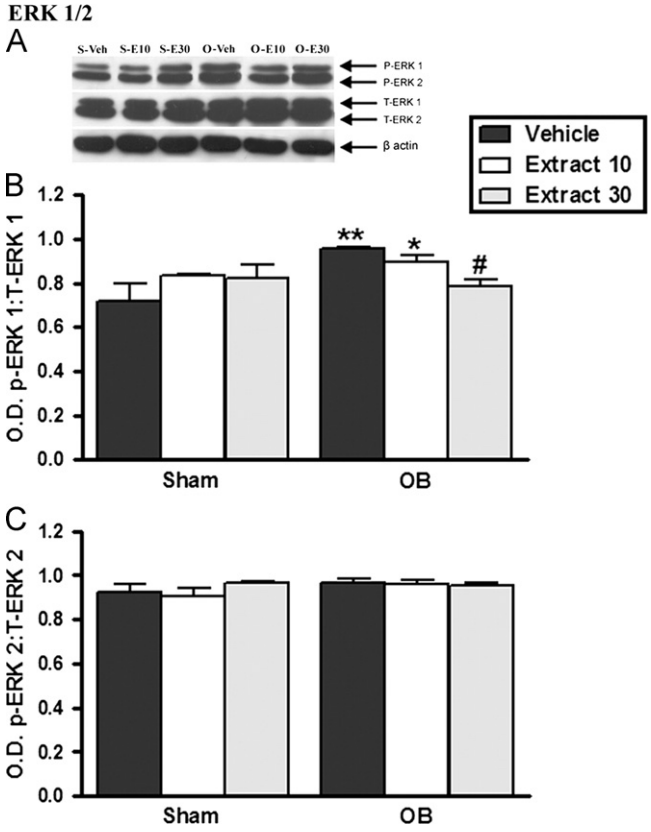

Fig. 7. Effect of repeated (14 days) treatment of bulbectomized mice with EET (dose range 10–30 mg/kg, p.o.) on ERK1 (panels A and B) and ERK2 (panels A and C) phosphorylation. Panel A shows a representative western blot of phosphorylated and total forms of ERK1 and ERK2. Quantitative analyses are illustrated in panels B and C. The data are expressed as ratio between phosphorylated (P-ERK1 and P-ERK2) and total (T-ERK1 and T-ERK2) forms. Each column represents the mean+S.E.M. of 4 independent experiments. Statistical analysis was performed by two-way ANOVA, followed by Duncan's test. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with the control group (Sham-vehicle); #$P < 0.05$ as compared with the OB-vehicle group. Abbreviations: sham/vehicle (S-Ct), sham/extract 10 mg/kg (S-E10), sham/extract 30 mg/kg (S-E30), OB/vehicle (B-Ct), OB/extract 10 mg/kg (B-E10) and OB/extract 30 mg/kg (B-E30) groups.

model employed in the present study reproduced some of the behavioral changes reported in the literature, particularly the hyperlocomotion in the open-field test, the anhedonic-like behavior in the splash test and the increased immobility time in the TST (Freitas et al., 2012; Machado et al., 2012a,b). In the present study, the treatment for 14 days with EET in bulbectomized mice was effective in preventing these behavioral alterations induced by olfactory bulbs ablation. OB caused a significant increase in CREB (Ser^{133}) and ERK1 phosphorylation levels and BDNF immunocontent. This result concurs with the findings of a previous study from our group (Freitas et al., 2012). EET *per se* caused an augmentation of both CREB (Ser^{133}) and GSK-3β (Ser^{9}) phosphorylation. In addition, it was able to abolish the enhancement in ERK1 phosphorylation and BDNF immunocontent induced by OB. Neither EET nor OB caused alterations in Akt (Ser^{473}) phosphorylation. Altogether, the results indicate that the antidepressant-like action of EET is accompanied by modulation of GSK-3β, ERK1, CREB and BDNF-mediated signaling pathways which are well-known to be implicated in the neuroplasticity alterations evoked by antidepressants (Covington et al., 2010; Vialou et al., 2012).

The TST is a predictive animal test widely used for screening antidepressant activity of different classes of drugs (Porsolt et al., 1977; Cryan et al., 2005). This test is based on the observation that animals, after initial escape-oriented movements, develop an immobile posture when placed in an inescapable stressful situation. When antidepressant treatments are given prior to the tests, the subjects will actively persist engaging in escape-directed behavior for longer periods of time than after vehicle treatment (Cryan et al., 2005). Recently, our group showed that an acute administration of EET produced an antidepressant-like effect in the TST in mice (Freitas et al., 2010). In the present study, the treatment of mice for 14 days with EET at dose of 10 and 30 mg/kg (p.o.) produced a significant antidepressant-like effect in the TST in agreement with the fact that antidepressant drugs produce a reduction in the immobility time in this predictive test (Steru et al., 1985). This experiment show that no tolerance was verified following repeated treatment of EET at doses of 10 and 30 mg/kg (p.o.), since the extract administered acutely causes an antidepressant-like effect in the TST at the doses of 10, 30, 100 and 300 mg/kg (p.o.) (Freitas et al., 2010).

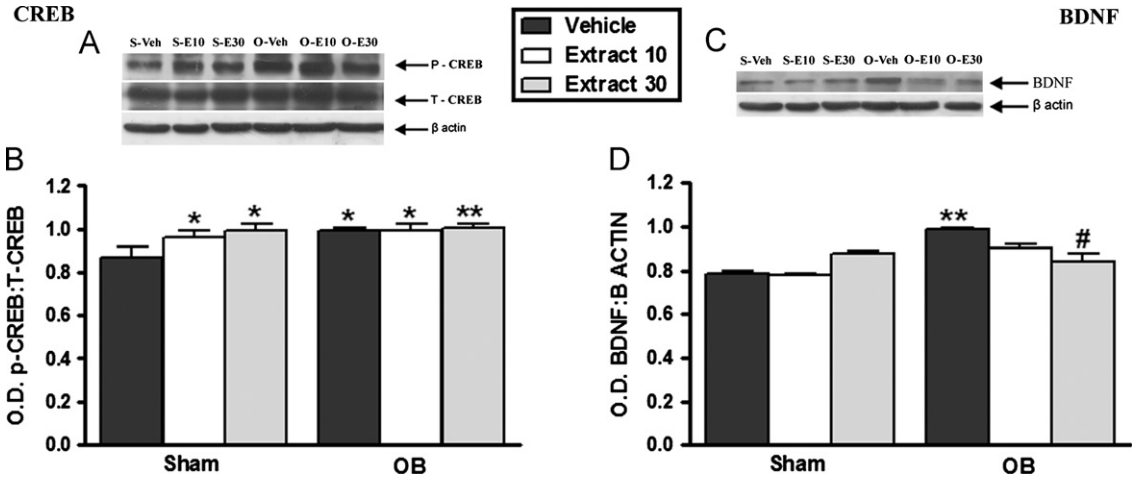

Fig. 8. Effect of repeated (14 days) treatment of bulbectomized mice with EET (dose range 10–30 mg/kg, p.o.) on CREB (panels A and B) phosphorylation and BDNF (panels C and D) immunocontent. Panels A and C show a representative western blot of total and phosphorylated forms of CREB. Quantitative analyses are illustrated in panels B and D. The data are expressed as ratio between phosphorylated (P-CREB) and total (T-CREB) forms of CREB and as ratio between BDNF content and β-actin. Each column represents the mean+S.E.M. of 4 independent experiments. Statistical analysis was performed by two-way ANOVA, followed by Duncan's test. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with the control group (Sham-vehicle); $\#P < 0.05$ as compared with the OB-vehicle group. Abbreviations: sham/vehicle (S-Ct), sham/extract 10 mg/kg (S-E10), sham/extract 30 mg/kg (S-E30), OB/vehicle (B-Ct), OB/extract 10 mg/kg (B-E10) and OB/extract 30 mg/kg (B-E30) groups.

The main contribution of the present study is to shown that EET was capable of reversing depressive-like behaviors induced by OB. Olfactory bulbs ablation induces learning and memory dysfunctions, psychomotor agitation, altered avoidance behavior and anhedonic-like behavior (Kelly et al., 1997). These effects do not appear to be due to anosmia, as selective ablation of the olfactory sensory receptors does not produce these OB-induced symptoms (Alberts and Friedman, 1972; Saitoh et al., 2006). In the present work, the OB produced a significant hyperlocomotion consistent with clinical symptoms of agitated depression that was prevented by the treatment (14 days) of OB-mice with EET at doses of 10 and 30 mg/kg (p.o.). The ability of EET to block symptoms of agitated depression reinforces the antidepressant-like property of this plant and corroborates with several studies that have shown that antidepressant compounds are able to prevent the hyperactivity induced by OB such as curcumin, the active constituent of *Curcuma longa* (Xu et al., 2005) and the hydroalcoholic extract from *Rosmarinus officinalis* L. (Machado et al., 2012a).

In the present work, OB produced a significant increase in the immobility time in the TST, an indicative of a depressive-like behavior. This result is in agreement with the findings of a previous study by our group (Carlini et al., 2012) that shows that olfactory bulbs ablation produces a depressive-related behavior in the TST. Of note, this behavioral alteration was prevented by EET at doses of 10 and 30 mg/kg (p.o.) once a day for 14 days, reinforcing the notion that EET exerts an antidepressant-like effect.

This study assessed anhedonic-like behavior that is related to the inability to experience pleasure, through the splash test in mice. In this test, an increase in the latency to start the grooming behavior as well as a decrease in the total grooming time indicates a loss of self-care and motivational behavior, suggestive of an anhedonic-like behavior (Kalueff and Tuohimaa, 2004). A study by Gambarana et al. (2001) shows that the hydroalcoholic extract from *Hypericum perforatum* was effective in preventing anhedonia in an animal model based on the finding that repeated stressors prevent the development of appetitive behavior induced by vanilla sugar in satiated rats fed ad libitum. In addition, Xu et al. (2008) showed that a mixture of honokiol and magnolol, the main constituents simultaneously identified in the barks of *Magnolia officinalis*, reversed chronic mild stress-induced reduction in sucrose consumption in rats. Our results are in line with the notion that EET (10 and 30 mg/kg, p.o.) act counteracting the loss in motivational and self-care behavior induced by OB. This suggests that components of EET are attractive candidates for further studies and, after confirming safety and effectiveness, clinical trial can address the usefulness for the management of depression associated with anhedonia.

A new class of putative antidepressant agents that act as GSK-3β inhibitors has been proposed. The primary mechanism of regulation of this enzyme involves enzyme inhibition through phosphorylation of Ser^{9} (Peineau et al., 2008) by kinases such as Akt/PKB,PKA, PKC, and ribosomal S6 kinase (Doble and Woodgett, 2003). Several studies have shown that treatment with antidepressants improve the phosphorylation of GSK-3β on Ser^{9} in cerebral cortex (Li et al., 2004) and hippocampus (Eom and Jope, 2009), causing enzyme inhibition. The novel GSK-3β inhibitor thiadiazolidinone NP031115, and the well-established GSK-3β inhibitor AR-A014418, were shown to produce antidepressant-like effects in the mouse FST (Rosa et al., 2008). Furthermore, a decrease in GSK-3β phosphorylation was also found in the prefrontal cortex of suicide victims and has been associated with major depressive disorders (Karege et al., 2006). Overall, these results corroborate the idea that GSK-3β is an important target for the action of antidepressant agents. Our results are consistent with this notion, since EET-treated sham-operated or OB-mice group (30 mg/kg, p.o.) presented a significant increase in Ser^{9} GSK-3β phosphorylation in mouse hippocampus, as compared with sham-vehicle or OB-vehicle group. The mechanism underling the phosphorylation of GSK-3β in response to EET was not identified in the present study, since this effect was not accompanied by activation of Akt, a well-known enzyme responsible for Ser^{9} GSK-3β phosphorylation.

ERK1/2 are members of mitogen activated protein kinases family activated by phosphorylation. ERK1/2 primarily regulates neuronal growth, cell differentiation and apoptosis, as well as synaptic plasticity (Stork and Schmitt, 2002; Thomas and Huganir, 2004). Recent studies have shown that the hyperactivity of Ras-ERK pathways may be implicated in the pathophysiology of depression (Galeotti and Ghelardini, 2011; Elomaa et al., 2012). Additionally, previous studies indicated that the antidepressant treatments may inhibit ERK activity (Fumagalli et al., 2005; Bravo et al., 2009). Our results support this notion, because the increase

in ERK1 phosphorylation caused by OB was significantly prevented by EET (10 and 30 mg/kg, p.o.). On the other hand, in the present study ERK2 phosphorylation was not altered in any experimental group.

A number of growth factors and hormones have been shown to stimulate the expression of genes involving Ser^{133} phosphorylation of the nuclear factor CREB (Tardito et al., 2006). This phosphorylation promotes the association of CREB with the CREB-binding protein, a co-activator protein that aids in the assembly of an active transcription complex enabling the activation of target genes (Lu et al., 2003). Data from the existing literature have shown that antidepressant compounds such as natural flavonols (Hou et al., 2010) and hyperforin (Gibon et al., 2012), one of the main bioactive compounds from *Hypericum perforatum*, up-regulates hippocampal pCREB. In the present study, treatment for 14 days with EET (10 and 30 mg/kg, p.o.) produced a significant increase in CREB phosphorylation in the hippocampus both in sham-operated and bulbectomized mice.

It is well known that hippocampal brain-derived-neurotrophic factor (BDNF) expression is dependent on CREB activation and this event may be a mediator of the therapeutic responses to antidepressants (Nair and Vaidya, 2006; Duman, 2009). Nevertheless, elevated hippocampal BDNF levels have been reported in bulbectomized mice (Hellweg et al., 2007; Freitas et al., 2012). In the present study, we identified a significant OB-induced up-regulation of BDNF content at hippocampal tissue that was blocked by treatment (14 days) of bulbectomized mice with EET at dose of 30 mg/kg (p.o.). It could be supposed that the increase in BDNF content may result from a compensatory up-regulation of this neurotrophin following OB that, interestingly, was prevented by EET.

5. Conclusion

The present study firstly indicates that the administration of *Tabebuia avellanedae* during 14 days in mice is able to produce an antidepressant-like effect in the TST that may be associated with CREB (Ser^{133}) and GSK-3β (Ser^{9}) phosphorylation. Furthermore, the repeated treatment with *Tabebuia avellanedae* was effective in reversing the hyperactivity, anhedonic behavior and increased immobility time in the TST induced by OB. This response is accompanied by modulation of signaling pathways related with neuronal survival, particularly ERK1 and BDNF. Finally, our results indicate that this plant could constitute an attractive tool for the treatment of depressive disorders, validating the traditional use of this plant.

Acknowledgments

This study was supported by the FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBN-Net/CNPq)", CNPq, FAPESC, CAPES/PROCAD and Núcleo de Excelência em Neurociências Aplicadas de Santa Catarina (NENASC) Project/ PRONEX Program CNPq/FAPESC (Brazil).

References

Alberts, J.R., Friedman, M.I., 1972. Olfactory bulb removal but not anosmia increases emotionality and mouse killing. Nature 238, 454–455.
Bourin, M., Chenu, F., Ripoll, N., David, D.J.P., 2005. A proposal of decision tree to screen putative antidepressants using forced swim and tail suspension tests. Behavioural Brain Research 164, 266–269.
Bravo, J.A., Díaz-Veliz, G., Mora, S., Ulloa, J.L., Berthoud, V.M., Morales, P., Arancibia, S., Fiedler, J.L., 2009. Desipramine prevents stress-induced changes in depressive-like behavior and hippocampal markers of neuroprotection. Behavioural Pharmacology 20, 273–285.
Carlini, V.P., Machado, D.G., Buteler, F., Ghersi, M., Ponzio, M.F., Martini, A.C., Schiöth, H.B., de Cuneo, M.F., Rodrigues, A.L.S., de Barioglio, S.R., 2012. Acute ghrelin administration reverses depressive-like behavior induced by bilateral olfactory bulbectomy in mice. Peptides 35, 160–165.
Cordova, F.M., Rodrigues, A.L.S., Giacomelli, M.B., Oliveira, C.S., Posser, T., Dunkley, P.R., Leal, R.B., 2004. Lead stimulates ERK1/2 and p38MAPK phosphorylation in the hippocampus of immature rats. Brain Research 998, 65–72.
Covington 3rd, H.E., Vialou, V., Nestler, E.J., 2010. From synapse to nucleus: novel targets for treating depression. Neuropharmacology 58, 683–693.
Cryan, J.F., Mombereau, C., Vassout, A., 2005. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neuroscience & Biobehavioral Reviews 29, 571–625.
Doble, B.W., Woodgett, J.R., 2003. GSK-3: tricks of the trade for a multi-task kinase. Journal of Cell Science 116, 1175–1186.
Duman, R.S., 2009. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. Dialogues in Clinical Neuroscience 11, 239–255.
Dzitoyeva, S., Imbesi, M., Uz, T., Dimitrijevic, N., Manev, H., Manev, R., 2008. Caffeic acid attenuates the decrease of cortical BDNF transcript IV mRNA induced by swim stress in wild-type but not in 5-lipoxygenase-deficient mice. Journal of Neural Transmission 115, 823–827.
Elomaa, A.P., Niskanen, L., Herzig, K.H., Viinamäki, H., Hintikka, J., Koivumaa-Honkanen, H., Honkalampi, K., Valkonen-Korhonen, M., Harvima, I.T., Lehto, S.M., 2012. Elevated levels of serum IL-5 are associated with an increased likelihood of major depressive disorder. BMC Psychiatry 12, 2–8.
Eom, T.Y., Jope, R.S., 2009. Blocked inhibitory serine-phosphorylation of glycogen synthasekinase-3alpha/beta impairs in vivo neural precursor cell proliferation. Biological Psychiatry 66, 494–502.
Freitas, A.E., Budni, J., Lobato, K.R., Binfaré, R.W., Machado, D.G., Jacinto, J., Veronezi, P.O., Pizzolatti, M.G., Rodrigues, A.L.S., 2010. Antidepressant-like action of the ethanolic extract from *Tabebuia avellanedae* in mice: evidence for the involvement of the monoaminergic system. Progress in Neuro-Psychopharmacology & Biological Psychiatry 34, 335–343.
Freitas, A.E., Machado, D.G., Budni, J., Neis, V. B., Balen, G.O., Lopes, M. W., Souza, L.F., Dafre, A.L.S., Leal, R.B., Rodrigues, A.L.S., 2012. Fluoxetine modulates hippocampal cell signaling pathways implicated in neuroplasticity in olfactory bulbectomized mice. Behavioural Brain Research 237, 176–184.
Fumagalli, F., Molteni, R., Calabrese, F., Frasca, A, Racagni, G., Riva, M.A., 2005. Chronic fluoxetine administration inhibits extracellular signal-related kinase 1/2 phosphorylation in rat brain. Journal of Neurochemistry 93, 1551–1560.
Galeotti, N., Ghelardini, C., 2011. Regionally selective activation and differential regulation of ERK, JNK and p38 MAP kinase signalling pathway by protein kinase C in mood modulation. International Journal of Neuropsychopharmacology 20, 1–13.
Gambarana, C., Tolu, P.L., Mais, F., Rinaldi, M., Giachetti, D., Morazzoni, P., De Montis, M.G., 2001. A study of the antidepressant activity of *Hypericum perforatum* on animal models. Pharmacopsychiatry 34, 42–44.
Gibon, J., Deloulme, J.C., Chevallier, T., Ladevèze, E., Abrous, D.N., Bouron, A., 2012. The antidepressant hyperforin increases the phosphorylation of CREB and the expression of TrkB in a tissue-specific manner. International Journal of Neuropsychopharmacology 9, 1–10.
Gómez Castellanos, J.R., Prieto, J.M., Heinrich, M., 2009. Red Lapacho (*Tabebuia impetiginosa*)—a global ethnopharmacological commodity? Journal of Ethnopharmacology 121, 1–13.
Hellweg, R., Zueger, M., Fink, K., Hörtnagl, H., Gass, P., 2007. Olfactory bulbectomy in mice leads to increased BDNF levels and decreased serotonin turnover in depression-related brain areas. Neurobiology of Disease 25, 1–7.
Hou, Y., Aboukhatwa, M.A., Lei, D.L., Manaye, K., Khan, I., Luo, Y., 2010. Anti-depressant natural flavonols modulate BDNF and beta amyloid in neurons and hippocampus of double TgAD mice. Neuropharmacology 58, 911–920.
Isingrini, E., Camus, V., Le Guisquet, A.M., Pingaud, M., Devers, S., Belzung, C., 2010. Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. PLoS One 5, e10404.
Jones, K., 1995. Pau D'Arco: Immune Power from the Rain Forest, first ed. Arts Press, Rochester, Healing.
Kalueff, A.V., Tuohimaa, P., 2004. Grooming analysis algorithm for neuro behavioural stress research. Brain research. Brain Research Protocols 13, 151–158.
Karege, F., Perroud, N., Burkhardt, S., Schwald, M., Ballmann, E., La Harpe, R., Malafosse, A., 2006. Alteration in kinase activity but not in protein levels of protein kinase B, glycogen synthase kinase-3beta in ventral prefrontal cortex of depressed suicide victims. Biological Psychiatry 61, 240–245.
Kelly, J.P., Wrynn, A.S., Leonard, B.E., 1997. The olfactory bulbectomized rat as a model of depression: an update. Pharmacology & Therapeutics 74, 299–316.
Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Archives of General Psychiatry 62, 593–602.
Leal, R.B., Cordova, F.M., Herd, L., Bobrovskaya, L., Dunkley, P.R., 2002. Lead-stimulated p38 MAPK dependent Hsp27 phosphorylation. Toxicology and Applied Pharmacology 178, 44–51.
Leonard, B.E., Tuite, M., 1981. Anatomical, physiological, and behavioral aspects of olfactory bulbectomy in the rat. International Review of Neurobiology 22, 251–286.

Li, X., Zhu, W., Roh, M.S., Friedman, A.B., Rosborough, K., Jope, R.S., 2004. In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. Neuropsychopharmacology 29, 1426–1431.

Lu, Q., Hutchins, A.E., Doyle, C.M., Lundblad, J.R., Kwok, R.P., 2003. Acetylation of cAMP-responsive element-binding protein (CREB) by CREB-binding protein enhances CREB-dependent transcription. Journal of Biological Chemistry 278, 15727–15734.

Lüebeck, W., 1999. The Healing Power of Pau d'Arco, first ed. Lotus Press, United States.

Machado, D.G., Bettio, L.E., Cunha, M.P., Capra, J.C., Dalmarco, J.B., Pizzolatti, M.G., Rodrigues, A.L.S., 2009. Antidepressant-like effect of the extract of *Rosmarinus officinalis* in mice: involvement of the monoaminergic system monoaminergic system. Progress in Neuro-Psychopharmacology & Biological Psychiatry 33, 642–650.

Machado, D.G., Cunha, M.P., Neis, V.B., Balen, G.O., Colla, A.R., Grando, J., Brocardo, P.S., Bettio, L.E., Dalmarco, J.B., Rial, D., Prediger, R.D., Pizzolatti, M.G., Rodrigues, A.L.S., 2012a. *Rosmarinus officinalis* L. hydroalcoholic extract, similar to fluoxetine, reverses depressive-like behavior without altering learning deficit in olfactory bulbectomized mice. Journal of Ethnopharmacology 143, 158–169.

Machado, D.G., Cunha, M.P., Neis, V.B., Balen, G.O., Colla, A., Grando, J., Brocardo, P.S., Bettio, L.E., Capra, J.C., Rodrigues, A.L.S., 2012b. Fluoxetine reverses depressive-like behaviors and increases hippocampal acetylcholinesterase activity induced by olfactory bulbectomy. Pharmacology Biochemistry & Behavior 103, 220–229.

Morilak, D.A., Frazer, A., 2007. Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. International Journal of Neuropsychopharmacology 7, 193–218.

Murray, C.J.L., Lopez, A.D., 1997. Alternative projections of mortality and disability by cause 1990–2020: global burden of disease study. The Lancet 349, 1498–1504.

Nair, A., Vaidya, V.A., 2006. Cyclic AMP response element binding protein and brain-derived neurotrophic factor: molecules that modulate our mood? Journal of Biosciences 31, 423–434.

Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z.A., Wang, Y.T., Collingridge, G.L., 2008. The role of GSK-3 in synaptic plasticity. British Journal of Pharmacology 153, S428–437.

Pereira, I.T., Burci, L.M., da Silva, L.M., Baggio, C.H., Heller, M., Micke, G.A., Pizzolatti, M.G., Marques, M.C., Werner, M.F., 2012. Antiulcer effect of bark extract of *Tabebuia avellanedae*: activation of cell proliferation in gastric mucosa during the healing process. Phytotherapy Research. In press. http://dx.doi.org/10.1002/ptr.4835.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Analytical Biochemistry 83, 346–356.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Archives Internationales de Pharmacodynamie et de Thérapie 229, 327–336.

Posser, T., de Aguiar, C.B., Garcez, R.C., Rossi, F.M., Oliveira, C.S., Trentin, A.G., Neto, V.M., Leal, R.B., 2007. Exposure of C6 glioma cells to Pb(II) increases the phosphorylation of p38 (MAPK) and JNK1/2 but not of ERK1/2. Archives of Toxicology 81, 407–414.

Rosa, A.O., Kaster, M.P., Binfaré, R.W., Morales, S., Martín-Aparicio, E., Navarro-Rico, M.L., Martinez, A., Medina, M., García, A.G., López, M.G., Rodrigues, A.L.S., 2008. Antidepressant-like effect of the novel thiadiazolidinone NP031115 in mice. Progress in Neuro-Psychopharmacology & Biological Psychiatry 32, 1549–1556.

Saitoh, A., Hirose, N., Yamada, M., Yamada, M., Nozaki, C., Oka, T., Kamei, J., 2006. Changes in emotional behavior of mice in the hole-board test after olfactory bulbectomy. Journal of Pharmacological Sciences 102, 377–386.

Sánchez-Maldonado, A.F., Schieber, A., Gänzle, M.G., 2011. Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. Journal of Applied Microbiology 111, 1176–1184.

Saravanakumar, M., Raja, B., 2011. Veratric acid, a phenolic acid attenuates blood pressure and oxidative stress in L-NAME induced hypertensive rats. European Journal of Pharmacology 671, 87–94.

Simić, A., Manojlović, D., Segan, D., Todorović, M., 2007. Electrochemical behavior and antioxidant and prooxidant activity of natural phenolics. Molecules 12, 2327–2340.

Singh, N., Jabeen, T., Pal, A., Sharma, S., Perbandt, M., Betzel, C., Singh, T.P., 2006. Crystal structures of the complexes of a group IIA phospholipase A2 with two natural anti-inflammatory agents, anisic acid, and atropine reveal a similar mode of binding. Proteins 64, 89–100.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Stork, P.J., Schmitt, J.M., 2002. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. Trends in Cell Biology 12, 258–266.

Takeda, H., Tsuji, M., Inazu, M., Egashira, T., Matsumiya, T., 2002. Rosmarinic acid and caffeic acid produce antidepressive-like effect in the forced swimming test in mice. European Journal of Pharmacology 49, 261–267.

Takeda, H., Tsuji, M., Miyamoto, J., Masuya, J., Iimori, M., Matsumiya, T., 2003. Caffeic acid produces antidepressive- and/or anxiolytic-like effects through indirect modulation of the alpha 1A-adrenoceptor system in mice. Neuro Report 14, 1067–1070.

Takeda, H., Tsuji, M., Yamada, T., Masuya, J., Matsushita, K., Tahara, M., Iimori, M., Matsumiya, T., 2006. Caffeic acid attenuates the decrease in cortical BDNF mRNA expression induced by exposure to forced swimming stress in mice. European Journal of Pharmacology 534, 115–121.

Tardito, D., Perez, J., Tiraboschi, E., Musazzi, L., Racagni, G., Popoli, M., 2006. Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. Pharmacological Reviews 58, 115–134.

Thomas, G.M., Huganir, R.L., 2004. MAPK cascade signalling and synaptic plasticity. Nature Reviews Neuroscience 5, 173–183.

Twardowschy, A., Freitas, C.S., Baggio, C.H., Mayer, B., dos Santos, A.C., Pizzolatti, M.G., Zacarias, A.A., dos Santos, E.P., Otuki, M.F., Marques, M.C., 2008. Antiulcerogenic activity of bark extract of *Tabebuia avellanedae*, Lorentz ex Griseb. Journal of Ethnopharmacology 118, 455–459.

Vialou, V., Feng, J., Robison, A.J., Nestler, E.J., 2012. Epigenetic mechanisms of depression and antidepressant action. Annual Review of Pharmacology and Toxicology. In press. http://dx.doi.org/10.1146/annurev-pharmtox-010611-134540.

Xu, Q., Yi, L.T., Pan, Y., Wang, X., Li, Y.C., Li, J.M., Wang, C.P., Kong, L.D., 2008. Antidepressant-like effects of the mixture of honokiol and magnolol from the barks of *Magnolia officinalis* in stressed rodents. Progress in Neuro-Psychopharmacology & Biological Psychiatry 32, 715–725.

Xu, Y., Ku, B.S., Yao, H.Y., Lin, Y.H., Ma, X., Zhang, Y.H., Li, X.J., 2005. Antidepressant effects of curcumin in the forced swim test and olfactory bulbectomy models of depression in rats. Pharmacology Biochemistry & Behavior 82, 200–206.

Yang, J., Cron, P., Good, V.M., Thompson, V., Hemmings, B.A., Barford, D., 2002. Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. Nature Structural & Molecular Biology 9, 940–944.

Zueger, M., Urani, A., Chourbaji, S., Zacher, C., Roche, M., Harkin, A., Gass, P., 2005. Olfactory bulbectomy in mice induces alterations in exploratory behavior. Neuroscience Letters 374, 142–146.