International Journal of Biological Macromolecules 111 (2018) 534–541

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Involvement of the dopaminergic system in the antidepressant-like effect of the lectin isolated from the red marine alga *Solieria filiformis* in mice

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ARTICLE INFO

Article history:
Received 27 July 2017
Received in revised form 11 December 2017
Accepted 26 December 2017
Available online 28 December 2017

Keywords:
Antidepressant
Lectin
Solieria filiformis

ABSTRACT

This study aimed at evaluating the antidepressant-like action of the marine alga *Solieria filiformis* lectin (SfL) and to investigate the participation of the monoaminergic system in this action. For this, male Swiss mice ($n = 10$) were pretreated with intravenous injections (i.v.) of SfL (1, 3 or 9 mg/kg) and submitted to open field (OFT), tail suspension (TST), forced swimming (FST), elevated plus-maze (EPMT) and hole-board tests (HBT). As controls, mice received sterile saline (i.v.), imipramine (10 or 30 mg/kg; intraperitoneally - i.p.) or diazepam (1 mk/kg; i.p.). To assess the involvement of the monoaminergic system in SfL effects, the FST was conducted in mice pretreated with PCPA, an inhibitor of serotonin synthesis, or noradrenergic and dopaminergic receptors specific antagonists. The results showed that SfL has an antidepressant-like effect, with no psychostimulant and anxiolytic-like effects. When denatured or combined with mannan, SfL lost the ability to reduce the immobility time in the FST. In addition, SfL antidepressant-like effect was inhibited by the pretreatment of mice with SCH 23390, a dopamine D_1 receptor antagonist, and by sulpiride, a dopamine D_2 receptor antagonist. Thus, SfL produced an antidepressant-like effect, which is probably dependent on its interaction with the dopaminergic system.

1. Introduction

Marine algae play a major role in the ocean by being primary producers, since they are used by other marine organisms to achieve energy requirements along the food web [1]. Algae are sources of fiber, minerals, antioxidants, vitamins, pigments, steroids, halogenated compounds, polyketides, polysaccharides, mycosporine-like amino acids, proteins, polyunsaturated fatty acids and other lipids. Based on these properties various alga species are consumed in many countries [2]. Focusing on bioproducts, recent trends in drug research from natural sources suggest that algae are a promising group to the development of novel biochemically active substances, among these substances the lectins are of great importance [3].

Lectins are carbohydrate-binding proteins which lack enzymatic activity on their ligand and are distinct from antibodies and free mono- and oligosaccharide sensor/transport proteins [4]. Marine algal lectins are especially interesting for biological applications because they have lower molecular weights compared to most plant lectins and, therefore, the algae lectins can be less antigenic than the larger plant lectins [5]. Recent studies have shown purified lectins from marine algae with important biological activities, such as: antiviral [6], anti-inflammatory and antinociceptive [7–9], insecticide [10] and immunostimulant activities [11].

The *S. filiformis* lectin (SfL) was firstly purified by Benevides et al. [12]. In this previous study, the authors showed that SfL presents a molecular weight of 29 kDa and exhibits affinity to carbohydrate mannan. Indeed, carbohydrate mannan, in the concentration of 19.5 $mg \cdot L^{-1}$, inhibited SfL hemagglutinating activity against heparinized rabbit erythrocytes. Posteriorly, Abreu et al. [13] determined that the N-terminal amino acid sequence of SfL was composed of 37 amino acid residues.

Regarding SfL biotechnological potential, previous studies have shown that this protein interferes with the growth of pathogenic eubacteria [14], has anti-inflammatory and antinociceptive activities

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https://doi.org/10.1016/j.ijbiomac.2017.12.132
0141-8130/

in vivo [13], and presents immunostimulatory activity *in vitro* [11]. Furthermore, when administered intravenously for 7 days in mice, this lectin showed no toxicity in the analyzed parameters [13]. However, there are no reports in the literature showing the effects of this lectin in mood disorders, such as depression.

Depression is a mental disorder characterized by low energy and fatigue, psychomotor retardation or agitation, inability to experience pleasure, with great harmfulness and recurrence rate. These symptoms can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. Depression also presents high suicide rates [15]. This disorder results from a complex interaction of social, psychological and biological factors. Depression alone accounts for 4.3% of the global disease burden and is among the largest single causes of disability worldwide, particularly for women [16].

It is well known that monoamine neurotransmitters such as serotonin, noradrenaline and dopamine in the central nervous system play a key role in the pathophysiology of depression [17]. Thus, the monoamine hypothesis of depression predicts that the major neurochemical process in depression is the impairment of monoaminergic functions manifested by decreased levels of serotonin, noradrenaline or dopamine [18]. Furthermore, the antidepressant compounds currently available act by restoring the brain levels of one or some of the monoamines [19].

The antidepressant bupropion presents a dopaminergic mechanism of action [20–23]. Dopamine exerts its effects on the postsynaptic neuron through its interaction with 1 of 5 subtypes of dopamine receptors, divided into 2 families, the dopamine 1 (D_1) family (comprising the D_1 and D_5 subtypes) and the D_2 family (comprising the D_2, D_3, and D_4 subtypes) [22]. In fact, there is a preliminary evidence suggesting that pharmacological agents which also possess pro-dopaminergic activity may offer advantages over other non-dopaminergic monoamine-based pharmacotherapies in alleviating certain symptoms including fatigue, anhedonia, excessive sleepiness, and psychomotor retardation [23].

The treatment of depression with conventional antidepressants (monoamine oxidase inhibitors, tricyclics, selective serotonin reuptake inhibitors and selective noradrenaline reuptake inhibitors) has several drawbacks related to the adverse effects of these drugs, such as severe orthostatic hypotension, sedation and gastrointestinal disturbances and sexual dysfunction [24]. Therefore, the development of alternative and efficacious medications to treat depressive disorders is a high priority, and within these alternative medications are compounds of natural origin.

Therefore, this study aims at evaluating the antidepressant-like action of a lectin isolated from the red marine alga *Solieria filiformis*, and to investigate, by the use of pharmacological tools, the possible participation of the dopaminergic, serotonergic and noradrenergic systems in SfL antidepressant-like action. Our results show, as far as we know for the first time, an alga marine lectin with antidepressant-like effect in mice, by a mechanism dependent on the dopaminergic system.

2. Materials and methods

2.1. Marine alga

The alga *Solieria filiformis* (Kützing) P.W. Gabrielson was collected from a growing area situated on Flecheiras beach (Trairí, Ceará, Brazil). After collection, the material was cleaned of epiphytes, washed with distilled water (1:2 m/v) and stored at −20 °C until use. The voucher specimens of *S. filiformis* (N° 35.682) were deposited in the Prisco Bezerra Herbarium at the Department of Biological Sciences, Federal University of Ceará (UFC), Brazil.

2.2. Animals

Male adult *Swiss* mice (25–30 g; 6–8 weeks old) from the Animal Care Unit of the UFC, at Fortaleza, Ceará, Brazil, were used throughout the experiments. They were housed 10 per cage in standard polypropylene cages (49 × 34 × 16 cm) in a temperature-controlled room (23 + 1 °C) with free access to water and food (NUVILAB®) on a 12/12 h light/dark cycle. This study was conducted with the approval of the Ethics Committee of the UFC (CEPA n° 12/15).

2.3. Drugs and reagents

The following drugs were used: imipramine (IMP; Tofranil®, Novartis Biociências S.A.), bupropion (BUP; Cloridrato de bupropiona®, Eurofarma), fluoxetine (FLU; Fluoxetina®, Medley), sulpiride (SUL; Equilid®, Aventis Pharma), prazosin (PRA; Minipress®, Pfizer), yohimbine (YOH; Yomax®, Apsen Farmacêutica), (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H benzazepinehydrochloride (SCH 23390; Sigma-Aldrich, Brazil); *p*-chlorophenylalanine methyl ester (PCPA; Sigma-Aldrich, Brazil) and diazepam (DZP; União Química Farmacêutica Nacional S/A). Drugs and SfL were solubilized in 0.9% sterile NaCl (saline).

2.4. Purification of Solieria filiformis lectin (SfL)

The *Solieria filiformis* lectin (SfL) was obtained by extraction with Tris-HCl buffer 25 mM (pH 7.5), precipitation with ammonium sulfate (70%) and sequential chromatography of ion exchange on a DEAE-cellulose gel and gel filtration on a column of Sephadex G-100, as described previously by Abreu et al. [11].

2.5. Experimental protocol

Mice were tested during the light period and were observed in a closed room, at a constant temperature (23 ± 1 °C) illuminated with normal light, except for the open field test, that was poorly illuminated with a 15-V red light.

Initially, in order to evaluate the SfL effect on locomotor and exploratory activities, the open field test was performed [25]. Thereafter, for assessing the SfL antidepressant-like activity, the tail suspension and forced swimming tests were carried out. These models are predictive of antidepressant-like action and have been used extensively for the development of new therapeutic compounds with antidepressant-like activity [26–29].

2.6. Behavioral tests

2.6.1. Open field test (OFT)

This test was based on the methodology described by Siegel [30] and validated by Archer [25] and allows assessment of the SfL effect on locomotor and exploratory activities of the mice. In order to perform this test, the animals were initially divided into four groups, which received the following treatments: sterile saline (NaCl 0.9%), intravenously (i.v.); or SfL at doses of 1, 3 or 9 mg/kg (i.v.). After 30 min, the mice were individually placed in the center of an acrylic open field arena (60 cm × 60 cm × 60 cm), with the floor divided into 9 quadrants. These animals remained in this open field for 6 min. The parameters for observation were: number of squares crossed with the four legs (spontaneous movement); the number of grooming, which is defined as a self-cleaning behavior; and the number of rearing, which is defined as the animal standing upright on its hind legs. All these parameters were observed and recorded for 5 min. After each trial, the arena was cleaned with a 10% ethanol solution in order to eliminate the presence of any olfactory cues.

2.6.2. Tail suspension test (TST)

This test was carried out, following the methodology of Steru et al. [31]. Mice were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for one hour before testing. For the test, the animals were divided into five groups, which received the following treatments: sterile saline (NaCl 0.9%; i.v.), or SfL at doses of 1, 3 or 9 mg/kg (i.v.); or imipramine (IMP; 30 mg/kg; intraperitoneally - i.p.), a tricyclic antidepressant – as positive control [32]. 30 min after injection, the mice were suspended by the tail on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded over a period of 6 min.

2.6.3. Forced swimming test (FST)

This test was conducted in a tank with 22 cm in diameter and 40 cm in height with a rounded lid containing freshwater at 25 °C at a depth of 20 cm [33]. For the test, the mice were divided into five groups, which received the following treatments: sterile saline (NaCl 0.9%; i.v.); or SfL at doses of 1, 3 or 9 mg/kg (i.v.); or IMP (10 mg/kg; i.p.), as positive control [32]. After 30 min, the animals were placed in the tank and left there for 5 min to observe the immobility time. A mouse was considered immobile when it remained floating on the water, without struggling, making only very slight movements necessary to keep its head above the water. Each animal was used only once.

To evaluate the importance of the three-dimensional structure of the SfL over the possible antidepressant-like effect of this lectin, its thermal inactivation was carried out (denaturation), following Benevides et al. [12] methodology. In this regard, SfL, at a dose of 9 mg/kg, was subjected to heating (90 °C, 30 min) and applied to mice by i.v. route. After 30 min, the animals were submitted to the FST. To assess the importance of the lectinic domain in the possible antidepressant-like effect of SfL, the incubation of SfL was performed, at a dose of 9 mg/kg, with its mannan hapten (39 mg/L) for 12 h at 37 °C [12]. After that, the application was performed to mice by i.v. route. To discard a possible effect of mannan in depression, this carbohydrate (39 mg/L) was also applied alone to the animals (i.v.). After 30 min of these applications, the animals were submitted to the FST.

Finally, in order to assess the possible involvement of the noradrenergic, dopaminergic, and serotonergic systems in the SfL antidepressant-like effect, the experiments were performed as described below [32]:

- For the evaluation of the participation of noradrenergic system: mice were pretreated with prazosin (PRA; 1 mg/kg; i.p.), an α_1-adrenoreceptor antagonist; or yohimbine (YOH; 1 mg/kg; i.p.), an α_2-adrenoreceptor antagonist, and 30 min later, were treated with SfL (9 mg/kg; i.v.); or sterile saline (i.v.), as negative control; or IMP (10 mg/kg; i.p.), as positive control. Thirty minutes after the application of SfL, saline or IMP, the animals were submitted to the FST.
- For the evaluation of the participation of the serotonergic system: mice were pretreated with PCPA (100 mg/kg; i.p.), an inhibitor of serotonin synthesis, once a day for four consecutive days. On the fourth day, 30 min after PCPA administration, the animals were treated with SfL (9 mg/kg; i.v.); or sterile saline (i.v.), as negative control; or fluoxetine (FLU; 35 mg/kg; i.p.), as positive control. Thirty min after SfL application, saline or FLU, the animals were submitted to the FST.
- For the evaluation of the participation of the dopaminergic system: mice were pretreated with SCH 23390 (15 μg/kg; i.p.), a dopamine D1 receptor antagonist; or sulpiride (SUL; 50 mg/kg; i.p.), a dopamine D2 receptor antagonist. Thirty min after were treated with SfL (9 mg/kg; i.v.); or sterile saline (i.v.), as negative control; or bupropion (BUP; 30 mg/kg; i.p.), as positive control. Thirty min after the application of SfL, saline or BUP, the animals were submitted to the FST.

2.6.4. Hole-board test (HBT)

The hole-board test for exploratory behavior of mice was performed as described previously [33]. The apparatus used was an Ugo Basile of 60 cm × 30 cm with 16 evenly spaced holes. For the test, the animals were divided into five groups, which received the following treatments: sterile saline (NaCl 0.9%; i.v.), or SfL at doses of 1, 3 or 9 mg/kg (i.v.); or diazepam (DZP; 1 mg/kg; intraperitoneally - i.p.), a benzodiazepine anxiolytic – as positive control [34]. 30 min after injection, the animals were placed at the center of the plaque and the number of head dips into the holes was counted for 5 min.

2.6.5. Elevated plus-maze test (EPMT)

The elevated plus maze consists of two perpendicular open arms (30 × 5 cm) and two closed arms (30 × 5 × 25 cm) also in the perpendicular position. The open and closed arms are connected by a central platform (5 × 5 cm). The platform and the lateral walls of the closed arms are made of transparent acrylic [35]. For the test, the animals were divided into five groups, which received the following treatments: sterile saline (NaCl 0.9%; i.v.), or SfL at doses of 1, 3 or 9 mg/kg (i.v.); or DZP (1 mg/kg; i.p.), as positive control. 30 min after treatment, the animal was placed at the center of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: the number of entries in the open arms (NEOA), and the time of permanence in the open arms (TPOA). The ratios 'number of entries into open arms/number of entries into all (i.e. open and closed) arms' and 'time spent in the open arms/time spent in all arms' were calculated and multiplied by 100 to yield the percentages of entries into open arms (PEOA) and the percentage of time spent in the open arms (PTOA).

2.7. Statistical analysis

The results are presented as mean ± S.E.M. (standard errors of the mean). Data were analyzed by one-way ANOVA followed by Student–Newman–Keuls *post hoc* test or two-way ANOVA followed by Tukey's *post hoc* test, for data that presented more than one factor. In this analysis, the two factors used were: "antidepressant groups" (negative control, SfL-9 or IMP) and "pretreatment groups" (SAL, PRA-1, YOH-1, PCPA-100, SUL-50 or SCH-15). $P < 0.05$ was considered statistically significant. The statistical program used was GraphPad Prism 5.0 Version for Windows, GraphPad Software (San Diego, CA, USA).

3. Results

3.1. Open field test

SfL, at the doses of 1, 3 or 9 mg/kg (i.v.), did not alter mice locomotion in the OFT when compared to control group [$F(3,32) = 0.7809$, $P = 0.5134$] (Fig. 1A). Furthermore, this lectin did not alter the number of rearings [$F(3,35) = 0.4166$, $P = 0.7422$] and groomings [$F(3,35) = 0.7755$, $P = 0.5156$] in these animals, as compared to control group (Fig. 1B and C).

3.2. Tail suspension test

In this test, SfL, at the doses of 3 and 9 mg/kg (i.v.), reduced the immobility time by 50.9% and 49.8%, respectively, as compared to control group [$F(4,43) = 18.89$, $P < 0.0001$]. While the positive control, IMP (30 mg/kg; i.p.), reduced this time by 83.1% when compared to control group (Fig. 2).

3.3. Forced swimming test

In the FST test, mice treated with SfL at the doses of 3 and 9 mg/kg presented a reduction of the immobility time by 54.5% and 57.4%, respectively, as compared to control group [$F(4,44) = 10.91$, $P < 0.0001$]. Similarly, animals treated with IMP (10 mg/kg) showed a decrease

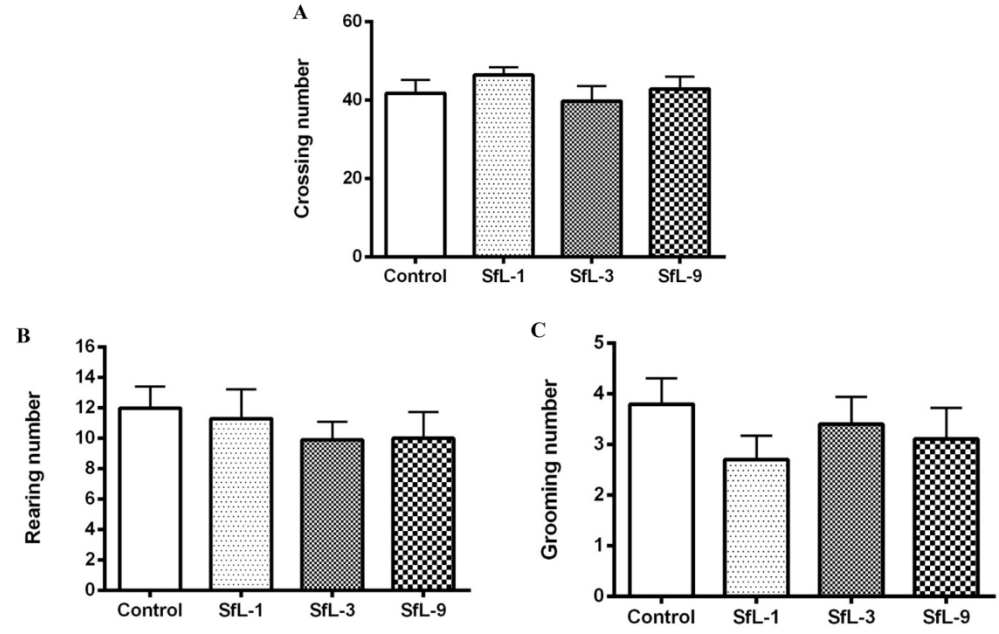

Fig. 1. Effect of the mice acute treatment with SfL in the open field test. Male mice ($n = 10$) were pretreated with SfL (1, 3 or 9 mg/kg; i.v.) or sterile saline (i.v.), as negative control. After 30 min, the animals were submitted to the test, in which the crossing (panel A), rearing (panel B) and grooming (panel C) numbers were observed for 5 min. Each column represents the mean ± S.E.M. Results were analyzed by one-way ANOVA followed by Student–Newman–Keuls as the *post hoc* test.

in the immobility time by 54.7%, as compared to the control group (Fig. 3A).

When denatured (90 °C/30 min) or when combined with its binding sugar mannan, SfL lost its ability to reduce the immobility time [$F(3,30) = 2.570$, $P = 0.0728$]. Moreover, the administration of mannan (39 mg/L; i.v.), as a negative control, did not modify the immobility time of mice, as compared to control (Fig. 3B).

Once the antidepressant-like action of SfL in the FST was observed at the doses of 3 and 9 mg/kg, the tests using the pharmacological antagonists in order to investigate the possible participation of the noradrenergic, serotonergic and dopaminergic systems in this action were performed with animals pretreated with 9 mg/kg SfL.

3.3.1. Involvement of the noradrenergic system

Regarding the pretreatment with PRA (1 mg/kg; i.p.), two-way ANOVA revealed significant interaction between "antidepressant groups" and "pretreatment groups" [$F(2,45) = 17.85$, $P < 0.0001$]. The *post hoc* test showed that PRA-1 pretreatment did not prevent SfL effect in the FST ($P < 0.01$) (Fig. 4A). In relation to YOH (1 mg/kg; i.p.) pretreatment, two-way ANOVA revealed significant interaction of "antidepressant groups" and "pretreatment groups" [$F(2,47) = 26.47$, $P < 0.0001$]. Similarly to PRA, the *post hoc* test showed that YOH-1

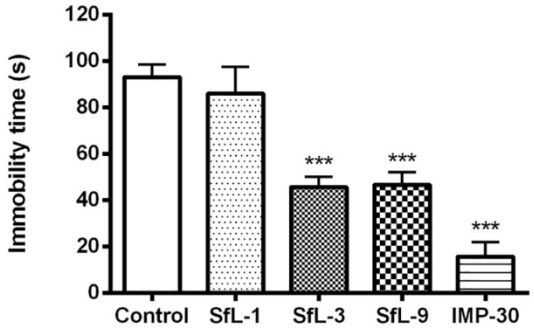

Fig. 2. Effect of the mice acute treatment with SfL on the immobility time (s) in the tail suspension test. Male mice (n = 10) were pretreated with SfL (1, 3 or 9 mg/kg; i.v.); sterile saline (i.v.), as negative control; or imipramine (30 mg/kg; i.p.), as positive control. After 30 min, these animals were submitted to this test and the immobility time (s) of each mouse was observed, for 6 min. Each column represents the mean (s) ± S.E.M. Results were analyzed by one-way ANOVA followed by Student–Newman–Keuls as the *post hoc* test. ***$P < 0.001$ as compared to control group.

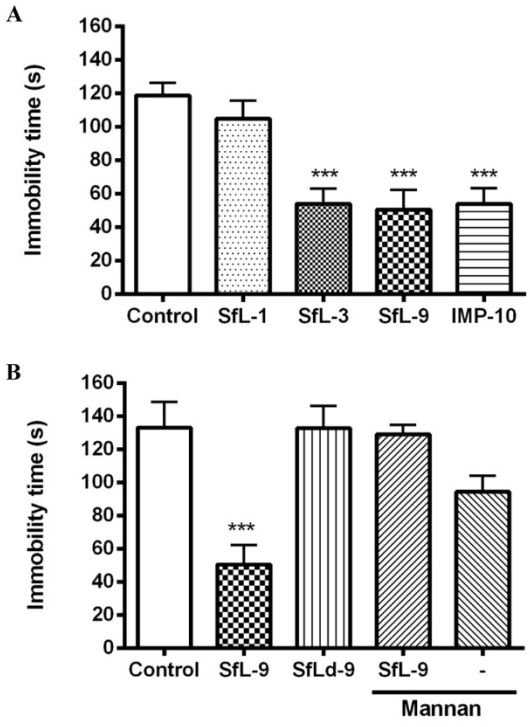

Fig. 3. Effect of the mice acute treatment with SfL on the immobility time (s) in the forced swimming test. Panel A: male mice ($n = 10$) were pretreated with SfL (1, 3 or 9 mg/kg; i.v.); sterile saline (i.v.), as negative control; or imipramine (10 mg/kg; i.p.), as positive control. Panel B: male mice (n = 10) were pretreated with sterile saline (i.v.); SfL (9 mg/kg; i.v.); denatured SfL (9 mg/kg; 90 °C/30 min; i.v.); SfL (9 mg/kg) associated with mannan (39 mg/L; i.v.); or mannan (39 mg/L; i.v.). After 30 min, these animals were submitted to this test and the immobility time (s) of each mouse was observed, for 5 min. Each column represents the mean (s) ± S.E.M. Results were analyzed by one-way ANOVA followed by Student–Newman–Keuls as the *post hoc* test. ***$P < 0.001$ as compared to control group.

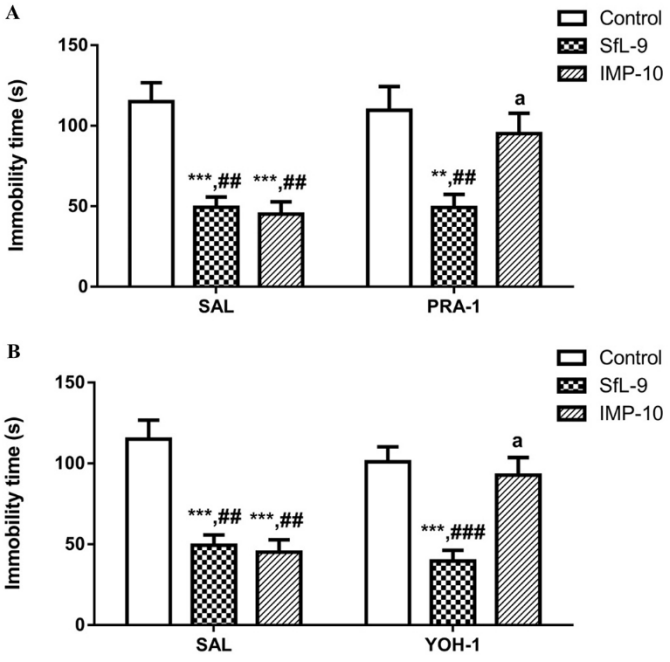

Fig. 4. Evaluation of the noradrenergic system involvement on the SfL-induced immobility time reduction in the forced swimming test. Male mice ($n = 10$) were pretreated with prazosin (PRA; 1 mg/kg; i.p.; panel A), yohimbine (YOH; 1 mg/kg; i.p.; panel B) or sterile saline (SAL; i.p.), as negative control, and 30 min later, they were treated with sterile saline (i.v.), as negative control; SfL (9 mg/kg; i.v.); or IMP (10 mg/kg; i.p.), as positive control. 30 min after saline, SfL or IMP administration, the animals were submitted to the FST. Each column represents the mean (s) ± S.E.M. Results were analyzed by two-way ANOVA followed by Tukey's test as the *post hoc*. $^{***}P < 0.001$ as compared to SAL + Control group; $^{**}P < 0.01$ as compared to SAL + Control group; $^{\#\#\#}P < 0.001$ as compared to YOH-1 + Control; $^{\#\#}P < 0.01$ as compared to PRA-1 + Control or YOH-1 + Control; $^{a}P < 0.05$ as compared to SAL + IMP-10.

pretreatment did not inhibit the effect of SfL in this test ($P < 0.001$) (Fig. 4B). Different from the observed with SfL, the effect of the positive control IMP (10 mg/kg; i.p.) was prevented by PRA and YOH administrations (Fig. 4).

3.3.2. Involvement of the serotonergic system

Two-way ANOVA revealed significant interaction between "antidepressant groups" and "pretreatment groups" [F (2,47) = 19.68, $P < 0.0001$]. The *post hoc* test showed that the anti-immobility effect of SfL (9 mg/kg; i.v.) was not significantly prevented by the pretreatment with PCPA, an inhibitor of serotonin synthesis (100 mg/kg; i.p.) ($P < 0.05$). On the other hand, the pretreatment with PCPA prevented the antidepressant-like effect of the positive control fluoxetine (35 mg/kg; i.p.) in the FST (Fig. 5).

3.3.3. Involvement of the dopaminergic system

Regarding SUL (50 mg/kg; i.p.) pretreatment, two-way ANOVA revealed significant interaction between "antidepressant groups" and "pretreatment groups" [F (2,49) = 14.15, $P < 0.0001$]. The *post hoc* test showed that SUL pretreatment prevented the reduction in the immobility time caused by the SfL in the FST, as shown in Fig. 6A. In relation to SCH 23390 (15 μg/kg; i.p.) pretreatment, two-way ANOVA revealed significant interaction between "antidepressant groups" and "pretreatment groups" interaction [F (2,49) = 18.91, $P < 0.0001$]. The *post hoc* test showed that SCH 23390 pretreatment (15 μg/kg; i.p.) also prevented the antidepressant-like effect of SfL in this test (Fig. 6B). Similarly, SUL and SCH 23390 pretreatments prevented the reduction in the immobility time caused by the positive control bupropion (30 mg/kg; i.p.), as shown in Fig. 6.

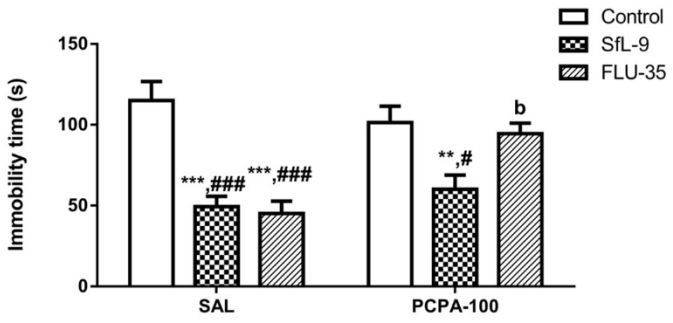

Fig. 5. Evaluation of the serotonergic system involvement on the SfL-induced immobility time reduction in the forced swimming test. Male mice ($n = 10$) were pretreated with PCPA (100 mg/kg; i.p.) or sterile saline (SAL; ip.), as negative control, once a day for four consecutive days. On the fourth day, 30 min after PCPA or SAL administrations, the animals were treated with sterile saline (i.v.), as negative control; SfL (9 mg/kg; i.v.); or fluoxetine (FLU; 35 mg/kg; i.p.), as positive control. 30 min after saline, SfL or FLU administration, the mice were submitted to the FST. Each column represents the mean (s) ± S.E.M. Results were analyzed by two-way ANOVA followed by Tukey's test as the *post hoc*. $^{***}P < 0.001$ as compared to SAL + Control group; $^{**}P < 0.01$ as compared to SAL + Control group; $^{\#\#\#}P < 0.001$ as compared to PCPA-100 + Control group; $^{\#\#}P < 0.01$ as compared to PCPA-100 + Control group; $^{b}P < 0.01$ as compared to SAL + FLU-35 group.

3.4. Hole-board test

The treatment with SfL, at the doses of 1, 3 or 9 mg/kg (i.v.), did not significantly alter the number of head dips, when compared to control. Alternatively, the positive control DZP (1 mg/kg; i.p.) increased the

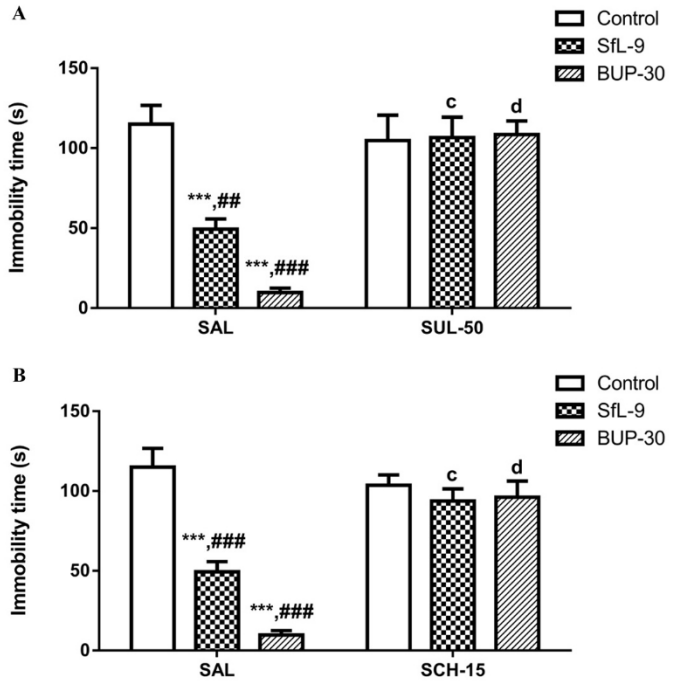

Fig. 6. Evaluation of the dopaminergic system involvement on the SfL-induced immobility time reduction in the forced swimming test. Male mice (n = 10) were pretreated with sulpiride (SUL; 50 mg/kg; i.p.; panel A), SCH 23390 (15 μg/kg; i.p.; panel B) or sterile saline (SAL; i.p.), as negative control, and 30 min later, they were treated with sterile saline (i.v.), as negative control; SfL (9 mg/kg; i.v.); or bupropion (BUP; 30 mg/kg; i.p.), as positive control. 30 min after saline, SfL or BUP administration, the animals were submitted to the FST. Each column represents the mean (s) ± S.E.M. Results were analyzed by two-way ANOVA followed by Tukey's test as the *post hoc*. $^{***}P < 0.001$ as compared to SAL + Control group; $^{\#\#\#}P < 0.001$ as compared to SUL-50 + Control or SCH-15 + Control; $^{\#\#}P < 0.01$ as compared to SUL-50 + Control; $^{c}P < 0.01$ as compared to SAL + SfL-9; $^{d}P < 0.001$ as compared to SAL + BUP-30.

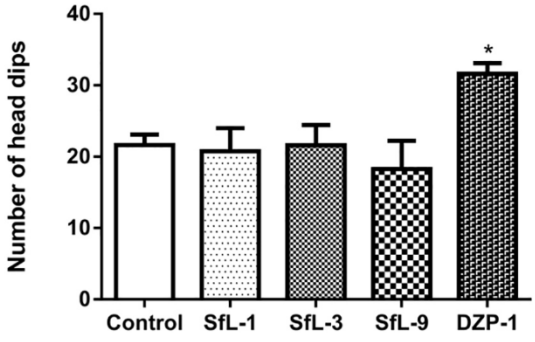

Fig. 7. Effect of the mice acute treatment with SfL in the hole-board test. Male mice (n = 10) were pretreated with SfL (1, 3 or 9 mg/kg; i.v.) or sterile saline (i.v.), as negative control, or DZP (1 mg/kg; i.p.), as positive control. After 30 min, the animals were submitted to the test, and the number of head dips into the holes was counted for 5 min. Each column represents the mean ± S.E.M. Results were analyzed by one-way ANOVA followed by Student–Newman–Keuls as the *post hoc* test. $^{*}P < 0.05$ as compared to control group.

number of head dips, when compared to control [F (4,43) = 3.113, $P = 0.0246$], as shown in Fig. 7.

3.5. Elevated plus-maze test

SfL, at doses of 1, 3 or 9 mg/kg (i.v.) did not alter the NEOA [F (4, 45) = 27.98, $P < 0.0001$] (Fig. 8A), the PEOA [F (4, 45) = 54.53, $P < 0.0001$] (Fig. 8B), the TPOA [F (4, 45) = 26.92, $P < 0.0001$] (Fig. 8C) and the PTOA [F (4, 45) = 25.56, $P < 0.0001$] (Fig. 8D), when compared to control. Differently, the positive control DZP (1 mg/kg; i.p.) increased all these analyzed parameters (Fig. 8).

4. Discussion

The present study was carried out in order to evaluate the antidepressant and anti-anxiety effects of the *Solieria filiformis* lectin (SfL) in mice, by using behavioral tests. Our results revealed that SfL caused a decrease in the immobility time in both TST and FST in mice, an effect consistent with an antidepressant-like action [36,37]. This lectin was devoid of anxiolytic-like activity and did not change the animal's locomotion pattern, since no significant change in the OFT was observed. This study also analyzed some of the possible mechanisms related to the observed antidepressant-like effects. To our knowledge, this is the first report of an antidepressant-like action of a lectin obtained from a marine alga.

Animal models predictive of antidepressant action have been extensively used in the development of novel therapeutic compounds and for understanding the neural substrates underlying depressive-like behavior [38]. These models are typically based on the exposure of animals to a stressful condition (a potential or actual threatening situation) and to a specific test for measuring behavioral responses. The two most widely used animal models for antidepressant screening are TST and FST [32]. Both of these tests are based on the observation that mice, after initial escape-oriented movements, develop an immobile posture when placed in a short-term inescapable, stressful situation. This immobility, which is referred in animals to a behavioral despair, is believed to reproduce a condition similar to human depression [39]. Thus, the reduction in the total duration of the immobility indicates an antidepressant-like effect [40]. Furthermore, both tests are sensitive to all major classes of antidepressant drugs, including tricyclics, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and atypical [31,36,41], although it seems that FST presents a major sensitivity to dopaminergic drugs. In this study, we provide evidence that SfL produces antidepressant-like effect, since this lectin, at doses of 3 and 9 mg/kg, reduced the immobility time of the mice in both TST and FST (Figs. 2 and 3A).

It is known that some drugs with psychostimulant activity may give a false positive effect in the TST and FST [24]. Thus, in order to rule out the possibility that the reduction in the immobility time elicited by SfL is due to an enhancement in the locomotor activity, the OFT was conducted. In this test, SfL, at the tested doses, did not change any parameter used for evaluation. Therefore, these results suggest that the reduction of the immobility time induced by SfL is not due to a psychostimulant action.

The SfL action in the FST was shown to be dependent on the integrity of the protein structure, because high temperature denaturation

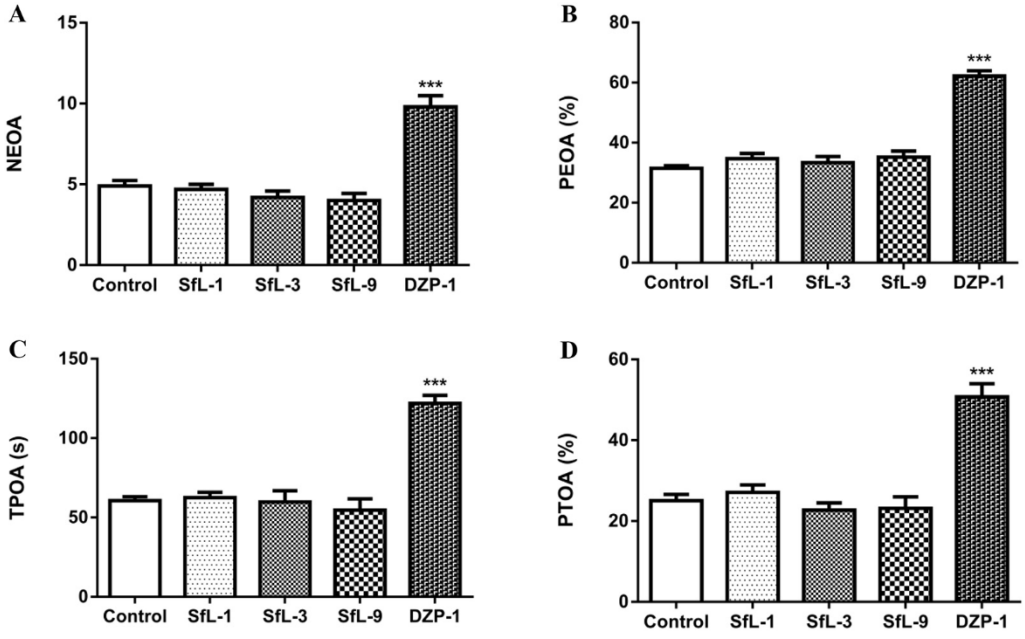

Fig. 8. Effect of the mice acute treatment with SfL in the elevated plus-maze test. Male mice (n = 10) were pretreated with SfL (1, 3 or 9 mg/kg; i.v.) or sterile saline (i.v.), as negative control, or DZP (1 mg/kg; i.p.), as positive control. After 30 min, the animals were submitted to the test, in which were analyzed number of entries in open arms (panel A), percentage of entries into open arms (panel B), time of permanence in open arms (panel C) and percentage of time spent in the open arms (panel D), for 5 min. Results were analyzed by one-way ANOVA followed by Student–Newman–Keuls as the *post hoc* test. $^{***}P < 0.001$ as compared to control group.

blocked its anti-immobility effect (Fig. 3B). Furthermore, another factor analyzed was whether the antidepressant-like effect of SfL, which is a mannan binding lectin [12], is dependent on its sugar-binding site. Indeed, when inhibited by mannan, SfL completely lost its ability to reduce the immobility time of the mice in the FST. Thus, our findings indicate that the interaction of this lectin with the cell surface carbohydrates is probably a fundamental step in its antidepressant-like activity. The ability to bind to carbohydrates and glycoconjugates makes lectins valuable tools in several physiological and pathological events [9]. Sugar chains are found at the cell surface and in extracellular compartments, playing important roles in the regulation of nervous system development, as well as in the synaptic activity and neuroplasticity [42].

As previously mentioned, the monoaminergic system is an important target of antidepressant treatment [43]. Therefore, we investigated the involvement of serotonergic, noradrenergic and dopaminergic systems in the anti-immobility effects of SfL in the FST. The FST shows a strong sensitivity to alterations in monoamine activity and for this reason, this test provides a useful model to study neurobiological mechanisms underlying stress and antidepressant responses, mainly those related to alterations in dopaminergic neurotransmission [37,44].

Initially, the involvement of the noradrenergic system was investigated. For that purpose, prazosin (an α_1-adrenoreceptor antagonist) and yohimbine (an α_2-adrenoreceptor antagonist) [45] were used. In our study, both PRA and YOH did not reverse SfL antidepressant-like effect. However, the effects of the tricyclic antidepressant imipramine were prevented by these antagonists. Tricyclic antidepressants inhibit serotonin and noradrenaline reuptake resulting in an increase in these neurotransmitter levels [46]. These results suggest that SfL anti-immobility effect is not related to the lectin interaction with the α_1 and α_2-adrenoceptors.

Subsequently, we evaluated the involvement of serotonin in the antidepressant-like effect of SfL. To this end, mice were treated along four consecutive days with PCPA, a tryptophan hydroxylase inhibitor. According to previous reports, PCPA is able to successfully deplete the endogenous serotonin store without affecting noradrenaline and dopamine levels [47]. Here we observed that the pretreatment with PCPA did not prevent the SfL anti-immobility effect, but did prevent the effect of fluoxetine, a selective serotonin reuptake inhibitor [48]. Thus, our results suggest that the serotonergic system is not involved in the anti-immobility effect of SfL.

Finally, the dopaminergic system was investigated by using SCH 23390 and sulpiride, which are respectively dopamine D_1 and D_2 receptor antagonists [49]. Our results showed that the dopaminergic system is involved in the antidepressant-like action of SfL, since the pretreatment of mice with SCH 23390 or SUL prevented the antidepressant-like effect evoked by this lectin. Similarly, the pretreatment with SUL or SCH 23390 prevented the reduction in the immobility time caused by bupropion, an antidepressant that inhibits dopamine and norepinephrine reuptake [50]. Dopamine is the most abundant monoamine neurotransmitter in the brain and plays a critical role in the regulation of emotions, mood, motivation, cognition, reward circuits and reinforcement behavior [51]. Dysfunctions in the central dopaminergic neurotransmission have been associated to depression [22,52]. Thus, an increase in dopaminergic neurotransmission might counteract the anhedonia, a core symptom of depression [21,38]. Medications which increase dopamine levels in the brain by inhibiting dopamine reuptake, such as bupropion, or by acting as dopaminergic agonists, such as pramipexole, have proven to be potent antidepressants [53,54]. The investigation of novel antidepressant agents that act on this system is justified to improve outcomes for patients with treatment-resistant and non-remitting depression symptoms [22,24]. It is important to highlight that despite presenting antidepressant-like effects by interfering with dopaminergic neurotransmission, SfL did not cause alterations in the locomotor activity of the animals. This suggests that this lectin may not be related to psychomotor agitation, a common side effect of conventional antidepressant therapy and a main contributor to the early discontinuation of pharmacotherapy.

The results obtained in this study do not allow us to indicate that the action of SfL occurs directly *via* dopaminergic receptors or by increases in dopamine levels in the synaptic cleft, which, in turn, activates these receptors. However, it is known that dopaminergic receptors have, at least, one N-glycosylated domain [55]. In addition, it was observed in the present study that SfL, which is a mannan-binding lectin, loses its antidepressant activity when its lectinic site is inhibited by this carbohydrate. Therefore, we can suggested that the effect of this lectin is related to the interaction of its lectinic site with the N-glycosylated domains of the dopaminergic receptors, although further studies are needed to address this issue. In addition, it is noteworthy that SfL presented antidepressant-like effect similar to classical antidepressants but at lower doses what shows a possible safer profile of action.

Since depression and anxiety are often comorbid psychiatric disorders [56], we tested whether SfL presented anxiolytic-like effect. For this, elevated plus-maze and hole-board tests, which are widely used in the anxiolytic drugs screening [57], were performed. The EPMT is based on the premise that rodents avoid open and closed sites, and, when confined there, show signs of fear, such as immobility, defecation, and urination [58]. Drugs with anxiolytic action increase both the percentage of open arms entrances and the time of permanence in the open arms [57]. Whereas the HBT is an ethological model that evaluates the anxiety-like behavior in rodents, through its exploratory activity, when it is exposed to an unfamiliar environment [59]. Since the SfL did not change any parameters evaluated in EPMT and HBT, it is suggested that this lectin does not present anxiolytic nor anxiogenic effects.

Despite the promising results obtained here, this study presents some limitations such as the route of administration of the lectin and the absence of tests with serotonergic antagonists. Thus, further studies are needed to better elicit the neurochemical mechanisms involved in the SfL antidepressant-like effect to better elucidate its potential as a new therapeutic agent for the treatment of mood disorders.

In conclusion, the present study indicates, for the first time, that the lectin from the red marine alga *Solieria filiformis*, a mannan binding lectin, produces a specific antidepressant-like effect in the TST and FST that are animal models of predictive value for the study of antidepressant activity. Furthermore, this lectin did not change the animal locomotion pattern and was devoid of anxiolytic-like effect. Our findings also indicate that the interaction of SfL with cell surface carbohydrates and the integrity of its three-dimensional structure are, probably, fundamental steps in this antidepressant-like activity. In addition, this work provides evidence that the antidepressant-like effect of this lectin is dependent on the interaction with the dopaminergic system, possibly through D_1- and D_2- receptors.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by Brazilian grants from ConselhoNacional de Pesquisa (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (23038001422/2014-84) and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP). BENEVIDES, N.M.B. is senior investigator of CNPq/Brazil.

References

[1] K. Samarakoon, Y.-J. Jeon, Bio-functionalities of proteins derived from marine algae – a review, Food Res. Int. 48 (2012) 948–960.
[2] F.A.E. Torres, T.G. Passalacqua, A.M.A. Velásquez, R.A. de Souza, P. Colepicolo, M.A.S. Graminha, New drugs with antiprotozoal activity from marine alga: a review, Rev. Bras 24 (2014) 265–276.

[3] K.H.M. Cardozo, T. Guaratini, M.P. Barros, V.R. Falcão, A.P. Tonon, N.P. Lopes, S. Campos, M.A. Torres, A.O. Souza, P. Colepicolo, E. Pinto, Metabolites from algae with economical impact, Comp. Biochem. Physiol. C 146 (2007) 60–78.

[4] H.J. Gabius, S. André, J. Jiménez-Barbero, A. Romero, D. Solís, From lectin structure to functional glycomics: principles of the sugar code, Trends Biochem. Sci. 36 (2011) 298–313.

[5] D.J. Rogers, K. Hori, Marine algal lectins: new developments, Hydrobiology 260 (261) (1993) 589–593.

[6] Y. Sato, K. Morimoto, M. Hirayama, K. Hori, High mannose-specific lectin (KAA-a) from the red alga *Kappaphycus alvarezii* potently inhibits influenza virus infection in a strain-independent manner, Biochem. Biophys. Res. Commun. 405 (2011) 291–296.

[7] R.L.C. Rivanor, H.V. Chaves, D.R. Do Val, A.R. De Freitas, J.C. Lemos, J.A.G. Rodrigues, K.M.A. Pereira, I.W.F. De Araújo, M.M. Bezerra, N.M.B. Benevides, A lectin from the green seaweed *Caulerpa cupressoides* reduces mechanical hyper-nociception and inflammation in the rat temporomandibular joint during zymosan-induced arthritis, Int. Immunopharmacol. 21 (2014) 34–43.

[8] L.M.C.M. Silva, V. Lima, M.L. Holanda, P.G. Pinheiro, J.A.G. Rodrigues, M.E.P. Lima, N.M.B. Benevides, Antinociceptive and anti-inflammatory activities of lectin from marine red alga *Pterocladiella capillacea*, Biol. Pharm. Bull. 33 (2010) 830–835.

[9] E.S.O. Vanderlei, K.K.N.R. Patoilo, N.A. Lima, A.P.S. Lima, J.A.G. Rodrigues, L.M.C.M. Silva, M.E.P. Lima, V. Lima, N.M.B. Benevides, Antinociceptive and anti-inflammatory activities of lectin from the marine green alga *Caulerpa cupressoides*, Int. Immnunopharmacol. 10 (2010) 1113–1118.

[10] Y.F.M.M. Leite, L.M.C.M. Silva, R.C.N. Amorim, E.A. Freire, D.M.M. Jorge, T.B. Grangeiro, N.M.B. Benevides, Purification of a lectin from the marine red alga *Gracilaria ornata* and its effect on the development of the cowpea weevil *Callosobruchus maculates* (Coleoptera: Bruchidae), Biochim. Biophys. Acta 1724 (2005) 137–145.

[11] T.M. Abreu, L.M.C.M. Silva, E.S.O. Vanderlei, C.M.L. Melo, V.R.A. Pereira, N.M.B. Benevides, Cytokine production induced by marine algae lectins in BALB/c mice splenocytes, Protein Pept. Lett. 19 (2012) 975–981.

[12] N.M.B. Benevides, A.M. Leite, A.L.P. Freitas, Atividade hemaglutinante na alga vermelha *Solieria filiformis*, R. Bras. Fisiol. Veg. 8 (1996) 117–122.

[13] T.M. Abreu, N.A. Ribeiro, H.V. Chaves, R.J.B. Jorge, M.M. Bezerra, H.S.A. Monteiro, I.M. Vasconcelos, E.F. Mota, N.M.B. Benevides, Antinociceptive and anti-inflammatory activities of the lectin from marine red alga *Solieria filiformis*, Planta Med. 82 (2016) 596–605.

[14] M.L. Holanda, V.M.M. Melo, L.M.C.M. Silva, R.C.N. Amorim, M.G. Pereira, N.M.B. Benevides, Differential activity of a lectin from *Solieria filiformis* against human pathogenic bacteria, Braz. J. Med. Biol. Res. 38 (2005) 1769–1773.

[15] Y. Ren, C. Zhu, J. Wu, R. Zheng, H. Cao, Comparison between herbal medicine and fluoxetine for depression: a systematic review of randomized controlled trials, Complement, Ther. Med. 23 (2015) 674–684.

[16] WHO, World Health Organization, Mental Health: Action Plan 2013-2020. Geneva, 2013 (48 p).

[17] A.S. Elhwuegi, Central monoamines and their role in major depression, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 28 (2004) 435–451.

[18] P.L. Delgado, Monoamine depletion studies: implications for antidepressant discontinuation syndrome, J. Clin. Psychiatry 67 (2006) 22–26.

[19] A.E. Gonçalves, C. Burger, S.K. Amoah, R. Tolardo, M.W. Biavatti, M.M. De Souza, The antidepressant-like effect of *Hedyosmum brasiliense* and its sesquiterpene lactone, podoandin in mice: evidence for the involvement of adrenergic, dopaminergic and serotonergic systems, Eur. J. Pharmacol. 674 (2012) 307–314.

[20] J. Mahmoudi, M. Farhoudi, M. Talebi, B. Sabermarouf, S. Sadigh-Eteghad, Antidepressant-like effect of modafinil in mice: evidence for the involvement of the dopaminergic neurotransmission, Pharmacol. Rep. 67 (2015) 478–484.

[21] P.S. D'Aquila, M. Collu, G.L. Gessa, G. Serra, The role of dopamine in the mechanism of action of antidepressants drugs, Eur. J. Pharmacol. 405 (2000) 365–373.

[22] B.W. Dunlop, C.B. Nemeroff, The role of dopamine in the pathophysiology of depression, Arch. Gen. Psychiatry 64 (2007) 327–337.

[23] G.I. Papakostas, Dopaminergic-based pharmacotherapies for depression, Eur. Neuropsychopharmacol. 16 (2006) 391–402.

[24] D.G. Machado, V.B. Neis, G.O. Balen, A. Colla, M.P. Cunha, J.B. Dalmarco, M.G. Pizzolatti, R.D. Prediger, A.L.S. Rodrigues, Antidepressant-like effect of ursolic acid isolated from *Rosmarinus officinalis* L. in mice: Evidence for the involvement of the dopaminergic system, Pharmacol. Biochem. Behav. 103 (2012) 204–211.

[25] J. Archer, Tests for emotionality in rats and mice: a review, Anim. Behav. 21 (1973) 205–235.

[26] J.F. Cryan, A. Markou, I. Lucki, Assessing antidepressant activity in rodents: recent developments and future needs, Trends Pharmacol. Sci. 23 (2002) 238–245.

[27] M. Geyer, A. Markou, The role of preclinical models in development of psycotropic drugs, in: K. Davis, D. Charney, J. Coyle, S. Nemeroff (Eds.), Neruropsychopharmacology: The Fifth Generation of Progress, Raven Press, New York 2002, pp. 445–455.

[28] P.V. Holmes, Rodent models of depression: reeaxamining validity without anthropomorphic inference, Crit. Rev. Neurobiol. 15 (2003) 143–174.

[29] P. Willner, P.J. Mitchell, Animal models of depression: a diathesis/stress approach, in: H. D'haenen, J. Den Boer, P. Willner (Eds.), Biol. Psychiatry, Wiley, New York 2002, pp. 703–726.

[30] P.S. Seigel, A simple electronic device for the measurement of gross bodily activity of small animals, J. Psychol. 21 (1946) 227–236.

[31] L. Steru, R. Chermat, B. Thierry, P. Simon, Tail suspension test: a new method for screening antidepressants in mice, Psychopharmacology 85 (1985) 367–370.

[32] C.T.V. Melo, A.M.R. De Carvalho, B.A. Moura, C.P.L. Teixeira, L.F. Vasconcelos, M.L. Feitosa, G.V. De Oliveira, J.M. Barbosa-Filho, M.M.F. Fonteles, S.M.M. Vasconcelos, F.C.F. De Sousa, Evidence for the involvement of the serotonergic, noradrenergic, and dopaminergic systems in the antidepressant-like action of riparin III obtained from *Aniba riparia* (Nees) Mez (Lauraceae) in mice, Fundam. Clin. Pharmacol. 27 (2013) 104–112.

[33] G. Clark, A.G. Koster, D.W. Person, Exploratory behavior in chronic disulfoton poisoning in mice, Psychopharmacology 20 (1971) 169–171.

[34] I. Lucki, K. Rickels, M.A. Giesecke, A. Geller, Differential effects of the anxiolytic drugs, diazepam and buspirone, on memory function, Br. J. Clin. Pharmacol. 23 (1987) 207–211.

[35] R.G. Lister, The use of a plus-maze to measure anxiety in the mouse, Psycopharmacology 29 (1987) 180–185.

[36] R.D. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmacodyn. Ther. 229 (1977) 327–336.

[37] B. Petit-Demouliere, F. Chenu, M. Bourin, Forced swimming test in mice: a review of antidepressant activity, Psychopharmacology 177 (2005) 245–255.

[38] J.C. Capra, M.P. Cunha, D.G. Machado, A.D.E. Zomkowski, B.G. Mendes, A.R.S. Santos, M.G. Pizzolatti, A.L. Rodrigues, Antidepressant-like effect of scopoletin, a coumarin isolated from *Polygala sabulosa* (Polygalaceae) in mice: evidence for the involvement of monoaminergic systems, Eur. J. Pharmacol. 643 (2010) 232–238.

[39] O.D. Can, Ü.D. Özkay, U.I. Üçel, Anti-depressant-like effect of vitexin in BALB/c mice and evidence for the involvement of monoaminergic mechanisms, Eur. J. Pharmacol. 699 (2013) 250–257.

[40] K. Pytka, K. Podkowa, A. Rapacz, A. Podkowa, E. Zmudzka, A. Olczyk, J. Sapa, B. Filipek, The role of serotonergic, adrenergic and dopaminergic receptors in antidepressant-like effect, Pharmacol. Rep. 68 (2016) 263–274.

[41] M.J. Detke, M. Rickels, I. Lucki, Active behavior in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants, Psychopharmacology 121 (1995) 66–72.

[42] T. Endo, Glycans and glycan-binding proteins in brain: galectin-1-induced expression of neurotrophic factors in astocytes, Curr. Drug Targets 6 (2005) 427–436.

[43] S. Porcelli, A. Drago, C. Fabbri, A. Serretti, Mechanisms of antidepressant action: an integrated dopaminergic perspective, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35 (2011) 1532–1543.

[44] S.H. Lin, M.L. Chou, W.C. Chen, Y.S. Lai, K.H. Lu, C.W. Hao, L.Y. Sheen, A medicinal herb, *Melissa officinalis* L. ameliorates depressive-like behavior of rats in the forced swimming test via regulating the serotonergic neurotransmitter, J. Ethnopharmacol. 175 (2015) 266–272.

[45] S.C. Barauna, M.P. Kaster, B.T. Heckert, K.S. Do Nascimento, F.M. Rossi, E.H. Teixeira, B.S. Cavada, A.L.S. Rodrigues, R.B. Leal, Antidepressant-like effect of lectin from *Canavalia brasiliensis* (ConBr) administered centrally in mice, Pharmacol. Biochem. Behav. 85 (2006) 160–169.

[46] P. Rojas, N. Serrano-Garcia, O.N. Medina-Campos, J. Pedraza-Chaverri, S.O. Ögren, C. Rojas, Antidepressant-like effect of a *Ginkgo biloba* extract (EGb761) in the mouse forced swimming test: role of oxidative stress, Neurochem. Int. 59 (2011) 628–636.

[47] C. Girish, V. Raj, J. Arya, S. Balakrishnan, Evidence for the involvement of the monoaminergic system, but not the opioid system in the antidepressant-like activity of ellagic acid in mice, Eur. J. Pharmacol. 682 (2012) 118–125.

[48] D.T. Wong, F.P. Bymaster, E.A. Engleman, Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication, Life Sci. 57 (1995) 411–441.

[49] M. Zheng, Y. Fan, D. Shi, C. Liu, Antidepressant-like effect of flavonoids extracted from *Apocynum venetum* leaves on brain monoamine levels and dopaminergic system, J. Ethnopharmacol. 147 (2013) 108–113.

[50] E. Poleszak, A. Szopa, E. Wyska, S. Wosko, A. Wlaz, M. Pieróg, A. Wróbel, P. Wlaz, The influence of caffeine on the activity of moclobemide, venlafaxine, bupropion and milnacipran in the forced swim test in mice, Life Sci. 136 (2015) 13–18.

[51] Z. Li, Y. He, J. Tang, X. Zong, M. Hu, X. Chen, Molecular imaging of striatal dopamine transporters in major depression - a meta-analysis, J. Affect. Disord. 174 (2015) 137–143.

[52] R. Moraga-Amaro, H. Gonzalez, R. Pacheco, J. Stehberg, Dopamine receptor D3 deficiency results in chronic depression and anxiety, Behav. Brain Res. 274 (2014) 186–193.

[53] G. Camardese, D. Di Giuda, M. Di Nicola, F. Cocciolillo, A. Giordano, L. Janiri, R. Guglielmo, Imaging studies on dopamine transporter and depression: a review of literature and suggestions for future research, J. Psychiatr. Res. 51 (2014) 7–18.

[54] L. Lattanzi, L. Dell'Osso, P. Cassano, S. Pini, P. Rucci, P.R. Houck, A. Gemignani, G. Battistini, A. Bassi, M. Abelli, G.B. Cassaso, Pramipexole in treatment-resistant depression: a 16-week naturalistic study, Bipolar Disord. 4 (2002) 307–314.

[55] RCSB PDB, RCSB Protein Data Bank, http://www.rcsb.org/pdb/home/home.do 2016, Accessed date: 24 January 2016.

[56] C. Tunvirachaisakul, R.L. Gould, M.C. Coulson, E.V. Ward, G. Reynolds, R.L. Gathercole, H. Grocott, T. Supasitthumrong, A. Tunvirachaisakul, K. Kimona, R.J. Howard, Predictors of treatment outcome in depression in later life: a systematic review and meta-analysis, J. Affect. Disord. 227 (2017) 164–182.

[57] D. Gupta, M. Radhakrishnan, Y. Kurhe, Anxiolytic-like effects of alverine citrate in experimental mouse models of anxiety, Eur. J. Pharmacol. 742 (2014) 94–101.

[58] A.A. Walf, C.A. Frye, The use of the elevated plus-maze as an assay of anxiety-related behavior in rodents, Nat. Protoc. 2 (2007) 322–328.

[59] R.G. Lister, Ethologically-based animal models of anxiety disorders, Pharmacol. Therapeut. 46 (1990) 321–340.