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Myricitrin exhibits antidepressant-like effects and reduces IL-6 hippocampal levels in the chronic mild stress model

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ABSTRACT

The flavonoid myricitrin showed an antidepressant-like effect in the tail suspension test and increased hippocampal neurogenesis, as well as demonstrating anti-inflammatory effects. Interestingly, inflammation has been linked to depression, and anti-inflammatory drugs showed promising results as antidepressant-like drugs. Thus, the present study evaluated the effects of myricitrin in the chronic mild stress (CMS) model, a translational and valid animal model of depression, using the mini-experiment design to improve the reproducibility of the findings. The sucrose preference test (SPT), forced swim test (FST), and tail suspension test (TST) were the readouts of depressive-like phenotypes induced by CMS. Relative adrenal weight was employed as an index of the hypothalamus–pituitary–adrenal (HPA) axis activation. Interleukin (IL)−6 and tumor necrosis factor (TNF)-alpha levels were measured in the hippocampus. Myricitrin (10 mg/kg, intraperitoneally, for 14 days) reversed depressive-like behaviors induced by CMS (increased immobility in the FST, the TST and anhedonia), as well as decreased adrenal hypertrophy and hippocampal levels of IL-6 in stressed mice. Similar results were observed by imipramine (20 mg/kg, intraperitoneally, for 14 days), a serotonin and norepinephrine reuptake inhibitor (positive control). A significant correlation was observed between immobility time in the TST, and hippocampal IL-6 levels. Hippocampal TNF-α levels were not affected by CMS or drug treatment. In conclusion, myricitrin exhibited an antidepressant-like profile in CMS, and this effect may be associated with its anti-inflammatory activity.

1. Introduction

Major depression is a heterogeneous and complex disorder with a high prevalence and recurrence rate, marked by worsening in subsequent episodes [1]. Up to 60% of the patients with major depression can present some resistance to treatment, and monoaminergic antidepressants are not effective in about 15% of the patients [1,2]. Therefore, the development of new antidepressant drugs is necessary.

Different lines of evidence indicate a relationship between inflammation and depression [3–5]. For example, depressive patients present higher levels of pro-inflammatory cytokines [4,6]. Moreover, these dysfunctions might be related to the hypothalamic-pituitary-adrenal axis (HPA axis) hyperactivation [1,4,5]. In this line, prolonged and/or repeated stress exposure would lead to HPA dysfunction with increasing

Abbreviations: CMS, chronic mild stress; FST, forced swimming test; OFT, open-field test; TST, tail suspension test; SPT, sucrose preference test.

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high, long-lasting levels of corticosteroids, decreasing the negative cortisol feedback loop and making the HPA axis unresponsive to an increase of glucocorticoids [1,4,5]. Reports from clinical and pre-clinical research showed an association between the increase in interleukin 1 (IL-1), interleukin 6 (IL-6), and TNF-α and depression [1,4,5,7]. Moreover, antidepressant drugs show anti-inflammatory effects and reduced peripheral levels of pro-inflammatory cytokines (e.g., IL-6), while anti-inflammatory medications exhibited clinical antidepressant effects [4,5,8]. In turn, the rise of pro-inflammatory cytokines would shift the tryptophan pathway towards the kynurenic pathway, thereby decreasing levels of serotonin and increasing oxidative stress, due to the production of neuroactive compounds (e.g., 3-hydroxykynurenine or 3-HK) [1,3,5].

Based on the hypothesis that compounds with anti-inflammatory effects are good candidates for the development of new antidepressants, myricitrin (myricetin 3-O-alpha-L-rhamnoside), a flavonoid with anti-inflammatory and antioxidant effects, seems to be an interesting compound as a potential drug for the treatment of depression [9–11]. Myricitrin exhibited anxiolytic and antipsychotic-like effects in animal models [12,13]. Regarding its antidepressant effect, Meyer and coworkers [11] showed a decrease in immobility and an increase in neurogenesis induced by repeated myricitrin administration to naïve mice submitted to the tail suspension test. This initial exploratory study should be followed by confirmatory studies evaluating myricitrin in an animal model that captures several facets of clinical depression which has good translational value, allowing the survey its putative mechanisms of action. The chronic unpredictable mild-stress (CMS) model of depression reproduces several features of depression on behavior (e.g., anhedonia), physiology (hypothalamic-pituitary-adrenal axis activation), and neurochemistry (e.g., changes in monoaminergic neurotransmission and an increase of pro-inflammatory cytokines) [14–16]. The CMS has been considered a valuable component of antidepressant drug discovery protocols. It has contributed to studying the psychobiological processes related to depression and evaluating antidepressant-like effects of drugs [14,15].

In biomedical research, there is a growing concern with reproducibility and the “mini-experiments” design, which split the study subjects into different cohorts tested a few weeks apart, which is proposed to increase reproducibility [17]. This design would introduce a systematic and controlled heterogeneity, reducing the risk of non-reproducible findings in single laboratory studies [17].

Therefore, the objective of the present study was to assess the antidepressant-like effects of myricitrin in the forced swim test, tail suspension test, and sucrose preference test of mice submitted to CMS. The mini-experiment design (three different cohorts of subjects) was used to reduce bias and increase the reproducibility of the results. Moreover, in one cohort, the effects of myricitrin on IL-6 and TNF-α (pro-inflammatory markers) in the hippocampus and the relative adrenal weight were also evaluated in mice submitted to CMS.

2. Methods

2.1. Animals

Adult male albino Swiss Webster mice (60–90 days old, 30–45 g) from our breeding stock were used (n = 115 mice). One week before the experiments, the mice were housed individually in polypropylene cages (15.2×26×12 cm) with wood shavings as bedding. They were kept in a controlled environment of 12/12 h light/dark cycle (lights on at 7:00 AM) and controlled temperature (22 °C) with free access to water and food throughout the experiment – except during some stressors of CMS procedure. All mice were handled when necessary for animal care (e.g., cleaning the cages) and treatment (e.g., weighing, tail marking, and drug administration).

2.2. Ethics

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the National Council for Control of Animal Experimentation (CONCEA, Brazil). The Institutional Committee on the Ethical Use of Animals of the Federal University of Paraná (CEUA) approved the protocol (CEUA certificate number 385). All efforts were made to minimize the suffering of mice and to reduce the number of mice utilized for the experiments.

2.3. Drugs

Imipramine (20 mg/kg; dissolved in distilled water), used as a positive control, myricitrin (10 mg/kg; dissolved in tween 80 plus saline, 2% v/v), or vehicle (control group) were administered intraperitoneally (i.p.). Myricitrin (purity of >98% - determined by high-performance liquid chromatography by the Department of Chemistry, Federal University of Santa Catarina) was extracted from the leaves of *Eugenia uniflora* [9,13]. Imipramine was purchased from Sigma-Aldrich (Saint Louis, USA). All treatments were administered in a constant volume of 10 ml/kg of body weight and the doses were based on previous studies [11,13,18].

2.4. Experimental design

Based on the results of the first sucrose preference test (SPT; Fig. 1), mice were divided by stratified randomization into two experimental groups: control and CMS. The mice in the CMS group were submitted to repeated unpredictable mild stress for 5 weeks, while mice on the control group were left undisturbed in their home-cage. Using SPT results in week 5, the mice were distributed in treatment groups according to stratified randomization. Then they were treated with vehicle, myricitrin, or imipramine for 2 weeks (daily, between 9 and 11 AM), and the sucrose preference test was evaluated weekly. On weeks 5, 6, and 7 the mice were also submitted to the forced swim test (FST) and on weeks 5 and 7 to the open-field test (cohort 1). A second cohort (cohort 2) of mice were submitted to the same experimental protocol except that the FST was replaced by the tail suspension test (TST) and no test was conducted at week 6 of CMS (since the results from cohort 1 showed robust results after 2 weeks of treatment). A third cohort (cohort 3) was submitted only to the sucrose preference test, since performing the TST or the FST could be considered as significant stress, and they could influence the SPT results. The experiments in all cohorts are outlined in Fig. 1. For the balanced subjects’ allocation baseline SPT and SPT after 5 weeks of CMS were used for stratified randomization using a table of random numbers [19]. The primary behavioral outcome of CMS was the sucrose preference in the SPT, which was measured in all cohorts. The mice were euthanized by deep anesthesia (isoflurane) followed by the immediate decapitation after the experiments (on cohort 2, no anesthesia was used to avoid any interference in IL-6 and TNF-α measurements).

2.5. Behavioral tests

2.5.1. Chronic mild stress procedure

CMS consisted of a variety of mild stressors delivered daily using a pseudo-randomized sequence and was initiated at different times to avoid habituation to the stressors. The CMS battery was composed of 45 degrees of tilt box, exposure to constant light or darkness (maximum of 24 h of exposure), water or food deprivation (maximum of 18 h of duration, avoiding it on the day before the SPT), and wet bedding (maximum of 24 h of exposure). The animals were exposed to a SPT on a weekly basis to evaluate the development of anhedonic-like behavior, which was defined as a reduction in sucrose preference [18].

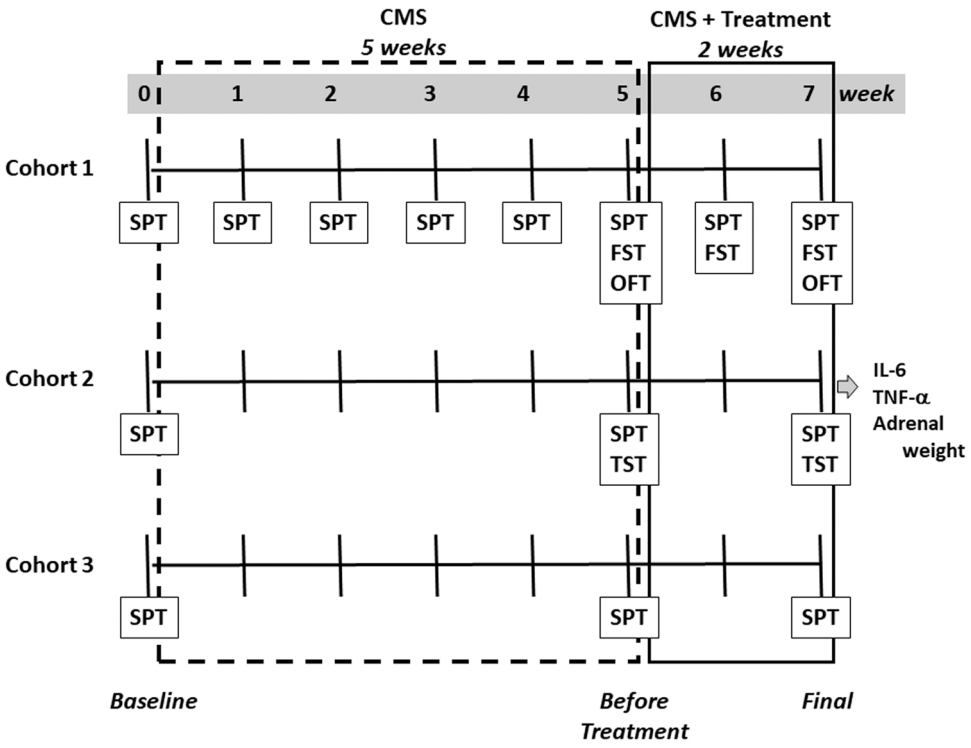

Fig. 1. Experimental protocol for the 3 cohorts of mice. CMS - chronic mild stress [stressors: 45° tilt box, 24 h exposure to constant light or darkness; water or food deprivation (18 h); wet bedding (24 h)]. SPT: sucrose preference test; FST: forced swimming test; TST: tail suspension test; OFT: open-field test. Treatments: vehicle, myricitrin (10 mg/kg, ip) and imipramine (20 mg/kg, ip).

2.5.2. Sucrose preference test

The anhedonic-like behavior was evaluated using the SPT conducted as previously described [20]. The animals could choose between two bottles (one containing water and the other with a sucrose solution – 1% w/v) while having free access to food. Food/ water deprivation did not occur immediately before the SPT. The test lasted 24 h. The bottles were weighed before and after the test, and the sucrose preference was calculated using the following equation:

% of sucrose preference = consumption of sucrose x 100 / [consumption of water + consumption of sucrose]

Sucrose preference was measured in three cohorts of mice.

2.5.3. Locomotor activity (open field test)

In the OFT, the mice were allowed to explore a circular arena for 5 min. The apparatus consisted of a white circular arena (40 cm diameter and 28 cm height) with black lines on the floor forming three concentric circles with perpendicular lines forming 25 areas. The illumination on the floor was approximately 40 lux. The number of zones crossed by the mice with its 4 paws, was recorded and was used as an indicator of locomotor activity. The arena was wiped with a water-alcohol (10%) solution before each behavioral test. Locomotor activity was measured in cohort 1.

2.5.4. Forced swim test

In the FST, the time of immobility (measured in seconds) of the mice is taken to indicate depressive-like behavior. Mice were judged immobile when they stopped swimming and performed minimal movements to stay afloat with their heads above the water level [21,22]. Treatment with antidepressants should decrease the immobility time without a stimulant effect on locomotor activity. The FST apparatus consisted of a glass cylinder (12.5 cm diameter and 25 cm height) filled with 19 cm of water at 24 ± 2 °C. The test duration was 6 min, and the immobility time was recorded during the last 4 min of the test. The water was changed between animals. After the test, the mice were dried and placed in a warm environment for 30 min to recover before being placed back in their individual cages at the animal facility. The FST was employed in cohort 1.

2.5.5. Tail suspension test

In the TST, the immobility time is also related to a depressive-like phenotype. Treatment with antidepressants reduced this behavior at a dose that did not increase locomotor activity [11,23,24]. Immobility is considered when the mice were hanging passively and motionless. In this test, mice were suspended 50 cm above the floor with adhesive tape placed approximately 1 cm from the tip of the tail, and immobility was measured during the last 4 min of the test. The TST was employed in cohort 2.

2.5.6. Relative adrenal gland weight

The relative weight of the adrenal gland was used as an indirect measure of the HPA-axis activity since the high activity in this axis will induce hypertrophy of the gland [25]. After euthanasia, the adrenal glands were dissected, and the relative weight was calculated with the following equation:

Relative adrenal weight = adrenal weight/animal weight

The mice used in this measurement were from cohort 2.

2.5.7. Levels of pro-inflammatory markers

After euthanasia, brains were removed and the hippocampus was immediately dissected and homogenized with a PBS buffer containing 0.05% Tween 20, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA, and 20 IU aprotinin A. The homogenates were centrifuged at 3000g for 10 min, and the supernatants were stored at − 70 °C until assays for the determination of levels of TNF-α and IL-6. TNF-α levels were measured using mouse TNF-α DY410 (R&D Systems, Inc. - Minneapolis, MN) and IL-6 levels were measured using

Mouse IL-6 Elisa Standard Sets (BioLegend – San Diego, CA). These samples were collected from cohort 2.

2.6. Statistical analysis

Data at week 5 were analyzed by the Student t-test to evaluate the impact of CMS. Four animals did not develop anhedonia (reduction in sucrose preference) after five weeks of stress, and they were excluded from further experiments (they did not enter the treatment phase). Data from week 7 of the SPT were analyzed first by three-way ANOVA with cohort (1, 2, and 3), treatment (vehicle, imipramine, or myricitrin), and CMS (unstressed and stressed) as between factors. Since the cohort factor did not show an interaction with drug treatment and CMS (table S2 and Fig. 1S), the data of the three cohorts were merged in one data file that was analyzed by two-way ANOVA with treatments (vehicle, imipramine, or myricitrin) and CMS (unstressed and stressed) as between factors. The Newman-Keuls *posthoc* test was applied for all between-group comparisons. The FST and TST data were also analyzed by two-way ANOVA followed by the Newman-Keuls *posthoc* test. Since IL-6 and locomotor activity data showed heteroscedasticity, these data were log-transformed [ln(raw data+1)] before statistical analysis. Finally, the correlation between IL-6 (log-transformed) and the immobility time in the TST was evaluated using the Pearson correlation coefficient. The software Statistica 7.0 (Statsoft, Tulsa, USA) was used and significance was set at $p < 0.05$.

3. Results

3.1. Induction of depressive-like behavior by chronic mild stress (CMS) on the sucrose preference test (SPT)

Five weeks of CMS exposure induced a statistically significant reduction in sucrose preference (t_{113} =13.31; $p < 0.001$; table S1). Regarding treatment, two-way ANOVA showed a significant effect for CMS ($F_{1,109} = 35.79$; $p < 0.001$), treatment ($F_{2,109} = 27.98$; $p < 0.001$) and interaction between factors ($F_{2,109} = 32.53$; $p < 0.001$). After 14 days of treatment, imipramine and myricitrin reversed the anhedonia induced by CMS observed in the vehicle-treated group (Fig. 2A).

As described in methods, these data are from 3 different cohorts of mice merged for the SPT data analysis. The same result pattern and statistical data observed with whole data were found in each cohort separately (Table S1, Fig. S1). No effect was observed for the cohort factor (Table S2).

3.2. Depressive-like behavior measured by the forced swim test (FST) or the tail suspension test (TST)

In the FST, two-way ANOVA showed a significant effect for CMS ($F_{1,15} = 37.22$; $p < 0.001$), treatment ($F_{2,25}$ =35.48; $p < 0.001$) and interaction between factors ($F_{2,25} = 35.88$; $p < 0.001$). CMS increased immobility time and myricitrin and imipramine reversed this increase (Fig. 2B).

The analysis of TST showed significance for CMS ($F_{1,36} = 67.17$; $p < 0.001$), treatment ($F_{2,36} = 23.49$; $p < 0.001$), and interaction between factors ($F_{2,36} = 28.87$; $p < 0.001$). Again, CMS increased immobility time and 2 weeks of imipramine and myricitrin administration reversed it (Fig. 2C).

3.3. Locomotor activity (OFT)

After 5 weeks of CMS, no effect was seen on the number of squares crossed in the OFT ($t_{31} = -1.070$, NS): unstressed: 236 ± 16; CMS: 290 ± 51 (mean ± SEM; n = 14–17 mice/ group). Treatment with myricitrin and imipramine for 2 weeks did not change locomotor activity (CMS: $F_{1,25} = 0.001$; NS; treatment: $F_{2,25} = 0.029$; NS; or interaction: $F_{2,25}$ =2.971; NS): unstressed + vehicle: 216 ± 26; unstressed + myricitrin: 197 ± 32; unstressed + imipramine: 151 ± 10; CMS + vehicle: 154 ± 18; CMS + myricitrin: 176 ± 11; CMS + imipramine: 271 ± 97 (mean ± SEM of the number of squares crossed; n = 4–6 mice/ group).

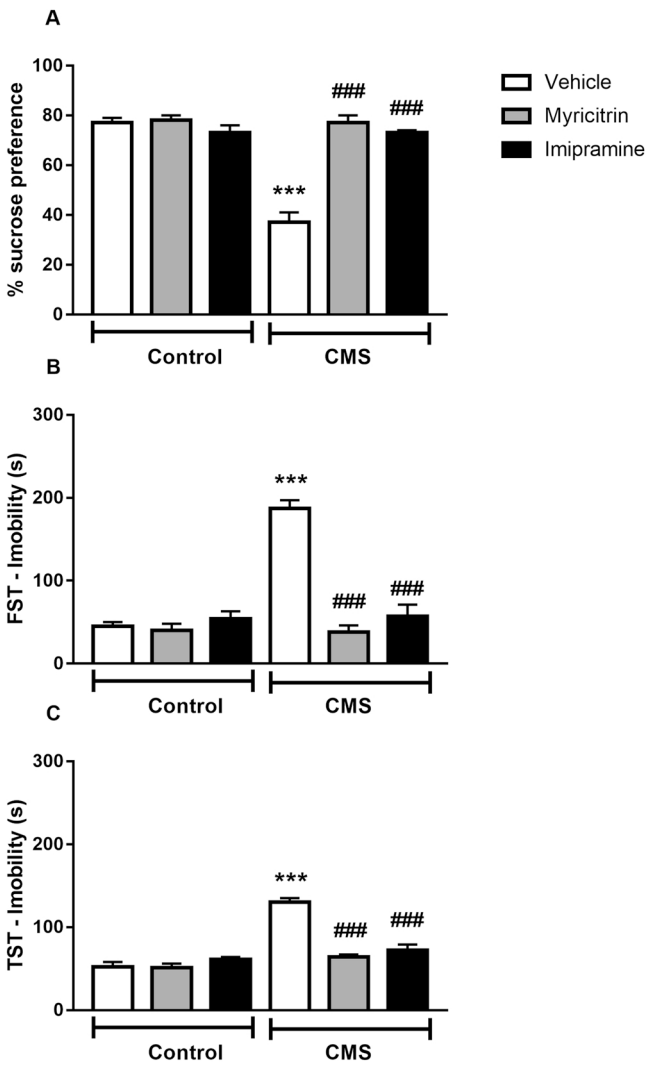

Fig. 2. Behavioral effects of repeated administration (14 days) of myricitrin and imipramine in mice submitted to chronic mild stress (CMS). (A) Anhedonia-induced by CMS measured by the sucrose preference test (SPT, measured as % of sucrose preference); (B) Immobility time (s) in the Forced Swimming Test (FST); (C) Immobility time (s) in the Tail Suspension Test (TST). Myricitrin (10 mg/kg, IP), imipramine (20 mg/kg, IP). Drug treatment started following 5 weeks of stress and it was administered daily for 2 weeks. Data represent mean + SEM (SPT: n = 18–20/ group; FST: n = 5–6/ group; TST: n = 7/ group). *** $p < 0.001$ compared to control non-stressed; ### $p < 0.001$ compared to stressed mice treated with vehicle.

3.4. Relative adrenal weight

On relative adrenal weight, ANOVA showed a significant effect of CMS ($F_{1,36} = 5.85$; $p < 0.05$), treatment ($F_{2,36} = 20.51$; $p < 0.001$) and interaction between factors ($F_{2,36} = 13.89$; $p < 0.001$) (Fig. 3A). Both imipramine and myricitrin-treated CMS animals presented lower relative adrenal weights when compared to the vehicle-treated stressed mice. This latter group also showed increased relative adrenal weights compared to the non-stressed vehicle mice.

3.5. Levels of pro-inflammatory markers

On hippocampal level of IL-6 (log transformed), two-way ANOVA

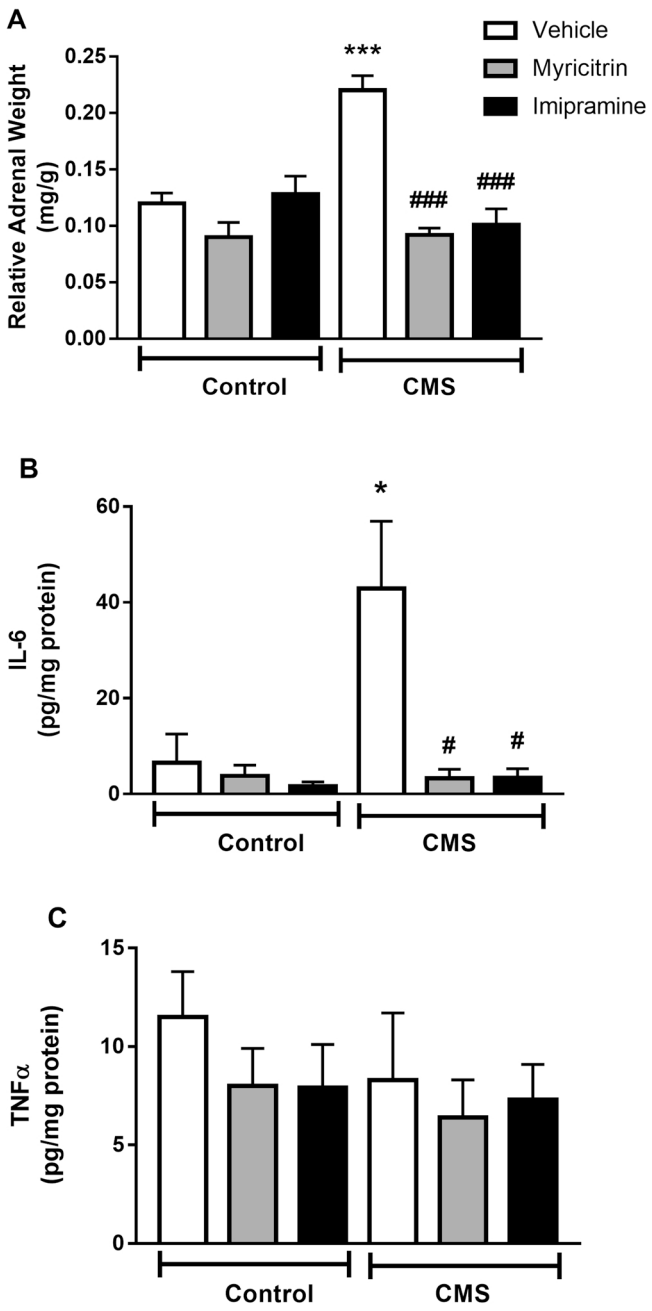

Fig. 3. Neurochemical and physiological effects of repeated administration (14 days) of myricitrin and imipramine in mice submitted to chronic mild stress (CMS). (A) Relative adrenal gland weight (adrenal/ body weight); (B) Hippocampal interleukine-6 (IL-6) levels (pg/mg protein); (C) Hippocampal tumor necrosis factor α (TNFα) level (pg/mg protein). Myricitrin (10 mg/kg, IP), imipramine (20 mg/kg, IP). Drug treatment started following 5 weeks of stress and it was administered for 2 weeks. Data represent mean + SEM (relative adrenal weight: n = 7/group; IL-6: n = 7/group; TNFα= 6–7 group). * $p < 0.05$ and *** $p < 0.001$ compared to control non-stressed; $^{\#}p < 0.05$ $^{\#\#\#}p < 0.01$ compared to stressed mice treated with vehicle.

showed a significant effect for interaction between CMS and treatment factors ($F_{2,32} = 3.727$; $p < 0.05$) but not for CMS ($F_{1,32} = 3.628$; $p = 0.065$) or treatment ($F_{2,32} = 3.124$; $p = 0.057$) factors. CMS induced a significant increase in IL-6 levels in the vehicle treated mice, which was reversed by myricitrin and imipramine treatments (Fig. 3B). No significant changes were observed in the levels of TNF-α in the hippocampus (CMS: $F_{2,34} = 0.90$, NS; Treatment: $F_{1,34} = 0.78$, NS; Interaction: $F_{2,34} = 0.16$, NS; Fig. 3C).

3.6. Correlations

A significant correlation was observed between the time of immobility in the TST and IL-6 ($r = 0.54$; $p < 0.001$; Fig. S3).

4. Discussion

Repeated myricitrin administration reversed depressive-like behaviors induced by CMS (anhedonia and increased immobility in the FST and TST). Myricitrin also blocked IL-6 and the relative adrenal gland weight increases induced by CMS. Thus, myricitrin exhibited antidepressant-like effects in several features of depression induced by the CMS model. Moreover, a significant correlation between immobility in the TST and IL-6 was observed, which suggests an association of myricitrin antidepressant-like effects with its anti-inflammatory activity.

One of the main objectives of the present study was to evaluate the antidepressant-like effects of myricitrin in the CMS model using a translational and reproducible experimental design, moving from an initial exploratory study [11] to confirmatory studies [26]. The CMS model presents a good face, construct, and predictive validity [14,15]. It is frequently used to induce depressive-like behaviors (e.g., anhedonia) and physiological changes (HPA axis activation and an increase in pro-inflammatory cytokines) as observed in clinical depression [14–16]. Moreover, the CMS procedure mimics the time course of clinical depression development and its remission after successful treatment: it takes weeks to induce depressive-like behaviors, and it is reversible by repeated (but not by single) administration of standard antidepressant treatments [15,18]. These characteristics make CMS an excellent procedure to study the pathophysiology of depression and its reversion by antidepressant drugs, providing it with high translational potential [14,15]. Furthermore, CMS is considered a reliable model for the study of neuroinflammation in depression [27,28]. In the present study, mice exposed to CMS presented depressive-like behaviors characterized by low sucrose preference (anhedonia) and an increase in immobility behavior in the FST and TST. CMS also activated the HPA axis, indicated by adrenal gland hypertrophy. Moreover, stressed mice also showed an increased level of IL-6 in the hippocampus. Thus, the procedure used in the present study was able to reproduce several alterations found in depression.

Fourteen days of treatment with myricitrin and imipramine (serotonin and norepinephrine reuptake inhibitor) reversed depressive-like behaviors induced by CMS. Importantly, this effect of myricitrin was seen at a dose that did not alter locomotor activity in the open field test. This antidepressant-like effect was seen in three different cohorts of mice using three different readouts of depressive-like behaviors (the sucrose´ preference, forced swim test, and tail suspension test), indicating its reproducibility and consistency. Thus, the initial findings of Meyer and coworkers [11] employing naïve mice in the FST were corroborated here in three different cohorts of mice using a translational model of depression. These drug treatments were started after the establishment of anhedonia, which helped to distinguish between an antidepressant-like effect, and an anxiolytic-like effect [15]. These data reinforce the putative antidepressant-like effects of myricitrin.

In the present study, with regards to reproducibility, the sample was split into 3 cohorts (“mini-experiment” design), a strategy proposed to introduce a systematic and controlled heterogeneity and to reduce the risk of non-reproducible findings in single laboratory studies [17]. Here, this mini-experiment design was done evaluating different cohorts of mice some weeks apart and included slight differences in the experimental design in the cohorts (for example, one cohort was conducted without the FST or TST). Thus, this approach gives robustness to the results suggesting an antidepressant-like effect of myricitrin. Moreover, this method helps to control the influence of a variety of different tests on the same subject. In addition, methods were also included to reduce bias (e.g., randomization and baseline characterization), which would

increase its reproducibility and translation [29–31]. In this line, the stratified randomization using a relevant baseline characteristic (sucrose preference) would improve the balance of the mice's assignment to the treatment groups [32].

Although the main endpoints of the present study are the behavioral parameters (primary: sucrose preference; secondary: immobility in the FST and TST), two physiological parameters were also analyzed: cytokines levels in the hippocampus and the relative adrenal gland weight. Depression has been linked to neuroinflammation, and the inflammatory process can be a target for new antidepressant drugs [3–5,7]. It had been reported that there are elevated levels of TNF-α and IL-6 in samples from depressive patients, as well as the effectiveness of antidepressants (including imipramine) on reducing them [4,6,8,33]. IL-6 is a pro-inflammatory cytokine, and it is proposed to play an essential role in stress and depressive disorders [6,28,34]. For example, meta-analyses showed that antidepressants decreased peripheral levels of IL-6 in patients with major depressive disorder [6,8]. Moreover, different treatment modalities (e.g., drugs, electroconvulsive therapy, exercise) affected IL-6 levels. [34]. In this line, Sakić et al. [35] observed a decrease in sucrose preference by increasing IL-6 expression in non-stressed animals. This is consistent with the proposal that inflammatory mediators are linked to anhedonia [33]. Pre-clinical studies targeting IL-6 (e.g., IL-6 antagonists) showed potential antidepressant effects, and clinical studies reinforce this proposal [4,6]. In the present study, CMS increased IL-6 levels in the hippocampus, and repeated administration of myricitrin and imipramine blocked this increase. In this line, CMS reduced sucrose preference and increased IL-6 levels in the hippocampus, which were blocked by the administration of fluoxetine [28,36]. This anti-inflammatory effect of myricitrin can be related to down-regulation of NF-κB and MAPK signaling pathways, since it is observed that myricitrin inhibits TLR4/MyD88 expression induced by LPS [37]. Interestingly, TLR4 gene expression increase in the hippocampus has been associated with depressive-like behaviors [38].

Prolonged HPA activation has also been linked to depression. Its normalization has been proposed to contribute to the antidepressant effect of drugs [3,4]. As expected, CMS caused hypertrophy of the adrenal gland [25,39], which was not observed in the groups treated with myricitrin or imipramine. This effect was also seen with the administration of fluoxetine in mice submitted to CMS [25]. The relative adrenal weight measurement is an interesting indirect index of the HPA axis activity since it is affected only after repeated stress (e.g., [40]). However, the absence of plasma corticosterone measurement could be a potential limitation of the HPA axis evaluation in the present study. As reviewed above, the HPA axis, stress, and neuroinflammation are associated with depression, and they are probably mutually interconnected. For example, a persistent inflammatory status contributes to hyperactivity of the HPA and glucocorticoid resistance [3,5,7]. HPA dysfunction is correlated with high LPS-induced secretion of IL-6 from peripheral blood mononuclear cells in patients with major depression [41]. Thus, hyperactivation of the HPA axis and increased immune response can form a positive feedback loop, which can play a significant role in the development of depression. In the present study, a significant correlation was observed between the immobility in the TST and the hippocampal levels of IL-6, thus, suggesting an association between the coping behavior to stress and inflammatory status. Consequently, breaking this positive feedback loop acting on one or more of these processes can be relevant for depression treatment, and the antidepressant-like effects of myricitrin can be related to these targets (IL-6 and the HPA axis). Interestingly, neuroinflammation and HPA activation can decrease neurogenesis [5], and myricitrin increased neurogenesis [11]. Moreover, increasing neurogenesis can attenuate anhedonia and HPA changes induced by CMS [42]. Unfortunately, there is no study evaluating the direct molecular effect of myricitrin on HPA axis activity.

Interestingly, myricetin also showed an antidepressant-like effect in mice submitted to repeated restraint stress [43]. Myricetin, similarly to myricitrin (myricetin 3-O-alpha-L-rhamnoside), reduced immobility in the FST and TST in the stressed mice and improved antioxidant activity. Moreover, both compounds act on hippocampal neuroplasticity since myricitrin increases neurogenesis while myricetin reverses stress-induced BDNF decrease [11,43].

It is worth mentioning that the absence of the anti-immobility effect of myricitrin and imipramine in unstressed mice was an unexpected finding, since previous studies observed that these drugs reduced immobility time in naïve mice [11]. However, this pattern was not new. For example, Mutlu et al. [44] observed that the repeated administration of fluoxetine (35 days) reduced the immobility time in the TST in the stressed mice only. On the other hand, Zhao et al. [45] observed that the repeated administration of fluoxetine (4 weeks) reduced the immobility in the FST time only in the stressed mice, while this drug reduced the immobility time in the TST both in the stressed and unstressed mice. Pesarico et al. [46] also observed an antidepressant-like effect of 7-fluoro-1,3-diphenylisoquinoline-1-amine only in the stressed mice, even though they previously published data demonstrating the antidepressant-like effects of the same compound in stress-naïve mice [47]. Thus, studies focusing on this intriguing aspect of CMS are clearly needed.

In conclusion, myricitrin reversed behavioral and physiological changes induced by chronic mild stress, a translational model of depression. Moreover, myricitrin may act through decreasing neuroinflammation and HPA axis hyperactivity.

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CRediT authorship contribution statement

Marcela Pereira and Roberto Andreatini designed the study and wrote the protocol; Marcela Pereira, Roberto Andreatini, and Alexandra Acco wrote the first draft of the manuscript, Marcela Pereira, Diego Correia, and Isadora P Siba conducted the experiments, Fernanda R Lapa, and Adair RS Santos performed the biochemical analysis, Ana P Ruani and Moacir G Pizzolatti isolated and analyzed the myricitrin sample used. All authors contributed to previous versions of the manuscript and have approved its final version.

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Declaration of interest

All authors declare that they have no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2022.113905.

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