Journal of Integrative Neuroscience

J. Integr. Neurosci. **2024**; 23(3): 51
https://doi.org/10.31083/j.jin2303051

Original Research

Involvement of the $GABA_A$ Receptor in the Antidepressant-Like Effects Produced by Low and High Doses of the Flavonoid Chrysin in the Rat: A Longitudinal Study

Gabriel Guillén-Ruiz[1,2,3,*,†], Blandina Bernal-Morales[2,3,*], Ana Karen Limón-Vázquez[3,4], Oscar Jerónimo Olmos-Vázquez[5], Juan Francisco Rodríguez-Landa[2,3,†]

[1]Researchers Program by Mexico, CONAHCYT –Neuroethology Institute, Universidad Veracruzana, 91190 Xalapa, Veracruz, Mexico
[2]Neuropharmacology Laboratory, Neuroethology Institute, Universidad Veracruzana, 91190 Xalapa, Veracruz, Mexico
[3]Faculty of Pharmaceutical and Biological Chemistry, Universidad Veracruzana, 91000 Xalapa, Veracruz, Mexico
[4]PhD Program in Neuroethology, Neuroethology Institute, Universidad Veracruzana, 91190 Xalapa, Veracruz, Mexico
[5]Faculty of Pharmaceutical and Biological Chemistry, Inter-American University Center, 94470 Cordoba, Veracruz, Mexico
*Correspondence: gguillen@uv.mx (Gabriel Guillén-Ruiz); bbernal@uv.mx (Blandina Bernal-Morales)
[†]These authors contributed equally.

Academic Editors: Domenico De Berardis and Gernot Riedel
Submitted: 1 September 2023 Revised: 7 November 2023 Accepted: 8 December 2023 Published: 4 March 2024

Abstract

Background: The flavonoid chrysin produces rapid and long-lasting anxiolytic- and antidepressant-like effects in rats. However, it is not known whether low and high doses of chrysin produce differential anti-immobility effects through the Gamma-Aminobutyric Acid subtype A ($GABA_A$) receptor. The goal of this work was therefore to compare low and high doses of chrysin for their effects on depression-like behavior in a longitudinal study. Moreover, chrysin was compared with the serotonergic fluoxetine and Gamma-Aminobutyric Acid (GABA)ergic allopregnanolone, and its involvement with the $GABA_A$ receptor after chronic treatment was also investigated. **Methods**: Male Wistar rats were assigned to five groups (n = 8 each): vehicle, 1 mg/kg chrysin, 5 mg/kg chrysin, 1 mg/kg fluoxetine, and 1 mg/kg allopregnanolone. In the first experiment, treatments were injected daily and the effects on locomotor activity and the forced swim test were evaluated at 0, 1, 14, and 28 days of treatment, and 48 h after the final treatment. In the second experiment, similar groups were treated for 28 days with injection of 1 mg/kg picrotoxin to investigate the role of the $GABA_A$ receptor. Depending on the experimental design, one- and two-way analysis of variance (ANOVA) tests were used for statistical analysis, with $p < 0.05$ set as the criteria for significance. **Results**: In both experiments, the treatments did not alter locomotor activity. However, low and high doses of chrysin, allopregnanolone, and fluoxetine gradually produced antidepressant-like effects in the forced swim test, and maintained this effect for 48 h post-treatment, except with low dose chrysin. Picrotoxin blocked the antidepressant-like effects produced by low dose chrysin, but did not affect those produced by high dose chrysin, allopregnanolone, or fluoxetine. **Conclusions**: The differential antidepressant-like effects caused by low and high doses of chrysin are time-dependent. Low dose chrysin produces a rapid antidepressant-like effect, whereas high dose chrysin produces a delayed but sustained the effect, even 48 h after withdrawal. The effect with high dose chrysin was similar to that observed with allopregnanolone and fluoxetine. The mechanism for the antidepressant-like effect of low chrysin appears to be GABAergic, whereas the effect of high dose chrysin may involve other neurotransmission and neuromodulation systems related to the serotonergic system.

Keywords: $GABA_A$ receptor; chrysin; fluoxetine; immobility; stress; log-term effect

1. Introduction

The flavonoid chrysin (5,7-dihydroxyflavone) is found in high concentrations in propolis and honeybee, and particularly also in plants such as *Passiflora coerulea*, *Passiflora incarnata* and *Matricaria chamomilla* [1]. It exerts antioxidant, anticancer and anti-inflammatory activity, as well as important pharmacological effects in several disorders involving the central nervous system, such as epilepsy, Parkinson's disease, multiple sclerosis, Alzheimer's disease, depression and anxiety [1,2]. Preclinical studies have demonstrated anxiolytic-like effects of chrysin in different anxiety models in mice and rats. For example, the injection of 1 mg/kg chrysin increases the time spent by male mice [3] and rats [4] on the open arms of the elevated plus maze (EPM). It also increases the time spent by male rats [5] and zebrafish [6] in the illuminated compartment in the light/dark test (LDT). Similarly, the administration of 2 mg/kg chrysin in ovariectomized [7] and cycling [8,9] female rats produces an anxiolytic-like effect in the EPM and LDT tests. The effect is similar to that produced by anxiolytic drugs as diazepam [8] and some neurosteroids such as 17β-estradiol [10,11], pregnanolone [12] and allopregnanolone [13]. Additionally, intrahippocampal microinjection of 0.5 µg of chrysin or allopregnanolone produces anxiolytic-like effects in female rats during the diestrus phase [9]. These effects are blocked by prior

administration of Gamma-Aminobutyric Acid sub-type A ($GABA_A$) receptor antagonists such as flumazenil, bicuculline and picrotoxin, without affecting locomotor activity [9]. Overall, these findings suggest that chrysin shares the same mechanism of action with anxiolytic drugs and neurosteroids, both of which involve the Gamma-Aminobutyric Acid (GABA)ergic system.

In experimental anxiety models, daily administration for 14–28 days of 5–20 mg/kg chrysin reduced the total time of immobility in forced swim and tail suspension tests [14,15]. This was accompanied by higher consumption of sucrose, suggesting an antidepressant-like effect. The effects were also associated with an increased serotonin concentration in the prefrontal cortex, hippocampus and nucleus accumbens, which could be blocked by pretreatment with p-chlorophenylalanine, a selective inhibitor of the tryptophan hydroxylase enzyme involved in serotonin biosynthesis [14,15]. At the clinical level, abnormalities in serotonin neurotransmission during depression can now be more reliably evaluated due to advances in measurement techniques. However, there is still much to learn about serotonin activity and its potential role in the causation of illness [16]. Novel research approaches go beyond the serotonin hypothesis of depression and have moved to molecular integration involving diverse receptors and biochemical systems, some of which could become novel targets for antidepressant drugs. For example, daily oral administration of 20 mg/kg chrysin or of 10 mg/kg fluoxetine for 28 days increased the levels of the neurotrophins Brain Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF) compared to controls in the hippocampus and prefrontal cortex of both non-stressed and stressed mice [17]. This was associated with a decrease in depression-like behavior [18,19], and suggests the potential for developing new drugs from their dipeptide mimetics [20]. Related to the above findings, the flavonoid chrysin, similar to the neuro-steroid allopregnanolone, exerts anxiolytic- and antidepressant-like effects through actions on GABAergic and serotonergic systems. These actions are likely to be dependent on the doses administered and the duration of treatment (21 to 28 days) [21,22]. Therefore, we hypothesized that low and high doses of chrysin may produce antidepressant-like effects through different mechanisms of action. The goal of this study was to examine the effects of 1 mg/kg and 5 mg/kg chrysin after 0, 1, 14, and 28 days of treatment, and 48 h post-treatment (day 30) in the forced swim test. The results were compared with those of fluoxetine (a serotonergic drug) and allopregnanolone (a GABAergic drug) as pharmacological controls of antidepressant-like activity in adult male rats. In addition, the involvement of the $GABA_A$ receptor in the long-term action of chrysin was also explored.

2. Materials and Methods

2.1 Ethics

All experimental procedures were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council [23] and the Mexican law for the use and care of laboratory animals [24]. All efforts were made to minimize animal discomfort and to reduce the number of animals, according to the 3R's principles of preclinical research [25]. The experimental protocol received authorization from the Committee for the Use and Care of Laboratory Animals of the Biomedical Research Center from Universidad Veracruzana with the approval number CLCIB2023/2.

2.2 Animals

Adult male Wistar rats (260–280 g) aged 2.5 months were used in the study. The rats were bred in the vivarium of the Institute of Neuroethology of the Universidad Veracruzana (Xalapa, Veracruz, Mexico) and weaned at 21 days postnatal. They were housed in Plexiglas cages, at 4 rats per cage (44 cm width $\times$ 33 cm length $\times$ 20 cm height) in a room with a 12-h/12-h light/dark cycle (lights ON at 7:00 AM) and an average temperature of 25 °C ($\pm$2 °C). Animals had *ad libitum* access to purified water and food (Standard chow pellets, Agribrands Purina Co., Mexico City, Mexico), except during the experimental intervention periods. All experimental sessions were conducted between 10:00 and 13:00 h.

The rats were randomly assigned to different groups using a free online program (https://random.org). The sample size per group (n = 8) was based on previous studies that found 7–8 rats per group [26–28] gave sufficient statistical power to detect antidepressant-like effects of the substances evaluated in the present study in the forced swim test (FST).

2.3 Drugs

Solutions of chrysin, allopregnanolone, and fluoxetine (FLX) were prepared daily in 35% 2-hidroxypropyl-γ-cyclodextrin and injected intraperitoneally at a volume of 1 mL/kg. Picrotoxin was freshly prepared in 0.9 % NaCl solution and injected intraperitoneally at a volume of 1 mL/kg. All reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.4 Experimental Groups and Treatments

2.4.1 Experiment 1. Effect of Chrysin on Depression-Like Behavior

Male rats were assigned to 5 independent groups, with 8 rats per group. The vehicle group (VEH) received the 35% 2-hidroxypropyl-γ-cyclodextrin solution in which the drugs were dissolved. Two groups (C1 and C5) received 1 mg/kg and 5 mg/kg chrysin, respectively. Another group (ALLO) received 1 mg/kg allopregnanolone, and the fifth group received 1 mg/kg fluoxetine (FLX). Allopregnanolone and fluoxetine were included as pharmacologi-

cal controls of antidepressant-like effects with GABAergic and serotonergic activity, respectively. The dosage was based on previous studies in which intraperitoneal administration of 1 mg/kg and 5 mg/kg chrysin, 1 mg/kg fluoxetine [29], and 1 mg/kg allopregnanolone [30] produced antidepressant-like effects in the FST, without significant effects on general motor activity in the locomotor activity test (LAT). The rationale for using allopregnanolone as a pharmacological control of antidepressant-like activity was because this substance reduces immobility in the FST through its action on the $GABA_A$ receptor, which may be blocked by antagonists such as picrotoxin and bicuculline [28,31,32]. Chrysin also exerts anxiolytic and antidepressant-like effects through its action on the $GABA_A$ receptor, and can also be blocked by picrotoxin and bicuculline [28,33]. Moreover, both chrysin and allopregnanolone activate some neurotrophic factors (BDNF and NGF) and the serotonergic system. Thus, allopregnanolone was selected because of its pharmacological action rather than because of its structural similarities with chrysin.

Before pharmacological administration, all rats were subjected to a 5-min pre-test in the LAT, and subsequently to a 15-min pre-test in the FST. This was for habituation to novel situations, and to trigger the development of behavioral despair, respectively. Pre-test sessions were not considered in the statistical analysis. Twenty-four hours after the pre-tests (defined as day 0), rats were subjected to a 5-min test session in the LAT and subsequently in the FST in order to evaluate the baseline behavior activity. The pharmacological treatments were started after the test sessions on day 0. Behavioral effects were evaluated on the 1st, 14th, and 28th day of treatment, at 1 hour after drug injection. To assess the effect of treatment withdrawal, all rats were also tested at 48 hours after the last injection (day 30). The experimental design is shown in Fig. 1A.

2.4.2 Experiment 2. Involvement of the $GABA_A$ Receptor in the Antidepressant-Like Effect of Chrysin

The second experiment evaluated the role of the ion chloride channel of the $GABA_A$ receptor in the antidepressant-like effect of chronic chrysin and of the pharmacological controls. The same 5 groups as in experiment 1 were compared with 5 other independent groups, each comprising 8 rats per group. The first 5 groups received 0.9% NaCl as a vehicle for picrotoxin, whereas the second 5 groups received a single dose (1 mg/kg) of the antagonist picrotoxin. Hence the second 5 groups were: 1 mg/kg picrotoxin (P), C1+P, C5+P, ALLO+P, and FLX+P. The picrotoxin was injected 30 min before the last injection of vehicle, chrysin, allopregnanolone, or fluoxetine at day 28 of treatment. The effects in the LAT and the FST were then evaluated 1 hour after the last injection of drugs. Fig. 1B shows the design of the second experiment. For this experiment, the animals treated with picrotoxin were measured only once. However, to maintain the same stress conditions as the animals treated with picrotoxin at day 28, they were subjected to the same number of sessions in the FST and LAT at days 0, 1 and 14. These sessions were not measured, and the variables were only evaluated at day 28. The treatment schedule for picrotoxin was based on a previous study in which 1 mg/kg of picrotoxin antagonized the anti-immobility effects of allopregnanolone in the FST, but without producing any effects in the LAT [31].

2.5 Behavioral Tests

On the test days, rats were brought to the experimental room at 09:00 AM and left for 1 h to acclimatize to the novel surroundings. Behavioral evaluations were performed between 10:00 AM and 01:00 PM. A digital video camera (Sony DCR-SR42, 40× optical zoom, Carl Zeiss lens, Tokio, Japan) was installed above the locomotor activity cage, and another one was installed in front of the forced swim pool. Two independent observers used *ex profeso* software (1.0 version, Neuroethology Institute, Xalapa, Veracruz, Mexico) to record the number and time (in seconds) of each behavioral variable. This was carried out until >95% agreement was reached between the observers, and was performed blind to the treatment group. After each individual 5-min test, the apparatus was carefully cleaned with 15% ethanol solution to remove the scent of the previously evaluated rat, thereby avoiding any influence on the spontaneous behavior of subsequent rats.

2.5.1 Locomotor Activity Test (LAT)

Rats were placed individually in a Plexiglas cage (44 cm length × 33 cm width × 20 cm height). This was done to identify hyperactivity, hypoactivity or no changes in the animal groups that could interfere with interpretation of the behavioral variables measured in the FST. The floor of the cage was delineated into 12 squares (11 cm × 11 cm) to evaluate spontaneous locomotor activity (crossing), grooming, and rearing. At the beginning of the test, the rat was placed gently in one of the corners of the cage. The following variables were then measured: (a) the number of crossings, with a crossing considered to be when the rat passed from one square to another with its hind legs; (b) time spent rearing (in seconds) was when the rat acquired a vertical posture relative to the cage floor; and (c) time spent grooming (in seconds) included paw licking, nose/face grooming, head washing, body grooming/scratching, leg licking, and tail/genital grooming [34]. Crossings are used to exclude the possible influence of locomotor activity on swimming behavior, and is thus considered to be an indication of mobility. This horizontal ambulation is not exacerbated by substances with anti-immobility effects [26,28,35,36], unlike stimulants [37–39].

2.5.2 Forced Swim Test (FST)

In the FST, rats were individually forced to swim in a rectangular pool (50 cm × 30 cm × 60 cm) with 24 cm deep

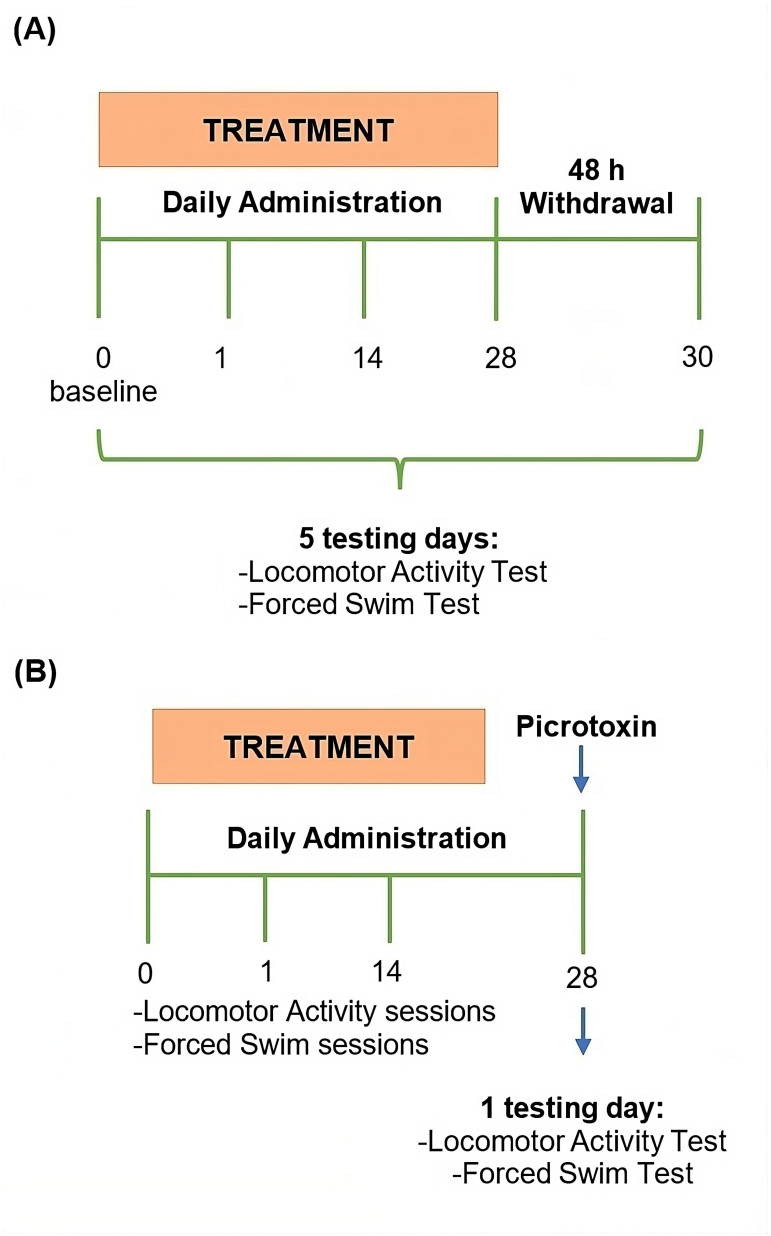

Fig. 1. Experimental design. (A) Experiment 1: Effect of chrysin on depression-like behavior. (B) Experiment 2: Involvement of the $GABA_A$ receptor on the antidepressant-like effect of chrysin. $GABA_A$, Gamma-Aminobutyric Acid sub-type A.

water at 25 °C ± 1 °C. This test has been validated for evaluating the antidepressant-like effects of clinically effective, antidepressant drugs including clomipramine, desipramine, and fluoxetine [26,40], neuro-steroids such as progesterone and allopregnanolone [31,41], and some flavonoids such as chrysin [28,42] and plant extracts [43,44]. The following variables were evaluated in the FST: latency to first immobility, and total immobility time (i.e., when the rat floated without making vigorous movements that led to displacement, and only maintained its head above the water surface for more than 2 s). These parameters have been used reliably to detect the antidepressant-like effects of antidepressants drugs in the FST.

2.6 Statistical Analysis

Data from experiment 1 were analyzed using two-way mixed analysis of variance, with treatment (between subjects) and days of treatment (within subjects) as factors. Data from experiment 2 were analyzed using one-way analysis of variance (one-way ANOVA), with treatment as a single factor. Data were transformed to satisfy the assumption for the normality test and equal variance test. The level of significance was set at $p \leq 0.05$. The Student-Newman-Keuls *post hoc* test followed ANOVA's when p reached statistical significance. The results are expressed as mean ± standard error. All analyses were conducted using SigmaPlot (version 12.0; Systat Software, Chicago, IL, USA).

3. Results

3.1 Experiment 1. Effects of Chrysin on Depression-Like Behavior

3.1.1 Locomotor Activity Test

Analysis of the number of crossings did not reveal any significant differences according to treatment [F (4,140) = 0.655, $p = 0.627$] or to the interaction of factors [F (16,140) = 1.028, $p = 0.431$] (Fig. 2A). However, significant differences were found according to the number of days of treatment [F (4,140) = 160.780, $p < 0.001$]. The *post hoc* test showed that the number of crossings decreased gradually and significantly with increasing days of treatment (day 1 = 39.9 ± 1.14; day 14 = 25.8 ± 1.00; day 28 = 17.1 ± 0.98) compared to the baseline (day 0 = 43.2 ± 1.04). This effect was maintained 48 h after the last administration on day 28 (48 h withdrawal = 15.0 ± 1.02). Similarly, the analysis of time spent rearing did not show any statistical differences according to treatment [F (4,140) = 0.976, $p = 0.433$] or to interaction of factors [F (16,140) = 1.695, $p = 0.054$] (Fig. 2B). However, a significant difference was found for the days of treatment [F (4,140) = 3.018, $p = 0.020$]. The time spent rearing was shorter on day 14 of treatment (14.2 ± 0.93) than on all other days of treatment (day 0 = 17.0 ± 0.64; day 1 = 16.5 ± 0.74; day 28 = 16.1 ± 0.85), but did not differ from the withdrawal period 48 h after the last administration on day 28 (48 h withdrawal =15.9 ± 0.86).

The time spent on grooming was significantly different according to treatment [F (4,140) = 36.368, $p < 0.001$], days of treatment [F (4,140) = 45.728, $p < 0.001$], and interaction of factors [F (16,140) = 12.635, $p < 0.001$]. The *post hoc* test revealed the VEH and C1 groups showed less time spent on grooming on all treatment days compared to baseline (day 0). This effect was maintained 48 h after the last administration on day 28. Grooming was lower on days 1 and 14 of treatment with C5 and FLX, but then increased on day 28 to be close to the baseline value. The longest time spent grooming was observed with ALLO, but this had decreased by day 14 and at 48 h after the last administration to levels similar to baseline (Fig. 2C). The C1, C5 and ALLO groups each spent more time grooming on day 1 of treatment compared to VEH. Grooming increased in all treatment groups from day 14 to day 28, but only C5 and FLX maintained this effect at 48 h post treatment (Fig. 2C).

3.1.2 Forced Swim Test

The latency to first immobility was significantly different between treatments [F (4,140) = 187.700, $p < 0.001$], days of treatment [F (4,140) = 64.577, $p < 0.001$], and interaction of factors [F (16,140) = 22.345, $p < 0.001$]. *Post hoc* analysis revealed that C5 was similar to FLX in showing increased latency to first immobility from day 14 of treatment (Fig. 3A). A significant increase in latency was evident with ALLO from day 1. Increased latency to first immobility was maintained at 48 h post-treatment in the C5, ALLO and FLX groups compared to the baseline and to the VEH group. The total time of immobility showed significant differences according to treatment [F (4,140) = 48.982, $p < 0.001$], days of treatment [F (4,140) = 47.150, $p < 0.001$], and interaction of factors [F (16,140) = 14.561, $p < 0.001$] (Fig. 3B). The *post hoc* test revealed that from day 1 of treatment, C1 and ALLO showed less immobility time compared to baseline and to VEH during all days of treatment. Similar to the effect observed for latency to first immobility, C5 and FLX showed reduced immobility time from day 14 of administration, as well as 48 h post-treatment. This effect was maintained by C5, ALLO and FLX, but C1 had lost its anti-immobility effect by 48 h after the last injection.

Table 1. Locomotor activity test to evaluate the involvement of the $GABA_A$ receptor in the antidepressant-like effects of chrysin.

	Crossings (n)	Rearing (s)
VEH	18.0 ± 2.95	14.0 ± 2.30
P	15.2 ± 1.26	15.4 ± 1.73
C1	16.0 ± 2.28	16.4 ± 1.18
C1+P	17.8 ± 1.07	17.3 ± 1.44
C5	21.6 ± 2.71	14.7 ± 1.49
C5+P	17.0 ± 1.76	19.5 ± 2.00
ALLO	18.2 ± 1.34	19.5 ± 2.00
ALLO+P	20.5 ± 1.29	18.6 ± 2.28
FLX	16.5 ± 1.37	15.4 ± 2.66
FLX+P	14.2 ± 2.19	16.0 ± 1.05

No significant differences in crossings or rearing were observed between treatments.

3.2 Experiment 2. Involvement of the $GABA_A$ Receptor in the Antidepressant-Like Effects of Chrysin

The involvement of $GABA_A$ receptor in the antidepressant-like effects of chrysin and of two pharmacological controls was investigated using picrotoxin, a non-competitive chloride ion channel antagonist. This was studied after 28 days of treatment.

3.2.1 Locomotor Activity Test

As shown in Table 1, no significant differences between treatments were observed for the number of crossings [H (9) = 11.104, $p = 0.269$] or for the time spent rearing [F (9,70) = 1.099, $p = 0.375$].

However, analysis of the time spent grooming revealed significant differences between the treatments [H (9) = 56.530, $p < 0.001$]. The *post hoc* test showed that all treatments, except picrotoxin, resulted in more time spent grooming compared to the VEH group (Fig. 4).

3.2.2 Forced Swim Test

Significant differences in the latency to first immobility were observed between treatments [F (9,70) = 80.249, p

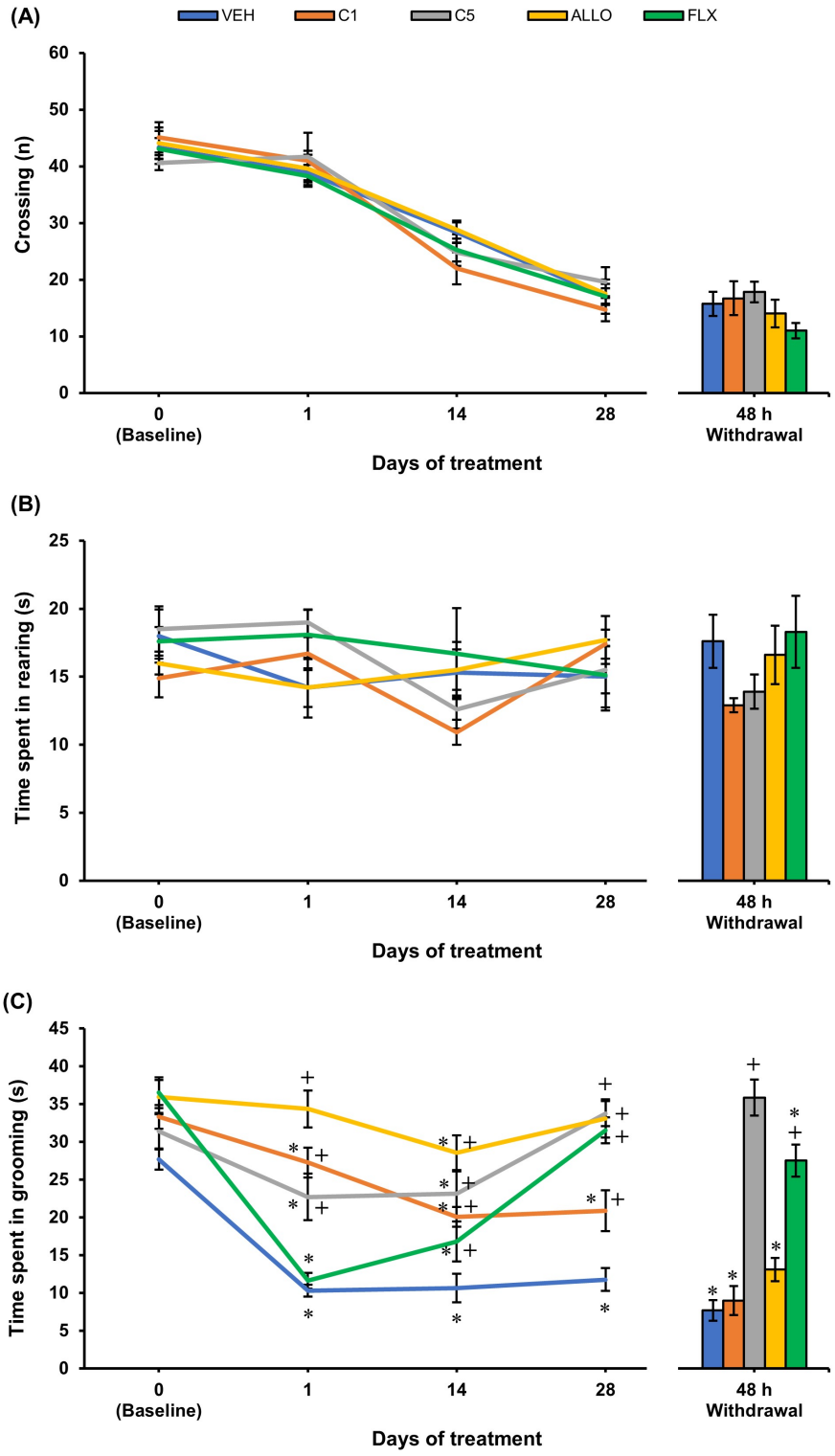

Fig. 2. Locomotor activity test. No differences between treatment groups were observed for the number of crossings (A), or time spent rearing (B). The C1, C5, and ALLO groups maintained grooming behavior from day 1 to 28 of treatment (C), while FLX showed a "U shape", with increased behavior from day 14 to 28 compared to the VEH group. At day 30 (48 h after treatment withdrawal), only C5 and FLX showed more time spent on grooming compared to the VEH group. *$p < 0.05$ vs baseline; +$p < 0.05$ vs vehicle same day. Student-Newman-Keuls *post hoc* test. VEH, vehicle; C1, 1 mg/kg chrysin; C5, 5 mg/kg chrysin; ALLO, 1 mg/kg allopregnanolone; FLX, 1 mg/kg fluoxetine.

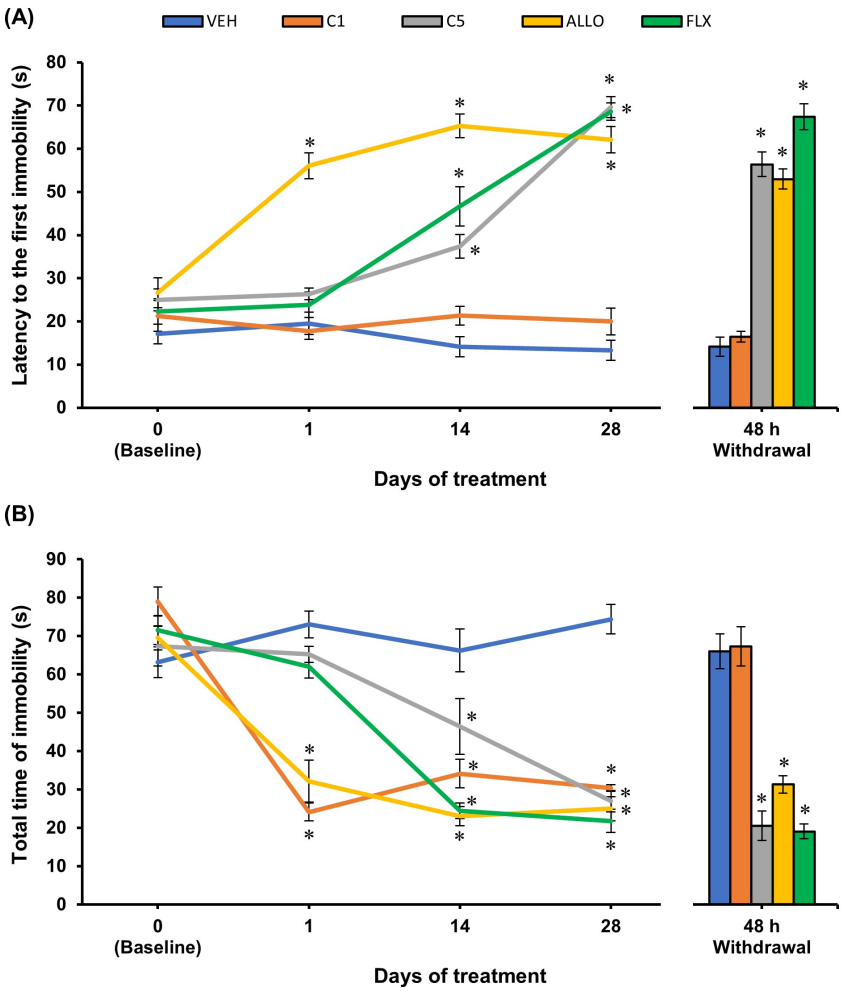

Fig. 3. Forced swim test. Effects of chronic treatment on latency to first immobility (A), and total time of immobility (B). C5, ALLO and FLX showed gradually increased latency to the first immobility, and decreased total time of immobility. Only C1 returned to the baseline level of immobility time 48 h after the last treatment. *$p < 0.05$ vs baseline and VEH in the respective session. Student-Newman-Keuls *post hoc* test.

< 0.001]. *Post hoc* test revealed that C5, ALLO and FLX produced a four-fold longer latency compared to the VEH, P, C1 and C1+P groups. This effect was attenuated by picrotoxin in the C5+P and ALLO+P groups, but not in the FLX+P group (Fig. 5A). Analysis of the total time of immobility also revealed significant differences between groups [$F(9,70) = 62.461$, $p < 0.001$]. The *post hoc* test revealed the C1, C5, ALLO and FLX groups had a shorter total time of immobility (Fig. 5B) compared to the VEH group. Only in the C1+P group was this effect blocked by prior injection of picrotoxin (Fig. 5B).

4. Discussion

The present study compared the effects of low and high doses of chrysin on depression-like behavior in adult male rats with those of fluoxetine and allopregnanolone. In addition, we investigated the mechanism involving the $GABA_A$ receptor in the antidepressant-like effects produced by chronic administration of chrysin. This was studied by antagonizing the $GABA_A$ receptor with picrotoxin. We evaluated the anti-immobility effects of low and high doses of chrysin at different times during the chronic treatment. Picrotoxin attenuated the latency to first immobility at high dose chrysin, and the total time of immobility at low dose chrysin.

The evaluation of crossings with the LAT allows the detection of changes in general locomotor activity caused by treatment. It is well known that stimulants of the central nervous system reduce immobility times [37], but the LAT can discriminate motor effects from motivational effects. This can occur with a substance that has antidepressant activity, which reduces immobility in the FST without significant changes in locomotion [26,28,35]. For this reason, we and others run the LAT before the FST [45]. When longitudinal studies are carried out using LAT, reductions in crossings may occur even when anti-immobility effects

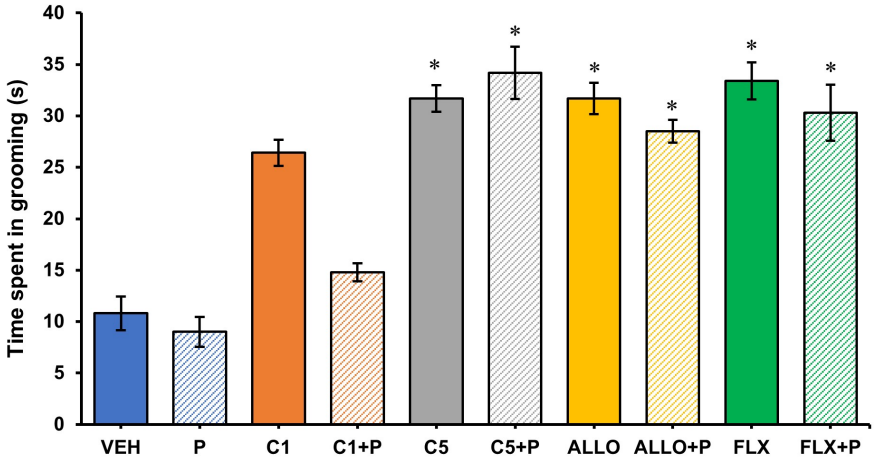

Fig. 4. Locomotor activity test. Involvement of the $GABA_A$ receptor on the effects of chrysin. The time spent grooming increased with all treatments except picrotoxin. Pretreatment with picrotoxin did not block this effect. *$p < 0.05$ vs vehicle. Student-Newman-Keuls *post hoc* test. VEH, vehicle; P, 1 mg/kg picrotoxin; C1, 1 mg/kg chrysin; C1+P, 1 mg/kg chrysin + 1 mg/kg picrotoxin; C5, 5 mg/kg chrysin; C5+P, 5 mg/kg chrysin + 1 mg/kg picrotoxin; ALLO, 1 mg/kg allopregnanolone; ALLO+P, 1 mg/kg allopregnanolone + 1 mg/kg picrotoxin; FLX, 1 mg/kg fluoxetine; FLX+P, 1 mg/kg fluoxetine + 1 mg/kg picrotoxin.

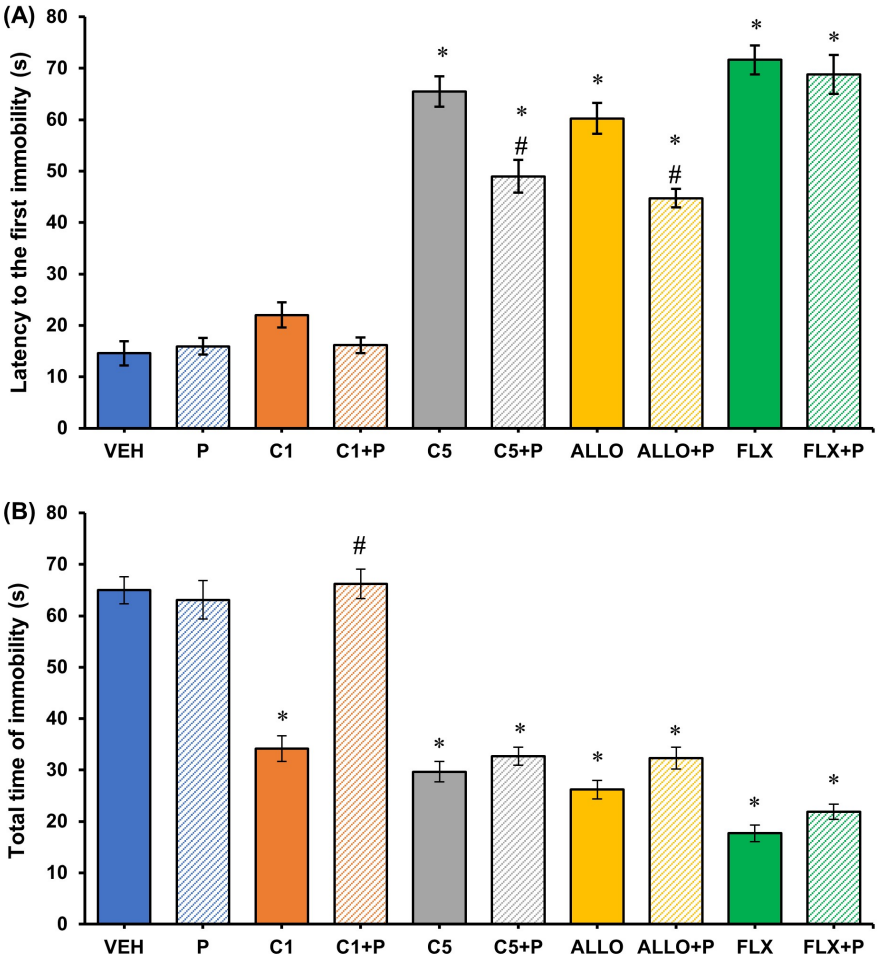

Fig. 5. Forced swim test. Involvement of the $GABA_A$ receptor on the antidepressant-like effects of chrysin. Latency time to the first immobility (A), and total time of immobility (B). *$p < 0.05$ vs VEH group, $^{\#}p < 0.05$ vs C1. Student-Newman-Keuls *post hoc* test.

are exerted by the substance with anti-despair properties [27]. Therefore, in the present study it is possible to disregard any motor influence on behavior detected by the FST. This demonstrates that chrysin, similar to allopregnanolone and fluoxetine, produces an antidepressant-like effect associated with motivation of the animals, rather than with a motor component.

Also in relation to the LAT, the time spent vertically exploring the environment (rearing) did not change with chrysin treatment, but differences were observed in grooming. Rearing and grooming are behavioral indicators of the emotional state of rats when exposed to novel environments [46]. Rearing is a measure of the active exploration carried out by the rat on the environment in which it is located. This can vary depending on the experimental situation. Some studies have reported that rearing increases after acute administration of drugs such as benzodiazepines, e.g., diazepam [32,47,48], or substances with anxiolytic potential such as neuro-steroids, e.g., progesterone or allopregnanolone, or flavonoids such as chrysin [7,28]. However, other studies have reported no changes with the same treatments [4,6,9,49]. There are no reports of increases in rearing after long-term administration of the abovementioned treatments. Indeed, no changes in this exploratory behavior are often reported [4,27,50], as found in the present study.

Grooming behavior is an indicator of animal motivation [51]. It may increase in mildly stressful situations, but is drastically reduced under conditions of severe stress [27,52,53]. Reduction of grooming time is prevented by anxiolytics, antidepressants and neuro-steroids, which reestablish this behavior to levels similar to those found in undisturbed animals [36,53–55]. As expected, our results showed that grooming decreased during the course of treatment compared to the baseline value in the VEH group. However, all treatments maintained a value similar to baseline from day 1 of administration, except for fluoxetine. This treatment showed a "U-shaped" effect, with an increase from day 14, and similar values to the baseline at day 28. Interestingly, 48 hours after the last administration, only the C5 and FLX-treated groups maintained grooming behavior at levels similar to baseline. This may reflect increased motivation consistent with reduced total immobility time, and is possibly also associated with changes in brain plasticity produced by prolonged treatment with antidepressants [53]. It is worth noting this effect was not observed with low dose chrysin, thus supporting the hypothesis that low doses exert "anti-stress" actions through effects on the $GABA_A$ receptor. This was supported by the results with picrotoxin, which canceled the pro-grooming effect observed with low dose chrysin, but not with high dose chrysin, allopregnanolone, or fluoxetine. This also supports the idea that chrysin exerts GABA-mediated effects in the short term, but that its effects in the long term involve other neurotransmission systems such as serotonin, as reported previously [28]. Therefore, the permanent effect of allopregnanolone and chrysin over picrotoxin during chronic administration may involve the actions of combined neurotransmission systems beyond GABA. Long-term treatment with allopregnanolone and chrysin may also produce significant changes in the BDNF, serotonin and dopamine neurotransmission systems [17,56], since allopregnanolone maintained the reduced immobility 48 h after withdrawal. This may be due to plasticity after 28 days of administration.

In the FST, low and high doses of chrysin produced antidepressant-like effects similar to fluoxetine and allopregnanolone. Picrotoxin prevented the effects of low dose chrysin, but not of the higher dose, suggesting a differential mechanism for chrysin action depending of the dose. The mechanism for higher doses could involve the activation of other neurotransmitter systems (e.g., serotonin) and neurotrophic factors, as identified in previous studies with chronic administration of chrysin [17,29,56]. The FST has been validated as a behavioral model and reveals substances with antidepressant activity because they produce longer latency and shorter immobility time, similar to clinically effective antidepressant drugs [26,37,57–59]. However, the terms "depression-like" and "despair" may be incorrect and FST behavior in general may represent a copying behavior [60,61]. Neural networks involved in the stress sessions of FST are heterogeneously complex and are linked by relevant factors to depression and to other conditions related to stress. The present work used naive rats to study the response to chrysin, and the findings with allopregnanolone and fluoxetine support the robust antidepressant-like effects observed with chrysin. In particular, 14 days of administration with high dose chrysin increased the latency to the first immobility, similar to the antidepressant drug fluoxetine and the neuro-steroid allopregnanolone. Moreover, the effect was maintained 48 h after ending the treatment, suggesting there was a long-term increase in motivation associated with changes in brain neuroplasticity, as occurs after treatment with antidepressant substances [29,62].

As expected, the total immobility time decreased with both low and high doses of chrysin, but important differences were noted. Low dose chrysin produced a rapid (day 1) anti-immobility effect, but was no longer effective 48 h after the last administration. This finding suggests that 1 mg/kg chrysin may act through ionotropic receptors to exert a reduction in immobility that is unrelated to antidepressant-like effects. Instead, it may be an "anti-stress" effect that allows the rat to quickly deal with the urgent situation, as previously reported for allopregnanolone [30]. Low dose chrysin may act in a manner similar to the acute administration of neuro-steroids such as progesterone or allopregnanolone. These reduce immobility through actions on ionotropic receptors, exhibiting rapid (30 min) but short-lasting (6 h) activity after injection [53]. In contrast, chronic application of 5 mg/kg chrysin may instead involve neuronal plasticity changes that take time to establish, as

reported in previous studies [63,64]. This flavonoid continues to produce anti-immobility effects 48 h after the suspension of 28-day treatment. Such long-term effects are similar to those seen with antidepressant drugs such as fluoxetine, which require 2–3 weeks for their effects to occur [26], as seen here with the FLX group. This effect could be related to chrysin-induced changes in the level of metabotropic serotonergic receptors such as 5-hydroxytryptamine subtype 1A (5-HT_{1A}) receptor [29], and in the production of serotonin. The establishment of long-term therapeutic effects could also involve changes in neuronal plasticity associated with the production of neurotrophic factors such as BDNF and NGF in structures involved with depression [65], such as the prefrontal cortex and the hippocampus [33,66,67]. Thus, it appears likely that 5 mg/kg chrysin induces neuronal plastic changes of long duration, as reported by other authors with fluoxetine [68,69]. This allows animals to maintain reduced immobility, even after the suspension of treatment. Pretreatment with picrotoxin, a non-competitive antagonist that blocks the $GABA_A$ receptor chloride channel, inhibits the reduction in immobility produced by 1 mg/kg chrysin and returns the values to control levels. This effect was not observed with high dose chrysin, allopregnanolone or fluoxetine, presumably because they involve the actions of combined neurotransmission systems beyond GABA. These treatments maintained the reduced immobility 48 h after withdrawal and may be involved in more permanent effects, such as those observed with the antidepressant phytomedicine Hiperikan® [70]. In summary, we propose that chrysin at higher doses may activate other neurotransmission systems (serotonergic, dopaminergic and norepinephrinergic) to increase the production of neurotrophic factors such as BDNF and NGF [14,15,29,71]. Additionally, chrysin exerts antioxidant and anti-inflammatory effects on the central nervous system (CNS), which may also contribute antidepressant effects [33], as occurs with conventional antidepressant drugs. This may explain why the effects of chrysin were not blocked by the $GABA_A$ receptor antagonist and highlights a crucial point in psychopharmacology. Substances such as many of the flavonoids, especially those found naturally and with pleiotropic effects, often do not have a single, clearly defined mechanism of action. Instead, their effects are the result of a complex interplay between multiple physiological systems. Therefore, single antagonism of the $GABA_A$ receptor is not sufficient to block the effects of allopregnanolone and 5 mg/kg chrysin on immobility.

Many flavonoids also act in a biphasic manner by enhancing the actions of GABA at low concentrations, and inhibiting it at high concentrations [72]. In particular, chrysin has agonist actions on the GABA receptor, producing anxiolytic effects similar to diazepam, that disappear with the administration of GABAergic antagonists [3]. In the present study, the absence of an antidepressant-like effect with high dose chrysin on day 1 may be due to the overstimulation of $GABA_A$ receptors. An excessive amount of drug or agonist substance may lead to overstimulation of the $GABA_A$ receptor, resulting in decreased ability of the brain to regulate neural activity. A similar phenomenon has been reported for other substances, such as benzodiazepines [54,73], neuro-steroids and flavonoids [72,74]. Furthermore, most antidepressants including fluoxetine exhibit an initial delay of two or more weeks before establishing a therapeutic effect [75,76]. At the preclinical level in rats, fluoxetine requires more time (14 to 21 days) to reduce immobility in the FST and to increase latency to the first immobility [26,43]. This is similar to the observed delay in humans. The delay in therapeutic effects of antidepressant drugs has been related to synaptic and neuronal plasticity, including changes in brain-derived neurotrophic factor and in the number of dendrites or synaptic receptors [29,62].

A limitation of the present study was that antagonism through a non-competitive mechanism using picrotoxin was only measured after 28 days of treatment. It would also be preferable to study the antagonism at days 1 and 14 of treatment. In addition, it would be preferable to antagonize the recognition site in the $GABA_A$ receptor using the competitive antagonist bicuculline. With regard to the differential effects of low and high doses of chrysin, further work should investigate selective antagonism of the benzodiazepine recognition site in the $GABA_A$ receptor using flumazenil. Futures studies should also investigate female rats in order to explore possible differences in the anti-immobility effects of low and high doses of chrysin according to the estrous phase. It was previously shown in ovariectomized rats that bicuculline blocked the anti-immobility effect of 1 mg/kg chrysin [26]. There is increasing interest in the use of chrysin as a potential therapeutic application for anxiety disorders, with many studies now being published on its anxiolytic effects. However, there has been less research on the antidepressant-like effects of chrysin and on its combined mechanism of action in the central nervous system. These areas clearly require further study.

5. Conclusions

The anti-immobility effects of low dose chrysin are time-dependent and different to those of high dose chrysin. Low dose chrysin produced a rapid onset, anti-immobility effect in the FST. In contrast, high dose chrysin produced a delayed but sustained anti-immobility effect during chronic treatment and 48 h after withdrawal, similar to the antidepressant fluoxetine. The mechanism underlying the antidepressant-like effects of low dose chrysin is GABAergic, while the antidepressant-like effects of high dose chrysin may be via other neurotransmission and neuromodulation systems likely related to serotonergic actions. Possible applications of chrysin for the treatment of comorbid anxiety-depression will first require the completion of relevant preclinical and clinical studies with this flavonoid.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

JFRL designed the research study. AKLV and OJOV performed the research. BBM provided help and advice on technical elements. GGR and JFRL analyzed the data. GGR, BBM, AKLV, OJOV and JFRL wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experimental protocol received authorization from the Committee for the Use and Care of Laboratory Animals of the Biomedical Research Center from Universidad Veracruzana with the approval number CLCIB2023/2.

Acknowledgment

To SIREI project no. DGI: 26650202346 to J.F.R-L. and CONAHCYT (CVU 121508, 285449, 121609, 863438, 635392).

Funding

This research was funded by SEP-PROMEP (grant no. 103.5/05/1955, UVER-PTC-155), High Quality Postgraduate Academic Strengthening Program (I010/458/2013, C-703/2013), CONAHCYT (grant 1840).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Naz S, Imran M, Rauf A, Orhan IE, Shariati MA, Iahtisham-Ul-Haq, *et al*. Chrysin: Pharmacological and therapeutic properties. Life Sciences. 2019; 235: 116797.

[2] Goyal A, Singh G, Verma A. A Comprehensive Review on Therapeutic Potential of Chrysin in Brain Related Disorders. CNS & Neurological Disorders Drug Targets. 2023; 22: 789–800.

[3] Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from Passiflora coerulea. Pharmacology, Biochemistry, and Behavior. 1994; 47: 1–4.

[4] Germán-Ponciano LJ, Puga-Olguín A, De Jesús Rovirosa-Hernández M, Caba M, Meza E, Rodríguez-Landa JF. Differential effects of acute and chronic treatment with the flavonoid chrysin on anxiety-like behavior and Fos immunoreactivity in the lateral septal nucleus in rats. Acta Pharmaceutica (Zagreb, Croatia). 2020; 70: 387–397.

[5] Zanoli P, Avallone R, Baraldi M. Behavioral characterisation of the flavonoids apigenin and chrysin. Fitoterapia. 2000; 71: S117–S123.

[6] German-Ponciano LJ, Dutra Costa BP, Feitosa LM, Campos KDS, da Silva Chaves SN, Cueto-Escobedo J, *et al*. Chrysin, but not flavone backbone, decreases anxiety-like behavior in animal screens. Neurochemistry International. 2020; 140: 104850.

[7] Rodríguez-Landa JF, Hernández-López F, Cueto-Escobedo J, Herrera-Huerta EV, Rivadeneyra-Domínguez E, Bernal-Morales B, *et al*. Chrysin (5,7-dihydroxyflavone) exerts anxiolytic-like effects through $GABA_A$ receptors in a surgical menopause model in rats. Biomedicine & Pharmacotherapy. 2019; 109: 2387–2395.

[8] Rodríguez-Landa JF, Guillén-Ruiz G, Hernández-López F, Cueto-Escobedo J, Rivadeneyra-Domínguez E, Bernal-Morales B, *et al*. Chrysin reduces anxiety-like behavior through actions on $GABA_A$ receptors during metestrus-diestrus in the rat. Behavioural Brain Research. 2021; 397: 112952.

[9] Rodríguez-Landa JF, Hernández-López F, Martínez-Mota L, Scuteri D, Bernal-Morales B, Rivadeneyra-Domínguez E. $GABA_A$ /Benzodiazepine Receptor Complex in the Dorsal Hippocampus Mediates the Effects of Chrysin on Anxiety-Like Behaviour in Female Rats. Frontiers in Behavioral Neuroscience. 2022; 15: 789557.

[10] Charoenphandhu J, Teerapornpuntakit J, Nuntapornsak A, Krishnamra N, Charoenphandhu N. Anxiety-like behaviors and expression of SERT and TPH in the dorsal raphé of estrogen- and fluoxetine-treated ovariectomized rats. Pharmacology, Biochemistry, and Behavior. 2011; 98: 503–510.

[11] Rodríguez-Landa JF, Cueto-Escobedo J, Puga-Olguín A, Rivadeneyra-Domínguez E, Bernal-Morales B, Herrera-Huerta EV, *et al*. The Phytoestrogen Genistein Produces Similar Effects as 17β-Estradiol on Anxiety-Like Behavior in Rats at 12 Weeks after Ovariectomy. BioMed Research International. 2017; 2017: 9073816.

[12] Gunter BW, Jones SA, Paul IA, Platt DM, Rowlett JK. Benzodiazepine and neuroactive steroid combinations in rats: anxiolytic-like and discriminative stimulus effects. Psychopharmacology. 2016; 233: 3237–3247.

[13] Mòdol L, Darbra S, Pallarès M. Neurosteroids infusion into the CA1 hippocampal region on exploration, anxiety-like behaviour and aversive learning. Behavioural Brain Research. 2011; 222: 223–229.

[14] Filho CB, Jesse CR, Donato F, Del Fabbro L, Gomes de Gomes M, Rossito Goes AT, *et al*. Chrysin promotes attenuation of depressive-like behavior and hippocampal dysfunction resulting from olfactory bulbectomy in mice. Chemico-biological Interactions. 2016; 260: 154–162.

[15] Bortolotto VC, Pinheiro FC, Araujo SM, Poetini MR, Bertolazi BS, de Paula MT, *et al*. Chrysin reverses the depressive-like behavior induced by hypothyroidism in female mice by regulating hippocampal serotonin and dopamine. European Journal of Pharmacology. 2018; 822: 78–84.

[16] Jauhar S, Cowen PJ, Browning M. Fifty years on: Serotonin and depression. Journal of Psychopharmacology (Oxford, England). 2023; 37: 237–241.

[17] Filho CB, Jesse CR, Donato F, Giacomeli R, Del Fabbro L, da Silva Antunes M, *et al*. Chronic unpredictable mild stress decreases BDNF and NGF levels and Na(+),K(+)-ATPase activity in the hippocampus and prefrontal cortex of mice: antidepressant effect of chrysin. Neuroscience. 2015; 289: 367–380.

[18] Almeida FB, Nin MS, Barros HMT. The role of allopregnanolone in depressive-like behaviors: Focus on neurotrophic proteins. Neurobiology of Stress. 2020; 12: 100218.

[19] Diniz DM, Calabrese F, Brivio P, Riva MA, Grandjean J, Homberg JR. BDNF Overexpression in the Ventral Hippocampus Promotes Antidepressant- and Anxiolytic-Like Activity in Serotonin Transporter Knockout Rats. International Journal of Molecular Sciences. 2021; 22: 5040.

[20] Mezhlumyan AG, Tallerova AV, Povarnina PY, Tarasiuk AV,

Sazonova NM, Gudasheva TA, *et al*. Antidepressant-like Effects of BDNF and NGF Individual Loop Dipeptide Mimetics Depend on the Signal Transmission Patterns Associated with Trk. Pharmaceuticals (Basel, Switzerland). 2022; 15: 284.

[21] Molina-Hernández M, Tellez-Alcántara NP, García JP, Lopez JIO, Jaramillo MT. Antidepressant-like actions of intra-accumbens infusions of allopregnanolone in ovariectomized Wistar rats. Pharmacology, Biochemistry, and Behavior. 2005; 80: 401–409.

[22] Evans J, Sun Y, McGregor A, Connor B. Allopregnanolone regulates neurogenesis and depressive/anxiety-like behaviour in a social isolation rodent model of chronic stress. Neuropharmacology. 2012; 63: 1315–1326.

[23] National Research Council, Guide for the Care and Use of Laboratory Animals. 8th edn. National Academy Press: Washington DC. 2011.

[24] Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. NOM-062-ZOO-1999, Especificaciones Técnicas para la Producción, Cuidado y Uso de los Animales de Laboratorio. 1999. Available at: https://www.gob.mx/cms/uploads/attachment/file/203498/NOM-062-ZOO-1999_220801.pdf (Accessed: 15 December 2023).

[25] Russell WMS, Burch RL, Hume CW. The principles of humane experimental technique. Johns Hopkins Bloomberg School of Public Health. Baltimore: USA. 2005.

[26] Contreras CM, Rodriguez-Landa JF, Gutiérrez-García AG, Bernal-Morales B. The lowest effective dose of fluoxetine in the forced swim test significantly affects the firing rate of lateral septal nucleus neurones in the rat. Journal of Psychopharmacology (Oxford, England). 2001; 15: 231–236.

[27] Rodríguez-Landa JF, Cueto-Escobedo J, Flores-Aguilar LÁ, Rosas-Sánchez GU, Rovirosa-Hernández MDJ, García-Orduña F, *et al*. The Aqueous Crude Extracts of Montanoa frutescens and Montanoa grandiflora Reduce Immobility Faster Than Fluoxetine Through $GABA_A$ Receptors in Rats Forced to Swim. Journal of Evidence-based Integrative Medicine. 2018; 23: 2515690X18762953.

[28] Cueto-Escobedo J, Andrade-Soto J, Lima-Maximino M, Maximino C, Hernández-López F, Rodríguez-Landa JF. Involvement of GABAergic system in the antidepressant-like effects of chrysin (5,7-dihydroxyflavone) in ovariectomized rats in the forced swim test: comparison with neurosteroids. Behavioural Brain Research. 2020; 386: 112590.

[29] German-Ponciano LJ, Rosas-Sánchez GU, Ortiz-Guerra SI, Soria-Fregozo C, Rodríguez-Landa JF. Effects of chrysin on mRNA expression of 5-HT1A and 5-HT2A receptors in the raphe nuclei and hippocampus. Revista Brasileira de Farmacognosia. 2021; 31: 353–360.

[30] Rodríguez-Landa JF, Contreras CM, García-Ríos RI. Allopregnanolone microinjected into the lateral septum or dorsal hippocampus reduces immobility in the forced swim test: participation of the GABAA receptor. Behavioural Pharmacology. 2009; 20: 614–622.

[31] Rodrìguez-Landa JF, Contreras CM, Bernal-Morales B, Gutièrrez-Garcìa AG, Saavedra M. Allopregnanolone reduces immobility in the forced swimming test and increases the firing rate of lateral septal neurons through actions on the GABAA receptor in the rat. Journal of Psychopharmacology (Oxford, England). 2007; 21: 76–84.

[32] Rodríguez-Landa JF, Hernández-Figueroa JD, Hernández-Calderón BDC, Saavedra M. Anxiolytic-like effect of phytoestrogen genistein in rats with long-term absence of ovarian hormones in the black and white model. Progress in Neuro-psychopharmacology & Biological Psychiatry. 2009; 33: 367–372.

[33] Rodríguez-Landa JF, German-Ponciano LJ, Puga-Olguín A, Olmos-Vázquez OJ. Pharmacological, Neurochemical, and Behavioral Mechanisms Underlying the Anxiolytic- and Antidepressant-like Effects of Flavonoid Chrysin. Molecules (Basel, Switzerland). 2022; 27: 3551.

[34] Guillén-Ruiz G, Cueto-Escobedo J, Hernández-López F, Rivera-Aburto LE, Herrera-Huerta EV, Rodríguez-Landa JF. Estrous cycle modulates the anxiogenic effects of caffeine in the elevated plus maze and light/dark box in female rats. Behavioural Brain Research. 2021; 413: 113469.

[35] Estrada-Camarena E, Contreras CM, Saavedra M, Luna-Baltazar I, López-Rubalcava C. Participation of the lateral septal nuclei (LSN) in the antidepressant-like actions of progesterone in the forced swimming test (FST). Behavioural Brain Research. 2002; 134: 175–183.

[36] Rodríguez-Landa JF, Olmos-Vázquez OJ, Dutra da Costa BP, Lima-Maximino M, Maximino C, Guillén-Ruiz G. Actions of progesterone on depression-like behavior in a model of surgical menopause are mediated by GABAA receptors. Salud Mental. 2020; 43: 43–53.

[37] Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977; 266: 730–732.

[38] Vieira C, De Lima TCM, Carobrez ADP, Lino-de-Oliveira C. Frequency of climbing behavior as a predictor of altered motor activity in rat forced swimming test. Neuroscience Letters. 2008; 445: 170–173.

[39] Costa APR, Vieira C, Bohner LOL, Silva CF, Santos ECDS, De Lima TCM, *et al*. A proposal for refining the forced swim test in Swiss mice. Progress in Neuro-psychopharmacology & Biological Psychiatry. 2013; 45: 150–155.

[40] Contreras CM, Martínez-Mota L, Saavedra M. Desipramine restricts estral cycle oscillations in swimming. Progress in Neuropsychopharmacology & Biological Psychiatry. 1998; 22: 1121–1128.

[41] Martínez-Mota L, Contreras CM, Saavedra M. Progesterone reduces immobility in rats forced to swim. Archives of Medical Research. 1999; 30: 286–289.

[42] German-Ponciano LJ, Rosas-Sánchez GU, Cueto-Escobedo J, Fernández-Demeneghi R, Guillén-Ruiz G, Soria-Fregozo C, *et al*. Participation of the Serotonergic System and Brain-Derived Neurotrophic Factor in the Antidepressant-like Effect of Flavonoids. International Journal of Molecular Sciences. 2022; 23: 10896.

[43] Lozano-Hernández R, Rodríguez-Landa JF, Hernández-Figueroa JD, Saavedra M, Ramos-Morales FR, Cruz-Sánchez S. Antidepressant-like effects of two commercially available products of Hypericum perforatum in the forced swim test: a long-term study. Journal of Medicinal Plants Research. 2010; 4: 131–137.

[44] Gunn BG, Cunningham L, Mitchell SG, Swinny JD, Lambert JJ, Belelli D. GABAA receptor-acting neurosteroids: a role in the development and regulation of the stress response. Frontiers in Neuroendocrinology. 2015; 36: 28–48.

[45] Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. Journal of Visualized Experiments: JoVE. 2015; 52587.

[46] Gilad VH, Rabey JM, Eliyayev Y, Gilad GM. Different effects of acute neonatal stressors and long-term postnatal handling on stress-induced changes in behavior and in ornithine decarboxylase activity of adult rats. Brain Research. Developmental Brain Research. 2000; 120: 255–259.

[47] Thakur P, Rana AC. Effect of Cissampelos Pareira Leaves on Anxiety-like Behavior in Experimental Animals. Journal of Traditional and Complementary Medicine. 2013; 3: 188–193.

[48] Shastry R, Ullal SD, Karkala S, Rai S, Gadgade A. Anxiolytic activity of aqueous extract of *Camellia sinensis* in rats. Indian

Journal of Pharmacology. 2016; 48: 681–686.
[49] Carro-Juárez M, Rodríguez-Landa JF, Rodríguez-Peña MDL, Rovirosa-Hernández MDJ, García-Orduña F. The aqueous crude extract of Montanoa frutescens produces anxiolytic-like effects similarly to diazepam in Wistar rats: involvement of GABAA receptor. Journal of Ethnopharmacology. 2012; 143: 592–598.
[50] Flores-Aguilar LÁ, Cueto-Escobedo J, Puga-Olguín A, Olmos-Vázquez OJ, Rosas-Sánchez GU, Bernal-Morales B, *et al*. Behavioral Despair Is Blocked by the Flavonoid Chrysin (5,7-Dihydroxyflavone) in a Rat Model of Surgical Menopause. Molecules (Basel, Switzerland). 2023; 28: 587.
[51] Kalueff AV, Tuohimaa P. Grooming analysis algorithm for neurobehavioural stress research. Brain Research. Brain Research Protocols. 2004; 13: 151–158.
[52] Perrot-Sinal TS, Gregus A, Boudreau D, Kalynchuk LE. Sex and repeated restraint stress interact to affect cat odor-induced defensive behavior in adult rats. Brain Research. 2004; 1027: 161–172.
[53] Contreras CM, Rodríguez-Landa JF, Bernal-Morales B, Gutiérrez-García AG, Saavedra M. Timing of progesterone and allopregnanolone effects in a serial forced swim test. Salud Mental. 2011; 34: 309–314.
[54] Bateson AN. Basic pharmacologic mechanisms involved in benzodiazepine tolerance and withdrawal. Current Pharmaceutical Design. 2002; 8: 5–21.
[55] Bernal-Morales B, Rodríguez-Landa JF, Ayala-Saavedra DR, Valenzuela-Limón O, Guillén-Ruiz G, Limón-Vázquez AK. Grooming behavior in stressed rats and the restorative effect of fluoxetine. Revista Conductual. 2020; 8: 1–14. (In Spanish)
[56] Chen S, Gao L, Li X, Ye Y. Allopregnanolone in mood disorders: Mechanism and therapeutic development. Pharmacological Research. 2021; 169: 105682.
[57] Espejo EF, Miñano FJ. Prefrontocortical dopamine depletion induces antidepressant-like effects in rats and alters the profile of desipramine during Porsolt's test. Neuroscience. 1999; 88: 609–615.
[58] Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, *et al*. Preclinical models: status of basic research in depression. Biological Psychiatry. 2002; 52: 503–528.
[59] Castagné V, Porsolt RD, Moser P. Use of latency to immobility improves detection of antidepressant-like activity in the behavioral despair test in the mouse. European Journal of Pharmacology. 2009; 616: 128–133.
[60] Commons KG, Cholanians AB, Babb JA, Ehlinger DG. The Rodent Forced Swim Test Measures Stress-Coping Strategy, Not Depression-like Behavior. ACS Chemical Neuroscience. 2017; 8: 955–960.
[61] Armario A. The forced swim test: Historical, conceptual and methodological considerations and its relationship with individual behavioral traits. Neuroscience and Biobehavioral Reviews. 2021; 128: 74–86.
[62] D'Sa C, Duman RS. Antidepressants and neuroplasticity. Bipolar Disorders. 2002; 4: 183–194.
[63] Sarkaki A, Farbood Y, Mansouri SMT, Badavi M, Khorsandi L, Dehcheshmeh MG, *et al*. Chrysin prevents cognitive and hippocampal long-term potentiation deficits and inflammation in rat with cerebral hypoperfusion and reperfusion injury. Life Sciences. 2019; 226: 202–209.
[64] Ghaderi S, Komaki A, Salehi I, Basir Z, Rashno M. Possible mechanisms involved in the protective effects of chrysin against lead-induced cognitive decline: An in vivo study in a rat model. Biomedicine & Pharmacotherapy. 2023; 157: 114010.
[65] Yang T, Nie Z, Shu H, Kuang Y, Chen X, Cheng J, *et al*. The Role of BDNF on Neural Plasticity in Depression. Frontiers in Cellular Neuroscience. 2020; 14: 82.
[66] Crupi R, Mazzon E, Marino A, La Spada G, Bramanti P, Battaglia F, *et al*. Hypericum perforatum treatment: effect on behaviour and neurogenesis in a chronic stress model in mice. BMC Complementary and Alternative Medicine. 2011; 11: 7.
[67] Rubio FJ, Ampuero E, Sandoval R, Toledo J, Pancetti F, Wyneken U. Long-term fluoxetine treatment induces input-specific LTP and LTD impairment and structural plasticity in the CA1 hippocampal subfield. Frontiers in Cellular Neuroscience. 2013; 7: 66.
[68] Ampuero E, Rubio FJ, Falcon R, Sandoval M, Diaz-Veliz G, Gonzalez RE, *et al*. Chronic fluoxetine treatment induces structural plasticity and selective changes in glutamate receptor subunits in the rat cerebral cortex. Neuroscience. 2010; 169: 98–108.
[69] Djordjevic A, Djordjevic J, Elaković I, Adzic M, Matić G, Radojcic MB. Fluoxetine affects hippocampal plasticity, apoptosis and depressive-like behavior of chronically isolated rats. Progress in Neuro-psychopharmacology & Biological Psychiatry. 2012; 36: 92–100.
[70] Rodríguez-Landa JF, Cueto-Escobedo J, Aguirre-Chiñas JD, Pérez-Vázquez MO. A commercially available product of Hypericum perforatum acts on GABAA receptors to produces anxiolytic-like, but not antidepressant-like effects in Wistar rats. In Howard RD (ed.) Hypericum: botanical sources, medical properties and health effects. 1st edn. Nova Science Publisher: New York, USA. 2015.
[71] Abd Al Haleem EN, Ahmed HI, El-Naga RN. Lycopene and Chrysin through Mitigation of Neuroinflammation and Oxidative Stress Exerted Antidepressant Effects in Clonidine-Induced Depression-like Behavior in Rats. Journal of Dietary Supplements. 2023; 20: 391–410.
[72] Johnston GAR. Flavonoid nutraceuticals and ionotropic receptors for the inhibitory neurotransmitter GABA. Neurochemistry International. 2015; 89: 120–125.
[73] Rosas-Gutiérrez I, Simón-Arceo K, Mercado F. Cellular and molecular mechanism of the benzodiazepines addiction. Salud Mental. 2013; 36: 325–329. (In Spanish)
[74] German-Ponciano LJ, Rosas-Sánchez GU, Rivadeneyra-Domínguez E, Rodríguez-Landa JF. Advances in the Preclinical Study of Some Flavonoids as Potential Antidepressant Agents. Scientifica. 2018; 2018: 2963565.
[75] Blier P. The pharmacology of putative early-onset antidepressant strategies. European Neuropsychopharmacology: the Journal of the European College of Neuropsychopharmacology. 2003; 13: 57–66.
[76] Opal MD, Klenotich SC, Morais M, Bessa J, Winkle J, Doukas D, *et al*. Serotonin 2C receptor antagonists induce fast-onset antidepressant effects. Molecular Psychiatry. 2014; 19: 1106–1114.