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Anxiolytic-like effects of ursolic acid in mice

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ABSTRACT

Ursolic acid is a pentacyclic triterpenoid that possesses several biological and neuropharmacological effects including antidepressant-like activity. Anxiety disorders represent common and disability psychiatric conditions that are often associated with depressive symptoms. This work investigated the anxiolytic-like effects of ursolic acid administration in different behavioral paradigms that evaluate anxiety in mice: open field test, elevated plus maze test, light/dark box test and marble burying test. To this end, mice were administered with ursolic acid (0.1, 1 and 10 mg/kg, p.o.) or diazepam (2 mg/kg, p.o.), positive control, and submitted to the behavioral tests. The results show that ursolic acid (10 mg/kg) elicited an anxiolytic-like effect observed by the increased total time in the center and decreased number of rearings responses in the open field test and an increased percentage of entries and total time spent in the open arms of elevated plus maze, similarly to diazepam. No significant effects of ursolic acid were shown in the light/dark box and marble burying test. These data indicate that ursolic acid exhibits anxiolytic-like effects in the open field and elevated plus maze test, but not in the light/dark box and marble burying test, showing the relevance of testing several behavioral paradigms in the evaluation of anxiolytic-like actions. Of note, the results extend the understanding on the effects of ursolic acid in the central nervous system and suggest that it may be a novel approach for the management of anxiety-related disorders.

1. Introduction

Ursolic acid ((3β)-3-hydroxyurs-12-en-28-oic acid, Fig. 1) is a pentacyclic triterpenoid widely found in different medicinal herbs and in several foods, thereby constituting an integral part of human diets. Ursolic acid possesses many biological effects, including antitumor, anti-inflammatory and antioxidant activities (Alqahtani et al., 2013; Lee et al., 2008; Sultana and Saify, 2012). Moreover, its administration is able to produce neuropharmacological effects in rodents including improvement in cognitive deficits elicited by different insults (Lu et al., 2011; Wang et al., 2011; Wu et al., 2013), neuroprotective (Li et al., 2013), antinociceptive (Verano et al., 2013) and antidepressant-like effects in the tail suspension test and forced swimming test (Colla et al., 2014; Machado et al., 2012b). However, the anxiolytic profile of this compound is not demonstrated so far.

Anxiety disorders are the most common psychiatric condition in the primary care practitioners, with a prevalence estimated in 13% (Kessler et al., 2005). This disorder causes significant costs in terms of healthcare use, disability, loss of productive and quality of life of patients (Combs and Markman, 2014). Furthermore, suicide risk increases with acute and chronic anxiety disorders (Khan et al., 2002). The medication side effects are a major limitation to adherence and successful treatment of the patients and draw attention to the need for new treatment alternatives (Thronson and Pagalilauan, 2014).

Different behavioral tasks are used for the study of the neurobiological basis of anxiety and in the screening for novel anxiolytic compounds. The open field test, the elevated plus maze test, the light/dark box test and the marble burying test are the main tests used to evaluate anxiolytic responses in mice and rats (Ennaceur, 2014; Kedia and Chattarji, 2014; Walf and Frye, 2007).

The first-line pharmacologic treatment of anxiety disorders aims to prevent future symptoms, and consist in the use of antidepressants that inhibit the reuptake of serotonin, norepinephrine or both (Combs and Markman, 2014; Thronson and Pagalilauan, 2014). Ursolic acid elicits an antidepressant-like effect in mice dependent on the activation of monoaminergic systems, due to its ability to inhibit the reuptake of monoamines (Colla et al., 2014; Machado et al., 2012b). Therefore, this study aims to investigate the possible anxiolytic-like activity of ursolic acid administration in different behavioral tasks: open field, elevated plus maze, light/dark box, and marble burying test, in mice.

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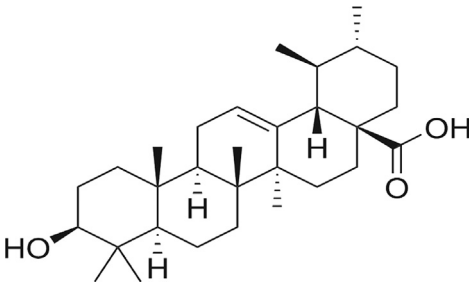

Fig. 1. Chemical structure of ursolic acid.

2. Materials and methods

2.1. Animals

Male Swiss mice (40–50 g, 50–60 days old) obtained from the Central Biothery of Universidade Federal de Santa Catarina (UFSC) were used. The animals were housed in groups of 15 animals per plastic cage under controlled conditions of light (12:12 h, lights on at 07:00 h) and temperature (20–22 °C) with free access to water and food. Mice were allowed to acclimatize to the holding room for 24 h before the behavioral procedure. All manipulations were conducted in the light phase ($n=8$ animals per group). All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Institution.

2.2. Drugs and treatment

Ursolic acid ($C_{30}H_{48}O_3$, Sigma Chemical Co, St. Louis, MO, USA) was dissolved in distilled water with 10% of Tween 80, and diazepam (positive control) (Produtos Roche Químicos e Farmacêuticos S.A., Rio de Janeiro, Brazil) was dissolved in distilled water. The control group received distilled water. Ursolic acid in the doses of 0.1, 1 and 10 mg/kg, or diazepam in the dose of 2 mg/kg, was administered orally 1 h before the behavioral tests in a constant volume of 10 ml/kg body weight. Independent groups of mice were tested in each test.

2.3. Open field test

The open field test besides providing measures of exploratory behavior is a valid initial screening test for anxiety-related behavior in rodents (Deacon et al., 2002). In this test, mice were individually submitted to the circular open field, an arena of 30 cm of diameter with the floor divided into 8 spaces. The number of squares crossed with the four paws was registered during a 6 min period and was considered as a parameter of locomotor activity. The increased time in the central zone of the apparatus, decreased number of rearings and groomings (washing of the coat) were considered as an anxiolytic-like effect (Kalueff et al., 2007a; Machado et al., 2012a). The floor of the apparatus was cleaned with 10% ethanol between tests.

2.4. Elevated plus maze test

Elevated plus maze test is the most employed test to evaluate anxiolytic activity of drugs (Walf and Frye, 2007). The maze consists of a central platform (6 × 6 cm), and two open arms (30 × 6 cm) aligned perpendicularly to two closed arms (30 cm × 6 cm × 16 cm). The open arms had 1 cm high plexiglass rim to prevent fall. The entire apparatus was elevated to a height of 50 cm above the floor. Mice were individually placed into the central square of the maze, facing between open arm and closed arm and their spontaneous behavior was recorded for 5 min. The percentage of the total entries and the total time spent in the open arms were measured. Increased entries into the open arms and increased time spent in the open arms were considered indices of anxiolytic profile (Budzynska et al., 2013; Lapmanee et al., 2013). The floor of the elevated plus maze apparatus was cleaned with 10% ethanol between tests.

2.5. Light/dark box test

In this test the exploratory activity is influenced by hazard and risk avoidance (Bourin and Hascoet, 2003). The apparatus consists in a box with one-third for the dark compartment and two thirds for the light compartment interconnected with an exterior size of 46 × 20 × 30 cm. The test is based on the observation that although rodents naturally tend to explore a novel environment, the safe area to mice is the small dark compartment and the aversive area is the illuminated compartment (Bourin and Hascoet, 2003). Mice were placed in the light area and the latency time for the first entry into the dark compartment as well as the total time spent in the light compartment was registered for 5 min. These parameters were used to infer about anxiolytic behavioral responses. Anxiolytic drugs increase the latency time to entry in the dark area and the total time in the light area (Bourin and Hascoet, 2003; Costall et al., 1989; Imaizumi et al., 1994). The floor of the light/dark box apparatus was cleaned with 10% ethanol between tests.

2.6. Marble burying test

The marble burying test is supposed to reflect repetitive and perseverative behavior, likely related to compulsions and/or anxiety disorders (Kedia and Chattarji, 2014). A cage (17.5 × 10 × 5.5 cm) was filled approximately 5 cm deep with husk bedding material that was evenly distributed into a flat surface across the whole cage. Twenty glass marbles (1.4 cm in diameter) were then spaced evenly in a 4 × 5 grid on the surface of the bedding. During the testing phase each mouse was placed in the cage and allowed to explore it for 20 min. At the end of the test, mice were removed from the cage and the number of marbles buried with bedding up to 2/3 of their depth was counted (Kedia and Chattarji, 2014). Rodents use bedding material to bury harmless objects and the inhibition of object burying is considered as an anxiolytic profile (Albelda and Joel, 2012).

2.7. Statistical analysis

All experimental results are given as mean ± S.E.M. Comparisons between experimental and control groups were performed by one-way ANOVA followed by Newman–Keuls posthoc test when appropriate. A value of $P<0.05$ was considered to be significant.

3. Results

3.1. Effect of ursolic acid in the open field test

The results presented in Fig. 2A show that neither ursolic acid nor diazepam administrations induced changes in the spontaneous locomotion in mice. However, the total time spent in the center of the apparatus and the total number of the rearing were reduced by ursolic acid (10 mg/kg), in a way similar to diazepam (2 mg/kg, positive control) (Fig. 2B and C, respectively). The number of groomings was decreased only by diazepam administration (Fig. 2D). The one-way ANOVA revealed no significant effects of treatment on the number of crossings [$F(4,35)=1.95$, $P=0.12$]. On the other hand, significant effects of treatment were

shown on the total time spent in the center [$F(4,35)=3.94$, $P<0.01$], number of rearings [$F(4,35)=6.48$, $P<0.01$] and groomings [$F(4,35)=4.62$, $P<0.01$] in the open field test.

3.2. Effect of ursolic acid in the elevated plus maze

The results depicted in Fig. 3 show that the acute treatment with ursolic acid (10 mg/kg) as well as diazepam (2 mg/kg) was able to increase the percentage of open arms entries (A) and total time spent in the open arms (B) of the elevated plus maze. The one-way ANOVA showed a significant effect of treatment [$F(4,35)=3.16$, $P<0.05$] on the open arms entries and a significant effect of treatment [$F(4,35)=4.76$, $P<0.05$] on the total time spent in the open arms of the elevated plus maze.

3.3. Effect of ursolic acid in the light/dark box

Fig. 4 shows that the treatment with ursolic acid was not able to alter the parameters analyzed in the light/dark box. The positive control, diazepam (2 mg/kg), increased the latency for the first entry in the dark area (A) and the total time spent in the light area of the apparatus (B). The one-way ANOVA showed a significant effect of treatment [$F(4,35)=3.50$, $P<0.05$] on the latency time to enter in the dark area and a significant effect of treatment [$F(4,35)=3.62$, $P<0.05$] on the total time spent in light area of the light/dark box.

3.4. Effect of ursolic acid in the marble burying test

Fig. 5 shows that the treatment with ursolic acid was not able to alter the number of marbles buried in the marble burying test. Conversely, the positive control diazepam (2 mg/kg) decreased the number of marbles buried in this test. The one-way ANOVA showed a significant effect of treatment [$F(4,35)=3.65$, $P<0.05$] on the total number of marbles buried in the marble burying test.

4. Discussion

Anxiety disorders are severe psychiatric conditions that affect performance in daily tasks and represent a high cost to public health (Campos et al., 2013; Combs and Markman, 2014). Given the costs in

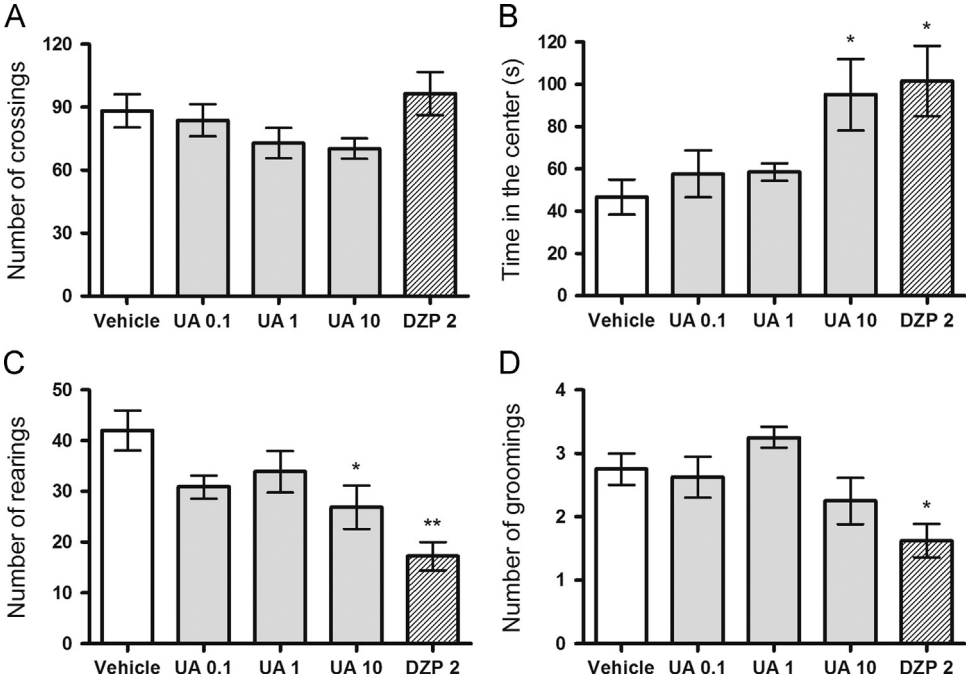

Fig. 2. Effect of treatment with ursolic acid (UA, 0.1, 1 and 10 mg/kg) or diazepam (DZP, 2 mg/kg) in the number of crossings (A), total time spent in the center (B), number of rearings (C), and number of groomings (D) in the open field test. Each column represents the mean ± S.E.M ($n=8$). $^{*}P<0.05$ and $^{**}P<0.01$ when compared with vehicle-treated control (one-way ANOVA followed by Newman–Keuls posthoc test).

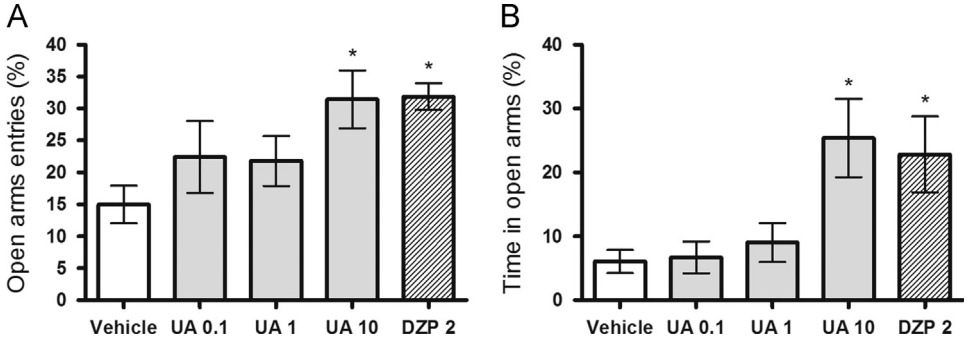

Fig. 3. Effect of treatment with ursolic acid (UA, 0.1, 1 and 10 mg/kg) or diazepam (DZP, 2 mg/kg) in the percentage of open arms entries (A) and total time spent in the open arms (B) in the elevated plus maze test. Each column represents the mean ± S.E.M ($n=8$). $^{*}P<0.05$ compared with the vehicle-treated control (one-way ANOVA followed by Newman–Keuls posthoc test).

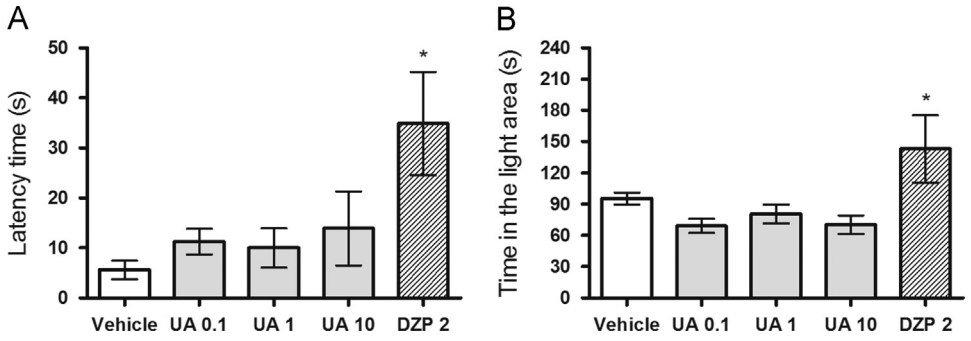

Fig. 4. Effect of treatment with ursolic acid (UA, 0.1, 1 and 10 mg/kg) or diazepam (DZP, 2 mg/kg) in the latency time for the first entry in the dark area (A) and total time spent in the light area (B) in the light/dark box test. Each column represents the mean ± S.E.M ($n=8$). *$P<0.05$ compared with the vehicle-treated control (one-way ANOVA followed by Newman–Keuls posthoc test).

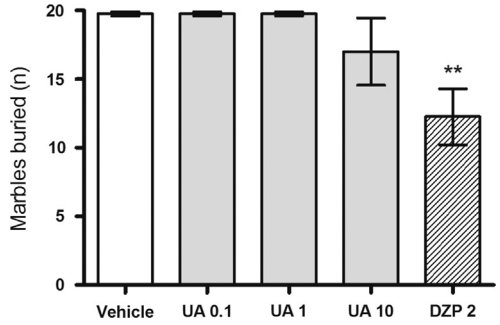

Fig. 5. Effect of treatment with ursolic acid (UA, 0.1, 1 and 10 mg/kg) or diazepam (DZP, 2 mg/kg) in the number of the marbles buried in the marble burying test. Each column represents the mean ± S.E.M ($n=8$). **$P<0.01$ compared with the vehicle-treated control (one-way ANOVA followed by Newman–Keuls posthoc test).

terms of debilitation and associated financial burden, and increased risk of suicide, the search for new and effective treatment of anxiety disorders is imperative (Combs and Markman, 2014). Despite their drawbacks, animal models are invaluable tools for investigation of the neurobiology of anxiety-related disorders (Campos et al., 2013). Among the available tests to assess anxiety-like behavior, the open field test, the elevated plus maze, and the light/dark box are the most commonly used (Kliethermes, 2005). In this study, we evaluated the possible anxiolytic-like effect of ursolic acid administration in different behavioral paradigms, the open field test, elevated plus maze, light/dark box and marble burying test. Our results demonstrate that acute administration of ursolic acid (10 mg/kg) produced an anxiolytic-like effect in the open field test and elevated plus maze. Interestingly, the effective anxiolytic dose is the same one that presents other neuropharmacological effects such as antidepressant-like effect in the forced swimming test (Machado et al., 2012b), and antioxidant and anti-inflammatory properties in mice (Lu et al., 2007, 2010). Otherwise, studies regarding its antitumor property show that higher doses of this compound (50–200 mg/kg) are necessary to elicit antitumoral action (De Angel et al., 2010; Shanmugam et al., 2011; Shih et al., 2013).

The open field test is a widely used procedure for examining the behavioral effects of drugs in locomotor activity and anxious behavior (Choleris et al., 2001; Rodrigues et al., 1996). To evaluate the effect of treatments (ursolic acid and diazepam) in the locomotor activity, the number of crossings was measured in the open field test. Ursolic acid and diazepam caused no alterations in the spontaneous locomotion of animals, ruling out the possibility that psychostimulant effect may account for the observed behavioral responses indicative of anxiolytic action in the tests employed. Besides the locomotor parameter, the open field test allows making inferences about anxious behavior, since anxiolytic compounds lead to an increase in the total time spent in the center of apparatus, and a decrease in the number of rearings and groomings (Costa et al., 2014; Kalueff et al., 2007a; Lakshmipathy Prabhu et al., 2012; Machado et al., 2012a). Our results showed that the ursolic acid administration, at dose of 10 mg/kg, similar to diazepam (2 mg/kg), was able to produce an increase in the total time spent in the center of open field and a reduction in the number of rearings, suggesting an anxiolytic-like effect of the ursolic acid administration. The number of groomings was reduced only in the diazepam group. In fact, the number of grooming was reported to be reduced by anxiolytic drugs; however, grooming behavior has been proposed as a parameter related more specifically with obsessive–compulsive disorder than unconditioned anxiety (Ting and Feng, 2011; Yang and Lu, 2011).

To reinforce the notion that ursolic acid exhibits anxiolytic properties, we tested its effect in the elevated plus maze that is a well-established and the most used test to detect anxiolytic/anxiety-like effect (Hogg, 1996; Walf and Frye, 2007). Similar to the open field test, the elevated plus maze is based on the natural conflict between the drive to explore a new environment and the tendency to avoid a potentially dangerous area (Ramos, 2008). The most classical indices of anxiolytic-like behavior in elevated plus maze test are the increased percentage in open arms entries and total time spent in the open arms (Jindal et al., 2013; Rodgers and Dalvi, 1997). Our results demonstrate that the acute administration of ursolic acid, at dose of 10 mg/kg, the same dose that presented anxiolytic-like behavior in the open field test, presents an anxiolytic-like effect in the elevated plus maze, since it increased the percentage of open arms entries and the total time spent in the open arms, similar to the result produced by diazepam, used as a reference drug. The elevated plus maze can be considered a very valuable tool in drug screening and in the study of the neurobiology of anxiety (Rodgers and Dalvi, 1997), and our results strengthen the notion that ursolic acid administration may elicit an anxiolytic-like effect in mice.

The light/dark box is a test based on the innate aversion of rodents to places with bright light. It generates an inherent conflict between the exploratory drive of rodents and their avoidance of the lit compartment (Campos et al., 2013). In our study, no dose of ursolic acid was able to induce anxiolytic behavior in this test. Conversely, diazepam administration increased the latency to first entry in the dark side and the total time spent in the light compartment. The literature data reported that the administration of anxiolytics increases the latency to enter in the less aversive side of the box and increases the time spent in the lit compartment, more aversive side (Kliethermes, 2005). Indeed, benzodiazepines are reliably detected in this behavioral paradigm. On the contrary, antidepressant compounds, especially those that act on the serotonergic system, have been shown to elicit a mild response or even no response in the light/dark box in mice (Bourin and Hascoet, 2003). Interesting, ursolic

acid was previously reported to exert an antidepressant-like effect by a serotonergic-mediated mechanism (Colla et al., 2014), a finding that could explain the lack of response of ursolic acid in the light/dark box. Moreover, ursolic acid exhibited a significant inhibition of monoamine oxidase B (Kim et al., 2012), and literature shows that monoamine oxidase B inhibitors, such as selegiline, exert no anxiolytic-like effect in this test (De Angelis and Furlan, 2000).

Rodents use bedding material to bury noxious as well as harmless objects and the inhibition of object burying is suggested as a screening test for anxiolytic activity (Albelda and Joel, 2012). In our study, ursolic acid administration did not produce anxiolytic profile in the marble burying test, since it was not able to cause a decrease in the marble buried. Interestingly, ursolic acid administration displayed anxiolytic effects in the open field test and elevated plus maze, but not in the light/dark box or marble burying test. Notably, marble burying test has been suggested as a more specific test to study obsessive–compulsive disorder than generalized anxiety (Albelda and Joel, 2012; Kalueff et al., 2007b). Furthermore, ursolic acid besides not causing significant effects in marble burying test produced no change in the number of groomings in the open field test, a parameter that has been also proposed to investigate obsessive–compulsive disorder-related behavior (Fineberg et al., 2011; Graybiel and Saka, 2002). Despite open field test, elevated plus maze, light/dark box, and marble burying test are supposed to evaluate anxiety-like behavior in rodents; it is feasible that these behavioral tests detect different aspects of the multifaceted and complex nature of anxiety, since the neurobiological mechanisms underlying the behavioral responses in each test may be different (Albelda and Joel, 2012; Gavioli et al., 2007; Kalueff et al., 2007b; Kedia and Chattarji, 2014). In line with this, our study shows the relevance of testing several behavioral paradigms in the evaluation of anxiolytic-like actions.

Altogether, the results of the present study demonstrate for the first time the anxiolytic-like effect of ursolic acid administration in the open field test and elevated plus maze, behavioral tests broadly used for the screening of anxiolytic compounds. Preclinical studies show that not only benzodiazepines, but also antidepressants are able to reduce the typical anxiety-like behaviors in rodents in these behavioral paradigms (Bourin and Hascoet, 2003; Choleris et al., 2001; Jindal et al., 2013). Consistently, ursolic acid, a compound with safe pharmacological profile (Aggarwal et al., 2004), has been shown by our group to exert antidepressant-like effect (Colla et al., 2014; Machado et al., 2012b). The diagnosis of depression is often associated with the presence of others disorders, particularly with anxiety disorders, being the most prevalent comorbidity (Brown et al., 2001; Hecht et al., 1990), and selective serotonin reuptake inhibitors and serotonin–norepinephrine reuptake inhibitors effectively prevent anxiety symptoms, reduce the risk for relapse, and have a better safety profile in comparison with benzodiazepines, which confer no long-term preventive benefit (Baldwin et al., 2011; Thronson and Pagalilauan, 2014). Considering the high anxiety/depression comorbidity, the need to develop more efficacious pharmacological approaches to the management of this condition (Baldwin et al., 2011), the previously reported antidepressant-like effect of ursolic acid and its anxiolytic-like effect demonstrated in this work suggest that the pharmacological action of ursolic acid may offer a novel therapeutic strategy for the treatment of comorbidity depression/anxiety.

5. Conclusions

In summary, the results of this study show for the first time that the acute oral administration of ursolic acid elicits an anxiolytic-like effect in widely and well-established tests to evaluate generalized anxiety in mice, the open field test and elevated plus maze. The results suggest that it may be a novel approach for the management of anxiety-related disorders.

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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