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In vivo stress model points (-)-α-Bisabolol as an alternative to relieve effects on neuropsychiatric disorders

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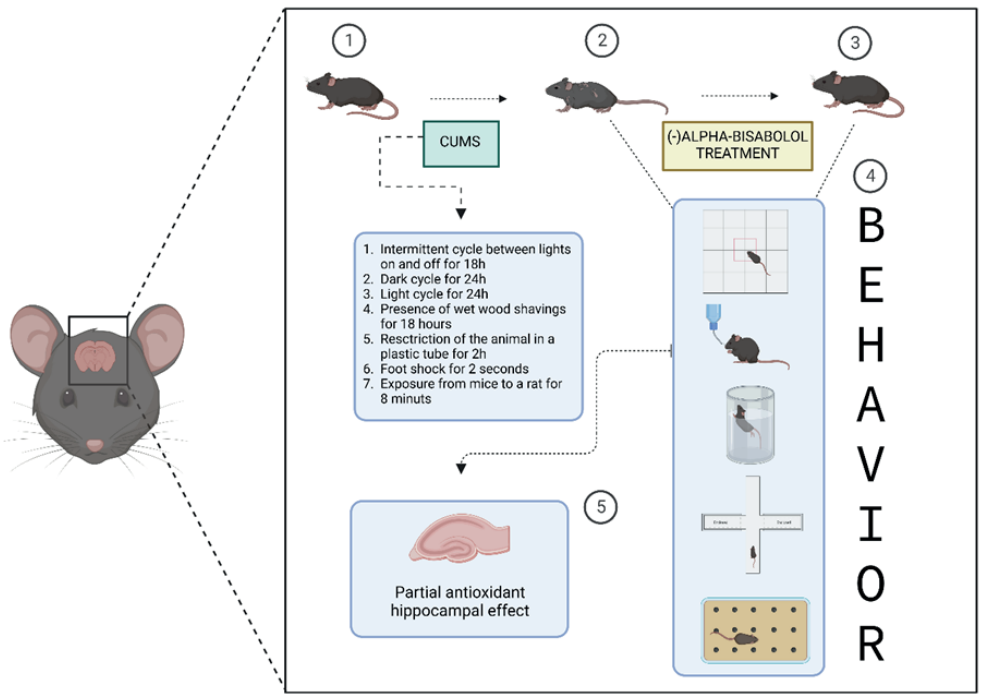
Graphical abstract



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Abstract

Several mechanisms are often associated with depression, including the role of chronic and oxidative stress. The present study aimed to investigate the antidepressant and anxiolytic effect of (-)-α-bisabolol (BIS) and its possible mechanisms of action related to alterations on products of oxidative stress, using the model of depression induced by Chronic Unpredictable Mild Stress. The male C57BL/6 mice were treated with CUMS for 28 days to

observe the depressive and anxiety-like behaviors at the end of the protocol. The depressive and anxious behavior was successfully attenuated after BIS administration, evidenced in forced swimming tests and preference for sucrose solution results, with exciting results when compared to fluoxetine, the reference drug for depression. The antioxidant profile as a mechanism indicated a significant reduction of thiobarbituric acid reactive substances (TBARS) levels in the hippocampus area. In brief, the study revealed that BIS relieve depressive and anxiety-like state by mitigating oxidative stress.

psychological disorders; monoterpene; chronic stress; animal model; oxidative stress

INTRODUCTION

Depression is a neuropsychiatric illness that affects more than 300 people worldwide and is considered the leading cause of disability and a public health concern [1]. The multifactorial cause of depression leads to chronic stress and anxiety, modifying physiological conditions and affecting people's behavior socially and cognitively [2, 3].

Although we have a significant group of antidepressant drugs in the pharmaceutical market, more than 30% of patients are resistant to treatment, present considerable adverse effects, have long latency periods for observation, and drug withdrawal [4, 5]. Thus, the search for alternative and safer substances is essential to reduce the impact caused by classic antidepressants and depression.

In this way, chronic and unpredictable stress (CUMS) is widely used to mimic in rodents what happens in depressed patients. Furthermore, this behavioral *in vivo* model can be applied to search for new antidepressant drugs [3, 6].

The pathophysiology of depression is associated with enhancing proinflammatory response, elevation of proinflammatory cytokines, ROS production, oxidative stress, and lipid peroxidation, which are important [7]. In this way, natural compounds, such as those that reduce inflammation events, lipidic peroxidation, and oxidative stress, are exciting candidates as antidepressant agents. Thus, (-)-α-bisabolol, a sesquiterpene alcohol from *Matricaria chamomilla* L., which has described anti-inflammatory, antioxidant, and neuroprotective effects[8–13], is a noteworthy agent in CUMS model of depression.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (22-25 g, age: five weeks, considered as young adults) were used in this study. The animals were kept at a controlled temperature (22 ± 1°C), with a 12-hour light/dark cycle, and received water and food *ad libitum*. The experiments followed current legislation and with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals and under the consent and surveillance of the Ethics Committee of the Department of Physiology and Pharmacology of the Federal University of Ceará (Protocol N°. 9361/2018).

Drugs

(-)-α-bisabolol (BIS; Sigma®, St. Louis, USA), diluted in 3% polysorbate (Tween 80 ®) with 0.9% NaCl saline solution, was administered orally by gavage for 14 consecutive days. The applied dose of BIS (50 mg kg^{-1}) followed previous work with other experimental models [14]. The reference drug, Fluoxetine (FLU; Sandoz®, Barleben, Alemão), was diluted in 0.9% saline solution and administered orally (10 mg kg^{-1}) by gavage following the same time protocol.

In silico ADMETox for distribution and metabolism description

The isomeric SMILES of (-)-α-bisabolol was extracted from PubChem® for ADMETox prediction. The prediction was analyzed considering the algorithm developed by [15]. The pharmacologic patterns considered each pharmacokinetic phase's predicted and reference values. The

distribution was analyzed to evaluate the prediction related to permeability of BBB and CNS, while liver metabolism and toxicity results were compared to fluoxetine.

Chronic Unpredictable Mild Stress (CUMS) procedure and experimental design

The CUMS procedure considered the previously described by He [16] with minor modifications. Initially, the mice were housed for seven days to adapt to their environment.

The study had six experimental groups (8 animals/group): 1 – saline solution (CONTROL); 2 – saline and (-)-α-bisabolol (CONTROL+BIS); 3 – saline and fluoxetine (CONTROL+FLU); 4 – CUMS and saline solution (CUMS); 5 – CUMS and (-)-α-bisabolol (CUMS+BIS) and 6 – CUMS and fluoxetine (CUMS+FLU). The protocols had a total of 28 days, 14 days to stressor protocol followed for 14 days with pharmacological treatment.

The CONTROL, CONTROL+BIS, and CONTROL+FLU groups were left in their cages separately. The CUMS groups received different stressors (intermittent cycle between lights on and off for 18 h, presence of wet wood shavings for 18 h, dark cycle for 24 h, light cycle for 24 h, restriction of the animal in a plastic tube for 2 h, exposure from mice to a rat, separated by an iron grid for 8 minutes and foot shock for 2 seconds. All stressors were applied randomly and unpredictably in all the CUMS groups.

On the 15th day, at the end of the stressors protocol, the pharmacological treatment was started with (-)-α-bisabolol, fluoxetine, or saline. Twenty-four hours (24h) after the last treatment, were applied the behavioral tests: Open Field Test (OFT), Forced Swimming Test (FST), Sucrose Preference Test (SPT), Elevated Plus Maze (EPM), and Holeboard test (HB). The tests were carried out in three phases, and after the last behavioral test, the animals were euthanized. The brain areas (prefrontal, cortex, and hippocampus) were sampled to measure the reactive substances of thiobarbituric acid (TBARS), nitrite, and reduced glutathione (GSH).

Behavioral tests

Open Field Test (OFT)

The OFT was performed in an open acrylic field device (transparent walls and black bottom, 30 x 30 x 15 cm) divided into nine equal quadrants. This test assesses the animal's exploratory activity for 5 minutes after a 1-minute acclimatization period. The crossing parameter was assessed by the number of times the mice crossed each quadrant. The test was conducted in a controlled environment, with a silenced temperature and adequate lighting [17].

Forced Swimming Test (FST)

The experimental procedure consisted of placing the animals individually in acrylic cylinders (height: 35 cm; diameter: 24 cm) containing 13.5 cm^3 of water for 5 min. The FST evaluates the animal immobility time, considering this parameter when the animal makes only the minimum movements to keep its head out of the water, which means depressive-like behavior [18].

Sucrose Preference Test (SPT)

The SPT was performed to evaluate the anhedonia-like behavior [19]. On the twenty-seventh day of the experimental protocol, the mice were submitted to an adaptation phase (48h). The water bottles were replaced with 2% sucrose solution for 24 h; after that, one bottle was replaced again for water, and the bottles were available for another 24 h.

After adaptation, all animals were deprived of food and water for 18 hours. After that, the animals were placed in individual cages and exposed to two bottles, one with 2% sucrose solution and the other with water. After 3 hours, the volume of consumption sucrose solution and water were measured. Sucrose preference was calculated as the amount of sucrose solution consumed by the total fluid intake as percentual, using the following formula (**Sucrose preference** $= {}^{\text{sucrose consumption}}/_{\text{(water consumption + sucrose consumption)}} \text{x}100$).

Hole board (HB)

The hole-board test, consists of a 20 x 20 cm apparatus with 16 evenly spaced holes used to evaluate the exploratory behavior in mice. The

parameter analyzed was the number of times the animal placed its head in the holes (head dips) during the 5 min period [20].

Elevated Plus Maze (EPM)

The EPM is a plus-shaped device with four arms at right angles to each other, as described by Lister [21]. The animal was placed in the center of the plus maze facing one of the closed arms and observed for 5 min, according to the following parameters: number of entries in the open and closed arms and the time spent in each arm. The arena was cleaned with 5% ethanol solution between each animal tested. The criterion for visiting the arm was considered when the animal decisively moved all four limbs on one arm. The time spent in the open and closed arms and the number of entries in each were recorded live by two blinded experimenters. The percentage of open entries and the time spent in the open arms were calculated from these data.

Neurochemical test

Thiobarbituric Acid Reactive Substances (TBARS)

The degree of lipid peroxidation in brain areas was measured by determining the levels of thiobarbituric acid reactive substances (TBARS) [22]. The protocol was performed using the brain areas after making a 10% homogenate (pH 7.4, 50 mM monobasic potassium phosphate buffer), adding 35% perchloric acid, and then centrifugation. Thiobarbituric acid was added to the supernatant, kept in a water bath, and read at 535 nm. Results were expressed as micrograms per gram (μg/g) of tissue.

Reduced Glutathione (GSH) concentrations

GSH levels were evaluated to estimate endogenous defenses against oxidative stress. The method is based on the reaction of Ellman's reagent (DTNB). Brain areas were diluted in 0.02 M EDTA buffer (10% w/v) and added to a 50% trichloroacetic acid solution. The supernatant was collected after centrifugation (3000 RCF; 15 min, (22 ± 1 °C). The samples were mixed with 0.4 M Tris-HCl buffer, pH 8.9, and 0.01 M DTNB. The GSH was determined spectrophotometrically at 412 nm, calculated based on a standard glutathione curve, and expressed as micrograms per gram (μg/g) of fresh tissue [23].

Determination of nitrite concentrations

The measurement of nitrite levels was performed using the Griess Reagent (5% phosphoric acid, 1% sulfonylamide in 5% phosphoric acid, 0.1% NEED, and distilled water). The solution was added to the supernatant and incubated at room temperature for 10 min [24]. The standard curve was drawn considering different concentrations of NaNO2 (ranging from 0.75 to 100 mM) under the same conditions. The absorbance was measured in a microplate reader at 540 nm, and the result was expressed in nanomolar nitrite per gram (nM/g) of tissue.

Statistical analysis

All values were calculated in medians (range). The Shapiro-Wilk normality test was used to analyze the values away from an approximate Gaussian distribution. The Kruskal-Wallis test assessed the difference between groups, considering $p < 0.05$ as a significant difference. The Mann-Whitney test (U) was performed to monitor this analysis, which evaluated the difference between the pairs using the Bonferroni correction. In addition, the z score (number of standard deviations) and the effect size (r) were demonstrated. All the statistical analyses were performed using SPSS 23.0 software (SPSS, Chicago, Illinois, USA).

Results

In silico ADMETox predictions

The ADMETox predictions indicated a similar pattern in Absorption parameters between the (-)-α-bisabolol and the positive control fluoxetine. The markable difference was observed in the inhibitor P-glycoprotein I, not predicted to (-)-α-bisabolol. Even with similar absorption profiles, the prediction indicates that distribution, metabolism, and excretion had different profiles, as shown in Table 1. The distribution profile indicates that the (-)-α – bisabolol could have a higher possibility to cross CNS (log

PS – 2.541) than fluoxetine (log PS – 1.329) besides the similar ability of cross BBB, supporting the hypothesis that the (-)-α-bisabolol isolated can have other effects in neuropsychiatric disorders further the inflammatory activity.

The metabolism results point to a different route of metabolization considering the CYP450 enzymes that also can be associated with the no predicted inhibition of P-glycoprotein I. The excretion results may indicate a faster excretion for (-)-α – bisabolol compared to fluoxetine, and this result may be related to the less predicted toxicity as indicated below.

The performed prediction supports the use of (-)-α-bisabolol *in vivo* to evaluate its effects on neuropsychiatric disorders such as depression and anxiety.

Table 1. ADMETox predictions to (-)-α-bisabolol compared to fluoxetine as a model for predicting distribution and toxicity patterns.; BBB – Blood-Brain Barrier; CNS – Central Nervous System

	Model name	(-)-α – bisabolol	Fluoxetine	Unit/Reference value
Absorption	Water solubility	-4.379	-4.455	Numeric (log mol/L)
	Caco2 permeability	1.505	1.764	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	93.014	91.371	Numeric (% Absorbed)
	Skin Permeability	-1.761	-2.482	Numeric (log Kp)
	P-glycoprotein I inhibitor	No	Yes	Categorical (Yes/No)
Distribution	VDss (human)	0.42	1.19	Numeric (log L/kg)
	Fraction unbound (human)	0.321	0.037	Numeric (Fu)
	BBB permeability	0.605	0.501	Numeric (log BB)
	CNS permeability	-2.541	-1.329	Numeric (log PS)
Metabolism	CYP3A4 substrate	No	Yes	Categorical (Yes/No)
	CYP1A2 inhibitor	No	Yes	Categorical (Yes/No)
	CYP2C19 inhibitor	Yes	Yes	Categorical (Yes/No)
	CYP2D6 inhibitor	No	Yes	Categorical (Yes/No)
Excretion	Total Clearance	1.363	0.694	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	Yes	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	Yes	Categorical (Yes/No)
	Hepatotoxicity	No	Yes	Categorical (Yes/No)
	Skin Sensitisation	Yes	No	Categorical (Yes/No)

Behavioral tests

Open field test (OFT)

Figure 1A showed no significant differences between the CONTROL, CONTROL+FLU, and CONTROL+BIS groups (p=0.079). The same was verified in comparisons between CUMS, CUMS+FLU and CUMS+BIS groups (p = 0.552). In addition, when comparing the CONTROL and CUMS groups, it was verified that stress also did not shift the horizontal locomotor activity (U=28.00; z=-0.771; p=0.481; r=-0.19).

Forced Swimming test (FST)

The evaluation of the control groups (CONTROL, CONTROL+BIS, and CONTROL+FLU) presented no differences (Figure 1B). This result shows that the pretreatment with BIS and FLU, without the stress model, did not change the immobility time (p=0.617). As expected, the CUMS group presented a higher immobility rate compared to the control group (U = 2.00; z = – 3.009; p = 0.001; r = – 0.75, Figure 1B).

The immobility time between stressed groups (CUMS, CUMS+BIS, and CUMS+FLU) was significantly affected by the treatment with both drugs BIS and FLU (p <0.0001). The CUMS+BIS group reduced immobility time when compared to the CUMS group (U=0.00; z=-3.243; p<0.0001; r=-0.81). The same was verified in the CUMS+FLU group, in which it presented a significant reduction (U=9.00; z =-2.201; p=0.029; r=-0.55) compared to the control group (Figure 1B).

Preference for the sucrose solution test (PST)

In the test of preference for the sucrose solution (Figure 1C), it was found that the comparison between CONTROL, CONTROL+FLU, and CONTROL+BIS groups did not have a significant difference (p=0.065). However, a decrease in sucrose consumption was observed in the animals-CUMS stressed compared to the control group (CONTROL) (U=8.00; z=-2.701; p=0.006; r =-0.67).

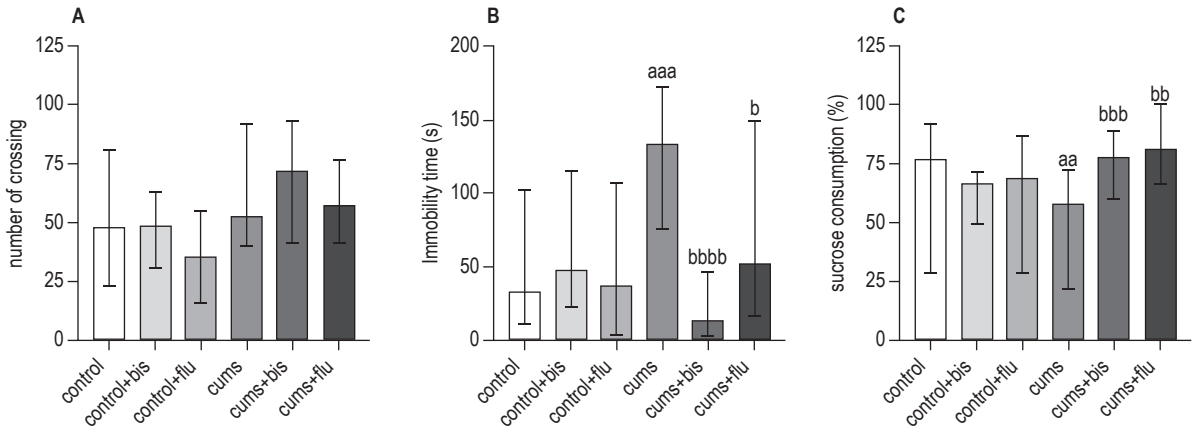

Figure 1. Screening of (-)-α-bisabolol effect in neurologic Central Nervous system after 14 days with saline solution, (-)-α-bisabolol (BIS, 50 mg kg-1) or fluoxetine (FLU, 10 mg kg-1) treatment while were submitted CUMS for 28 days. (A) Effect of (-)-α-bisabolol administration in open field test. (B) Effect of (-)-α-bisabolol administration in Forced Swimming Test (FST). (C) Effect of (-)-α-bisabolol administration in sucrose consumption. Values are represented as median (range). aap<0.01, aaap <0.001 vs CONTROL; bp <0.05, bbp <0.01, bbbp <0.001, bbbbp <0.0001 vs CUMS. Kruskal-Wallis, followed by Mann-Whitney (pairs) and Bonferroni correction. CUMS: Unpredictable Chronic Mild Stress.

(-)-α-bisabolol produced an anxiolytic-like effect in the unpredictable mild chronic stress (CUMS) model

The comparison among the group CUMS, CUMS+FLU, and CUMS+BIS groups showed a significative difference (x^2=11,792; df=2; p=0.003) (Figure 1C). The group treated with (-)-α-bisabolol (CUMS+BIS) increased the consumption of sucrose when compared to the group that received only saline (CUMS) (U=3.5; z=-2.998; p=0.001; r= – 0.74), the same was verified to the treatment with fluoxetine (CUMS+FLU) (U=4.5; z=-2.892; p =0.002; r =-0.72).

Elevated Plus Maze test (EPM)

The CUMS, CUMS+BIS, and CUMS+FLU groups analyzed the percentage of the open arms entries (Figure 2A) (p=0.049) and the percentage of time spent on the open arms (Figure 2B) (x^2 = 15,142; df = 2;p = 0.001) demonstrated a significant difference between them. When comparing the CONTROL and CUMS groups, in both parameters, there was a reduction in the open arms entries (U=2.0; z=-3.165; p = 0.001; r = – 0.79) and the time spent on open arms (U=0.0; z = – 3.466; p <0.0001; r = – 0.86) in the group subjected to stress.

In the evaluation of the open arms entries (%), the group treated with BIS (CUMS+BIS) showed a significant increase in this parameter when compared to the CUMS group (U=10.0; z=- .319; p=0.021; r=-0.57). The Fluoxetine treatment (CUMS+FLU) did not present differences compared to CUMS group (U = 17.0; z = – 1.588; p = 0.130; r = – 0.39).

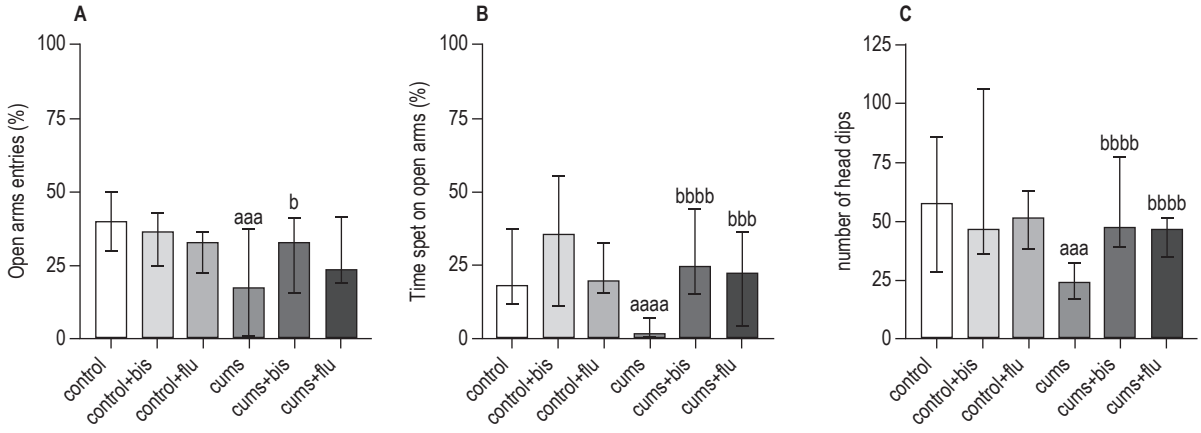

Figure 2. Anxiolytic-like effect of (-)-α-bisabolol after 14 days of saline solution, (-)-α-bisabolol (BIS;50mg/kg) or fluoxetine (FLU;10mg/kg) treatment while was submitted CUMS for 28 days. The effect of (-)-α-bisabolol administration in Elevated Plus Maze test (A) percentage number of entries. (B) The percentage time on open arms. (C) Effect of (-)-α-bisabolol administration in Hole-board test. Values are represented as median (range) aaa p <0.001; aaaa p <0.0001 vs CONTROL; bp <0.05; bbbp <0.001; bbbbp <0.0001 vs CUMS. Kruskal-Wallis, followed by Mann-Whitney (pairs) and Bonferroni correction. CUMS: Unpredictable Chronic Mild Stress.

Considering the time spent on open arms (%), the CUMS+BIS group showed an increase in time spent on open arms when compared to the CUMS group (U=0.0; z = – 3.363; p <0.0001; r = – 0.84). The same was observed in CUMS + FLU group (U=2.00; z = – 3.153; p = 0.001; r = – 0.78).

Hole-board test (HB)

The comparison between CONTROL, CONTROL+FLU, and CONTROL+BIS groups demonstrated no significant difference in the pretreatment with fluoxetine and (-)-α-bisabolol (p=0.422). The animals submitted to stress without treatment (CUMS) showed a significant reduction when compared to the CONTROL group (U = 2.00; z = – 3.153; p = 0.001; r = – 0.78) (Figure 2C).

The CUMS, CUMS+FLU and CUMS+BIS groups showed a significative difference between them (x^2 = 15,538; df = 2; p <0.0001). The treatment with BIS (CUMS+BIS) was able to reverse the evaluated parameter when compared with CUMS (U = 0.00; z = – 3.363; p <0.0001; r = – 0.84). The same results were found in the comparison between CUMS+FLU and CUMS (U = 0.00; z = – 3.363; p <0.0001; r = – 0.84) (Figure 2C).

Effect of (-) – α-bisabolol on TBARS levels of the hippocampus and prefrontal cortex in CUMS models

The comparison between CUMS and CONTROL groups showed significant differences in Hippocampus (U = 2.00; z = – 3.151; p = 0.001; r = – 0.78) and prefrontal cortex (U = 2.00; z = – 2.567; p = 0.009; r = – 0.64) (Figure 3A and 3a).

In the hippocampus area, it was found a significant difference among CUMS, CUMS+FLU, and CUMS+BIS groups (p <0.0001). The group treated with (-)-α-bisabolol (CUMS+BIS) significantly reduced thiobarbituric acid reactive substances (TBARS) levels when compared to CUMS group (U=0.00; z=-3.361; p <0.0001; r = – 0.84). The same was observed in CUMS+FLU group when compared to the CUMS group (U = 3.00; z = – 3.046; p = 0.001; r = – 0.76). In the prefrontal cortex, the comparison between the CUMS, CUMS+FLU, and CUMS+BIS groups did not evidence differences (p=0.238).

Effect of (-)-α-bisabolol on levels of reduced glutathione (GSH) of the hippocampus and prefrontal cortex in the CUMS

The comparison between CUMS and CONTROL groups showed a significative difference only in hippocampus (p=0.015). The analysis performed between the CUMS, CUMS + BIS, and CUMS+FLU groups showed no significant difference in both brain areas (Hippocampus: p = 0.994; prefrontal cortex: p = 0.238) (Figure. 3B and 3b).

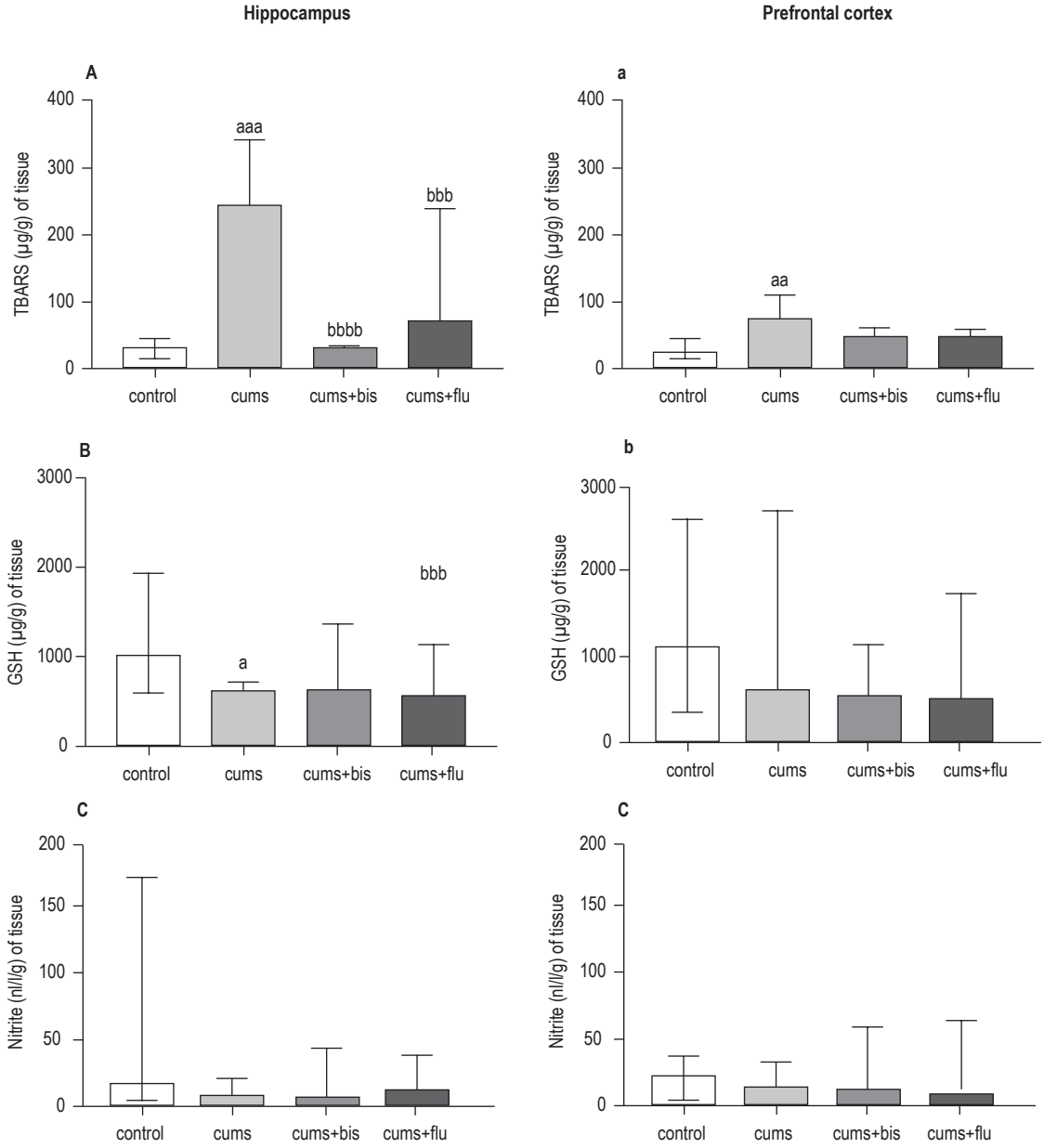

Figure 3: Effects of (-) – α-bisabolol on GSH pathway in Hippocampus and Prefrontal Cortex after 14 days with saline solution, (-)-α-bisabolol (BIS;50mg/kg) or fluoxetine (FLU;10mg/kg) treatment while exposed CUMS for 28 days. (A, a) Thiobarbituric acid reactive substances (TBARS), (B, b) Measurement of reduced glutathione (GSH) and (C, c) reduced nitrite. Values are represented as median (range). ap <0.5, aap <0.01 aaap <0.001; vs CONTROL; bbbp <0.001 bbbbp <0.001 vs CUMS Kruskal-Wallis or Mann-Whitney (pairs). CUMS: Unpredictable Chronic Mild Stress.

Effect of (-)-α-bisabolol on nitrite levels of the hippocampus and prefrontal cortex in the model of unpredictable mild chronic stress

No significative differences were found between CONTROL and CUMS groups in nitrite levels in the hippocampus (U =15.5; z =-1.739; p=0.083; r=-0.43; Figure 3C) and prefrontal cortex (U=21.00; z=-1.162; p=0.279; r=-0.28; Figure 3c). Additionally, the comparison between CUMS, CUMS+BIS, and CUMS+FLU groups showed no differences between them (Hippocampus: p = 0.624; prefrontal cortex: p = 0.912).

DISCUSSION

The present study showed that treatment with (-)-α-bisabolol reversed behavior and caused neurochemical changes in animals submitted to the CUMS model. Additionally, it was possible to detect the effectiveness of this model in inducing depressive-like behaviors, proving the relationship between stress and depression a critical relation that has been studied [25] and can bring new study lines to prevent depression.

The hypothesis of (-)-α-bisabolol comes considering his possible protective effects as an antioxidant [26] and the relation between stress and depression [6]. Besides the experimental approaches, studies have indicated using *in silico* predictions to point to the markable characteristics for molecules before *in vivo* studies, including ADMETox evaluation [27–30]; this kind of prediction can guide the drug behavior pattern before the tests.

The present studies compared the *in silico* ADMETox characteristics of (-)-α-bisabolol and the antidepressant drug fluoxetine, used as the positive control, to observe the expected patterns in pharmacokinetics parameters. The results indicated a similar pattern in Absorption parameters between the (-)-α-bisabolol and the positive control fluoxetine, indicating that the absorption pathway will not be a concern in the comparative experimental studies confirmed after the experimental tests. The markable differences between both compounds were observed in the inhibitor P-glycoprotein I not predicted to (-)-α – bisabolol and the CYP450 enzymes metabolism that probably point to a different route of metabolization between the compounds. The relation between P-glycoprotein I inhibitors and CYP450 enzymes is usually necessary in drug design experiments to minimize adverse reactions [31]. It can be the difference between the different toxicity levels for each drug. The *in silico* prediction for (-)-α-bisabolol indicates less toxicity than fluoxetine. One of the most important predictions is in distribution and points (-)-α-bisabolol with a more remarkable ability to CNS sensitization and similar ability to cross BBB than Fluoxetine. The predictions bring to the studies a point to expect protection by (-)-α-bisabolol in neurological disorders, such as those observed *in vivo* after the use of the mild unpredictable chronic stress model (CUMS).

The use of CUMS model was pointed out as an inducer of depressive-like behavior, considering that it presents criteria of reliability, efficacy, and mimics that occur in humans and animals. In this context, the animals were initially exposed exclusively to CUMS to observe their pattern of behavior and later submitted to the tests such as swimming on what showed depressive-like conduct indicated by the increase in immobility time. The motionless behavior ratifies the exposition of the animals to CUMS as an effective model to induce a depressive-like state.

Studies indicate that CUMS, besides causing behavioral and biochemical changes considered like those reported in patients with depression [26], also activates the hypothalamic-adrenal pituitary axis (HPA), causing a depressive phenotype. It is a substantial point that (-)-α-bisabolol had an absence of psychostimulant, sedative, or muscle relaxant effects, confirmed by the non-alteration of locomotor activity evaluated in the open field test (OFT). The OFT test is routinely used to evaluate locomotor and exploratory behavior in rodents [32]. The results observed in the OFT test show the relation drugs such as antidepressants and anxiolytics currently used in the clinic with psychomotor effects that can cause sedation or significant motor changes, limiting patient activities such as driving a vehicle or operating machinery [33]. Previous *in vivo* studies [25] using (-)-α-bisabolol also did not observe a relaxing effect in the Alzheimer model.

Classical test such as forced swimming (FST) is used to search for new drugs with antidepressant potential and are based on behavior observation after subjecting the animals to a situation with no possibility of escape. After a period of agitation, they tend to remain immobile, and this immobility and the decrease in active behavior are indicative of depressive-like symptoms in animals. In addition, antidepressants used in the clinic effectively reduce immobility time in these tests [34, 35].

The potential antidepressant effect of (-)-α-bisabolol in this study could be indicated by the decrease in immobility time observed in treated animals when exposed to FST. The reversion of the increased immobility time observed in CUMS-induced animals could be a sound in-

dicative of potential use in depression. Furthermore, it is essential to mention that some studies investigating other terpenoids, such as thymol, geraniol, and bilobalide, also observed a reduction in immobility time parameter in the same model, indicating antidepressant effects for the sesquiterpene alcohols such as (-)-α-bisabolol [1,36,37].

Among the symptoms presented in depression, one of the most evident is anhedonia, characterized by the loss of pleasure or interest in performing daily activities. This symptom is part of the diagnostic criteria for major depressive disorder (MDD), according to the Diagnostic and Statistical Manual of Mental Disorders-DSM V, and indicates a process of reward devaluation [32]. Anhedonia-like symptoms have been observed in animals that, after prolonged exposure to a series of unpredictable stressors, showed an impaired reward state. [3].

In this context, the animals submitted to CUMS in the present study showed an anhedonia-like effect evidenced by the decrease in consumption and preference for sucrose solution, and the previous treatment with (-)-α-bisabolol also reversed this effect induced by CUMS. It is important to emphasize that, according to several studies, not all clinically available antidepressants and anxiolytics are capable of reversing this symptom of anhedonia [38–41], thus emphasizing the vital relevance of (-)-α-bisabolol in the behavior tests performed and highlighting its crucial pharmacological potential.

Anxiety is another disorder frequently associated with depression, and the use of antidepressants to treat it is expected, including the use as "drugs of first choice" [42]. In animals, chronic exposure to physical stress is associated with anxiety-like behaviors, which can be assessed by using behavioral tests such as elevated plus maze (EPM) and hole board (HB) [36,43]. Both are classical tests based on the repulsion shown by animals when exposed to high and open places or to explore new environments. The degree of anxiety is measured by the animal's capability of exploring the apparatus. How much more significant is the exploration, the lower is the level of anxiety [44]. In this context, (-)-α-bisabolol also indicated an anxiolytic-like effect through reversion of the anxiogenic-like effect induced in animals previously submitted to CUMS, evaluated in the EPM and HB tests. Previous results demonstrating possible anxiolytic activity of (-)-α-bisabolol suggested that the mechanism likely involves GABAergic transmission [45].

Considering the positive behavioral results of an antidepressant potential of (-)-α-bisabolol, the investigation followed to evaluate neurochemical parameters related to depression. Therefore, we investigated the effects of CUMS and bisabolol on the lipid peroxidation process (TBARS), in addition to nitrite and GSH levels, which are essential parameters of oxidative stress, in critical brain areas related to depression such as the hippocampus and the prefrontal cortex.

There is evidence that oxidative stress is responsible for an increase in the number of neuropsychiatric diseases due to the deleterious effects caused by excess free radicals. Depression is, in fact, one of the causal factors in the development of these neurobehavioral disorders. It is well known that the brain is the main target organ of oxidative stress due to its high content of polyunsaturated fatty acids and iron. It is susceptible to the formation of free radicals and the increase in the lipid peroxidation process, which manifests itself, for example, by the formation of thiobarbituric acid reactive substances (TBARS) [45,46].

The treatment with (-)-α-bisabolol reversed the increase in TBARS levels in the hippocampus of animals submitted to CUMS, indicating the potential antioxidant effect that may be related to antidepressant and anxiolytic-like effects observed in the behavior *in vivo* evaluation. Suggesting that this substance could act as a protector of oxidative damage caused by reactive oxygen species (ROS). The reduction in TBARS levels in the hippocampus, provided by treatment with (-)-α-bisabolol, could be the mechanism of the reversing depression induced by (-)-α-bisabolol treatment and this finding could be helpful in further evaluations to prove the potential of this class to treat neuropsychiatric disorders. Interestingly, clinical data show that high levels of substances, such as malondialdehyde, a marker of oxidative stress, were previously found in some studies with depressive patients [47,48].

The reduction in GSH levels in the hippocampus of animals exposed to CUMS confirms a deleterious effect on this brain region, which, how-

ever, was not reversed by pretreatment with (-)-α-bisabolol. This detrimental action on the hippocampus after exposure to the CUMS model may involve microglial activation, a feature intrinsically related to depression [49], and the variability of GSH between the animals in all the groups. The inability to change nitrite levels by (-)-α-bisabolol also were observed in any of the brain areas studied. The level of nitrite observed was low, which could impact the differences observed between the groups. Despite that, there is evidence of low nitrite levels in stressed gastric tissue of animals submitted to the administration of absolute ethanol [12]. The lipophilic profile and high capability of sensibilization of CSN predicted *in silico* point the antioxidation ability of (-)-α-bisabolol probably related more directly to the reversion of lipid oxidation.

The findings suggest that (-)-α-bisabolol, at a dose of 50mg/kg, has a potential antidepressant and anxiolytic-like effect in animals submitted to the CUMS model, effects that may be related to a possible antioxidant action, more specifically to its ability to reduce TBARS levels in the hippocampus. Knowledge of the connection between oxidative stress and depression is undoubtedly a step forward to a better comprehension of the pathophysiology of depression.

CONCLUSION

This study points to a connection between oxidative stress and depression. In this sense, it evidences (-)-α-bisabolol as a promising alternative treatment for neuropsychiatric disorders after relieving depressant and anxiolytic behavior in mice. The findings indicated that the administration of (-)-α-bisabolol had antidepressant and anxiolytic effects in the chronic unpredictable mild stress (CUMS) model that were associated with an antioxidant effect reducing the levels of thiobarbituric acid reactive substances in the hippocampus of stressed animals. However, further studies are needed to explore deeper into the mechanism of action BIS related to the founded effects.

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Abbreviation list

BBB – Blood Brain Barrier
BIS – (-)-α-bisabolol
CNS – Central Nervous system
CUMS – Chronic Unpredictable Mild Stress
EPM – Elevated Plus Maze
FLU – Fluoxetine
FST – Forced Swimming Test
GSH – reduced glutathione
HB – Holeboard
MDD – Major depressive disorder
OFT – Open Field Test
PFC – Prefrontal cortex
ROS – Reactive oxygen species
SPT – Sucrose Preference Test
TBARS – thiobarbituric acid reactive substances

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