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Article

Antidepressant-Like Effect of *Lippia sidoides* CHAM (Verbenaceae) Essential Oil and Its Major Compound Thymol in Mice

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Abstract: Depression is a common disease affecting more than 300 million people worldwide. Since *Lippia sidoides* has shown central nervous system effects in previous works, we aimed to investigate the effect of *L. sidoides* essential oil and its major compound, thymol on a corticosterone-induced depression model in mice. Male mice (20–25 g) received corticosterone (20 mg/kg, subcutaneously), once a day for 22 days. From the 16th day on, mice were grouped to receive either corticosterone or *L. sidoides* essential oil (100 and 200 mg/kg), or thymol (25 and 50 mg/kg) or fluoxetine (35 mg/kg) by gavage. The forced swimming test, tail suspension, open field, elevated plus maze and sucrose preference tests were performed from the 19th to 22nd day. Data were analyzed by ANOVA followed by the Student-Newman-Keuls as a post hoc test and the results were considered significant when $p < 0.05$. It was shown that *L. sidoides* essential oil, thymol and fluoxetine decreased the immobility time in the tail suspension and forced swimming tests and none of these altered locomotor activity in the open field test. However, the drugs increased the amount of grooming. In the elevated plus maze, all drugs increased the number of entries and the time of permanence in the open arms. In the sucrose preference test, the *L. sidoides* essential oil, thymol and fluoxetine reversed anhedonia. These results suggest that the thymol and *L. sidoides* essential oil have an antidepressant-like effect, similar to fluoxetine. However, future studies should be encouraged to enhance understanding of the effects of essential oil and thymol for the treatment of depression.

Keywords: depression; thymol; antidepressant effect; *L. sidoides*; essential oil

1. Introduction

According to the World Health Organization (WHO), depression is the leading cause of mental illness and disability affecting more than 300 million people worldwide with an increase of more than 18% between 2005 and 2015 [1].

Depression is a heterogeneous multifactorial disease, with an unclear etiology and pathophysiology [2]. The main symptoms of depression are sad mood, self-deprecation, lack of concentration, energy and sleep deficits, psychosomatic disorders, anhedonia and suicidal thoughts [3,4]. The pathophysiology of depression remains poorly understood and is determined by genetic, epigenetic and physiological factors [3–5].

The standard form of treatment for depression generates several side effects, which often require the patient to discontinue using the drug [6]. In order to treat depression successfully, antidepressants are usually prescribed for at least four to six months. There may be a latency period of more than 3 weeks before their effects show, which requires understanding, interest and habituation to their use by patients [7]. However, it is estimated that 30–50% of depressed patients do not fully recover with currently available drug therapy [8].

Although drug treatment has proven effectiveness for depression, its effect is not always sufficient to prevent relapse in some patients [7]. As a result, drugs produced from medicinal plants can improve the antidepressants' actions and decrease side effects in refractory patients [8]. Also, medicinal plants are easily found in nature and present a variety of chemical compounds in their extracts or essential oil which show antidepressant-like actions, such as *Hypericum perforatum* L. (St. John's wort) which is widely used as herbal remedy for the treatment of mild to moderate depressive episodes [9]. Similarly, essential oils, such as the ones extracted from *Lippia* (Verbenaceae) species have shown pharmacological actions such as antimicrobial, antiparasitic, anesthetic, analgesic, anti-inflammatory and antitumor [10–14]. One of its species, *Lippia sidoides* Cham, known as rosemary, peppermint, rosemary-bravo, estrepa horse and rosemary-large, is popularly used to treat infections [15,16]. *L. sidoides* essential oil (LSEO) produces thymol monoterpene and its isomer, carvacrol; both have a powerful action in animals [17]. Carvacrol has been reported as showing anxiolytic [18] and antidepressant-like [19] effects and thymol has shown antimicrobial, larvicide, antioxidant, anti-inflammatory and analgesic activities [20–23].

Data has shown that several monoterpenes extracted from essential oils act on the central nervous system (CNS) [24]. Since carvacrol has presented anxiolytic and antidepressant-like effects in previous work, it seems relevant to investigate LSEO and thymol, the carvacrol isomer and also the major compound of LSEO, in animal models of depression and anxiety.

Studies on rodents regularly administered with corticosterone (CORT) have shown that this mimics the chronic stress-associated dysregulation of the hypothalamic-pituitary-adrenal axis [25,26]. Other groups have previously utilized this model to evaluate potential antidepressant candidates and have validated its application to study the neurobehavioral alterations as well as the pathophysiological changes associated with depression [27].

In this context, we aimed to evaluate the antidepressant efficacy of *Lippia sidoides* Cham. (Verbenaceae) essential oil and its major compound, thymol, against a mice model of CORT-induced depression. Our work is unprecedented, as in surveys conducted through websites, no other study using either *Lippia sidoides* essential oil or thymol in corticosterone-induced depression model was found.

2. Materials and Methods

2.1. Animals

Swiss mice, males, weighing 20–25 g, from INTA University Center—UNINTA animal house were used. The animals were kept in polypropylene cages with an alternating 12 h light/dark cycle, with lights on at 7 a.m., and they received specific balanced rodent chow and water ad libitum. The experimental protocols followed the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were evaluated by the Ethics Committee on Animal Use of UNINTA under protocol 2014.02.003-P on 16 June 2014.

2.2. Essential Oil of Lippia Sidoides Cham (Verbenaceae)—LSEO

2.2.1. Collection of Plant Material

The aerial parts (leaves and branches) of *Lippia sidoides* were collected in July 2014 from the Garden of Medicinal Plants, Farm of UNINTA, City of Cariré, Ceará, Brazil (3°49′51 82″ S and 40°24′37 85″ W). The plant was classified and a voucher specimen was deposited in the Francisco José de Abreu Matos Herbarium of the State University of Acaraú Valley (UVA) under the number: HUVA 17480.

2.2.2. Oil Extraction and Chemical Analysis

Approximately 1 kg of plant material (shoots) from the species *Lippia sidoides* Cham (rosemary pepper) was properly packaged and transported to INTA University Center, UNINTA experimental laboratory (NUBEM). The material was subjected to the extraction process using the hydro-distillation Cleavenger modified method for 3 h. The essential oil was dried over anhydrous sodium sulfate, maintained and kept under refrigeration until analysis of the outputs. The content of the essential oils was determined by the masses of oil in the analytical balance and expressed as a percentage weight/weight (1 g oil per 100 g of fresh weight). The extract was retained and kept in a refrigerator until analysis of the outputs. The chemical composition of the essential oil was investigated and described in previous work [28].

2.2.3. Preparation of Drugs

All drugs were administered in a volume of 0.1 mL/10 g body weight. CORT (20 mg/kg/day, subcutaneous (SC), Sigma®, São Paulo, SP, Brazil) was dissolved in a saline solution containing 0.2% dimethyl sulfoxide and 1% Tween-80 (Sigma®). Flu (35 mg/kg/day, per os—p.o., EMS®, Hortolândia, SP, Brazil) was diluted in distilled water. *L. sidoides* essencial oil (100 and 200 mg/kg/day, p.o.) was dissolved in 3% Tween 80 and diluted in distilled water. Thymol (25 and 50 mg/kg/day, p.o., Sigma®) was dissolved in 2% Tween 80 using distilled water as vehicle.

2.3. Experimental Protocol

For each set of experiments, thirty five animals were divided into 7 groups of 5 animals each: these included a control group (received saline water (s.c) + distilled water (p.o.)), corticosterone group (CORT) (CORT (s.c.) + distilled water (p.o.)), fluoxetine group (CORT (s.c.) + FLU 35 mg/kg (p.o.)), two thymol groups (TML) with doses of (CORT (s.c.) + TML 25 mg/kg (p.o.)) and (CORT (s.c.) + TML 50 mg/kg (p.o.)) and two *Lippia sidoides* essential oil (LSEO) groups at doses of (CORT (s.c.) + LSEO 100 mg/kg (p.o.)) and (CORT (s.c.) + LSEO 200 mg/kg, p.o.)). As the protocol has been performed twice, each group presented 10 animals. CORT (20 mg/kg, s.c.) was administered once daily, at 8 a.m, for 22 days to induce depressive behavior in mice. The experiments were performed from 9 a.m. to 3 p.m. On the first day of the experimental design, 30 mice (from 6 groups) received CORT (20 mg/kg/day) and 5 of them received vehicle. After 60 min, the forced swimming test (FST) was conducted for 5 min. From the 2nd day to the 14th day, mice were treated as described but were not involved in any experiments. On the 15th day, 60 min after treatment mice were again subjected to FST for 5 min.

From the 16th to 22nd days mice received respective drugs such as, vehicle, LSEO 100, LSEO 200, TML 25, TML 50 or FLU 35 after 30 min of CORT or vehicle administration. The experiments were conducted 60 min after the last treatment on the 19th, 20th, 21st and 22nd days of treatment. The sucrose preference test (SPF) was performed on the 19th, 20th, 21st days, the FST on the 20th day, the TST and OFT on the 21st day and PMT on the 22nd day. After all experiments had been finished, mice were sacrificed to obtain brain areas (Figure 1).

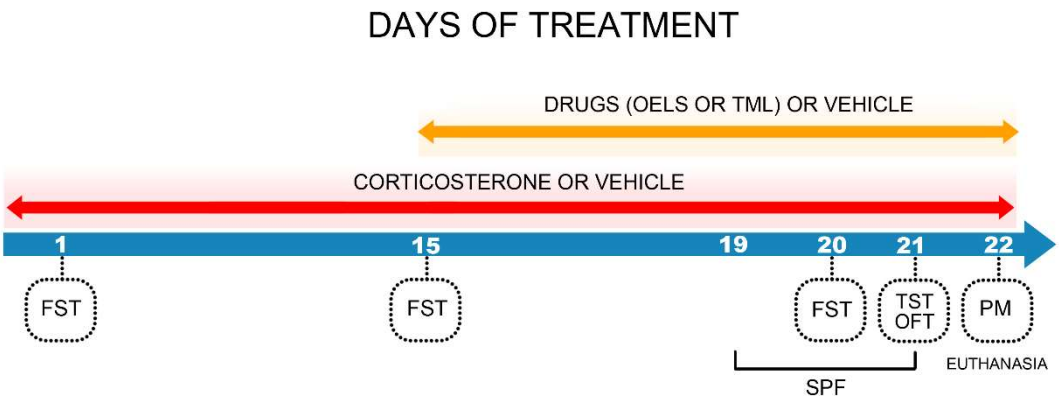

Figure 1. Experimental design. Numbers represent the days of treatment and setting experiments. FST = forced swim test, TST = tail suspension test, OFT = open field test, PM = elevated plus maze test, SPF = sucrose preference test, OELS = *Lippia sidoides* essential oil, TML = thymol.

2.4. Behavioral Tests

2.4.1. Forced Swim Test

Sixty minutes after treatment, mice were individually forced to swim in an open cylinder container (diameter 15 cm; height: 40 cm) filled with 20 cm of water at 24 ± 1°C. The amount of time in which the mouse remained immobile during a 5-min period was recorded. Immobility was defined as the animal floating in the water without struggling and making only slight movements to keep its head above the water [29].

2.4.2. Open Field Test

The animals were placed on an apparatus made of acrylic (transparent black walls and floor 30 × 30 × 15 cm) and divided into 9 equal quadrants. Sixty minutes after treatment by gavage, the animals were placed, one at a time, in the center of the open field area and they were observed for 5 min. The parameters recorded were the number of crossings (spontaneous locomotor activity), the number of rearing behaviors, and the number of grooming behaviors [30].

2.4.3. Tail Suspension Test

Sixty minutes after the last treatment by gavage, each mouse was suspended by the tail on the edge of a shelf placed 58 cm above the table top. The mouse was secured in place by adhesive tape placed 1 cm from the tip of the tail. The time that the mouse remained immobile, up to 6 min was recorded [31].

2.4.4. Elevated Plus Maze Test

Sixty minutes after treatment by gavage, the animals were placed one at a time, with heads facing one of the closed arms in the center of an elevated plus maze consisting of two opposing open arms (30 × 5 cm) and two closed ones (30 × 25 × 5 cm), also opposing, crosswise. The parameters observed were the number of entries and time spent either in the open or in the closed arms [32]. For statistical analysis, the parameters considered were those related to the open arms; number of entries in the open arms (NEOA), time spent in the open arms (TSOA) and their percentage, percentage of number of entries in the open arms (PEOA) and the percentage of time spent in the open arms (PTOA).

2.4.5. Sucrose Preference Test

First, a sucrose solution was prepared at 2% to 3 L of water using 60 g of sucrose. This solution was divided into 14 bottles of 200 mL of sucrose solution at all. On the first day of the test two bottles

with the solution were placed in each cage. On the second day, after 18 h, these bottles were removed, weighed and their volumes measured. After that, a bottle with sucrose solution, and another bottle with water, each containing 200 mL, were placed in the cage at the same time as the first day. On the third day, the bottles were removed and weighed to measure their respective volumes [33]. The sucrose preference was measured by the following equation:

$$\text{Sucrose preference } (\%) = \frac{\text{sucrose consumption (mL)}}{\text{sucrose consumption (mL)} + \text{water consumption (mL)}} \times 100\%$$

2.5. Statistical Analysis

The statistical analysis was performed using the software Graph Pad Prism version 5.02 for Windows, Graph Pad Software, San Diego, CA, USA (Copyright© 1992–2009 Graph Pad Software Inc.). The results, which followed a parametric distribution were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test as a post hoc test, and the values were represented by mean ± standard error of mean (SEM). Data were considered significant when* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

3. Results

3.1. Forced Swim Test

Immobility Time

Animals treated with CORT showed an increase in immobility time on the 14th day (75.50 ± 18.70, $n = 10$) and the 20th day (105.50 ± 14.91, $n = 10$) as compared to treatment in the 1st day (13 ± 15.41, $n = 10$) (Figure 2).

Either LSEO 100 mg/kg (52.82 ± 13.14, $n = 10$) or LSEO 200 mg/kg (32.64 ± 6.66, $n = 10$) decreased the immobility time in comparison to the CORT group (135.6 ± 13.45, $n = 10$) (Figure 3a). Similarly, TML associated with CORT, at both doses (TML 25 mg/kg = 61.70 ± 9.82, $n = 10$; TML 50 mg/kg = 51.78 ± 11.49, $n = 10$) also decreased the immobility time compared to CORT (135.6 ± 13.45, $n = 10$) (Figure 3b). FLU 35 mg/kg as standard, decreased the same parameter (69.82 ± 12.91, $n = 10$) (Figure 3a,b).

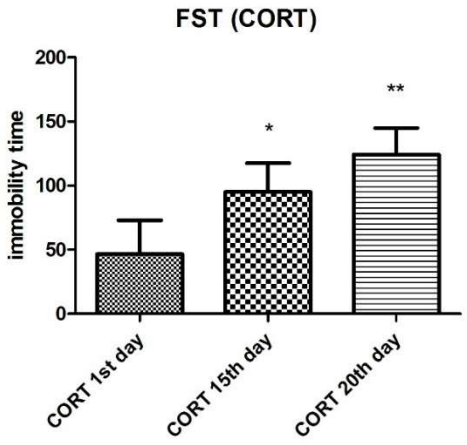

Figure 2. CORT immobility time in the forced swimming test (FST). Values are expressed as mean ± SEM ($n = 10$). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. Significant values: * $p < 0.05$, ** $p < 0.001$ vs. CORT 1st day.

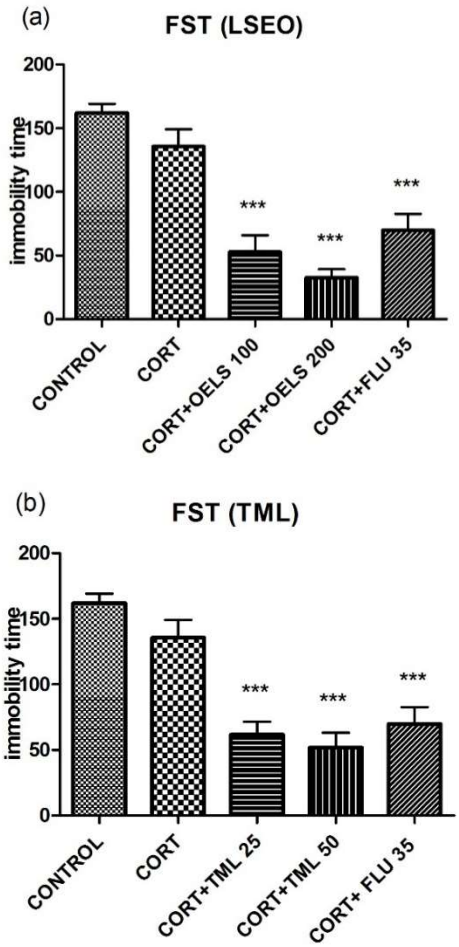

Figure 3. Effects of repeated administration of (**a**) LSEO (100 and 200 mg/kg; p.o.) and (**b**) TML (25 and 50 mg/kg; p.o.) in animals treated with CORT (22 days, 20 mg/kg, s.c.) on the immobility time, in seconds (s), on the FST. Values are expressed as mean ± SEM (n = 10). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. Significant values: *** $p < 0.0001$ vs. CORT. LSEO *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; FST = forced swimming test.

3.2. Tail Suspension Test

Immobility Time

LSEO at both doses, 100 mg/kg (40.0 ± 10.31, n = 10) and 200 mg/kg (42.73 ± 9 57, n = 10), decreased the immobility time compared to CORT (91.83 ± 4.73, n = 10) (Figure 4a). Regarding TML, the higher dose (TML 50 mg/kg = 56.50 ± 9.81, n = 10) but not the lower one (TML 25 mg/kg = 68.92 ± 12.15, n = 10) decreased this parameter as compared to CORT (91.83 ± 4.73, n = 10) (Figure 4b). FLU 35 mg/kg also decreased this parameter (43.4 ± 9.43, n = 10).

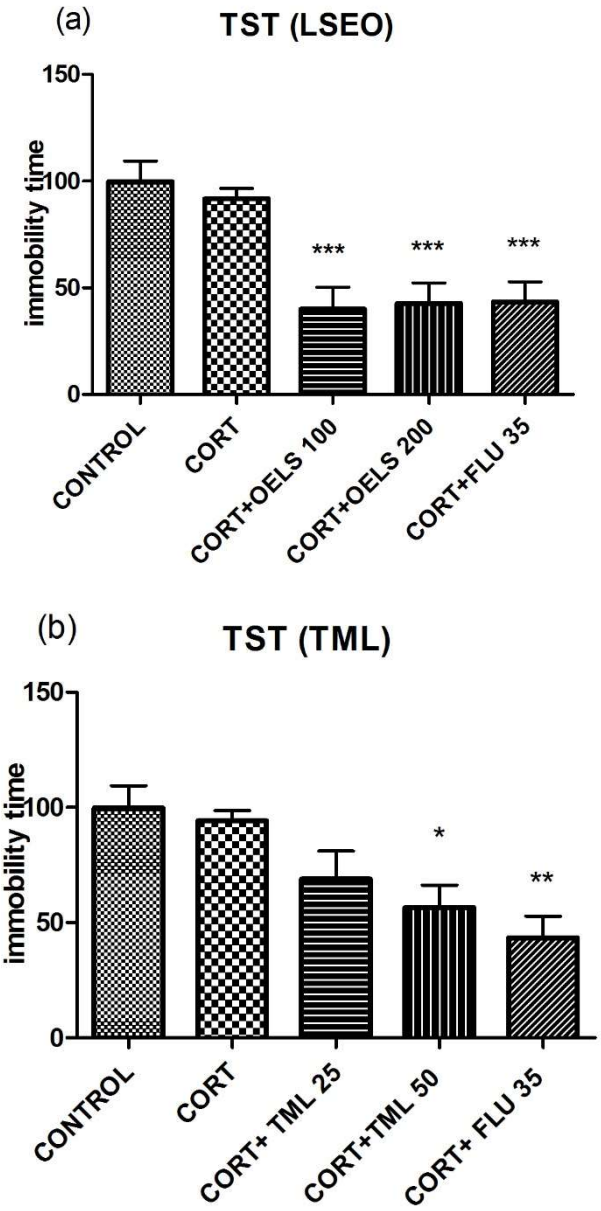

Figure 4. Effects of repeated administration of (**a**) LSEO (100 and 200 mg/kg; p.o.) and (**b**) TML (25 and 50 mg/kg; p.o.) in animals treated with CORT (22 days, 20 mg/kg, s.c.) on the immobility time, in seconds (s), on the TST. Values are expressed as the mean ± SEM (n = 10). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. Significant values: * $p < 0.05$, ** $p < 0{,}001$, *** $p < 0.0001$ vs. CORT. LSEO = *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; TST = tail suspension test.

3.3. Open Field

3.3.1. Number of Crossings

Both doses of LSEO, 100 mg/kg (56.13 ± 5.20, n = 10) and 200 mg/kg (49.67 ± 6.92, n = 10), were unable to alter the number of crossings compared with CORT (50 ± 4.11, n = 10) (Figure 5a). Similarly, TML either with the dose of 25 mg/kg (46.80 ± 5.53, n = 10) or with the dose of 50 mg/kg (55.64 ± 4.57, n = 10) did not alter this parameter compared with CORT (50 ± 4.11, n = 10) (Figure 5b). Also, the same pattern of behavior was shown for FLU (49.77 ± 6.16, n = 10).

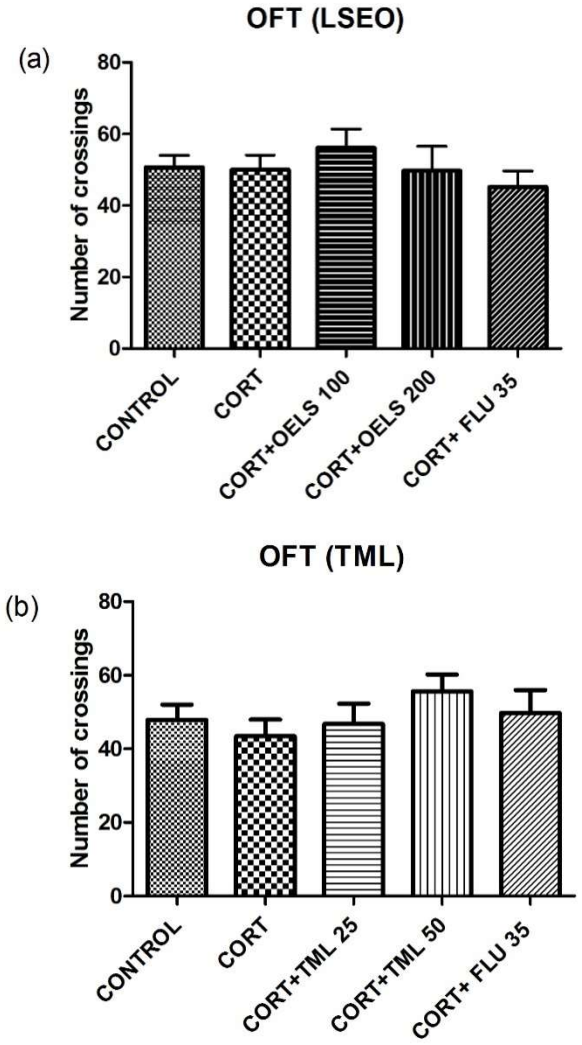

Figure 5. Effects of repeated administration of (**a**) LSEO (100 and 200 mg/kg; p.o.) and (**b**) TML (25 and 50 mg/kg; p.o.) in animals treated with CORT (22 days, 20 mg/kg, s.c.) on the number of crossings on the OFT. Values are expressed as the mean ± SEM (n = 10). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. There are no significant values vs. CORT. LSEO = *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; OFT = open field test.

3.3.2. Number of Rearing

Figure 6 shows that neither LSEO 100 mg/kg (12.77 ± 1.54, n = 10); LSEO 200 mg/kg (12.29 ± 2.68, n = 10), at both doses, nor TML 25 mg/kg (9.07 ± 1.73, n = 10); TML 50 mg/kg (18.60 ± 2.63, n = 10), at both doses, have changed the number of rearing as compared with CORT (12.47 ± 2.37, n = 10). Following the same pattern, FLU 35 mg/kg did not alter the parameter (9.93 ± 2.23, n = 10).

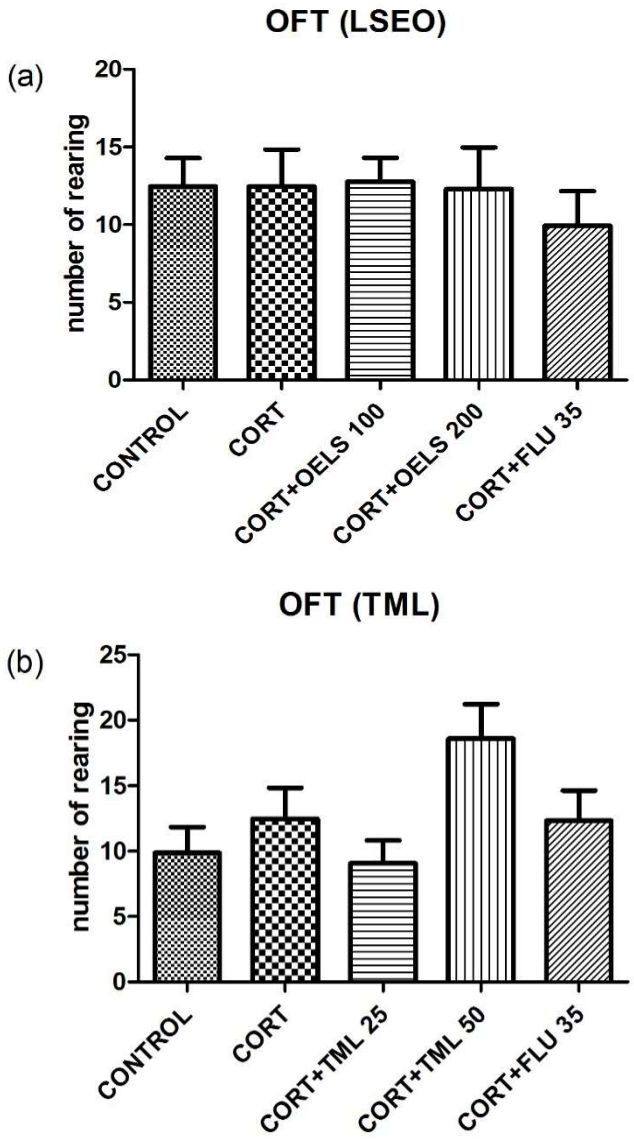

Figure 6. Effects of repeated administration of (**a**) LSEO (100 and 200 mg/kg; p.o.) and (**b**) TML (25 and 50 mg/kg; p.o.) in animals treated with CORT (22 days, 20 mg/kg, s.c.) on the number of rearings on the OFT. Values are expressed as the mean ± SEM (n = 10). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. There are no significant values vs. CORT. LSEO = *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; OFT = open field test.

3.3.3. Number of Grooming Behaviors

LSEO at both doses, 100 mg/kg (9.61 ± 1.92, n = 10) and 200 mg/kg (8.81 ± 1.76, n = 10), increased the amount of grooming in the open field test compared to CORT (2.08 ± 0.57, n =10) (Figure 7a). On the other hand, TML did not alter this parameter (TML 25 mg/kg = 3.21 ± 0.43, n = 10; TML 50 mg/kg = 2.92 ± 0.63, n = 10) compared to CORT (2.08 ± 0.57, n = 10) (Figure 7b). FLU 35 mg/kg, was the same as LSEO, it increased this parameter (8.45 ± 1.59, n = 10).

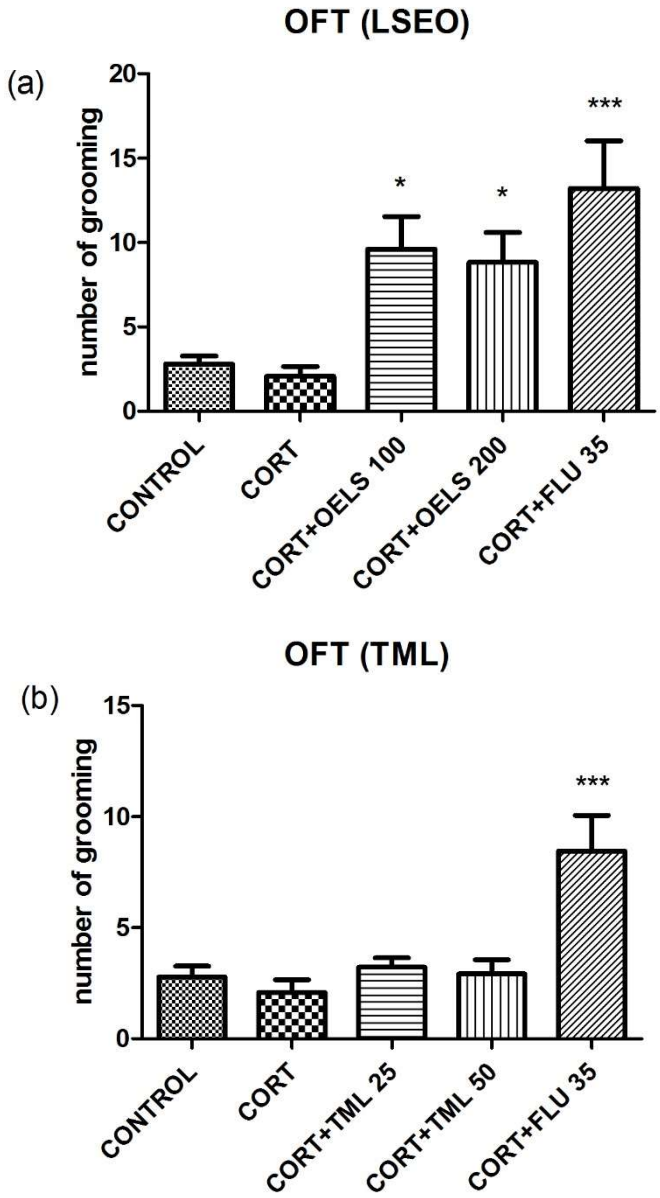

Figure 7. Effects of repeated administration of (**a**) LSEO (100 and 200 mg/kg; p.o.) and (**b**) TML (25 and 50 mg/kg; p.o.) in animals treated with CORT (22 days, 20 mg/kg, s.c.) on the number of grooming behaviors on the OFT. Values are expressed as the mean ± SEM (n = 10). Statistical analysis was determined by one-way ANOVA followed by Student-Newman-Keuls test. Significant values: * $p < 0.05$, *** $p < 0.0001$ vs. CORT. LSEO = *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; OFT = open field test.

3.4. Elevated Plus Maze Test (PM)

The parameters used to perform the statistical analysis were: number of entries in the open arms (NEOA), the percentage of entries in the open arms (PEOA), the time spent in the open arms (TSOA) and percentage of time spent in the open arms (PTBA).

3.4.1. NEOA and PEOA

LSEO at both doses, 100 mg/kg (9.33 ± 1.69, n = 10) and 200 mg/kg (9.30 ± 1.59, n = 10), increased NEOA in the plus maze test compared to CORT (2.26 ± 0.43, n = 10) (Figure 8a). Likewise, TML also increased this parameter at both doses (TML 25 mg/kg = 4.41 ± 0.84, n =10; TML 50 mg/kg = 4.66 ±

0.61, n = 10) as compared to CORT (2.26 ± 0.43, n = 10) (Figure 8b). On the other hand, FLU 35 mg/kg did not alter this parameter either in Figure 8a (4.33 ± 1.21, n = 10) or in Figure 8b (2.33 ± 0.65, n = 10).

Regarding PEOA, Figure 8c shows that LSEO increased it (100 mg/kg = 53.45 ± 3.05, n = 10; 200 mg/kg = 52.08 ± 4.52, n = 10), at both doses, compared to CORT (18.14 ± 4.05, n = 10). However, TML at the dose of 50 mg/kg (40.42 ± 4.76, n = 10) but not at the dose of 25 mg/kg (35.19 ± 2.73, n = 10), increased PEOA as compared to CORT (20.59 ± 4.46, n = 10). FLU 35 mg/kg did not alter this parameter (20.10 ± 4.18, n = 10) (Figure 8d).

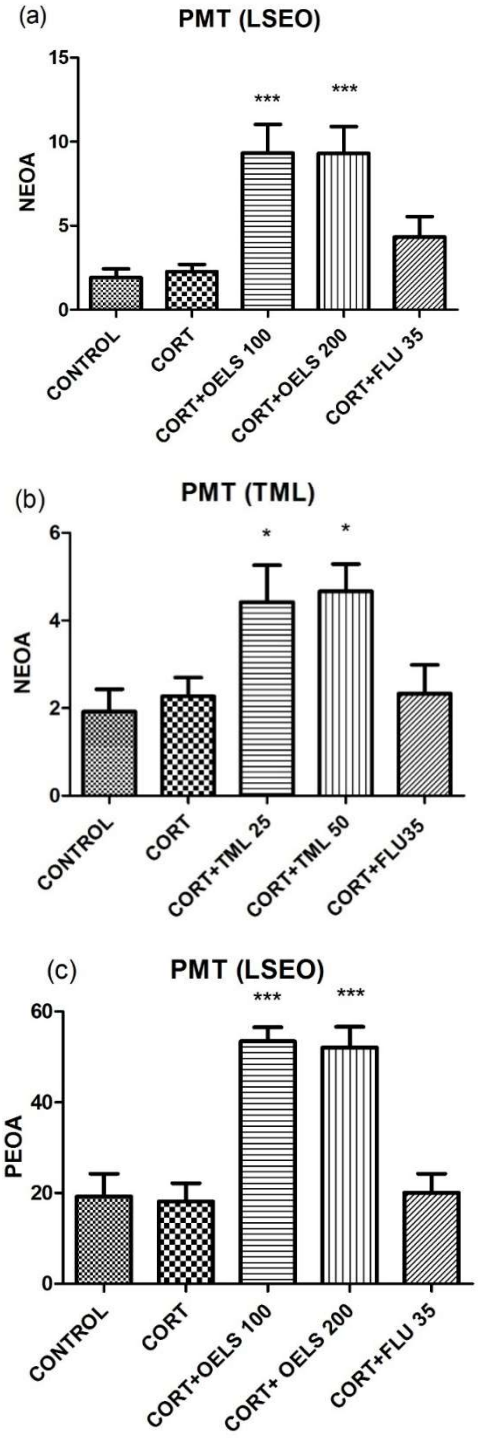

Figure 8. *Cont.*

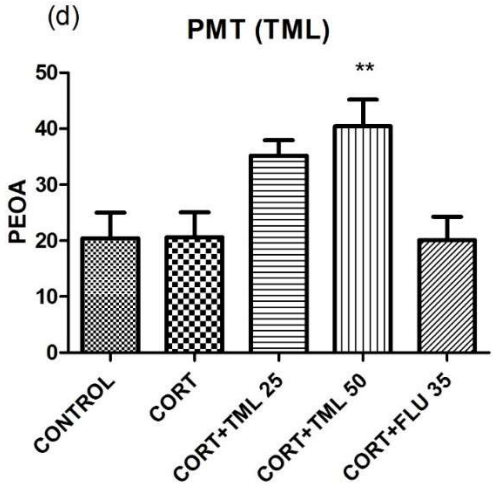

Figure 8. Effects of repeated administration of LSEO (100 and 200 mg/kg; p.o.) in the NEOA (**a**) and PEOA (**c**) and TML (25 and 50 mg/kg; p.o.) in the NEOA (**b**) and PEOA (**d**) on animals treated with CORT (22 days, 20 mg/kg, s.c.) in the plus maze test. Values are expressed as the mean ± SEM (n = 10). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. Significant values: * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ vs. CORT. LSEO = *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; PMT = plus maze test.

3.4.2. TSOA and PTOA

LSEO at both doses, 100 mg/kg (95.08 ± 14.72, n = 10) and 200 mg/kg (181.6 ± 21.57, n = 10), increased TSOA in the plus maze test compared to CORT (21.27 ± 6.48, n = 10) (Figure 9a). Likewise, TML also increased this parameter at both doses (TML 25 mg/kg = 62.70 ± 9.60, n = 10; TML 50 mg/kg = 91.10 ± 18.06, n = 10) as related to CORT (18.00 ± 5.87, n = 10) (Figure 9b). However, FLU 35 mg/kg did not alter this parameter either in Figure 9a (29.92 ± 10.83, n = 10) or in Figure 9b (30.25 ± 10.75, n = 10).

Figure 9c shows that LSEO increased PTOA (100 mg/kg = 48.34 ± 5.40, n = 10; 200 mg/kg = 54.20 ± 6.81, n = 10), at both doses, compared to CORT (8.87 ± 2.48, n = 10). Also, an increase in PTOA was observed with TML at both doses (25 mg/kg = 28.89 ± 4.40, n = 10; 50 mg/kg = 44.54 ± 8.29, n = 10) when related to CORT (8.87 ± 2.48, n = 10). FLU 35 mg/kg did not alter this parameter (20.10 ± 4.18, n = 10) (Figure 9d). FLU 35 mg/kg did not alter this parameter either in Figure 9c (13.83 ± 4.53, n = 10) or in Figure 9d (16.28 ± 6.30, n = 10).

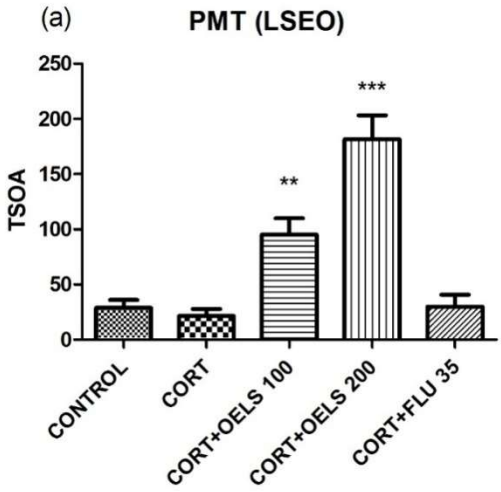

Figure 9. *Cont.*

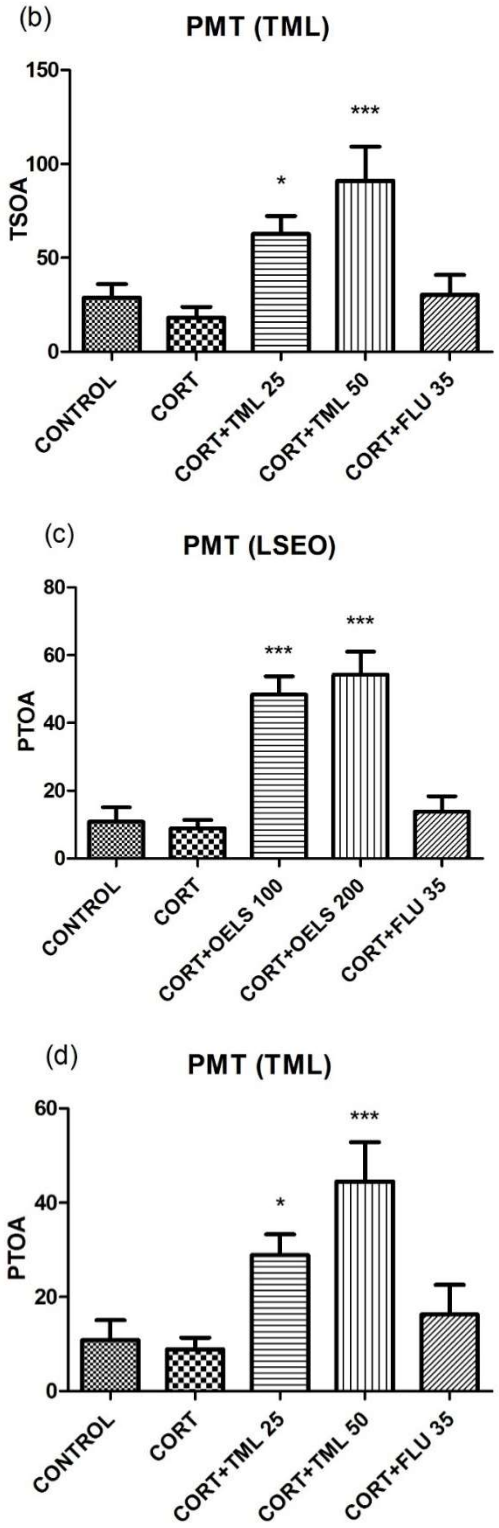

Figure 9. Effects of repeated administration of LSEO (100 and 200 mg/kg; p.o.) on the TSOA (**a**) and PTOA (**c**) and TML (25 and 50 mg/kg; p.o.) on the TSOA (**b**) and PTOA (**d**) in animals treated with CORT (22 days, 20 mg/kg, s.c.) in the plus maze test. Values are expressed as the mean ± SEM (n = 10). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. Significant values: * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ vs. CORT. LSEO = *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; PMT = plus maze test.

3.5. Sucrose Preference Test

CORT (66.68 ± 0.007, n = 10) decreased the sucrose preference compared to the control as shown in Figure 10a (76.94 ± 2.56, n = 10) and Figure 10b (82.43 ± 2.67, n = 10). In addition, LSEO (100 mg/kg = 75.02 ± 1.66, n = 10; 200 mg/kg = 87.97 ± 0.98, n = 10), at both doses (Figure 10a), and TML (25 mg/kg = 89.69 ± 1.68, n = 10; 50 mg/kg = 82.01 ± 1.83, n = 10), at both doses (Figure 10b) were able to revert CORT's effect (66.68 ± 0.007, n = 10). Likewise, FLU 35 mg/kg (80.13 ± 2.46, n = 10) also reverted CORT's effect.

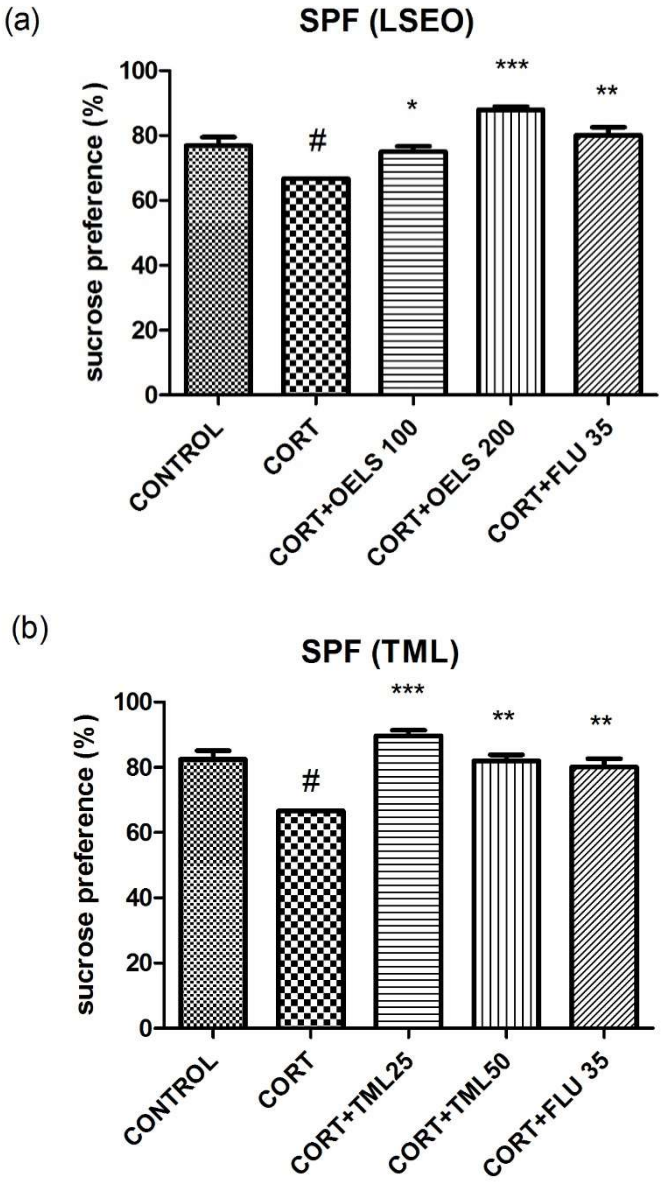

Figure 10. Effects of repeated administration of (**a**) LSEO (100 and 200 mg/kg; p.o.) and (**b**) TML (25 and 50 mg/kg; p.o.) in animals treated with CORT (22 days, 20 mg/kg, s.c.) on the sucrose preference (%). Values are expressed as the mean ± SEM (n =10). Statistical analysis was determined by one-way ANOVA followed by the Student-Newman-Keuls test. Significant values: * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ vs. CORT; # $p < 0.05$ vs. control. LSEO = *Lippia sidoides* essential oil; TML = thymol; CORT = corticosterone; SPF = sucrose preference test.

4. Discussion

This study is the first to investigate the antidepressant-like effects of LSEO and TML in the CORT-induced depression model in mice. It has been reported that repeated administration with high doses (20 mg/kg/day), but not low, of CORT produces depressant-like behavior in rodents [34,35]. The two most widely used animal models for screening new antidepressant drugs are the forced swimming (TNF) and tail suspension (TSC) tests. Several studies using naturally occurring substances in order to investigate possible antidepressant effects [36–38], have shown a reduction in immobility time in the TST and FST.

In this study, CORT increased immobility time on the 14th day and 20th day of treatment in the FST, showing depressant behavior. This result suggests that CORT administered repeatedly induces depressive behavior in animals. Thus, our results are consistent with other studies [39] where the mice receiving regular CORT treatment showed significant behavioral despair, as shown by significantly increased immobility time in the FST, which validates the model used for investigating the antidepressant-like effects of LSEO and TML.

Both doses of LSEO and TML associated with CORT for 22 days were able to reverse the CORT-induced immobility time in the FST and TST, suggesting an antidepressant-like effect of the two drugs in question. This effect is similar to a study in which resveratrol, a polyphenol found in the *Polygonum cuspidatum*, a Japanese plant that has neuroprotective activity, when administered daily was able to decrease the immobility time in the FST and TST, reversing the depressive action of CORT [40].

In our present study it was observed that LSEO at both doses associated with CORT were able to decrease the immobility time of mice in the TST. However, only the highest dose of CORT associated with TML was able to decrease the immobility time in this test. This is similar to the study by [41] which states that repeated injections of CORT are able to increase the immobility time in the TST in mice. It has been reported in [42] that after 21 consecutive days of treatment with magnolol, which is a compound that acts on $GABA_A$ receptors of rats, and FLU (20 mg/kg) the duration of immobility in the TST decreased compared to the CORT group. These studies support our findings, allowing us to state that LSEO and TML have antidepressant actions, as well as FLU.

The TST and FST exhibit acute sensitivity and specificity for antidepressants, and also, these tests are easy to perform and provide reliability [43]. Both animal tests provide a stressful environment, where mice cannot escape and after an initial period of struggling, the animals become motionless, which mimics a depressed and desperate behavior [44]. Also, the TST has been recognized as having great importance due to its major sensitivity in detecting selective serotonin reuptake inhibitor (SSRI) type antidepressants [45]. Since both LSEO and TML effectively showed antidepressant-like behavior in the TST, as did FLU, we suggest that these drugs may have similar actions to that class of drugs. However, further studies are needed to investigate their mechanism of action.

It is known that some drugs with psychostimulant activity may have a false positive effect in the TST and FST [46]. Thus, in order to rule out the possibility that the reduction in the immobility time elicited by LSEO and TML is due to enhancement in locomotor activity, the OFT was conducted. Neither LSEO nor TML were able to change the exploratory activity of animals, as seen in the number of crossings parameter, similar to FLU. Therefore, these results confirm the antidepressant effect of LSEO and TML, observed in the FST and TST, since it excludes the possibility of these drugs having a psychostimulant effect [46]. Besides, this pattern of behavior is similar to the effects of antidepressants, such as FLU. This result is confirmed by a study of *Pelargonium roseum* essential oil, which contains monoterpenes, and is similar to the essential oil of *Lippia sidoides* Cham. The locomotor activity of the animals did not change, which also demonstrates its antidepressant effect [47].

Rearing is the animals' ability to stand on its hind legs to explore the environment vertically and it is related to anxiety behaviors [48]. In our protocol, it was observed that neither LSEO nor TML doses were able to change this parameter. These results are similar to those found in the spontaneous locomotor activity parameter which determined horizontal exploratory activity, as was observed by

the number of crossings. Both results help to confirm LSEO and TML antidepressant-like activity by eliminating the psychostimulant effect.

Grooming is a behavior that develops in a cerebrospinal flow direction and consists of several steps [49]. It is considered a sensitive measure of stress and anxiety behavior when associated with an enhancement in the OFT [49]. Grooming is related to an anxiety behavior that can be mitigated with anxiolytic drugs and thus reduce this parameter, and may be potentiated by anxiogenic agents, which tend to increase this parameter [50]. In the present study, it was observed that both doses of LSEO associated with CORT increased the number of grooming behaviors, but none of the TML doses associated with CORT were able to do this. This effect suggests LSEO, but not TML might present anxiogenic effects. In order to clarify the anxiety profile of LSEO and TML, the PMT was conducted, because it is more sensitive and specific for the detection of drugs with benzodiazepine-like anxiolytic action than the open field test.

All PMT parameters were enhanced by either LSEO or TML but not by FLU, which was used as an antidepressant standard. This effect can be explained because the PMT is specific to benzodiazepine-like drugs, not to SSRIs. TML has been described as a $GABA_A$ agonist opening Cl-channels, resembling the anesthetic agent propofol (2,6-isopropyl-phenol) [51]. These findings confirm the effects of LSEO and TML in the PMT, which is more specific for anxiolytic drugs that act by activating the $GABA_A$ receptor, such as benzodiazepines. Thus, this effect can be better analyzed by this model than the open field test [18]. Hence, we suggest that LSEO and TML present, in addition to the antidepressant effect already described, an anxiolytic effect in animals. This is similar to a study carried out by [52], using a substance of natural origin, the monoterpene 1,4-cineol, which presented a potential anxiolytic effect in the PMT test.

The sucrose preference test measures a behavior similar to anhedonia, defined as the inability to perform pleasurable activities, a common symptom in depressed patients and which may be related to depression models in rodents [53]. In the present study, it was observed that both LSEO and TML doses associated with CORT increased the animals' sucrose preference, indicating a behavioral pattern reversal of anhedonia, in the same way that FLU did. It can be reported that animals subjected to chronic stress with CORT showed a decrease in sucrose preference when compared with control animals. Our findings are similar to a study conducted for two weeks with geniposide, a monoterpene, in which it was able to reverse sucrose preference [54] showing an anti-anhedonic effect. Hence, we suggest that LSEO and TML present the same effect.

In conclusion, our data suggest that LSEO and TML have an antidepressant-like effect, due to the decrease in immobility time in the FST and TST without altering the number of crossings and rearings, thus, discarding the psychostimulant effect. In addition, LSEO and TML reversed two very common symptoms related to depressive behavior, including anhedonia in the sucrose preference test, and anxiety in the PMT, a specific model for researching anxiolytic drugs. The main limitation of this work is that we can only assume the behavioral effects of the drugs tested but we cannot suggest how they act to provide the antidepressant-like effects. Despite those limitations, the results found provide strong evidence for their antidepressant-like effects. Therefore, many more studies can follow this one to investigate the LSEO and TML mechanisms of action; thus, contributing scientifically with new perspectives on the treatment of depression.

Author Contributions: The conceptualization was developed by C.T.V.d.M., T.S.d.M. and M.I.L. Methodology was carried out by M.S.R.P., F.R.C., N.A.C. and M.J.A. L.F.R.C. contributed with Software. Validation was conducted by C.T.V.d.M., R.M.P.S. and M.I.L. The formal analysis was performed by C.T.V.d.M. and M.S.R.P. The Investigation was conducted by T.S.d.M., M.S.R.P. and C.T.V.d.M. The Resources were provided by A.A.d.N. and F.E.A.C.J. Data curation was conducted by T.S.d.M. and M.I.L. The writing-original draft preparation was elaborated by M.S.R.P. The Writing-Review & Editing was carried out by C.T.V.d.M. T.S.d.M. and M.I.L. contributed with visualization. The supervision, project administration and funding acquisition was conducted by C.T.V.d.M.

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