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ORIGINAL ARTICLE

Antidepressant Effect of *Buddleja cordata* Methanolic Extract in Chronic Stress Mouse Model

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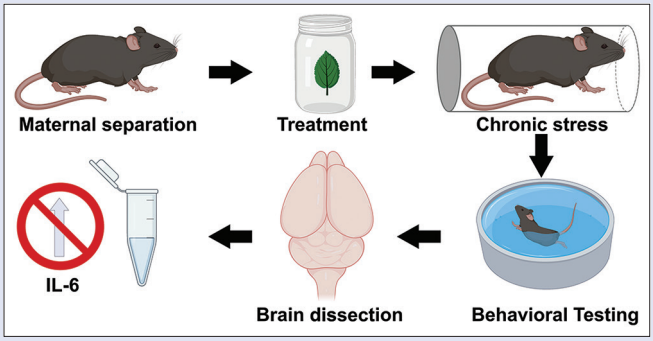
ABSTRACT

Background: Preclinical studies in animal models have shown anti-inflammatory, analgesic, antioxidant, and neuroprotective effects of *B. cordata*. **Objectives:** To explore the antidepressant effect of a methanolic extract of *Buddleja cordata* leaves (MEBc) in early maternal separation and chronic restraint stress mouse model. **Materials and Methods:** From day 2 to day 21, the litters were removed from the maternal cage for 3h/day. Based on the pharmacological treatments, four experimental groups were established: sham animals without maternal separation, chronic restrained stress and oral administration (S group) or 10 ml/Kg/day saline solution (SS group) or 100 mg/kg/day of MEBc (MEBc group) or 20 mg/kg/day of fluoxetine (F group). After 4 weeks of these treatments the antidepressant activity was evaluated through behavioral tests and also IL-6 determinations were performed. **Results:** Mice administered with MEBc showed significantly diminished immobility time in the forced swimming test ($P < 0.01$), tail suspension test ($P < 0.001$) and the time spent in the open arm of the elevated plus-maze ($P < 0.01$) versus SS group. By contrast, animals treated with fluoxetine only diminished immobility time in the tail suspension test ($P < 0.01$). The vegetal extract was more effective ($P < 0.05$) that fluoxetine in preventing the increase of interleukin 6 (IL-6) over the hippocampus and prefrontal cortex region (45 and 20 %, respectively). **Conclusion:** These results demonstrate the antidepressant and anxiolytic effects of MEBc over the chronic stress depression-induced model.

Key words: Buddleja cordata, chronic stress, depression, interleukin-6, medicinal plant

SUMMARY

- This study was conducted to evaluate the antidepressant effect of Buddleja cordata methanolic extract in early maternal separation and chronic restraint stress mouse model. This study supports the medicinal use of this plant. The methanolic extract demonstrated antidepressant and anxiolytic effects over the chronic stress depression-induced model



Abbreviations used: BCA: Bicinchoninic acid; F:fluoxetine; HPLC: high performance liquid chromatography; IFN: interferon: IL-10: Interleukin 10; IL-6: Interleukin 6; MAO-A: monoamine oxidase A; MAO-B: monoamine oxidase B; MEBc: methanolic extract from *Buddleja cordata*; MeOH: methanolic; MPP 1: methyl-4-phenylpyridinium; MPP+: 1-methyl 4-phenylpyridinium; MS: maternal separation group; NSAIDs: nonsteroidal anti-inflammatory drugs: S: sham group; SS: saline solution group; TNF: tumor necrosis factor; UASLP: Universidad Autónoma de San Luis Potosí; UVM: metastatic uveal melanoma; WHO: World Health Organization.

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INTRODUCTION

The World Health Organization mentions that the annual prevalence of depression is 4.4%; 5.1% in women and 3.6 in men[1,2] Nowadays, it is estimated that only 25% of depressive patient receives adequate pharmacological treatment. The conventional antidepressants are often associated with low compliance, response, remission rates and high risk of side effects, relapse, and tolerability.[3] Medicinal plants are being explored as alternative treatment to conventional antidepressants.

Buddleja cordata Kunth (Buddlejaceae) is called Tepozan in the Mexican traditional medicine. It is a plant distributed from northern Mexico to Guatemala.[4] *B. cordata* is applied topically for the treatment of tumors, cancer, skin burns, sores, arthritis, and other inflammatory diseases,[5] The Raramuris people (also known as Tarahumaras) in the north of Mexico use the bark and wood of *B. cordata* for treating inflammation.[6] Their

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ethnomedicinal effects could be attributed to some secondary metabolites present in the plant. Bioactive flavonoids (linarin), iridoid (aucubin), antifungal sesquiterpenoids, saponins, phenylpropanoids (Verbascoside; Hydrocinnamic acids: P-coumarin, caffeic, ferulic, and sinapic acids), and antibacterial phenylethanoids have been isolated and identified in previous chemical studies of B. cordata.[7-10] Preclinical studies have shown several pharmacological effects of *B. cordata*. Oral administration of 250 mg/kg MeOH extract from wild plant leaves for 28 days exerted an anti-inflammatory effect with a downregulation of lymphocites CD + producers of interleukin 1β (IL 1β) and TNF-α and increase in IL-10 levels, as well as, a decrease in leukocyte infiltration from ganglionic tissue during experimental arthritis model.[11] While the dried aqueous extract of *B. cordata* leaves and its main flavonoid glycoside, linarin, had showed significant analgesic, anti-inflammatory, and antipyretic effects in different animal models.[10,12] Particularly, the *B. cordata* methanolic extract showed antioxidant properties in UVM-induced skin damage assay.[13] While *in vivo* toxicity study has not shown lethality after a daily oral administration for 28 days at 1 g/kg.[11]

The neuroprotective effect of *B. cordata* methanolic extract has been demonstrated in the 1 methyl-4-phenylpyridinium (MPP^+) Parkinson disease rat model at doses of 50 or 100 mg/kg, every 24 h for 14 days. The rats were injected intracerebrally with the methanolic extract and treated for 7 days before and after neurotoxin administration. The methanolic extract showed a significant reduction of 75%–80% ipsilateral rotations (apomorphine test), that was correlated with the 60% dopamine level in striatum rat brain region. This antioxidant effect was neurochemically evident with a decrement of 90% for the lipidic fluorescent products formation in this brain region. On the other hand, the hydroxytyrosol (3,4-dihydroxyphenyl ethanol) is a phenylpropanoid compound, present either as an isolated form or chemically bound form by ester bonds to sugars, forming verbascoside, which is a compound that have been found in *B. cordata*. The neuroprotective effect of *B. cordata* has been attributed to the hydroxytyrosol compound.[14] This phenylpropanoid has inhibitory effect on MAO-A and MAO-B. Its intravenous administration at 1.5 mg/kg produced a significant inhibition of MAO-A and MAO-B isoforms by 50% and 40%, respectively, in the MPP^+ rat model.[15]

Given that the neuroprotective activity of the methanolic extract from the leaves from *B. cordata* has been demonstrated *in vivo,* the present study characterizes the antidepressant effect of the methanolic extract on chronic stress like early maternal separation (MS) and chronic restraint stress as an animal model of depression[16,17] by the behavioral tests (sucrose preference, forced swimming, elevated plus maze, and tail suspension tests) and anti-inflammatory effect (IL-6).

MATERIALS AND METHODS

Plant material

The collection of leaves of *B. cordata* Kunth (Buddlajeaceae), occurred in March 2016, in the forested area of Pedregal, located at the south of Mexico City. The plant was authenticated by Dr. Guillermo Avila Acevedo and a voucher (No. 42663) was deposited at the Universidad Nacional Autónoma de México (herbarium Itza). Leaves of *B. cordata* (300 g) were dried and extracted with hexane and methanol. The liquid methanolic extract of *B. cordata* (MEBc) was evaporated under reduced pressure at 55°C to obtain a solid residue (MEBc). The dry residue (34.55 g) was stored at 4°C. HPLC analysis and the total polyphenol content of the dry methanolic extract were determined and reported previously.[13]

Animals

C57BL/6J (C57) mice weighing 25–30 g were obtained from vivarium of Universidad Autónoma de San Luis Potosi (Medicine school).

Early maternal mice separation protocol

There were included litters ranged from 5 to 9 pups per litter. The litters were progressively incorporated to the assay. Each litter was housed in a single cage until 21 postnatal day with free access to standard laboratory food mice and water, in 12/12 light/dark cycle at 23°C ± 2°C. All efforts were made to minimize animal´s suffering in accordance with the national and international regulating animal research. On day 2, pups were divided into two groups: The MS group, and sham group (S group) [Figure 1]. From day 2 to day 21, the litters were removed from the maternal cage daily for 3 h, and the dams remained in their home cages. Pups were weaned at P22. For all the following procedures, we used only male offspring. After weaning, male mice were housed in standard cages and given free access to standard laboratory mice food and water, in 12/12 h light/dark cycle at 23°C ± 2°C.

Pharmacological treatment

The MS group of 8-week age, weighing 25–30 g were randomly divided into three groups with $n = 7–12$ animals per group; Group saline solution (SS), these animals were orally treated with vehicle SS 0.9% (10 ml/kg/day); Group F, mice were gavage with 20 mg/kg/day fluoxetine (Sigma-Aldrich) dissolved in SS 0.9%; Group MEBc, animals were treated orally with methanolic extract dissolved in SS 0.9% (100 mg/kg/day). A sham group (free stress) was used for the IL6 quantification assay [Figure 1]. Vehicle and pharmacological treatments were administered once-daily at 24-h intervals using a metallic cannula for 4 weeks (week 8–12 age).

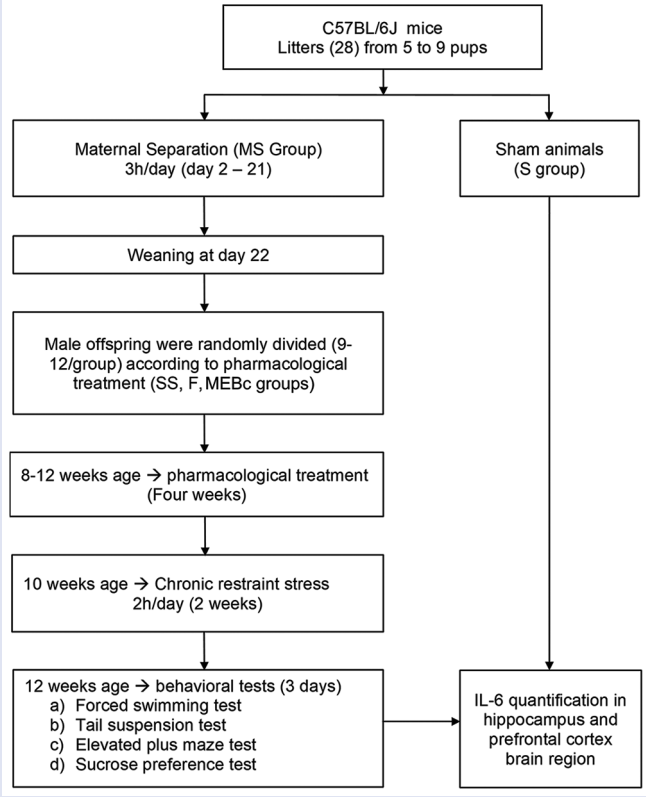

Figure 1: Experimental design diagram. Animals, pharmacological treatment, behavioral tests in SS, methanolic extract (methanolic extract of *Buddleja cordata*) and Fluoxetine (f) groups and interleukin measurement in Sham (s), SS, methanolic extract of *Buddleja cordata* and F groups

Chronic restraint stress

All 10-week-old mice of the three experimental groups were undergoing a second phase of stress called "chronic restraint stress," which consisted of introducing daily the animals for 2 h in acrylic tubes (restraint devices) for 2 weeks once they were administered with their treatment. At week 12 age, the pharmacological treatments and "chronic restraint stress" were finished and the mice were subjected to behavioral tests for 3 days.

Behavioral evaluation

Tests were performed in red light the next day after the last dosing according to the following schedule: First day, forced swimming test; second day, tail suspension test, and third day, elevated plus maze and sucrose preference tests.

a. Forced swimming test was performed the next day after the last dosing. The mice were placed in a glass with water (35 cm high × 20 cm diameter) at 25°C for 6 min, after that the mice were returned to the home cage. The immobility time was measured over the last 4 min and expressed as percentage of the total time. Immobility is the action to keep the head of each mouse above the water surface without any movements
b. Tail suspension test was used to measure depression-like behavior. The mice tails were fixed on the top of the tail suspension test cubicle. The mice were suspended to a hook (20 cm above the ground) for 6 min. The immobility time was measured over the last 4 min and expressed as percentage. Immobility time is the lack of any physical movement (passively hanging and not moving at all) or escape-oriented behavior
c. Elevated plus maze test to measure Anxiety-like behavior. Mice were placed at the center and the amount of time spent on the open arms was recorded during 5 min. The percentage of the total time spent in the open arms was registered
d. Sucrose preference test, the mice were given two bottles, one with a 1% sucrose solution and another without sucrose. At the beginning and end of the test, the weight of each bottle was measured, and the amount of solution consumed during 48 h was calculated. The position of the bottles in the cage was switched after 24 h. Sucrose consumption index was calculated using the next formula: % of sucrose preference = Sucrose solution intake (g)/water without sucrose intake (g) + sucrose solution intake × 100.

Euthanasia

At the end of sucrose preference test, the mice were euthanized by decapitation and the brain was dissected. All animals were handled in accordance with the Ethical permission for animal experimentation from the Animal Experimentation Committee of Sciences Faculty of UASLP (Universidad Autónoma de San Luis Potosi) to minimize discomfort.

Interleukin-6 determination

The animals were sacrificed by decapitation and their brain was quickly removed and dissected to extract prefrontal cortex and hippocampus regions. IL-6 levels were determined by Mouse IL-6 ELISA kit, Sigma-Aldrich, MO, USA in accordance with the manufacturer´s instructions. Briefly, dissected brain regions were homogenized (10c/w ratio) for 1 min in homogenization buffer containing 10 mM Trizma, pH 7.5, 0.05 mM EDTA, 0.1% Tween-20 and a protease inhibitor tablet (Roche, IN, USA). Then, each sample was centrifuged at 15,000 g for 20 min at 4°C. The supernatant was recovered and stored until use at −80°C. Aliquots of 50 µl for each sample were assayed by the ELISA kit (Mouse IL-6 ELISA Kit, RAB0309 Sigma Aldrich), and IL-6 levels were normalized according to the total protein levels (mg/ml) of each sample. Protein levels were analyzed by the BCA (Bicinchoninic acid) method at 540 nm using bovine serum albumin as standard reference (Sigma-Aldrich, MO, USA).

Statistical analysis

Statistical tests were carried out in MATLAB software (TheMathWorks, Natick, MA). Normality was assessed using Shapiro–Wilk test, then the statistical significance of the three-group comparison was determined by either ANOVA (analysis of variance) or Kruskal–Wallis test and thereafter corrected by Dunn-Sidak's *post-hoc* comparisons and $P = 0.05$ or less were used as the criterion for statistical significance.

Ethics approval

The ethical permission for animal experimentation was obtained on June 1, 2017 from the Animal Experimentation Committee of Sciences Faculty of UASLP Nº 0274 (Universidad Autónoma de San Luis Potosi). This investigation was performed in compliance with NOM-062-ZOO-1999 "Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio" (laboratory animals) and the US guidelines (NIH publication Nº 85-23 1985) for laboratory animal use and care and Directive 2010/63/EU of the European Parliament of the Council of the Eropean Union.

RESULTS

Forced swimming test

Forced swimming test [Figure 2, panel a] showed a significant difference among the three groups (χ^2 (2) = 12.37, $P = 0.0021$); the MEBc oral administration (100 mg/kg/day) diminished immobility time (6.83%) of the mice during the forced swimming activity. This value was different statistically ($P < 0.01$) when compared with the corresponding result for fluoxetine (F group) or (SS group) treatment, both groups showed a longer immobilization time (17.77% and 32.86%, respectively).

Tail suspension test

With respect to the immobility time in tail suspension test [Figure 2, panel b], there was a significant difference ($\chi^2 2 = 14.54$, $P = 6.9 \times 10^{-4}$) among the three groups. The animals that were orally administrated with plant extract (MEBc group) and with fluoxetine drug (F group) showed a significant decrease ($P < 0.01$) in the time of immobilization for this behavioral test (21.28% and 11.50%, respectively) when compared to the animals treated only with (SS group).

Plus maze test

The difference in the open-arms activity in the plus-maze among the three groups [Figure 2, panel c] was statistically significant ($\chi^2 = 26.54$, $P = 7.3 \times 10^{-6}$). Fluoxetine-treated rats spent significantly ($P < 0.01$) less time on the open arms (0.92%) than the MEBc group (8.39%). Furthermore, the SS group also showed significantly less open-arms activity (0.69%) than the MEBc group. The time spent in the open arm of the elevated plus-maze test was not significantly different between the fluoxetine and the saline groups.

Sucrose preference test

The sucrose preference test [Figure 2, panel d] revealed no statistically significant differences over the three experimental groups ($P > 0.05$).

Interleukin-6 determination

Regarding IL-6 determinations, a significant increase of IL-6 was observed after the (SS group) administration with 5.60 pg/g tissue for

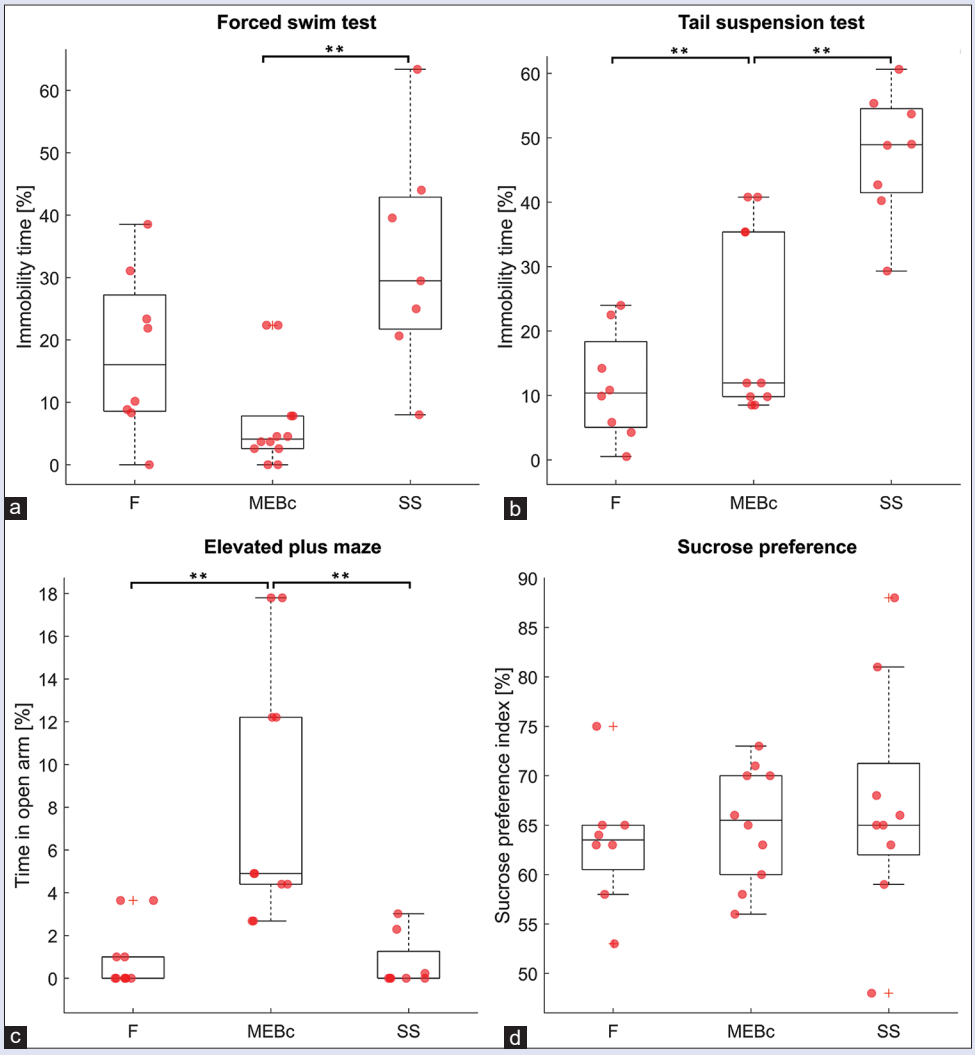

Figure 2: Behavioral testing results visualized with box plots Immobility time (percent) in the forced swimming test (a); Immobility time (percent) in the tail suspension test (b); Time (percent) spent in the open arm of the elevated plus-maze test (c); Sucrose preference index on 2 consecutive days of testing (d). Methanolic extract (methanolic extract of *Buddleja cordata*), saline (SS), and Fluoxetine (F) groups. Each boxplot is combined with a smoothed display of data distribution. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ were calculated by Kruskal–Wallis test followed by Dunn-Sidak's *post-hoc* comparisons

the hippocampus ($F[3,16] = -3.38$; $P < 0.001$) and 4.6 pg/g tissue for Cortex ($F[3,16] = -3.78$; $P < 0.05$). A similar increase was observed in fluoxetine group with 5.3 pg/g tissue for the hippocampus ($F[3,16] = -3.11$; $P < 0.001$) and 4.5 pg/g tissue for the Cortex ($F[3,16] = -3.28$; $P < 0.05$), both compared with the Sham mice (S group) [Figure 3]. The animals treated with MEBc showed a less increase in IL-6 levels in both brain regions 3.60 pg/g tissue for the hippocampus and 4.5 pg/g tissue for Cortex compared to the SS and F group. However, these levels are not statically significant respect to Sham group ($F[3,16] = -1.357$; $P = 0.815$ for Hippocampus and $F(3,16) = -2.28$; $P < 0.09$) [Figure 3].

DISCUSSION AND CONCLUSION

Depressive disorders are not yet fully understood however the inflammation is considered as an important risk factor. The prevalence of depressive disorders has increased, and a higher index of pharmacological resistance, adverse effects, and relapses has been described. Therefore, new alternative treatments have been studied; one of these alternative treatments is the use of medicinal plants and their isolated chemical compounds.[18,19]

In this study, a combination of depression model of early MS and restriction in adulthood was used. The use of these two types of chronic stress has induced a depressive-like behavior in rodents.[20-22] Early MS, besides being a validated murine model for the induction of depressive-like behaviors, can also induce neuroinflammation[23] with the affection of cerebral structures related to emotions as the hippocampus and prefrontal cortex.[24,25] This knowledge supports that the rodents could be more vulnerable to the subsequent stress and an inflammatory process is involved.[26,27]

The results show an antidepressant activity of the methanolic extract from the leaves of *B. cordata* (MEBc). For the evaluation of antidepressant activity, we used several tests developed for this purpose as described above. The tail suspension and forced swimming tests are useful when assessing the antidepressant effect of drugs products, active compounds, or whole plant extracts;[28-30] The results for both tests, tail suspension and forced swimming tests, showed a significant decrease of the immobility time percentage in MEBc and fluoxetine groups when compared to SS group. However, no statistically significant differences were found between negative controls, fluoxetine or MEBc groups with the sucrose preference test. It has been established using animal models

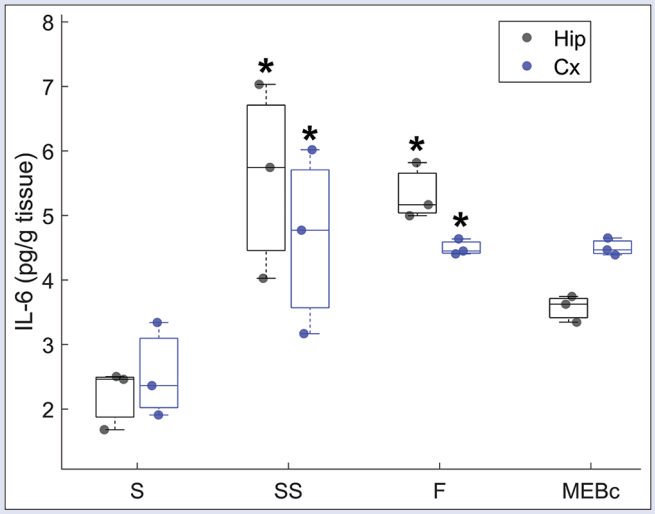

Figure 3: Quantification of IL-6 in the brain region of chronic stress mice treated with *Budleja cordata* methanolic extract. Effect of methanolic extract from *Buddleja cordata* (MEBc) (100 mg/kg/day) on interleukin-6 level in the hippocampus (Hip) and prefrontal cortex (Cx) compared to sham (S), saline (SS), and Fluoxetine (F) groups. Values are shown as the mean ± S.E.M. *$P < 0.05$; asterisk indicates statistical difference versus the Sham animals (S group)

that anhedonia can be induced in rodents exposed to chronic stress,[31,32] and that it can be relieved with antidepressant drugs.[32] Anhedonia can be evaluated by the decrease in the consumption of solutions with low concentrations of sucrose (0.1% and 0.2%), as a consequence of reduced reward sensitivity. However, some studies have reported an increased consumption of sucrose in rats exposed to chronic stress, suggesting that solutions with higher concentrations of sucrose (up to 32%) can promote its consumption,[33] and make more evident the difference in the preference for sucrose solutions. Thus, for the rodent with anhedonia, the reward obtained from sucrose solutions should be very high to promote its consumption, showing that anhedonia is related to low sensitivity for reward.[34] Early MS and moderate chronic stress in adulthood induce reward suppression in mice,[35] this change in the reward should be evidenced by 1%–2% sucrose consumption,[34] as this is the most commonly used test when assessing chronic stress impact and lack of motivation. The results didn't show statistically significant differences between study groups for sucrose preference, so we can infer that sucrose concentration was insufficient.

The elevate plus-maze behavioral test, validated for the evaluation of the anxiolytic effect of drugs,[36-38] showed that MEBc extract can produce an anxiolytic effect when compared to fluoxetine or SS. The antidepressant action of MEBc could be related to its anti-inflammatory effect which has been described for *B. cordata* extracts as a reduction in TCD4 + cell number and an increase in IL-10 concentrations, according to a rheumatoid arthritis animal model assay.[11] The study demonstrated that the MEBc treatment inhibited the IL-6 levels induced by chronic stress in mice reinforcing that the anti-inflammatory effect of *B. cordata* observed in other preclinical models has an influence on the antidepressant effect.

Regarding the potential relationship between inflammation and depression, nonsteroidal anti-inflammatory drugs as ibuprofen, indomethacin and celecoxib have been effective on reducing the depressive-like behavior induced in murine model of interferon alpha induced depression.[39] It has been shown that the chronic stress restriction model induces a systemic and cerebral inflammatory response, evidenced by increased levels of corticosterone, epinephrine, TNF-α, and IL-6.[40,41] Taking into consideration the reported evidence of increased levels of IL-6 in the hippocampus and prefrontal cortex,[42] we decided to perform IL-6 determinations in these two regions in our groups as part of our study. It was observed a reduction of hippocampal and prefrontal IL-6 concentrations for the MEBc treated group. While the fluoxetine and SS groups reported increased levels of IL-6 when compared to the MEBc group.

The objective of this study was to assay the effectivity of MEBc extract over the depressive-like symptoms and IL-6 levels in mice, taking into consideration that the well-known anti-inflammatory effect of MEBc may influence its therapeutic antidepressant activity. Many other medicinal plants, as the *Valeriana fauriei,* have been studied using this same approach to prove its antidepressant activity.[17] *Roman chamomile* has demonstrated to improve the antidepressant effect of clomipramine in patients with treatment-resistant depression.[43] Ethanolic extract of *Aurantiochytrium* sp. has also shown an antidepressant effect in conjunction with a neuroinflammation reduction.[41] The main compound of *Polygonum multiflorum* acts as neuroprotective factor with antidepressant, antioxidant, and anti-inflammatory (decreasing levels of inflammatory cytokines, in hippocampus and prefrontal cortex) effects[42] and flavonoids from *Trigonella Foenum Graecum* seed have shown antidepressant activity in a murine model of restriction-induced depression.[44]

In conclusion, using the forced swimming test, it was clear that MEBc can reduce the average of immobility time as compared to SS and fluoxetine. In the tail suspension test, both groups, fluoxetine and MEBc shown significant differences over the reduction of the immobility time as compared to SS group. In the elevated plus-maze, it was possible to prove the effect of the plant extract, as the MEBc group increased the percentage of time spent in the open arms as compared to the negative control (SS) and fluoxetine treatment. These results evidence the antidepressant and anxiolytic effects of MEBc over the chronic stress depression-induced model and suggest that this antidepressant effect could be mediated by its anti-inflammatory effect. It would be interesting, in future, to evaluate the *B. cordatha* effect about other diseases in which there is an inflammatory process and or depression.

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Conflicts of interest

There are no conflicts of interest.

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