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Acute effect of an infusion of *Montanoa tomentosa* on despair-like behavior and activation of oxytocin hypothalamic cells in Wistar rats

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ABSTRACT

Background and aim: In Mexican traditional medicine, *Montanoa tomentosa* (*Mt*) has been used as a remedy for reproductive impairments and mood swings. In pre-clinical research, both the extract and some of its active metabolites have produced oxytocinergic-like effects on female reproductive organs; however, there are no detailed studies of its effects on mood swing and brain structures. The aim of this study, was to analyze the behavioral effects of acute administration of a *Mt* infusion on male rats, during the Open Field (OFT) and Forced Swim (FST) Tests, and their association with the activation of oxytocin (OXT) cells, indicated by Fos protein (Fos/OXT) in the paraventricular (PVN) and supraoptic nuclei (SON).
Experimental procedure: 52 adult male Wistar rats were assigned to two conditions; with FST (n = 8), or without (n = 5). Each integrated condition included four groups [Control, Vehicle, Fluoxetine (Flx; 10 mg/kg), and *Mt* (50 mg/kg), *p.o.*].
Results and conclusion: Mt and Flx treatment produced an anti-despair-like effect on the FST, but no significant changes in locomotor activity. Also, the *Mt* infusion -but not Flx-significantly increased the number of Fos/OXT cells in the PVN and SON, regardless of the condition, compared to the control and vehicle groups. These results show that *Mt*, but not Flx, produces an anti-despair-like effect that could be associated with the activation of OXT cells in PVN and SON. This study thus contributes to our knowledge of the pharmacological activity of *Mt* infusions, which could be a natural antidepressant agent with future clinical relevance.

1. Introduction

All organisms strive to maintain dynamic equilibrium and homeostasis, which may be altered by certain physical and psychological stressors[1] that can trigger physiological and behavioral responses as the organism seeks to recover their homeostasis. This "stress response" is reflected in activation of the hypothalamic-pituitary-adrenal (HPA) axis.[2]

Many factors influence the pattern and magnitude of the stress response, including duration of exposure to stress. Repeated stress with HPA axis hyperactivity has been associated with the development of depression, a serious medical condition and public health concern. While causes may vary, the factors that can contribute to the onset and persistence of depressive episodes may be psychosocial, genetic and/or biological factors.[3]

Clinical and laboratory evidence suggests a role for OXT as an endogenous antidepressant/anxiolytic hormone.[4] Oxytocin is produced centrally by neurons in the paraventricular (PVN) and supraoptic nucleus (SON) of the hypothalamus, and is involved in various physiological processes. PVN oxytocinergic neurons project

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to several brain areas, including the hypothalamus. The oxytocin released from these projections acts as a neuromodulator in several brain regions, so it affects behaviors associated with stress, anxiety and mood swing.[5]

Oxytocin is released from the endocrine hypothalamic-neurohypophyseal system in response to numerous physical and psychological stressors to attenuate the activity of the HPA axis and modulate the stress response.[6] Therefore, shaking or forced swimming increase OXT release in hypothalamic nuclei[7] as a way to counteract the negative effects of stress. When this hormone is administered systemically, it produces anti-despair-like effects on the forced swim test,[4] while blocking the oxytocin receptors increases secretion of the adrenocorticotropic hormone (ACTH) and corticosterone into the bloodstream as a response to stressful situations, such as the elevated plus maze and FST.[8] Also, chronic intracerebroventricular injection of OXT attenuates anxiety-related behaviors. These studies suggest that central OXT exerts anxiolytic- and anti-depressant-like effects in pre-clinical research.[9]

Oxytocin can stimulate its own release, but interestingly, the release of OXT can also be promoted by neurosteroids,[10] oxytocin analogues, and oxytocin-receptor ligands produced by some plants.[11] Mexican traditional medicine uses several plants to treat mood swing, including *Montanoa tomentosa*, known in the Nahuatl language as *Zoapatle* or *Cihuapatli* (*cihuatl* = woman, *patli* = remedy or medicine.[12] Descriptions of this plant as a traditional remedy are found in the *Badianus Codex* or *Libellus de Medicinalibus Indorum Herbis* (1552), which lists botanical determinants, traditional recipes, and prescriptions, *La Historia General de las Cosas de Nueva España* (*Florentine Codex*) and *Historia Natural de la Nueva España* which describes a tea prepared from the leaves of *Mt* that facilitates parturition, ameliorates puerperium, serves as a contraceptive agent, and remedies mood disorders.[13]

Clinical data show that menstrual-like cramps, cervical dilatation, and uterine bleeding were observed when *Mt* was administered orally to women during early pregnancy.[14] Oral ingestion of the crude aqueous extract of this plant increases uterine contractions in both pregnant and non-pregnant women, but with no apparent side effects. This extract was also found to have a potent uterotonic effect that is stronger than any of the organic fractions tested alone.[15] Furthermore, *in vivo* and *ex vivo* animal studies have reported that the crude aqueous extract of *Zoapatle* or its purified fractions, exert a contraceptive effect, but without affecting subject's endocrine, hematological, blood lipid, protein, or electrolytic status, or liver, kidney, or thyroid gland function.[15,16] Those reports suggest that *Mt* extract could activate the oxytocinergic system, but specific studies that support or refute this hypothesis are not currently available.

Other work indicates that the crude extract of *Mt* crosses the blood-brain barrier to exert effects on the central nervous system. The main acute effect was a significant improvement in general motivation.[17] Extracts of *Mt* have also shown an oxytocin-like effect,[18] though it is unknown whether this involves actions on oxytocinergic cells at the level of the brain. In light of these findings, the present study was designed to explore whether the infusion of *Mt* acts as an oxytocinergic agent that promotes an anti-stress-like effect by acting on central structures involved in mediating the stress response.

2. Materials and methods

2.1. Ethics

All experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health[19] and the *Norma Oficial Mexicana para el Cuidado y Uso de Animales de Laboratorio*.[20] The study protocol was authorized by the Internal Ethics Committee of the Veterinary School at the *Universidad Autónoma de Tlaxcala*, Mexico (no. MVZ-189/12).

2.2. Animals

Adult male Wistar rats weighing 300–350 g at the beginning of the experiments were used. They were housed at random in Plexiglas cages (44 cm long × 33 cm wide × 20 cm high) under a 12/12 h light/dark cycle (lights on at 7:00 a.m.) at an average temperature of 25 ± 2 °C with *ad libitum* access to water and food (Harlan, México, S.A. de C.V.).

2.3. Preparing of the infusion

Mt was collected near Tlaxcala, Mexico, and authenticated by a specialist at the *Jardín Botánico Universitario* at the *Universidad Autónoma de Tlaxcala*[21] (serial no. MT-UATX10). The infusion process has been described previously by.[17] Briefly, leaves were collected, dried for 20 days, and then ground into a fine powder. An average of 1 g was mixed with 20 mL of purified water just before boiling. The infusion obtained was filtered and kept at 3 °C. The infusions were prepared 30 min prior to administration to prevent any modifications of the extract's original chemical properties.

2.4. Treatments

A total of 52 rats were randomly divided into two conditions (with or without forced swimming), and organized in four independent groups for each condition. The first group received no handling or treatment [control (Ctrl) group; n = 5]; the second was given only purified water [(Vh) group; n = 5], which was the vehicle in which *Mt* or Flx were dissolved for the remaining two groups; the third group received 50 mg/kg/mL of the infusion of *M. tomentosa* [*Mt* group; n = 5]; and the fourth 10 mg/kg/mL of fluoxetine [Flx group; n = 5]. The other set of animals received similar treatment schedules (Ctrl, Vh, Flx and *Mt* groups; n = 8 each group), but were also exposed to the open field test and forced swim tests, to measure effects on despair-like behavior.

All groups received the treatments *per os* route through an oral gavage, stainless steel curved cannula (18G X 3.0″ w/2.5 mm ball. Cadence, Inc. Staunton, Va., USA), coupled to a 1-ml disposable syringe (Terumo Medical de México, SA de CV, Mexico City, Mexico), in a volume equivalent to 1 mL/kg, administered 30 min before the behavioral tests. Effects were evaluated on both the open field and forced swim tests for 5 min each. Approximately 2 min elapsed between tests.

The dose of *Mt* was selected on the basis of a dose-response curve which detected that 50 mg/kg was the minimal effective dose to produce anti-despair-like effects on the FST.[22] The dose of Flx (fluoxetine chlorhydrate, Prozac®, was authenticated and elaborated by the Eli-Lilly Compañía de México, S.A. de C.V., (México, City Mexico; PubChem CID: 62857), and selected from reports in which 10 mg/kg produced anti-despair-like effects on the FST after acute administration.[23]

2.5. Behavioral tests

2.5.1. Locomotor activity test

The apparatus used was an opaque Plexiglas cage (44 × 33 cm) with 20-cm high walls. The floor was divided by lines into 12 squares (11 × 11 cm). This test was carried out to identify -or discard-possible hypoactivity, hyperactivity, or no treatment-associated changes that might interfere with the interpretation of

the FST results. A digital video camera (Fujifilm, Finepix Z70, 6.3 × digital zoom) was placed above the cage to record each rat's activity. Later, two independent observers measured the behavioral variables until they reached a consensus with >95% agreement. At the beginning of the test, the animals were gently placed in one corner of the cage to evaluate the following parameters 1) the number of squares crossed [*i.e.,* when a rat passed from one square to another with all four paws][24,25] 2) the time spent grooming, including all self-directed behaviors of cleaning from the head, to the ears, limbs and ano-genital region,[26] and 3) the time spent rearing [*i.e.,* when the rat explored the cage in a vertical position on its rear limbs]. After each test, the cage was cleaned thoroughly with a 15% alcohol solution to eliminate the odor of the previous rat. At the end of this test, the animals were evaluated in the FST.

2.5.2. Forced swim test (FST)

In a first pre-test session, the rats were placed individually in a rectangular glass tank (30 cm wide × 20 cm long × 50 cm high) for 15 min with water at 25 ± 1 °C. The water level was adjusted to the size of each animal so that it could touch the bottom of the tank with the tips of its hind limbs and maintain their heads above the water level. During this session, the animals confronted a new and threatening situation represented by immersion in water. This allowed us to ensure the development of "behavioral despair".[27]

Vh, *Mt* or Flx were administered 24 h after the pre-test session. Thirty minutes later, the rats were evaluated in a test session of the FST for 5 min. The same digital video camera was placed in front of the tank to record each rat's activity. Later, two independent observers measured the behavioral variables until they reached a consensus with >95% agreement. The behavioral measures assessed were: 1) latency to the first immobility period; and 2) total immobility time. Immobility was assumed when the rat touched the bottom of the tank, making at least two points of contact (*i.e.*, with one or both hind paws or the tail), or when it floated, making only the minimal movements necessary to maintain its head above the surface, but without displacing any water. Only the results from the test session were included in the statistical analysis. At the end of the FST, the rat was returned to its home cage. Ninety minutes later, each rat was anesthetized to extract their brains and process them for immunohistochemical studies.

2.6. Immunohistochemistry

Activation of OXT cells in the PVN and SON was evaluated by the presence of the Fos protein, as an index of the neural activation of OXT cells using immunohistochemical techniques. Briefly, the rats were euthanized with an overdose of sodium pentobarbital (Sedalpharma, Pet's Pharma, México; 44 mg/kg, i.p.) and then perfused transcardially with a 0.9% saline solution, followed by 4% paraformaldehyde in phosphate buffer (PB; pH 7.4). The animal's brains were removed immediately, cryoprotected in serial dilutions of sucrose (10%, 20%, and 30%) in PB, and coronally sectioned at 50 μm with a cryostat (Micron HM-520. Microm International GmbH. Walldorf, Germany) at −23 °C. Serial sections were collected in PB from the level of the opening of the organ vasculosum of the *lamina terminalis* to the mammillary bodies.

One of every four sets of sections (n = 5 per group) was used to double-label Fos and OXT. Tissue were washed in PB five times for 5 min each to remove excess aldehydes, and then exposed to a 0.5% hydrogen peroxide solution for 10 min to eliminate endogenous peroxidase activity. Non-specific tissue antibody reactions were blocked by placing the sections in 3% normal horse serum (Vector Labs, Burlingame, CA, USA) for 1 h at room temperature. The sections were then incubated for 72 h at 4 °C with a polyclonal Fos antibody (1:5000; Santa Cruz Biotechnology, Santa Cruz CA, USA) in 3% normal horse serum and 0.3% Triton X-100 (Sigma, St. Louis, MO, USA). Later, the tissue samples were placed in biotinylated horse anti-goat IgG (1:200; Vector Labs., Burlingame, CA) for 1 h. After three washes in PB, they were incubated in ABC solution (1:250; Vector Labs., Burlingame, CA) for 1 h, and then revealed with 0.05% diaminobenzidine (Polyscience, Warrington, PA, USA) in the presence of nickel sulfate (10 mg/mL, Fisher Scientific, Pittsburgh, PA, USA), cobalt chloride (10 mg/mL, Fischer Scientific, Pittsburgh, PA, USA), and 0.01% hydrogen peroxide, which produced a black-purple precipitate. After 10 min, the tissue was transferred to PB to stop the reaction. Following three washes in PB, the sections were incubated for 72 h at 4 °C using a monoclonal antibody against OXT (1:5000; Millipore, Billerica, MA, USA) in PB with 0.3% Triton X-100 and 3% normal horse serum. Next, they were exposed to a biotinylated horse anti-mouse IgG (1:200; Vector Labs., Burlingame, CA) the ABC kit, and finally revealed with 0.05% diaminobenzidine and 0.01% hydrogen peroxide, which produced a brown cytoplasmic precipitate. The sections were mounted on gelatin-coated slides, dehydrated, cleared with Hemo-De (Fisher Scientific), and then cover-slipped with Permount. In all cases, the tissue sections from different groups were incubated at the same time. The negative control was also processed as just described, but the primary antibody was omitted.

2.6.1. Cell-counting

To quantify the oxytocin-immunoreactive (OXT-ir) and double-labeled Fos/oxytocin cells (Fos/OXT-ir) in the PVN and SON, the sections were examined under bright-field illumination with an Olympus BX41 microscope at 10 × and 20 × magnification, and counted bilaterally by two observers who were blind to the experimental conditions. Oxytocin immunoreactivity was identified as a brown precipitate in the cytoplasm, while Fos immunoreactivity was determined as a black-purple precipitate in the cell nucleus. Double-labeled Fos/OXT-ir neurons had brown cytoplasm with a black nucleus. In the tissues that were processed without the primary antibody the immunoreactivity for Fos and OXT was completely absent.

The PVN and SON were examined from the rostral to caudal ends. For counting, three bilateral brain sections were included per condition of the animal (n = 5). For analysis, the number of Fos/OXT expressing cells was counted bilaterally in both nuclei at the level of bregma −0.60 to −1.92 mm, following[28] atlas of the rat's brain. Images were digitalized with a Cool snap Pro digital camera and processed with a computerized image analysis system (ImagePro Plus v.5; Media Cybernetics, Silver Spring, MD, USA).

2.7. Statistical analyses

The behavioral data were analyzed by a one-way analysis of variance (ANOVA). For cell-counting analysis, a two-way ANOVA was performed, followed by the Student-Newman-Keuls *post hoc* test, using SigmaStat 3.5 software. Data are expressed as mean ± standard error for each variable. The accepted significance was $p < 0.05$.

3. Results

3.1. Behavioral tests

3.1.1. Locomotor activity test

The crossings on the open field test revealed no significant differences among groups ($F_{3,28} = 0.167$, $p = 0.918$), so there were no differences in grooming [($F_{3,28} = 0.371$, $p = 0.775$) or rearing behaviors ($F_{3,28} = 2.331$, $p = 0.096$); Data not shown].

3.1.2. Forced swim test

The latency to the first immobility revealed significant between-group differences ($F_{3,28} = 7.445$, $p < 0.001$). The *Mt* infusion induced a significantly ($p < 0.01$) longer latency compared to results from the Ctrl, Vh and Flx groups (Fig. 1A). Total immobility time also revealed significant differences between groups ($F_{3,28} = 15.893$, $p < 0.001$), as Flx induced a significantly lower ($p < 0.001$) time than in the Ctrl, Vh and *Mt* groups. Finally, this variable decreased significantly in the *Mt* group ($p < 0.05$) with respect to Ctrl (Fig. 1B).

3.2. Oxytocin cell activation

3.2.1. Oxytocin immunoreactivity in the SON and PVN

Oxytocin immunoreactivity cells were expressed in the SON and PVN in all eight groups. The OXT-ir in the SON did not reveal significant effects of condition [(with or without forced swimming; $F_{1,32} = 1.104$, $p = 0.301$), treatment ($F_{3,32} = 0.074$, $p = 0.973$) or a condition × treatment interaction ($F_{3,32} = 0.499$, $p = 0.686$); Data not shown]. The OXT-ir in the PVN showed no significant effects of condition [(with or without forced swimming; $F_{1,32} = 3.954$, $p = 0.055$), treatment ($F_{3,32} = 0.829$, $p = 0.488$) or interaction ($F_{3,32} = 0.581$, $p = 0.632$) either; Data not shown].

3.2.2. Fos/OXT immunoreactivity in the SON and PVN

The number of double-labeled Fos/OXT-ir cells varied among the experimental conditions (with or without forced swimming) in both the SON and PVN.

3.2.2.1. Supraoptic nucleus. Fos/OXT immunoreactivity in the SON revealed a significant effect of condition ($F_{1,32} = 37.747$, $p < 0.001$), as the number of Fos/OXT-ir cells increased significantly ($p < 0.001$) in the swimming condition compared to the no-swimming condition. Similarly, the treatments showed a significant effect ($F_{3,32} = 11.710$, $p < 0.001$). The number of Fos/OXT-ir cells increased significantly ($p < 0.001$) in the 50 mg/kg *Mt* group between both conditions; with swimming showed an increased of 32% compared to Flx, Vh and Ctrl; 7.86, 16.25 and 14.44% respectively. So also, in the condition no-swimming *Mt* group increased an 8.51% compared to Flx, Vh and Ctrl; 0.79, 2.75 and 2.79% respectively, of the total number of oxytocin cells. To support these findings, significant differences in the interaction of factors were also detected ($F_{3,32} = 3.775$, $p = 0.020$). A clear increase in the number of Fos/OXT-ir cells was found in the *Mt*-treated group when compared to the other groups (Figs. 2 and 4A).

3.2.2.2. Paraventricular nucleus. The Fos/OXT-ir in the PVN revealed a significant effect of condition (*i.e.,* with or without forced swimming; $F_{1,32} = 58.84$, $p < 0.001$). The number of Fos/OXT-ir cells increased significantly ($p < 0.001$) in the forced swimming group compared to the no-forced swimming group. Similarly, the analysis of Fos/OXT-ir in the PVN revealed a significant effect of treatment ($F_{3,32} = 34.90$, $p < 0.001$). Since the number of Fos/OXT-ir cells increased significantly ($p < 0.001$) in the *Mt* group between both conditions; with swimming showed an increased of 28%; compared to Flx, Vh and Ctr; by, 0.96, 12.46 and 8.79% respectively. While, in the condition no swimming increased a 7.82% compared Flx, Vh and Ctrl; 0.23, 1.83 and 1.43% respectively, of the total number of oxytocin cells. The interaction of factors also showed significant differences ($F_{3,32} = 13.03$, $p < 0.001$; Figs. 3 and 4B), as observations of the swimming condition showed that the *M. tomentosa*-treated group had a higher number of Fos/OXT-ir cells than the other experimental groups.

4. Discussion

The present study explored the effects of a *M. tomentosa* infusion on the behavioral responses of rats subjected to the forced swim test, and their relation to the activation of oxytocin cells in hypothalamic nuclei. Acute administration of *Mt* (50 mg/kg), significantly increased latency to first immobility; suggesting a partial anti-despair-like effect in rats that did not disrupt general motor activity on the open field test. Also, oral administration of the *Mt* infusion (50 mg/kg) increased the number of Fos/OXT-ir cells in the SON and PVN. Taken together, these findings could suggest that the *Mt* infusion activated OXT cells in hypothalamic nuclei, which may be associated with a partial anti-despair-like effect. The present data support the hypothesis that *Mt* possesses some properties that modulate mood states, possibly associated with OXT-like properties, but distinct from the common antidepressant, fluoxetine.

The FST is a validated behavioral model for screening substances with potential antidepressant-like activity. In this model, rats develop a state of "despair," characterized by increased immobility[29] and a reduced latency to the first immobility period.[24] Latency to the first immobility is considered an indicator of the first effort that rat's make when they attempt to cope with a stressful situation, and as a measure of motivation.[30,31] An increase in this parameter is considered a motivational indicator that allows the rat to cope with the stressful situation of being forced to swim.[32] At the experimental level, an increase in latency to the first immobility is observed after administering antidepressant drugs or other agents with antidepressant or anti-stress effects.[24,32–34] Clinically-effective antidepressant drugs[40,46] and extracts from plants with antidepressant activity[34] reduce total immobility time in animals subjected to this paradigm. An increase in the latency to the first

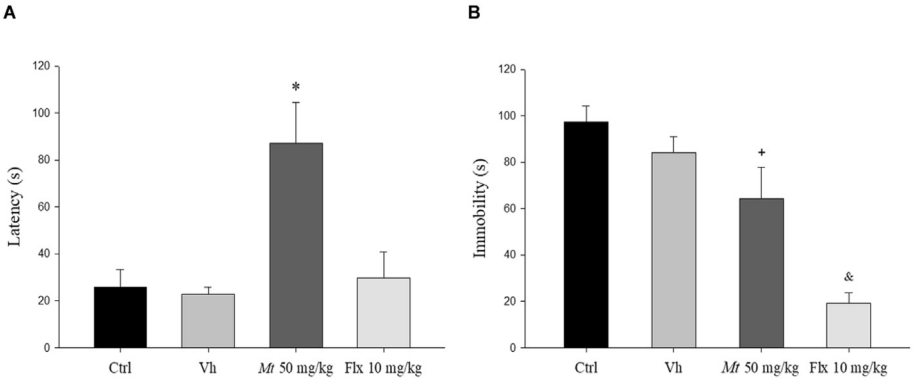

Fig. 1. Forced swim test: (A) Latency to first immobility. Only the *M. tomentosa* (*Mt*) group significantly increased latency to first immobility. **(B) Total immobility time:** the *Mt* and Flx groups significantly decreased total immobility time, but this effect was more marked in the Flx. Two-way ANOVA followed by Student-Newman-Keuls *post hoc* test. $^{*}p < 0.01$ *vs.* control (Ctrl), vehicle (Vh) and fluoxetine (Flx) groups; $^{\&}p < 0.001$ *vs.* Ctrl, Vh and *Mt* groups; $^{+}p < 0.05$ compared to Ctrl.

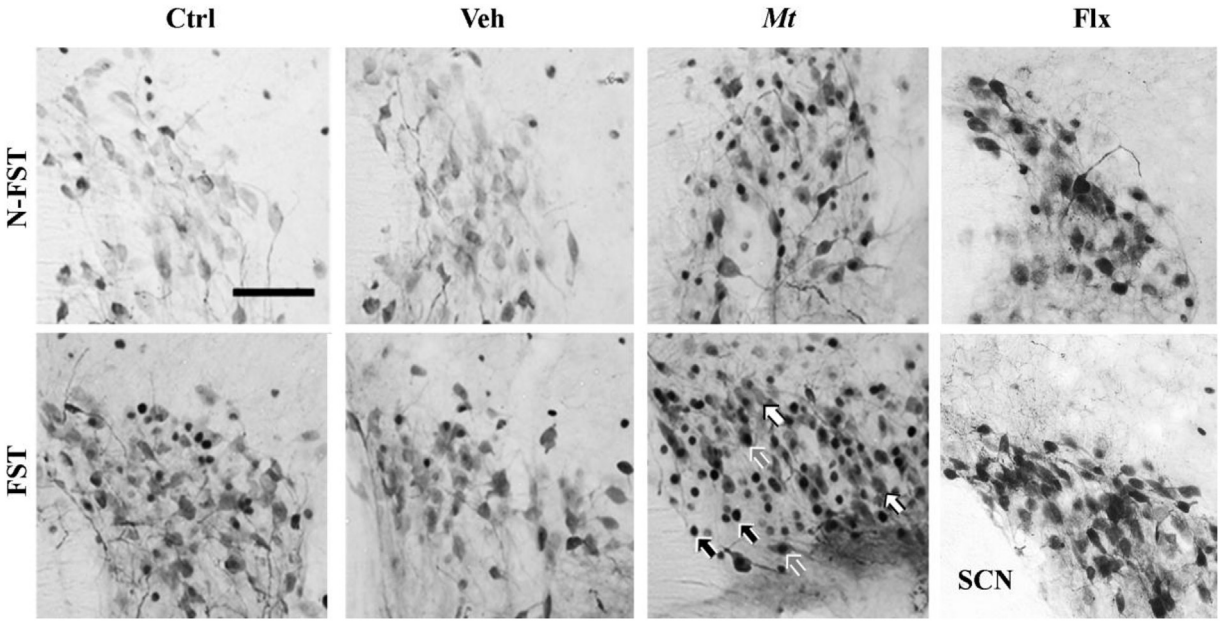

Fig. 2. Double-labeling of Fos/OXT-ir cells in the SON. (A, B) photomicrographs of representative sections that illustrating the expression of Fos (black arrow), OXT (white arrow), and Fos/OXT cells (grey arrow) at the level of the mid-portion of the SON under six different experimental conditions with or without forced swimming. Scale bar = 50 μm.

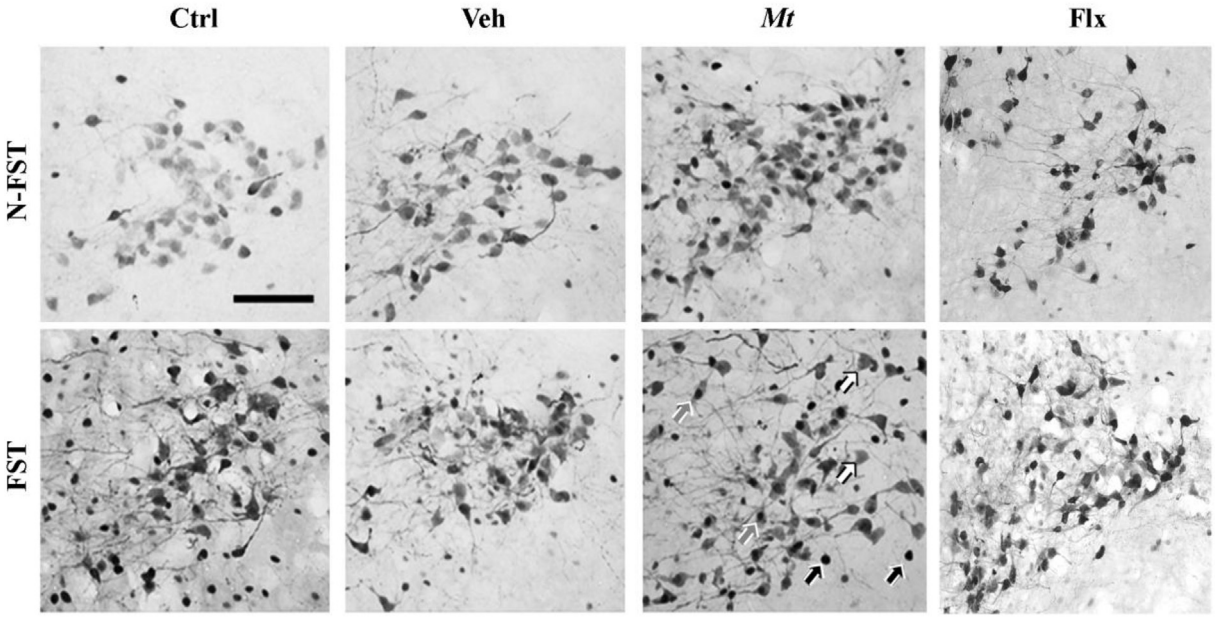

Fig. 3. Double-labeling of Fos/OXT-ir cells in the PVN. (A, B) Photomicrographs of representative sections illustrating the expression of Fos (black arrow), OXT (white arrow), and Fos/OXTcells (grey arrow) at the level of the mid-portion of the PVN under six different experimental conditions with or without forced swimming. Scale bar = 50 μm.

immobility, coupled with a reduction of immobility produced by antidepressants or neurosteroids in the FST, is considered an indicator of antidepressant-like activity,[29,31,33,35,36] but such effects can also be interpreted as possible indicators of a reduction of forced swim-induced stress.[33]

Our study found that acute administration of 50 mg/kg of the *Mt* infusion did not significantly reduce total immobility time, but significantly increased latency to the first immobility on the swimming test, suggesting a partial anti-despressant-like effect. Similar results have been reported with such antidepressant and anti-stress substances as neurosteroids and some extracts of medicinal plant.[24,33,34,36] We also found that the dose of 50 mg/kg of the *Mt* extract did not induce significant changes in motor activity. It has been reported that 75 mg/kg of the *Mt* extract increased latency to the first immobility and reduced total immobility time on the FST, but this dose also significantly increased the number of crossings in the open field tes.[22] An increase in locomotor activity may disguise the motivational or anti-depressant-like effects of antidepressant drugs, similar to stimulant drugs that activate the central nervous system, but also reduce immobility while increasing general locomotor activity.[29,35] This finding may be considered as an anti-depressant-like effect or, perhaps, a

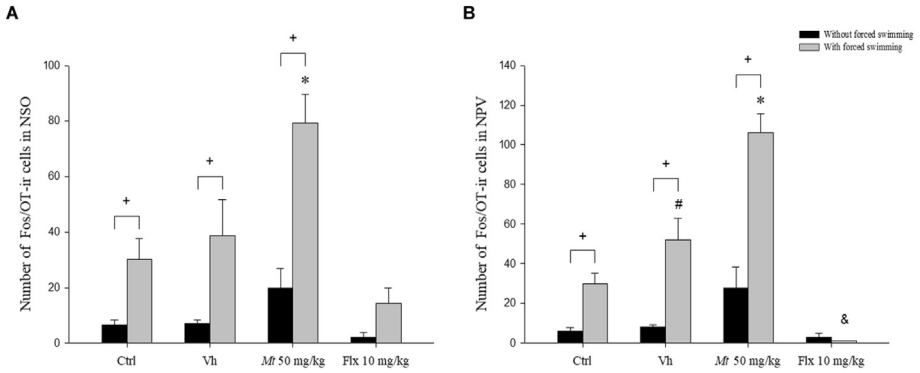

Fig. 4. Number of neurons with double-labeled Fos/OXT in the SON and PVN in groups with or without forced swimming. (A) SON: in groups with forced swimming ($^{*}p < 0.001$) *Montanoa tomentosa* (*Mt*) with respect to Control (Ctrl), Vehicle (Vh) and fluoxetine (Flx) groups. Between treatments ($^{+}p < 0.05$) *Mt,* Ctrl, Vh groups, with respect to with or without forced swimming. **(B) PVN**: in groups with forced swimming ($^{*}p < 0.01$) *Mt* with respect to Ctrl Vh and Flx groups; ($^{\#}p < 0.05$) Vh with respect to Ctrl; ($^{\&}p < 0.01$) Flx compared to the Ctrl and Vh groups. Between treatments ($^{+}p < 0.05$) *Mt*, Ctrl, Vh groups, with respect to with, or without, forced swimming. Two-way ANOVA followed by Student-Newman-Keuls *post hoc* test.

motor-stimulant effect. Thus, we suggest that the *Mt* extract possesses a partial anti-depressant-like profile.

We propose that this partial anti-depressant-like effect of *Mt* may be related to activation of the oxytocinergic system. Previous findings have shown that oxytocin acts locally on the activity of the HPA axis to modulate the stress response.[4,8,37] In the present study, we observed activation of oxytocinergic neurons in both the SON and PVN in response to acute administration of the *Mt* infusion, but not with the antidepressant fluoxetine. Interestingly, *Mt* induced activation of hypothalamic oxytocinergic neurons in both nuclei in rats subjected to the swimming test, as well as in those that were not forced to swim. The possible action mechanism of this central effect is unknown, but previous studies suggest that the compounds contained in the *Mt* infusion could cross the blood-brain barrier to directly target the central nervous system.[17] Considering that this *M. tomentosa* infusion promoted an anti-depressant-like effect in rats on the FST while simultaneously inducing activation of hypothalamic oxytocinergic neurons in both experimental and control rats, we suggest that the biologically-active compounds that it contains may act directly on the oxytocinergic system in the brain to produce its effects. The chemical compounds in the *Mt* infusion likely target other neural structures of the CNS to promote general anti-depressant-like activity, but targeted neuroanatomical and pharmacological studies are required to test this possibility.

Turning to fluoxetine, while an antidepressant effect was observed on the FST in the Flx group, it did not exert any significant influence on the activation of oxytocin cells as did the *Mt* infusion. This is likely because fluoxetine activates the serotonergic system directly and may subsequently activate the release of oxytocin[38,39]; whereas *Mt* seems to act primarily on the oxytocinergic system.

The present results suggest that the activation of OXT cells by acute administration of a *Mt* infusion prepared in the traditional manner may be involved in the action mechanism that underlies the antidepressant-like effect detected in the forced swimming in rats, similar to the release of oxytocin that is induced by some chemical compounds, such as neurosteroids.[10]

Although the main objective of this study was not to identify the bioactive components of the *Mt* infusion that are involved in its behavioral and neurochemical action, we may offer a possible explanation for its partial antidepressant-like effects. *Mt* infusion contains several bioactive compounds (e.g., flavonoids)[40] that exert beneficial effects against some central nervous system disorders, related to stress, for example; isoquercitrin had a partial anti-despair effect[41] associated with the activation of hypothalamic OXT neurons[42,43]. Besides some flavonoids also modulates GABAergic transmission increasing the firing of OXT neurons[44] and GABA receptors are the main target for anxiolytic and anti-stress agents, including benzodiazepines. Therefore, the anti-depressant-like effect and activation of OXT neurons produced by the *Mt* infusion could be related to the flavonoids contained in the extract, though this possibility requires additional research.

Based on the above information we hypothesize that the behavioral and neuronal effects of *Mt* could be related to the flavonoids previously identified in the extract of this plant, though further pharmacological studies are necessary to test this hypothesis. The results of several studies have shown that the effect of *Zoapatle* extract cannot be reproduced by only one of these compounds, thus suggesting that synergism between compounds may occur.[45]

In conclusion, the present results show that the partial anti-depressant-like effect of acute administration of the *M. tomentosa* infusion, could be associated with the activation of hypothalamic oxytocinergic neurons in rats, thus differing from the typical action mechanism involved in the antidepressant drugs like fluoxetine, which act on the serotonergic pathways.

Conflict of interest statement

This manuscript has been reviewed and approved by the authors, which state that they have no conflict of interest.

Author contributions

Substantial contributions to the conception of the work: OLM, MSV, FGO, MCJ, to study design: MJRH, MSV, JFRL, MCJ; to acquisition analyses: MJRH, FGO, MC; to data interpretation: MJRH, JFRL, OLM, APO, MCJ, MC; to drafting the manuscript: MJRH, JFRL; and to its critical review: MJRH, JFRL, APO, MC, MCJ. Final approval of the version proposed for publication and agreement on all aspects of the work: all authors.

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