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Chronic infusion of *Montanoa tomentosa* reduces despair-like behavior and activates hypothalamic oxytocin neurons in male Wistar rats

[La infusión crónica de *Montanoa tomentosa* reduce la conducta de desesperanza y activa las neuronas hipotalámicas de oxitocina en ratas Wistar macho]

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Abstract: *Montanoa tomentosa* is used in traditional Mexican medicine to treat reproductive and mood disorders. Preclinical studies support the idea that acute administration of *M. tomentosa* induces an antidepressant-like response that may be related to oxytocin activation in hypothalamic cells, however, it is unknown whether this behavioral and neuroendocrine effect is maintained when chronically administered. Here, 39 adults male Wistar rats were subjected to two conditions: without and with the forced swimming test (FST). Each group received for 28 consecutive days p.o., vehicle (1 mL/kg); fluoxetine (1 mg/kg); or *M. tomentosa* (50 mg/kg). *M. tomentosa* and fluoxetine treatments significantly decreased the total immobility time compared with that using vehicle without producing any significant change in locomotor activity. No significant between-treatment differences were found in the number of oxytocinergic neurons, indicating that chronic infusion of *M. tomentosa* exerts antidepressant-like effects, similar to those of Fluoxetine, independently of oxytocinergic activation.

Keywords: Antidepressant; Fos protein; Oxytocin; Zoapatle; Hypothalamic nuclei.

Resumen: *Montanoa tomentosa* es utilizada en la medicina tradicional mexicana para tratar trastornos reproductivos y de estado de ánimo. Estudios preclínicos, reportan que la administración aguda de *M. tomentosa* produce efectos tipo antidepresivo asociados con la activación de células hipotalámicas oxitocinérgicas, pero se desconoce si estos efectos conductual y neuroendocrino se mantienen después de un tratamiento crónico. Se incluyeron 39 ratas macho adultas Wistar bajo dos condiciones: sin y con inducción de estrés por nado forzado. Cada grupo recibió durante 28 días consecutivos p.o., vehículo (1 mL/kg); fluoxetina (1 mg/kg); o *M. tomentosa* (Mt; 50 mg/kg). Los tratamientos con *M. tomentosa* y fluoxetina disminuyeron significativamente el tiempo total de inmovilidad comparado con vehículo, sin cambio significativo en la locomoción. No hubo diferencias significativas en el número de neuronas oxitocinérgicas entre tratamientos, lo que indica que la infusión crónica de *M. tomentosa* ejerce efectos tipo antidepresivos similares a Fluoxetina, independientemente de la activación oxitocinérgica.

Palabras clave: Antidepresivo; Proteína Fos; Oxitocina; Zoapatle; Núcleos hipotalámicos

INTRODUCTION

The negative impact of stress depends on several factors, including individual susceptibility, that is, people's ability to cope with a stressor or their use of coping strategies (Mercier, 2003), and the nature of stressful stimuli, which can be classified both qualitatively (characteristics) and quantitatively (intensity and duration). Acute stress can trigger intense, rapid, and often violent responses that can cause cardiovascular disorders, immunosuppression, gastrointestinal pathologies, and reproductive system inhibition. However, prolonged or excessive exposure to a stressor can also induce the use of drugs for abuse, anxiety, and depression symptoms (Nadal & Armario, 2010).

Depression specifically develops in response to repeated high-intensity stress, which can trigger hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, characterized by low mood and loss of interest and/or feelings of pleasure (Remick, 2002). Clinical and preclinical studies have reported that oxytocin (OXT) produced in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei modulates the response of this axis (Windle *et al*., 1997; Neumann, 2002) to physical or psychological stressors (Gibbs, 1984; Higuchi *et al*., 1988; Sanders *et al*., 1990). OXT exerts antidepressant effects in animals subjected to the forced swimming test (FST) (Arletti & Bertolini, 1987). In addition, administration of OXT (Nowakowska *et al*., 2002), or the OXT agonist, carbetocin, have antidepressant-like effects (Chaviaras *et al*., 2010), while drugs, such as sildenafil, produce similar effects by activating OXT signaling in mice (Matsushita *et al*., 2012). One study showed that treatment with the serotonin reuptake inhibitor (SSRI) drug citalopram (20 mg/kg i.p.) had antidepressant effects related to an increase in plasma levels of OXT in Sprague–Dawley rats (Uvnäs-Moberg *et al*., 1999). As these findings suggest, OXT can enhance the antidepressant-like effects of citalopram in Wistar rats treated with corticosterone and subjected to the FST (Stanić *et al*., 2021). Modulation of the OXT system by serotonin (Vacher *et al*., 2002; Emiliano *et al*., 2007) indicates that OXT may mediate the antidepressant properties of SSRIs. For example, the use of antidepressants during pregnancy produces greater increases in oxytocin levels during the perinatal period (Galbally *et al*., 2021).

OXT and its receptors participate peripherally in ejaculation in males, smooth uterine muscle contractions during childbirth, and ejection of milk during lactation in females. In the central nervous system, they participate as neurotransmitters in complex social behaviors, such as trust, care, and maternal bonding, as well as stress, anxiety, and depression (Gruber *et al*., 2010). Its release depends not only on its concentration, which can stimulate secretion, but can also be promoted by OXT analogs and receptors, which can be reproduced by some natural products (Gruber *et al*., 2012).

In traditional Mexican medicine, some plants have been used to treat problems related to mood swings, including *Montanoa tomentosa* Cerv., called c*ihuapahtli* (*cihua* = woman; *patli* = medicine or remedy) or *zoapatle* in Nahuatl. It has been described in some pre-Columbian Mesoamerican codices, such as the *Libellus de Medicinalibus Indorum Herbis* (1552), *La Historia General de las Cosas de Nueva España*, and *Historia Natural de la Nueva España* (Derbez *et al*., 1945), as an infusion prepared from the leaves of this plant to aid in childbirth, as a contraceptive agent and analgesic, and to reduce symptoms of mood disorders (Ximenez, 2003).

Extracts of *M. tomentosa* have been used as a remedy for reproductive problems, such as inducing labor, stimulating postpartum bleeding, promoting menstruation, and facilitating milk secretion (Gallegos, 1983). It has also been used to induce abortions (Levine *et al*., 1981). Notably, these effects were similar to those of OXT (Strand, 1999; Moberg & Moberg, 2003). Pre-clinical studies have reported that acute treatment with an infusion of *M. tomentosa* produces an antidepressant-like effect in both female (Rodríguez-Landa *et al*., 2018) and male rats (Lagunes-Merino *et al*., 2020). Apparently, the effects detected in males were associated with the activation of oxytocinergic neurons in the PVN and SON (Lagunes-Merino *et al*., 2020); however, it is unknown whether chronic infusion of *M. tomentosa* exerts antidepressant-like effects associated with the activation of OXT neurons in these nuclei.

MATERIALS AND METHODS

Ethics

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (2011) and the Official Mexican Standard for the Care and Use of Laboratory Animals (NOM-062-ZOO-1999). Every effort was made to

minimize animal discomfort during the study.

Animals

A total of 39 adult male Wistar rats, weighing 250-300 g, were housed in plexiglass cages for this study (four per cage) under a 12/12 h light/dark cycle (lights on at 7:00 h), at an average temperature of 25 ± 2ºC, with *ad libitum* access to food and water (Nutricubo Harlan®, México, SA de CV).

Preparation of the infusion

M. tomentosa was collected near Tlaxcala, Mexico and authenticated by an expert taxonomist (Thiers, 2016, serial number MT-UATX10). The leaves were dried during twenty consecutive days under the sun in ambient conditions (Rovirosa-Hernández *et al*., 2024) and then ground to a fine powder in a mortar. The powder (1 g) was mixed into 20 mL of purified water just before the boiling point, then allowed to cool to room temperature, and filtered before use. The filtered infusion obtained was kept at 4°C until administration. Under these conditions a concentration 50 mg/mL was obtained (Rovirosa-Hernández *et al*., 2024). The doses and volume of administration were selected from previous dose-response curve studies (Rodríguez-Landa *et al*., 2014; Lagunes-Merino *et al*., 2020).

Treatments

The rats were assigned to two treatment groups: with and without the forced swim test (FST). They were then randomly divided into three independent groups, one for each condition, as follows: vehicle (Veh; 1 mL/kg of purified water), *M. tomentosa* (*Mt*; 50 mg/kg body weight), and fluoxetine (Flx; 1 mg/kg body weight). All treatments were applied orally through a curved, stainless steel cannula (18G × 3.0" w/2.5 mm ball, Cadence, Inc., Staunton, VA, USA) coupled to a 1-mL disposable syringe (Terumo Medical de Mexico, SA de CV, Mexico City) at a volume equivalent to 1 mL/kg. The dose was administered once daily (10:00) for 28 consecutive days. The number of rats per group was 8 and 5 with and without FST, respectively. The group size was in accordance with previous studies on immunohistochemical analysis (Caba *et al*., 2003; Lagunes-Merino *et al*., 2020) and for detecting neuronal immunoreactivity without compromising statistical power. Therefore, the low number of rats per group adhered to the 3R principles of preclinical research (Russell *et al*., 2005).

Behavioral tests

Locomotor activity

An opaque plexiglass box (44 × 33 cm) with 20 cm high walls. The base was divided into 12 squares (11 × 11 cm). This test was performed to rule out possible changes associated with hypo- or hyperactivity in rats that could interfere with the interpretation of the FST results. A digital video camera (Canon EOS 70D) was placed above the cage to record the behavior of each rat for 5 min. At the beginning of the trial, the rats were gently placed in a corner of the box. The variables evaluated were as follows: 1) number of crossings when a rat moved from one square to another with all four legs or a third of its body (Contreras et al., 2001; Rodríguez-Landa et al., 2012); 2) time spent in self-grooming, including all self-directed cleaning behaviors from head to ears, limbs, and the anogenital region (Kalueff & Touhimaa, 2005); and 3) time spent rearing when the rat explored the cage in an upright posture supported on its hind limbs. After each test, the cage was cleaned with a 15% alcohol solution to remove the odor from the previous rat. Each rat then underwent the FST.

FST

At 27 days post-administration of the treatments (Vh, *Mt*, and Flx), a pre-test was performed on all rats in the FST condition. This consisted in placing individual rats gently into a rectangular glass pond (30 cm wide × 20 cm long × 50 cm high) with water at 25 ± 1ºC for 15 min. The water level was adjusted to the size of each animal, that is, it could only touch the bottom of the tank with the tips of its hind paws to maintain its head above the water level. During this session, the animals were confronted with a novel threatening situation as they were immersed in water to foster the development of behavioral despair (Porsolt *et al*., 1978).

On day 28, 30 min after treatment administration, individual rats were evaluated again in an FST session that lasted for 5 min. All sessions were videotaped: 1) latency to first immobility after being placed in the water and 2) total immobility time. Immobility was considered when the rat touched the bottom of the pond with one or both hind legs and the tail or when it remained floating, making only the minimum movements necessary to keep its

head above the surface, without moving through the water. At the end of the FST, rats were returned to their cages. After 90 min, each rat was euthanized to remove the brain and processed for immunohistochemistry.

Perfusion and immunohistochemistry

The rats were euthanized with an *i.p*. overdose of sodium pentobarbital (40 mg/kg, i.p., Pisa Agropecuaria, SA de CV, Atitalaquia, Hidalgo, Mexico), and perfused transcardially with 0.9% saline solution, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB; pH 7.4). Brains were immediately removed and cryoprotected in serial sucrose dilutions (10, 20, and 30%). Subsequently, 50-µm coronal cuts were made in a cryostat at -23ºC (Leica CM1520). The serial sections were collected from the rostral border of the preoptic area (Bregma 0.48 mm, interaural 9.48 mm) to the rostral border of the mammillary bodies (Bregma -5.40 mm, interaural 3.60 mm), and placed in 0.1 M of PB. One of every four sets of sections (n=5 per group) was used for double-labeling Fos and OXT. The activation of OXT cells in the PVN and SON was evaluated by the presence of the Fos protein as a nuclear indicator of oxytocinergic cell activation, according to a previous study (Caba *et al*., 2003).

The tissues were washed several times with 0.1 M PB and exposed to 0.5% hydrogen peroxide solution to eliminate endogenous peroxidase activity. They were then incubated in a PB solution with 0.3% Triton X-100 (PBT, Sigma, St. Louis, MO, USA) with 3% normal horse serum, plus the primary anti-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 72 h at 4°C. Subsequently, they were incubated with a biotinylated horse anti-goat IgG antibody for 1 h (1:200, Vector Labs, Burlingame, CA, USA). Next, the cells were incubated in ABC solution (1:250, Vector Labs) for 1 h. Finally, they were exposed to 0.05% diaminobenzidine (Polyscience, Warrington, PA, USA) in the presence of nickel sulfate (10 mg/mL; Fisher Scientific, Pittsburgh, PA, USA) plus cobalt chloride (10 mg/mL; Fisher Scientific,), and 0.01% hydrogen peroxide, which together produced a black-purple precipitate. After 8-10 min, the tissues were transferred to PB to stop the reaction. At that point, they were washed in PB three times, and then incubated again in 0.3% PBT and 3% normal horse serum plus a monoclonal anti-OXT antibody for 72 h at 4ºC (1:5000, Millipore, Billerica, MA, USA). The tissues were incubated once more in biotinylated horse anti-mouse IgG antibody (1:200, Vector Labs) for 1 h, then in ABC solution for 1 h. The sections were then treated with 0.05% diaminobenzidine and 0.01% hydrogen peroxide. The reaction produced a brown cytoplasmic precipitate. Individual sections were mounted on gelatin-coated slides, dehydrated, and covered with coverslips using Permount (Fisher Scientific).

Cell-counting

Quantification of immunoreactive cells corresponding to OXT (OXT-ir) and double-labeled neurons (Fos/OXT-ir) in the PVN and SON was performed under bright-field illumination using a Leica DM microscope at magnifications of 10× and 20×. OXT-ir cells were identified as brown precipitates in the cytoplasm, whereas Fos was identified as a black-purple precipitate in the nucleus. Double-labeled Fos/OXT-ir neurons had a brown cytoplasm with a black nucleus. Neuron counts in the PVN and SON were performed bilaterally in three brain sections per animal and condition (n=5) (Caba *et al*., 2003).

Statistical analyses

A one-way analysis of variance (ANOVA) was applied to the behavioral data, and a two-way ANOVA to the neuron counts, followed by a Newman–Keuls *post hoc* test using SigmaStat 4.0. Data are expressed as mean $\pm$ standard deviation for each variable, at an accepted significance of $p<0.05$.

RESULTS

Behavioral tests

Locomotor activity test

No statistically significant differences were found in the number of crossings between the treatment groups [$F(2,23)=2.122$, $p=0.145$]. Similarly, no significant differences were observed in the time spent grooming [$F(2,23)=0.390$, $p=0.682$] or rearing [$F(2,23)=3.589$, $p=0.166$] (data not shown).

FST

The analysis of latency to first immobility did not reveal significant between-treatment differences [$F(2,23)=1.654$, $p<0.215$; Figure No. 1]. However, significant between-treatment differences in total immobility time were found [$F(2,23)=15.453$,

$p<0.001$]. The *post hoc* test showed that this variable was significantly lower in rats treated with *M. tomentosa* infusion than that in the Vh group. This effect was similar to that of Flx ($p<0.001$, Figure No. 2).

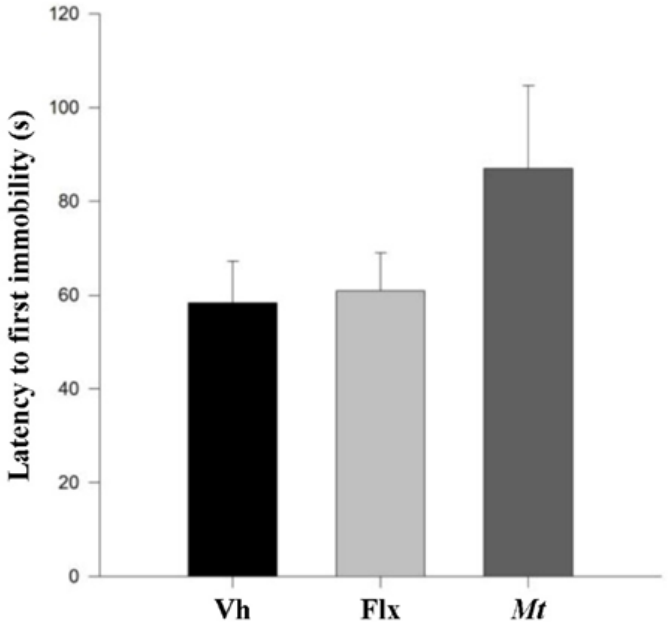

Figure No. 1
Latency to first immobility on the FST. The one-way ANOVA did not show between-treatment differences: vehicle (Vh), fluoxetine (Flx), *M. tomentosa* (*Mt*). (data are presented as mean ± SD). FST, forced swimming test

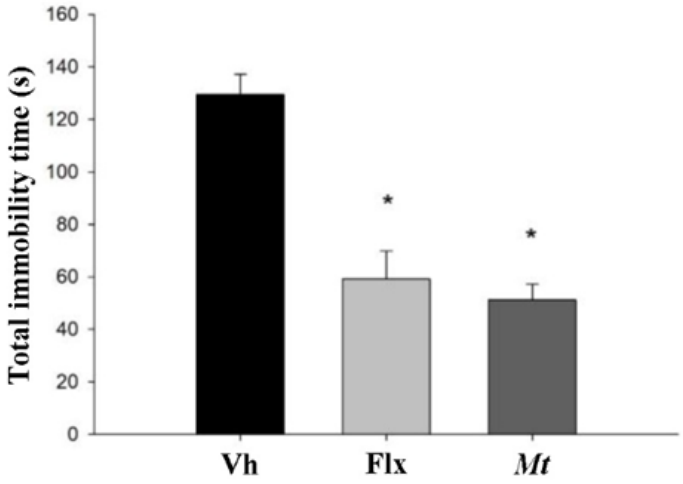

Figure No. 2
Total immobility time on the FST for the different treatments. *M. tomentosa* (*Mt*) and fluoxetine (Flx) produced significantly lower immobility times than that of the Vh group ($p<0.050$, Kruskal–Wallis *post hoc* test). (data are presented as mean ± SD). FST, forced swimming test

Oxytocin neuron activation

OXT-ir in the PVN and SON

Analysis of the total number of OXT cells in the PVN did not reveal any significant between-group differences [n=5; F(5,29)=1.248, p<0.305]. A similar effect was observed for the number of OXT cells in the SON group [n=5; F(5,29)=3.364, p<0.052]. None of the treatments changed the number of OXT neurons regardless of the condition (data not shown).

Fos/OXT-ir in the PVN and SON

The analysis of the Fos/OXT-ir double cell count did not reveal significant differences in the PVN [n=5; F(5,29)=2.818, p<0.080] (Figures No. 3 and No. 4) or SON [n=5; F(5,29)=0.825, p<0.450] (Figures No. 5 and No. 6) in relation to treatment or condition.

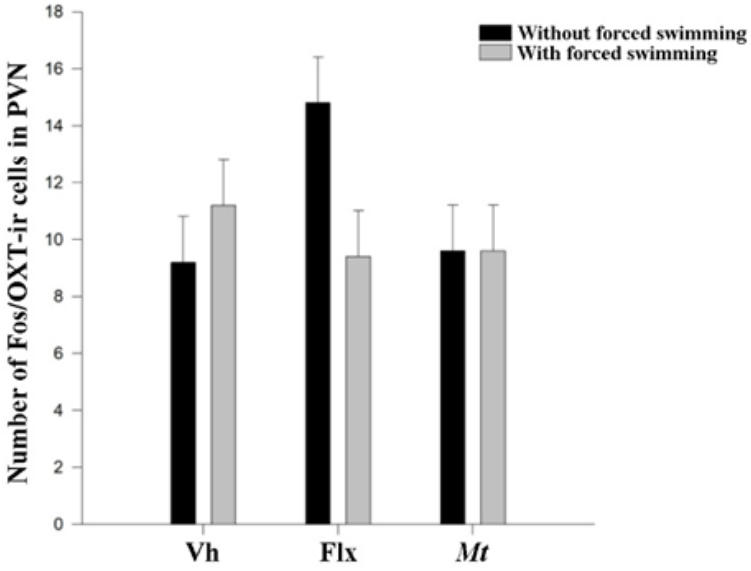

Figure No. 3

Number of Fos/OXT-ir cells in the PVN (Me ± SD) of the rats with and without the FST in the three treatment groups: vehicle (Vh), fluoxetine (Flx), *M. tomentosa* (*Mt*) (two-way ANOVA, p=0.080). FST, forced swimming test; OXT-ir, immunoreactive cells corresponding to oxytocin; PVN, paraventricular nuclei

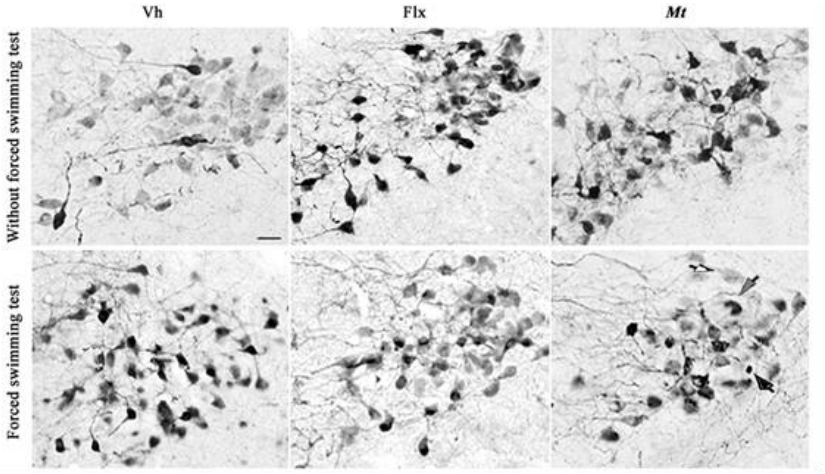

Figure No. 4

Micrographs showing double-labeled Fos/OXT-ir cells in the PVN of rats with and without the FST in three treatment groups: vehicle (Vh), fluoxetine (Flx), *M. tomentosa* (*Mt*). This image shows the expression of Fos (filled black arrow), OXT (filled white arrow), and Fos/OXT (filled grey arrow) according to treatment. Bar calibration was 50 µm, objective 20× magnification. FST, forced swimming test; OXT-ir, immunoreactive cells corresponding to oxytocin; PVN, paraventricular nuclei

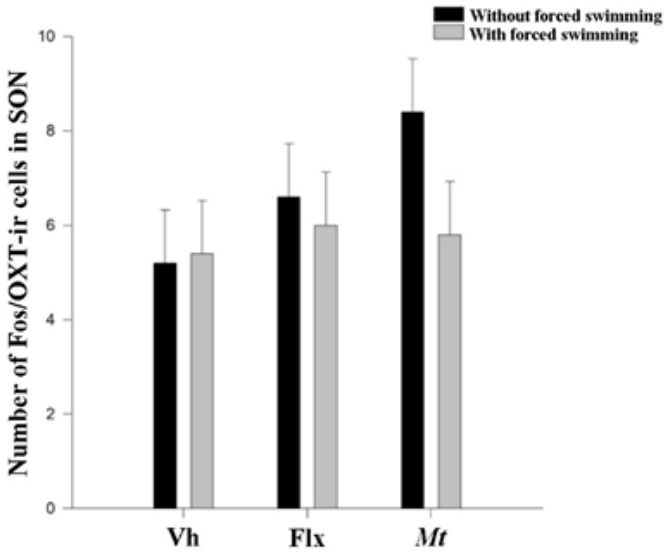

Figure No. 5

Number of Fos/OXT-ir cells in the SON (Me ± SD) of the rats with and without the FST in the three treatment groups: vehicle (Vh), fluoxetine (Flx), *M. tomentosa* (*Mt*) (two-way ANOVA, *p*=0.450). FST, forced swimming test; OXT-ir, immunoreactive cells corresponding to oxytocin; SON, supraoptic nuclei

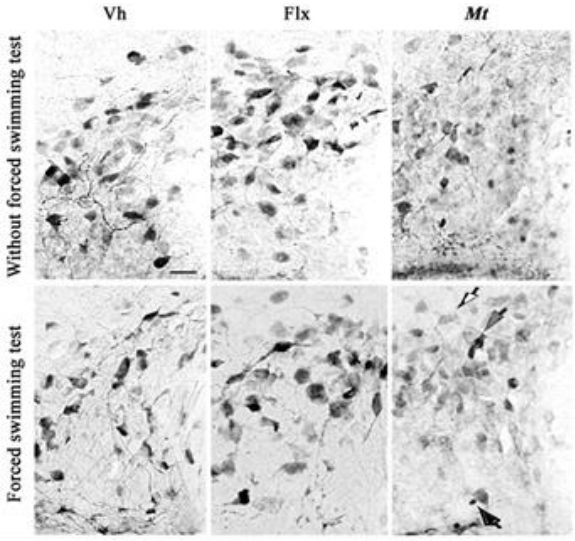

Figure No. 6

Micrograph of double-labeled Fos/OXT-ir cells in the SON of rats with and without FST in the three treatment groups: vehicle (Vh), fluoxetine (Flx), *M. tomentosa* (*Mt*). The image shows Fos (filled black arrow), OXT (filled white arrow), and Fos/OXT (filled gray arrow) expression according to treatment. Bar calibration was 50 µm, objective 20× magnification. FST, forced swimming test; OXT-ir, immunoreactive cells corresponding to oxytocin; SON, supraoptic nuclei

These results were used to calculate the activation percentages of the OXT-ir and Fos/OXT neurons in the PVN and SON (Table No. 1).

Without forced swimming				
SON	**Condition**	**Total Neurons OXT-ir me ± s.e.**	**Double labeled Fos/OXT-ir me ± s.e.**	**% double labeled**
	Vh	61.2 ± 4.5	5.2 ± 1.1	8.4
	Flx	80.6 ± 4.5	6.6 ± 1.1	8.18
	Mt	65 ± 4.5	8.4 ± 1.1	12.92
With forced swimming				
	Vh	65.6 ± 4.5	5.4 ± 1.1	8.23
	Flx	63 ± 4.5	6 ± 1.1	9.52
	Mt	65.8 ± 4.5	5.8 ± 1.1	8.81
Without forced swimming				
PVN	**Condition**	**Total Neurons OXT-ir me ± s.e.**	**Double labeled Fos/OXT-ir me ± s.e.**	**% double labeled**
	Vh	92.8 ± 10.4	9.2 ±1.6	9.91
	Flx	107.8 ± 10.4	14.8 ± 1.6	13.72
	Mt	87.2 ± 10.4	9.6 ± 1.6	11.0
With forced swimming				
	Vh	80 ± 10.4	11.2 ± 1.6	14.0
	Flx	90.2 ± 10.4	9.4 ± 1.6	10.42
	Mt	100.4 ± 10.4	9.6 ± 1.6	9.56

Table No. 1
Mean and error percentage of the immunoreactive neurons of the rats with and without the FST in the three treatments group: vehicle (Vh), fluoxetine (Flx), *M. tomentosa* (*Mt*).

DISCUSSION

The present study explored the effect of chronic treatment with an infusion of *M. tomentosa* on despair-like behavior and the activation of OXT cells in the PVN and SON of male Wistar rats. The observed effects were compared with those of the clinically effective antidepressant, fluoxetine. The FST has been used to induce a state of despair and evaluate the potential antidepressant effects of diverse substances (Porsolt *et al*., 1977; Anisman & Matheson, 2005). Despair behavior is characterized by an increase in total immobility time (Porsolt *et al*., 1977) and a lower latency to the first immobility period (Contreras *et al*., 2001). Substances with antidepressant-like activity reduced the total immobility time and increased the latency to the first immobility period.

The results of the present study showed that chronic administration of *M. tomentosa* infusion had no effect on the latency to first immobility, in contrast to the results reported when administered acutely (Lagunes-Merino *et al*., 2020). Latency to first immobility is considered an indicator of a rat's initial effort to cope with a stressful situation and is a measure of motivation (Contreras *et al*., 1998; Espejo & Miñano, 1999). An increase in this parameter is considered an indicator of stress reduction when faced with stressful situations, such as the FST (Castagné *et al*., 2009). Therefore, the present results showed that chronic administration of *M. tomentosa* infusions did not affect this variable.

Chronic administration of *M. tomentosa* significantly reduced the total immobility time in the FST to an effect similar to that of the antidepressant

fluoxetine, without inducing significant changes in locomotor activity. Similar results have been reported for the acute administration of *M. tomentosa* (Lagunes-Merino *et al*., 2020) and antidepressants and anti-stress substances, such as antidepressant drugs, neurosteroids, and extracts of some medicinal plants (Contreras *et al*., 2001; Rodríguez-Landa *et al*., 2009; Lozano-Hernández *et al*., 2010). Acute administration of *M. tomentosa* significantly activates OXT neurons in the PVN and SON, which may be associated with its antidepressant-like effects (Lagunes-Merino *et al*., 2020). However, the present study showed that when *M. tomentosa* was administered chronically, 9.56 and 8.81% of OXT cells were activated in the PVN and SON, respectively. These activation levels were not significantly different from those in the vehicle and fluoxetine groups.

The main effectors of the stress response are the PVN of the hypothalamus, anterior lobe of the pituitary gland, and adrenal gland, which comprise the HPA axis. Neurons in the medial parvocellular subdivision of the PVN synthesize and secrete corticotropin-releasing factor (CRF), the main regulator of this axis (Rivier & Vale, 1983). Additionally, the OXT produced in PVN parvo cells is released simultaneously or directly after a stressful stimulus via the somato-dendritic pathway in stress-sensitive areas of the brain, suggesting that this release of OXT could modulate or dampen stress responses (Winter & Jurek, 2019), a phenomenon that could be reflected in the antidepressant-like behavioral results observed herein.

Another likely explanation is that the medial parvocellular subdivision of the PVN receives afferent projections from γ-aminobutyric acid (GABA) (Roland & Sawchenko, 1993). These neurons express $GABA_A$ receptor subunits (Cullinan, 2000), therefore, when intracerebroventricular $GABA_A$ receptor agonists are administered, they inhibit glucocorticoid secretion after exposure to stressors (Cullinan *et al*., 1996; Cullinan & Wolfe, 2000). This suggests that GABA plays an important role in integrating hypothalamic stress and participates in antidepressant effects similar to those observed in the FST (Bernal Morales *et al*., 2018; Cueto-Escobedo *et al*., 2020). Finally, the participation of $GABA_A$ receptors in the anxiolytic-like effects of *M. tomentosa* has been reported in both female (Rodriguez-Landa *et al*., 2014) and male rats (Sollozo-Dupond *et al*., 2015).

Similarly, GABAergic neurons positive for OXT receptors exert inhibitory effects on CRF neuronal activity. Furthermore, CRF-positive neurons for the OXT-receptor can detect OXT release and establish a direct genomic effect by inhibiting CRF expression (Jureck *et al*., 2015).

In the present study, fluoxetine, similar to *M. tomentosa*, exerted an antidepressant-like behavioral effect, but did not significantly activate OXT cells in the PVN (10.42%) or SON (9.52%). Chronic treatment with fluoxetine acts on the serotonergic system by decreasing the sensitivity of $5\text{-}HT_{2A}$ receptors (Damjanoska *et al*., 2003). Serotonin acts on OXT neurons through the $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptors in the PVN and SON, where OXT is produced (Osei-Owusu *et al*., 2005). This low sensitization might have influenced the percentage of OXT-ir neurons observed in this study.

M. tomentosa is a member of the Asteraceae family, which contains bioactive compounds, such as alkaloids, polyacetylenes, polyphenols, and terpenoids (Heinrich *et al*., 1998), and some of the compounds identified in this plant may be responsible for its antidepressant-like effects (Saki *et al*., 2014; Bahramsoltani *et al*., 2015).

A limitation of the present study is the absence of phytochemical characterization of *M. tomentosa* infusion; however, it has been established in previous studies that the infusion of several species of the *Montanoa* genus showed the presence of flavonoids, alkaloids, sesquiterpene lactones, and terpenes, which are associated with its antidepressant-like effects (Rodríguez-Landa *et al*., 2018). Monoterpenes contained in some plant extracts interact with the $5HT_{1A}$ receptor (Chaouloff, 2000; Guzmán-Gutiérrez *et al*., 2012) and adrenergic receptors that participate in the regulation of stress (Pandey *et al*., 1995; Guzmán-Gutierrez *et al*., 2015). It has been suggested that $5\text{-}HT_{1A}$ receptors in the PVN mediate the release of the adrenocorticotropic hormone and OXT, and the low sensitivity of these receptors is associated with depression and other psychiatric disorders (Osei-Owusu *et al*., 2005). Additionally, sesquiterpenes exert antidepressant effects (Bahi *et al*., 2014) through their action on $GABA_A$ receptors (Tolardo *et al*., 2010). The flavonoid isoquercetin (Oshima et al., 1986) affects anxiety and depression (Guo *et al*., 2011). Their antidepressant activity is mediated by $5\text{-}HT_{1A}$ and

$5HT_{2A}$ receptors (Martinez-Hernández *et al*., 2021). These neurochemical interactions between plant phytochemicals and brain receptors are involved in the antidepressant-like effects produced by the *Montanoa* genus.

In conclusion, chronic oral administration of an infusion of *M. tomentosa* exerted antidepressant-like behavioral effects in male rats subjected to the FST, similar to the clinically effective antidepressant, fluoxetine, independent of OXT cell activation. Therefore, it has been suggested that the chronic administration of this infusion exerts its effects through other neurotransmission systems that could involve neuroplasticity, as has been reported for long-term antidepressant drugs.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

ETHICAL APPROVAL

This study was approved by the Internal Ethics Committee of the Instituto de Ciencias de la Salud, Universidad Veracruzana (CICUAL-ICS; Reg. No. 2019-003)

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