The Aqueous Crude Extracts of Montanoa frutescens and Montanoa grandiflora Reduce Immobility Faster Than Fluoxetine Through GABA<sub>A</sub> Receptors in Rats Forced to Swim

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Abstract

Background. Montanoa frutescens and Montanoa grandiflora have been indistinctly used for centuries in traditional Mexican medicine for reproductive impairments, anxiety, and mood disorders. Preclinical studies support their aphrodisiac and anxiolytic properties, but their effects on mood are still unexplored. Methods. The effects of 25 and 50 mg/kg of M frutescens and M grandiflora extracts were evaluated on days 1, 7, 14, 21, and 28 of treatment, and compared with fluoxetine (1 mg/kg) and Remotiv (7.14 mg/kg) in Wistar rats. The participation of GABA<sub>A</sub> receptor in the effects produced by the treatments was explored. Results. Montanoa extracts reduced immobility since day 1 of treatment, while fluoxetine and Remotiv required 14 days. The GABA<sub>A</sub> antagonism blocked the effects of Montanoa extracts, but not of fluoxetine or Remotiv. Conclusions. Montanoa extracts prevented quickly the stress-induced behaviors in the swimming test through action at the GABA<sub>A</sub> receptor, exerting a protective effect different to the typical antidepressants drugs.

Keywords

Montanoa frutescens, Montanoa grandiflora, antidepressant; GABA<sub>A</sub> receptor; forced swim test

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Depression is a worldwide stress-related psychiatric disorder. According to the World Health Organization, depression is the fourth largest contributor to the global burden disease and has been predicted to reach the second place by 2020. Pharmacological treatments of mood disorders as depression are based on a wide group of drugs including inhibitors of monoamine oxidase, tricyclic antidepressants and selective serotonin reuptake inhibitors as fluoxetine, among others. Despite clinical effectiveness of antidepressant drugs, patients also use alternative therapies based on extracts of plants or standardized herbal products, that is, Hypericum perforatum, with reputed antidepressant properties. Nowadays, Hypericum perforatum is used at clinical level exerting anxiolytic and antidepressant effects in humans, with probed effects on animal models similar to fluoxetine and other clinically effective antidepressant drugs. Nonetheless, long-term use is limited by some severe side effects.

In the ancient Mexican traditional medicine, the Badianus Codex or Libellus de Medicinalibus Indorum Herbis written in 1552 describes the use of Cihuapatli (“women’s medicine” in the Nahuatl language) for the treatment of mood and nervous disorders. Cihuapatli is the common name assigned to plants from the Montanoa genus (family: Asteraceae; tribe: Heliantheae), including Montanoa tomentosa, Montanoa tomentosa.
frutescens, and Montanoa grandiflora, among others. The aqueous crude extract from these plants has been used individually or mixed for centuries in traditional Mexican medicine as a remedy for reproductive impairments, anxiety and mood disorders. Preclinical studies, however, also show that the Montanoa tomentosa, Montanoa frutescens, and Montanoa grandiflora crude extracts facilitates expression of sexual behavior and increase the ejaculatory potency in male rats, suggesting a potent aphrodisiac effect that involves a positive motivational state. The extract of Montanoa frutescens produced anxiolytic-like effects similar to diazepam in male Wistar rats, through the modulation of γ-aminobutyric acid-A (GABA\textsubscript{A}) receptors. Similar anxiolytic-like effects can be observed in rats during metestrus-diestrus phase of the ovarian cycle treated with Montanoa grandiflora and Montanoa frutescens extracts. Interestingly, Montanoa tomentosa extract also produces anxiolytic-like effects in rats with long-term absence of ovarian hormones by action on GABA\textsubscript{A} receptors. In addition, a preliminary study identified the potential antidepressant-like effects of Montanoa tomentosa extract; however, the potential antidepressant-like effect of Montanoa frutescens and Montanoa grandiflora extracts remains to be explored. All these data support traditional use of Montanoa plants as potent aphrodisiac and anxiolytic agent, but its effect on depression symptoms remains to be further explored.

Preclinical and clinical studies support both anxiolytic and antidepressant effect of fluoxetine and Hypericum extracts. In the particular case of Montanoa frutescens and Montanoa grandiflora extracts the anxiolytic-like properties have been identified but the potential antidepressant-like effects have not been tested, which limit their use as a treatment for mood disorders. It is noteworthy that some agents that act on the GABA\textsubscript{A} receptors, in addition to their anxiolytic-like effects also produces antidepressant-like effects in experimental models as the forced swim test, for instance some neurosteroids as progesterone and allopregnanolone, which in turn may be blocked by previous administration of antagonist of the GABA\textsubscript{A} receptor. All these data together point out the necessity to evaluate Montanoa extracts to support or discard its traditional use as antidepressant agents. Therefore, the aim of the present study was (a) to study the probable antidepressant-like effects produced by a long-term treatment with Montanoa frutescens and Montanoa grandiflora extracts and compare it against fluoxetine and Remotiv, 2 clinically effective antidepressant drugs and (b) to explore the participation of GABA\textsubscript{A} receptors on the potential antidepressant-like effects of the extracts in rats.

**Methods**

**Animals**

Adult male Wistar rats weighing between 250 and 300 g at the beginning of the experiments were used. The rats were housed in Plexiglas cages (5 rats per cage), with a 12-hour/12-hour light/dark cycle (lights on at 07:00 hours), an average room temperature of 25°C (±1°C) and free access to water and food. All experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and, the Norma Official Mexicana para el Uso y Cuidado de Animales de Laboratorio. This protocol received authorization from the Ethical Internal Committee from Escuela de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tlaxcala (Reg. No. MVZ-189/12).

**Doses, Plant Material, and Preparation of Montanoa frutescens and Montanoa grandiflora Extracts**

The doses of the aqueous crude extracts of Montanoa frutescens and Montanoa grandiflora used in the present study (25 and 50 mg/kg) were selected from a dose response curve in which 25 and 50 mg/kg of Montanoa frutescens and Montanoa grandiflora produced anxiolytic-like effects, while higher doses (>75 mg/kg) produce motor hypactivity, for this reason no lower or higher doses were evaluated. Montanoa frutescens and Montanoa grandiflora (family: Asteraceae; tribe: Heliantheae) were collected on September 2014, in their habitat in the state of Tlaxcala, México. Specimens were authenticated by Dr José Luis Martinez y Pérez from Herbarium (TLXM) of the Universidad Autónoma de Tlaxcala, in this place voucher specimens are preserved (serial number of Montanoa frutescens TLXM MCarro02 and Montanoa grandiflora TLXM MCarro03).

The leaves of Montanoa frutescens and Montanoa grandiflora were collected and prepared for drying for 20 days under ambient conditions. Once dried, the material was ground into a fine powder average 1 g, which was mixed with 20 mL of purified water. The mixture was warmed for 10 minutes, just before boiling. The obtained infusion was filtered and oven-dried at 55°C, and the brownish residue of the extract yield was calculated as 80 to 85 mg. The dried extract of the plant was maintained at 3°C and then used to prepare the stock solutions. In the present study, a 50 mg/mL solution was initially prepared and then diluted to obtain equivalent solutions of 25 mg/mL. The extracts used in each dose were prepared daily, 40 minutes prior to administration, to avoid alterations of their chemical properties.

**Preliminary Phytochemical Tests**

The Montanoa frutescens and Montanoa grandiflora extracts were subjected to phytochemical analyses using preliminary qualitative methods through standardized techniques to detect the presence of secondary metabolite groups. The following qualitative tests were used: Dragendorff and Wagner reagents for alkaloids, Liebermann-Burchard and Salkowski tests for sterols and terpenes, Shinoda test for flavonoids, Molisch test for saponins, and Legal and Baljet reagents for sesquiterpene lactones. All qualitative tests were realized in duplicate.

**Drug and Dosage**

Two doses of the extract of Montanoa frutescens and 2 of Montanoa grandiflora were evaluated and compared with Remotiv (produced, authenticated, and elaborated by Max Zeller SOHNE AG Seeblickstrasse 4, CH Romanshorn, Suiza and distributed by Grunenthal de México, S.A. de C.V., Ciudad de México, México) and fluoxetine chlorhydrate (Prozac, authenticated and elaborated by Eli-Lilly Compañía de México, S.A. de C.V., Ciudad de México, México; PubChem CID: 62857). The dose of Remotiv used in the present experiment (7.14 mg/kg), was taken from a previous study that showed their...
antidepressant-like effects in the weekly repeated forced swim test, which is equivalent to that doses used for treatment of depression symptoms in humans. Fluoxetine has antidepressant-like effects in the swim test at doses of 1 mg/kg when it is administered during 21 consecutive days in rats.

All treatments were administered once per day (10:00 hours) during 28 consecutive days at a volume equivalent to 1 mL/kg of purified water 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, Ciudad de México, México). This route of administration was selected considering that is the most similar to the route used by humans, where the passage through digestive system could influence in the biotransformation of extracts to produce their pharmacological activity; also, under this condition a control of the quantity of extract consumed is possible.

The doses and treatment schedules with picrotoxin (1 mg/kg; PubChem CID: 57402144) in the present experiment were based on previous studies that reported the efficacy to antagonize the behavioral effects produced by anxiolytic and antidepressant drugs.

The effects of treatments were evaluated in the behavioral test, first in the locomotor activity test and subsequently in the forced swim test. The behavioral tests were performed between 11:00 and 13:00 hours.

Behavioral Tests

Locomotor Activity Test. To evaluate the effect of the treatments on the spontaneous locomotor activity, rats were individually placed in an opaque Plexiglas cage (44 x 33 cm, base) with walls 20 cm high and the floor delineated into 12 squares (11 x 11 cm). In this test, variables as crossing and time spent in grooming and rearing were evaluated. When the hind legs crossed the line of the squares, the rat was considered to have crossed from one square to another (crossing). Grooming included all self-directed behaviors of cleaning from head, ears, limbs, and anal-genital region. Rearing was also measured when rats explored the cage in a vertical position standing on its rear limbs. After each rat was tested in the locomotor activity cage, it was carefully cleaned with 10% alcohol solution to remove the scent of the previously evaluated rat. After the locomotor activity test, rats were subjected to the forced swim test.

Forced Swim Test. In this paradigm, rats were individually forced to swim in a rectangular pool (50 x 30 x 60 cm) with 25 cm deep water (25°C ± 1°C), which has been validated to detect antidepressant-like effects of clinically effective antidepressant drugs as clomipramine, desipramine, and fluoxetine; neurosteroids as progesterone and allopregnanolone, and some extracts from Mimosa pudica and Hypericum perforatum. The variables evaluated were: the latency to first immobility and total time of immobility. Latency to the first immobility was the elapsed time since the rat was introduced to the pool, until the first immobility episode. The immobility was considered when the rat floated for more than 2 seconds without making vigorous movements leading to displacements and only maintaining its head above the water surface. All experimental sessions were video-recorded and 2 independent observers, blind to treatment, measured the behavioral variables with a concordance level of at least 95%.

Experimental Groups

Experiment 1: Temporal Effects of Montanoa frutescens and Montanoa grandiflora Extracts. In a first experiment, rats were assigned to seven independent groups administered during 28 days and evaluated at 1st, 7th, 14th, 21st, and 28th day in the behavioral tests, a schedule successfully used to measure the time elapsed until the appearance of the behavioral effects of antidepressants. The control group received only the vehicle in which fluoxetine, Remotiv or the extracts were dissolved (1 mL/kg of purified water). Four additional groups received two different doses of Montanoa frutescens or Montanoa grandiflora (25 and 50 mg/kg, respectively). The fluoxetine group received 1 mg/kg of fluoxetine chloride and the last group received 7.14 mg/kg of Remotiv. Treatments were administered orally in a volume of 1 mL/kg. The groups were similar in size (n = 10), except Remotiv (n = 8).

Before any pharmacological administration, all rats were subjected to a 5-minute pretest in the locomotor activity test and subsequently a 15-minute pretest in the forced swim test. Results of these pretests were discarded from the statistical analysis, given that pretest is only used for habituation to the novel situation. In the forced swim pretest, rats confronted a stressful aversive situation represented by swimming that triggers the development of behavioral despair. Twenty-four hours later (defined as day 0), rats were subjected to a 5-minute test session in the locomotor activity and subsequently to forced swim test to evaluate baseline behavioral activity. After this test session, the pharmacological treatments were initiated and its effects were evaluated on the 1st, 7th, 14th, 21st, and 28th day of treatment, 1 hour after the corresponding administration on the test day, as previously performed. Day 28 was the last day of treatment. In order to measure the effect of treatment withdrawal, all rats were tested again 24 and 48 hours after the last administration.

Experiment 2: Antagonism of GABAA Receptor. In a second experiment, rats were assigned to 8 independent groups (n = 8 per group) administered during 28 consecutive days with vehicle, extracts or fluoxetine, but in this experiment, the behavioral effect only was evaluated at 28th day of administration. The vehicle group received purified water plus saline solution (vehicle of picrotoxin), 2 groups received 50 mg/kg of Montanoa frutescens or Montanoa grandiflora extracts followed by saline solution; another group received 1 mg/kg of fluoxetine plus saline solution, while other group received purified water followed by 1mg/kg of picrotoxin. Additionally, 3 groups received the same chronic treatments with Montanoa grandiflora, Montanoa frutescens, or fluoxetine, respectively, but were also administered with picrotoxin (1 mg/kg). The long-term treatment for 28 consecutive days (vehicle, Montanoa grandiflora, Montanoa frutescens, and fluoxetine) was administered orally in an equivalent volume of 1 mL/kg. The acute treatment with the antagonist was administered intraperitoneally, just once, 30 minutes before the last administration of the long-term treatment (at day 28 of treatment). The effects of treatments were evaluated on the locomotor activity and forced swim tests, 1 hour after the last administration of extracts or fluoxetine (day 28 of administration).

Statistical Analysis

In the first experiment, data were analyzed with 2-way repeated-measures analysis of variance (ANOVA) to evaluate the effect of treatments along time, with treatment (vehicle, extracts, Remotiv, and fluoxetine) and days of treatment as factors. Significant effects in the ANOVA were followed by the Student-Newman-Keuls post hoc test.

In the second experiment, a 1-way ANOVA was used to analyze the data from the antagonism study, with treatment as the only factor.
Significant effects in the ANOVA were followed by Student-Newman-Keuls post hoc test. Values of \( P \leq .05 \) were considered statistically significant. In cases of nonparametric distributions of the data, we used the Kruskal-Wallis post hoc tests. The results are expressed as mean ± standard error.

**Results**

**Preliminary Phytochemical Test**

The preliminary phytochemical analysis of the *Montanoa frutescens* and *Montanoa grandiflora* aqueous crude extract showed the presence of flavonoids, alkaloids, sesquiterpene lactones, and terpenes in both extracts. The presence of sterols only was positive in the *Montanoa grandiflora* extract, but not in *Montanoa frutescens* extract. The presence of saponins was not identified in any of the *Montanoa* extracts.

**Experiment 1: Temporal Effects of Montanoa frutescens and Montanoa grandiflora Extracts**

**Locomotor Activity Test.** The analysis of crossing revealed significant differences, \( F(6, 427) = 3.689, P < .003 \), by treatment; days of treatment, \( F(7, 427) = 484.555, P < .001 \); and interaction between factors, \( F(42, 427) = 1.957, P < .001 \). The post hoc test showed that from day 14 of treatment all groups, including vehicle, decreased crossing, even after treatment withdrawal. Only 50 mg/kg of *Montanoa frutescens* or *Montanoa grandiflora* at day 1, and Remotiv at day 21 had higher crossing than the vehicle group, but despite this increment, all groups decreased crossing along the treatment when compared with its respective day 0 (Figure 1).

The analysis of total time of grooming revealed significant, \( F(6, 427) = 95.946, P < .001 \), differences by treatment; days of treatment, \( F(7, 427) = 107.157, P < .001 \); and interaction between factors, \( F(42, 427) = 11.987, P < .001 \). The post hoc analysis showed that a decreased of grooming was produced in the control and most of the experimental groups: Rats treated with fluoxetine or Remotiv prevented this decrease since they had a longer time of grooming than control from day 21, but it was shorter compared with the first day of treatment in the same group. This effect lasted even 48 hours after treatment withdrawal. Groups treated with 50 mg/kg of *Montanoa frutescens* or *Montanoa grandiflora* also prevented the decrease of grooming from the first day of treatment, but the effect only persisted 24 hours after withdrawal; 48 hours after withdrawal the effects disappeared (Figure 2).

With regard to the total time spent in rearing, there were significant, \( F(6, 427) = 4.935, P < .001 \), effects of treatment; post hoc test showed that rats treated with Remotiv had more rearing independently of days of treatment. The factor days of treatments, \( F(7, 427) = 1.358, P = .221 \), nonsignificant, and the interaction between factors, \( F(42, 427) = 1.108, P = .303 \), were not significant (Figure 3).

**Forced Swim Test.** The analysis of latency to the first immobility revealed significant, \( F(6, 427) = 191.475, P < .001 \), differences by treatments; days of treatment, \( F(7, 427) = 32.155, P < .001 \); and interaction between factors, \( F(42, 427) = 24.450, P < .001 \). Post hoc test revealed that rats treated with fluoxetine increased the latency from day 14 of treatment, while Remotiv did it until day 21 respect their basal session and respective session of the control group; both effects prevailed 48 hours after treatment withdrawal. In contrast, all doses of extracts did not modify latency; nevertheless, from day 14, *Montanoa frutescens* (25 mg/kg) prevented the decrease of latency with regard to
the vehicle group, an effect that only lasted 24 hours after treatment withdrawal. Montanoa grandiflora (25 mg/kg) only prevented the decreased of latency on day 28 of treatment, which was maintained until 24 hours after conclusion of treatment with regard to vehicle group (Figure 4).

For total time of immobility, the statistical analysis revealed significant, $F(6, 427) = 52.879, P < .001$, differences by treatment; days of treatment, $F(7, 427) = 27.329, P < .001$; and interaction of factors, $F(42, 427) = 13.568, P < .001$. Post hoc analysis showed that the groups treated with fluoxetine or Remotiv decreased immobility from day 14 and 21 of treatment, respectively, an effect that continued even 48 hours after treatment withdrawal. On the other hand, experimental groups treated with the dose of 50 mg/kg of Montanoa frutescens or Montanoa grandiflora showed an immediate reduction of immobility (day 1 of treatment), but this effect disappear 24 hours after the interruption of treatments. Although 25 mg/kg of Montanoa frutescens was not effective to reduce
immobility, after 24 hours of treatment withdrawal a fortuitous increased of immobility was observed (Figure 5).

**Experiment 2: Antagonism of GABA<sub>A</sub> Receptors**

**Locomotor Activity Test.** The results of crossing, grooming, and rearing are presented in Table 1. The analysis of crossing revealed nonsignificant, $H(7) = 2.484$, $P < .928$, differences between treatments. With regard to statistical analysis of time spent in grooming, it revealed significant, $F(7, 56) = 19.66$, $P < .001$, differences between treatments. Post hoc test showed that rats treated with Montanoa frutescens, Montanoa grandiflora or fluoxetine all exhibited longer times of grooming compared with vehicle group. The treatment with picrotoxin has no effect on grooming itself; nevertheless, picrotoxin blocked the effect of Montanoa frutescens and Montanoa grandiflora but lacked an effect on the fluoxetine-treated rats. On the other hand, the analysis of time spent in rearing revealed

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**Figure 4.** Temporal effects of Montanoa frutescens and Montanoa grandiflora extracts on latency to the first immobility in the forced swim test. (A) Pharmacological control, fluoxetine and Remotive, versus vehicle; (B) Montanoa frutescens extracts versus vehicle group; (C) Montanoa grandiflora extracts versus vehicle group. Values are expressed as mean ± standard error. *P < .05, compared with day 0 of the same group; **P < .05, compared with the respective session of vehicle group. Two-way repeated-measures analysis of variance followed by Student-Newman-Keuls post hoc test.

**Figure 5.** Temporal effects of Montanoa frutescens and Montanoa grandiflora extracts on total immobility time in the forced swim test. A) Pharmacological control, fluoxetine and Remotive, versus vehicle group; (B) Montanoa frutescens extracts versus vehicle group; (C) Montanoa grandiflora extracts versus vehicle group. Values are expressed as mean ± standard error. *P < .05, compared with day 0 of the same group; **P < .05, compared with the respective session of vehicle group. Two-way repeated-measures analysis of variance followed by Student-Newman-Keuls post hoc test.
experiment, the participation of GABA A receptors in the interruption was also measured. Additionally, in a second effect of treatment withdrawal (24 and 48 hours after treatment erroneously applied in repeated sessions is used to evaluate gradual effect of treatments. Post hoc test revealed that rats treated with fluoxetine, had a longer latency to immobility, with regard to vehicle group, which was unaffected by picrotoxin. There were not significant differences in groups treated with Montanoa frutescens or Montanoa grandiflora extracts alone or combined with picrotoxin, compared with vehicle group (Figure 6A).

With regard to the total time of immobility, the statistical analysis reported significant, $F(7, 56) = 97.010$, $P < .001$, differences among treatments. Post hoc test revealed that rats treated with 50 mg/kg of Montanoa frutescens or Montanoa grandiflora, as well as with 1 mg/kg of fluoxetine had shorter times of immobility than vehicle-treated rats. Interestingly, the combination of treatment with picrotoxin blocked the effects of Montanoa extracts, but not of fluoxetine; while picrotoxin has no effect itself. A higher reduction of immobility ($P < .001$) was detected in fluoxetine-treated rats compared with Montanoa extracts (Figure 6B).

**Discussion**

The present study evaluated the effects of the aqueous crude extracts of Montanoa frutescens and Montanoa grandiflora at 2 different doses (25 and 50 mg/kg), during a long-term treatment of 28 consecutive days on depression-like behavior. The experimental design explored the immediate effect of treatments (1 day later) and continued every week until day 28; the effect of treatment withdrawal (24 and 48 hours after treatment interruption) was also measured. Additionally, in a second experiment, the participation of GABA A receptors in the actions of effective dose (50 mg/kg) of Montanoa extracts to reduce immobility time was evaluated by antagonizing GABA A receptors with picrotoxin. All effects were compared with fluoxetine and Remotiv, which are clinically effective synthetic and natural antidepressant drugs, respectively.

**Forced Swim in Experiment 1**

The forced swim is a widely used model, validated and employed to investigate drugs and substances with potential antidepressant effects. In this model, the stress of being forced to swim without any possibility of escape triggers immobility behavior, which is measured as an indicator of low levels of motivation of the animal and interpreted as despair. The latency to the first period of immobility is not significant differences, $H(7) = 3.696$, $P < .814$, between treatments.

**Forced Swim Test.** Statistical analysis of latency to the first immobility revealed significant, $H(7) = 38.684$, $P < .001$, differences between treatments. Post hoc test revealed that rats treated with fluoxetine, had a longer latency to immobility, which was unaffected by picrotoxin. There were not significant differences in groups treated with Montanoa frutescens or Montanoa grandiflora extracts, but not of fluoxetine. One-way analysis of variance, Student-Newman-Keuls post hoc test. Values are expressed as mean ± standard error.

### Table 1. Effects of Treatments and Antagonist on the Locomotor Activity Test.

| Groups                     | Crossing (n) | Rearing (s) | Grooming (s) |
|----------------------------|--------------|-------------|--------------|
| Vehicle                    | 44.6 ± 3.5   | 16.9 ± 1.6  | 12.4 ± 0.9   |
| Montanoa frutescens        | 44.6 ± 2.8   | 17.2 ± 1.1  | 29.1 ± 2.1   |
| Montanoa grandiflora       | 39.8 ± 2.8   | 18.0 ± 3.3  | 23.9 ± 2.0   |
| Fluoxetine                 | 45.0 ± 3.8   | 21.3 ± 3.3  | 27.3 ± 1.7   |
| PTX                        | 40.7 ± 4.8   | 17.8 ± 2.0  | 13.6 ± 1.6   |
| Montanoa frutescens–PTX    | 42.0 ± 3.4   | 15.1 ± 1.8  | 11.3 ± 1.3   |
| Montanoa grandiflora–PTX   | 42.8 ± 5.1   | 19.6 ± 1.9  | 14.5 ± 1.8   |
| Fluoxetine–PTX             | 43.6 ± 3.5   | 19.6 ± 3.1  | 28.7 ± 2.1   |

*Abbreviation: PTX, picrotoxin; n, number; s, seconds.

*No significant changes in crossing and rearing were associated with treatments. Differences between treatments. Post hoc test revealed that rats treated with fluoxetine, had a longer latency to immobility, which was unaffected by picrotoxin. There were not significant differences in groups treated with Montanoa frutescens or Montanoa grandiflora extracts, but not of fluoxetine. One-way analysis of variance, Student-Newman-Keuls post hoc test. Values are expressed as mean ± standard error.

$^*P < .001$ versus vehicle, PTX, M frutescens–PTX, and M grandiflora–PTX groups.
considered an indicator of the magnitude of the first effort of rats to solve the demanding situation represented by the forced swim test. A short latency is considered a complementary indicator of despair behavior, while a larger latency is associated with the antidepressant-like effect produced by some antidepressant drugs. An increase in the total time of immobility is considered a depression-like behavior, which can be reversed by antidepressant drugs. In this model, antidepressant-like effects can be obtained with high doses (10-20 mg/kg) of antidepressant drugs in a subchronic schedule of treatment: 24, 5, and 1 hour before the test; however, these rapid effects are not observable in humans.

At the clinical level, most antidepressants including fluoxetine have an initial delay of 2 or more weeks to establish its therapeutic effects. It is noteworthy that in rats, lower doses of fluoxetine (1 mg/kg) require more time, around 14 to 21 days to reduce immobility and to increase latency to the first immobility in the forced swim test, resembling the delay of therapeutic effects in humans. The delay of therapeutic effects of antidepressant drugs has been related with synaptic and neuronal plasticity, including changes in brain-derived neurotrophic factor, CREB (cAMP response element binding) protein, and number of dendrites or synaptic receptors that require of several days to instauration.

In the present experiment, long-term treatment with Montanoa frutescens and Montanoa grandiflora extracts (50 mg/kg) exerted behavioral effects (ie, reduction of total time of immobility), similar to antidepressant drugs, but opposed to the typical antidepressant fluoxetine and Remotiv that required at least 14 days of treatment, Montanoa extracts elicit a shorter latency to produce its effects as occurred at day 1 after the first administration, which rapidly disappeared when administrations were discontinued. These data suggest that the effects produced by the extracts here analyzed are established by action on ionotropic receptors, in contrast to antidepressants fluoxetine and Hypericum perforatum phytomedicine that require of metabotropic receptors and neuronal plasticity to establish their therapeutic effects in the long term.

In the present investigation, the effects of withdrawal were also evaluated. Results showed that reduction of immobility observed with Montanoa frutescens and Montanoa grandiflora extracts on day 28, disappeared after 24 hours, returning to values similar to the vehicle group. In fact, withdrawal in the groups treated with the extracts showed a tendency after treatment interruption to increase immobility compared with the previous days of treatment, until differences versus control disappeared; even so the dose of 25 mg/kg of Montanoa frutescens increased immobility significantly than the control animals, but this increase is not longer than 10 seconds respect to the other groups treated with extracts. Interestingly, the antidepressant-like effects produced by fluoxetine and Remotiv remained until 48 hours after the interruption of treatments. These results support the notion that Montanoa extracts exert their action on ionotropic receptors, with short-lasting activity on the reduction of immobility similar to neurosteroids. In the forced swim test, neurosteroids reduce immobility through actions on ionotropic receptors; exhibit a short-lasting activity, with a starting average of 30 minutes and a total approximate that let go in approximately 6 hours after administration. In contrast, fluoxetine and Remotiv probably produce neuronal plastic changes of long instauration as reported by other authors, which allows the permanence in the reduction of immobility even after suspension of treatments.

**Forced Swim in Experiment 2**

The second experiment demonstrated that, in contrast to fluoxetine, the antidepressant-like effects of Montanoa frutescens and Montanoa grandiflora extracts are mediated by GABA<sub>A</sub> receptors. The administration of picrotoxin, a noncompetitive antagonist that block chloride ion channels of this receptor, blocked the reduction of immobility produced by Montanoa frutescens and Montanoa grandiflora extracts, but lacked an effect on the reduction of immobility provoked either by fluoxetine or by Hypericum perforatum extracts, as previously reported. Thus, results suggest that the protective effects of the extracts of Montanoa plants against the stress induced by the forced swim test are mediated through GABA<sub>A</sub> receptor chloride ion channels and are not completely comparable to the typical effects of antidepressant drugs as fluoxetine, but in contrast a protective effect against the stress-induced behavioral changes produced by these extracts could be suggested. In line with this notion, the concept of “protective effect against the stress-induced behavioral changes” is referred to the property of some substances (ie, neurosteroids) to prevent the development of behavioral changes associated with the exposure of animals to physical stressors. Thus, for instance, progesterone prevents the behavioral changes produced by the immobilization-induced stress, while allopregnanolone precludes behavioral effects produced by prenatal-induced stress or exposure to conflict situations in rats. The acute effects of both Montanoa extracts on the immobility here reported are similar to those produced by neurosteroids as progesterone or allopregnanolone, which at low doses promptly reduce immobility in the forced swim test and whose effects are blocked by antagonists of the GABA<sub>A</sub> receptors, including picrotoxin, further supporting the notion that the Montanoa extracts exhibit a protective activity against behavioral effects produced by stress. Thus, present results confirm previous studies that demonstrated that pharmacological action of Montanoa extracts on emotional states are established by action on ionotropic GABA<sub>A</sub> receptors, as a result, the observed short permanency of its behavioral effects is naturally expected. In addition, present findings suggest that some of the chemical compounds contained in Montanoa extracts (probably flavonoids) apparently have pharmacological actions similar to neurosteroids. Specific studies are necessary to test this hypothesis.

**Locomotor Activity**

The locomotor activity was measured to discard or identify any motor effect that could interfere with immobility behavior in
the forced swim test. No significant changes in crossing allows us to discard motor disturbances on the immobility in the forced swim test; as a result, the effects in the forced swim test are only attributed to the motivational status, but not to motor interferences (ie, hyperactivity). In this animal model, rearing and grooming were also measured; these behaviors are emotional indicators of the animal exposed to a novel environment.60 Present data show that the vehicle groups displayed the lowest level of grooming, probably due to the stress induced by forced swim as reported in other studies after stressful sessions.53 Reduction of grooming can be prevented, returning to values of unstressed animals by administration of diazepam and other substances with well-characterized anxiolytic potency.62,63 In the present study, Montanoa frutescens and Montanoa grandiflora extracts maintained grooming behavior constant along the treatment, suggesting a low stress-related behavior that was in turn prevented by picrotoxin. Similarly, fluoxetine maintained grooming but it occurred only after 21 days of treatment and its effects were not antagonized by picrotoxin, which again sets important differences between the classical effects of antidepressant fluoxetine and the extracts here tested. Besides, rearing was not modified by treatments in both experiments, suggesting that this variable is not sensible enough to emotional disturbances as the forced swim test, which is the validated model measure such effects.

Finally, it has been hypothesized that the long latencies for the establishment of therapeutic effects of antidepressant treatments are characteristic of the drug but not of the disease; in accordance, research efforts are directed to test substances that exhibit a short latency of onset.64 Montanoa frutescens and Montanoa grandiflora seem to have antidepressant effects with short latencies; nonetheless the short term of its effects after the treatment withdrawal and their action modulated through the GABA<sub>A</sub> receptors, suggest that Montanoa extracts do not possess tangible antidepressant properties, but instead have protector effects that prevent the stress triggered by forced swim. This might be important to design specific phytomedicines destined to particular emotional or affective disorders in the future.

Plants of the Montanoa genus contain active compounds of medical importance such as triterpenes, diterpenes, sesquiterpene lactones, alkaloids, and flavonoids.65-67 In present study using qualitative phytochemical techniques was possible to identify the presence of terpenes, alkaloids, flavonoids and sesquiterpene lactones in both Montanoa extracts. The present study partially described the potential mechanism of action through Montanoa frutescens and Montanoa grandiflora exert its antidepressant-like actions, and even though the active compounds of the extracts inducing such activity were not isolated and identified, the qualitative phytochemical analysis confirm the presence of flavonoids and alkaloids, as reported in other studies,65-67 which could contribute to the behavioral actions reported in the present investigation. In line with this proposal, it has been widely reported that flavonoids from different sources may cross the hematoencephalic barrier and exert actions on the central nervous system,68 producing neuroprotective, antistress, anxiolytic, antidepressant and anticonvulsant effects at the GABA<sub>A</sub> receptors.69-72 Flavonoids have high affinity for GABA<sub>A</sub> receptors.73,74 When GABA<sub>A</sub> receptors are activated by its agonists the conductance of chloride ions increases, thus hyperpolarizing the neuron and consequently inhibiting neuronal activity. This neurophysiological effect that occurs through GABA<sub>A</sub> receptor chloride ion channels is associated with the psychopharmacological effects of several substances, including benzodiazepines, barbiturates, psychoactive drugs, some neurosteroids, and flavonoids.75,76 These latter 2 substances also quickly reduce immobility time in the forced swim test suggesting an antidepressant-like effect mediated by GABA<sub>A</sub> receptors.23,24,77,78 In the present study, pretreatment with picrotoxin did not produce intrinsic effects in the forced swim test but prevented the effects of the effective dose of Montanoa extracts to reduces immobility time. Therefore, the observed anti-immobility effects of both extracts appear to be mediated by GABAergic actions. This possibility opens new lines of investigation to find the specific bioactive compounds responsible of the effects here reported. At this moment, the present investigation contributes to partially support traditional use Montanoa extracts to ameliorate the impact of stress on mental health and prevent the development of mood disorders as people use it in their communities (ie, aqueous crude extract). Nonetheless, additional studies on toxicity and drug interactions are required before Montanoa extracts could be widely recommended for human use as a safety and efficient therapeutic alternative.

Conclusion
The results of the present study show that the extracts of Montanoa frutescens and Montanoa grandiflora (50 mg/kg) exert immediate protective effects against stress-induced behaviors in forced swim test through actions on the GABA<sub>A</sub> receptors, instead of a typical antidepressant-like effect.

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Author Contributions
JFR-L and MC-J conceived the project, the experimental design, and wrote the protocol. LAF-A, GUR-S, and FG-O carried out the experiment and measured the behavioral variables. JFR-L, JCE, and MJR-H realized the statistical analysis and interpretation of results. All the authors significantly reviewed and discussed the final version of the manuscript.

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Ethical Approval
This project was approved by the Ethical Internal Committee from Escuela de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tlaxcala (Reg. No. MVZ-189/12).

References
1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Washington, DC: American Psychiatric Association; 2013.
2. Marie N, Stojiljkovic D, Pavlovic Z, Jasovie-Gasic M. Factors influencing the choice of antidepressants: a study of antidepressant prescribing practice at University Psychiatry Clinic in Belgrade. Vojnosanit Pregl. 2012;69:308-313.
3. O’Leary OF, O’Brien RM, Cryan JF. Drugs, genes and the blues: pharmacogenetics of the antidepressant response from mouse to man. Pharmacol Biochem Behav. 2014;123:55-76.
4. Kasper S, Gastpar M, Müller WE, et al. Efficacy of St. John’s wort extract WS 5570 in acute treatment of mild depression: a reanalysis of data from controlled clinical trials. Eur Arch Psychiatr NeuropsychoClin Neurosci. 2008;258:59-63.
5. Caccia S, Gobbi M. St. John’s wort components and the brain: uptake concentrations reached and the mechanisms underlying pharmacological effects. Curr Drug Metab. 2009;10:1055-1065.
6. Brechner ML, Albright LD, Weston LA. Effects of UV-B on secondary metabolites of St. John’s wort (Hypericum perforatum L.) grown in controlled environments. Photochem Photobiol. 2011;87:680-684.
7. Guilhermano LG, Ortiz L, Ferigolo M, Barros HM. Commercially available Hypericum perforatum extracts do not decrease immobility of rats in the forced swimming test. Prog Neuropsychopharmacol Biol Psychiatry. 2004;28:49-55.
8. Clark DB, Birmaher B, Axelson D, et al. Fluoxetine for the treatment of childhood anxiety disorders: open-label, long-term extension to a controlled trial. J Am Acad Child Adolesc Psychiatry. 2005;44:1263-1270.
9. Rodríguez-Landa JF, Cueto-Escobedo J, Aguirre-Chiáas ID, Pérez-Vásquez MO. A commercially available product of Hypericum perforatum acts on GABAergic receptor to produce anxiolytic-like, but not antidepressant-like effects in Wistar rats. In: Howard RD, ed. Hypericum: Botanical Sources, Medical Properties and Health Effects. New York, NY: Nova Science; 2015:81-100.
10. Rodríguez-Landa JF, Contreras CM. A review of clinical and experimental observations about antidepressant actions and side effects produced by Hypericum perforatum extracts. Phytomedicine. 2003;10:688-699.
11. Codex de la Cruz-Badiano. Libellus de Medicinalibus Indorum Herbis 1552. Ciudad de México, Mexico: Instituto Mexicano del Seguro Social; 1964.
12. Ximenez F. Quatro libros de la naturaleza y virtudes de las plantas y animales que están recibidos en el uso de medicina en la Nueva España, y la método, y corrección y preparación que para administrarlas se requiere con lo que el doctor Francisco Hernández escribió en lengua latina, viuda de Diego López Dávalos. Ciudad de México, Mexico: Documento Histórico; 1615.
13. Levine SD, Hahn DW, Cotter ML, et al. The Mexican plant zopatle (Montanoa tomentosa) in reproductive medicine. Past, present and future. J Reprod Med. 1981;26:524-528.
14. Gallegos AJ. Revisited. Contraception 1985;31:487-497.
15. Carro-Juaréz M, Cervantes E, Cervantes-Méndez M, Rodríguez-Manzo G. Aphrodisiac properties of Montanoa tomentosa aqueous crude extract in male rats. Pharmacol Biochem Behav. 2004;78:129-134.
16. Carro-Juaréz M, Franco MA, Rodríguez-Peña ML. Increase of the ejaculatory potency by the systemic administration of aqueous crude extracts of Cihuapatli (Montanoa genus) plants genus in spinal male rats. J Evid Based Complement Alternat Med. 2014;19:43-50.
17. Carro-Juaréz M, Rodríguez-Landa JF, Rodríguez-Peña Mde L, Rovirosa-Hernández Mde J, García-Orduña F. The aqueous crude extract of Montanoa frutescens produces anxiolytic-like effects similarly to diazepam in Wistar rats: involvement of GABA receptor. J Ethnopharmacol. 2012;143:592-598.
18. Rodríguez-Landa JF, Vicente-Serna J, Rodríguez-Blanco LA, Rovirosa-Hernández MJ, García-Orduña F, Carro-Juaréz M. Montanoa frutescens and Montanoa grandiflora extracts reduce anxiety-like behavior during the metestrus-diestrus phase of the ovarian cycle in Wistar rats. Biomed Res Int. 2014;2014:938060.
19. Rodríguez-Landa JF, Rodríguez-Santiago MG, Rovirosa-Hernández MJ, García-Orduña F, Carro-Juaréz M. Aqueous crude extract of Montanoa tomentosa exerts anxiolytic-like effects in female rats with long-term absence of ovarian hormones. J Chem Biol Phys Sci. 2014;4:37-46.
20. Sollozo-Dupont I, Estrada-Camarena E, Carro-Juaréz M, López-Rubalcava C. GABA/benzodiazepine receptor complex mediates the anxiolytic-like effect of Montanoa tomentosa. J Ethnopharmacol. 2015;162:278-286.
21. Baldés-Baiabal C. Efecto de la infusión de Cihuapatli (Montanoa tomentosa) en ratas hembra de la cepa Wistar forzadas a nadar [bachelor’s thesis]. Xalapa, Mexico: Universidad Veracruzana; 2007.
22. Martínez-Mota L, Contreras CM, Saavedra M. Progesterone reduces immobility in rats forced to swim. Arch Med Res. 1999;30:286-289.
23. Rodríguez-Landa JF, Contreras CM, Bernal-Morales B, Gutiérrez-Garcia AG, Saavedra M. Allopregnanolone reduces the immobility in the forced swimming test and increases firing rate
of lateral septal neurons through actions on the GABA\textsubscript{A} receptor in the rat. *J Psychopharmacol*. 2007;21:76-84.

24. Rodríguez-Landa JF, Contreras CM, García-Rios RI. Allopregnanolone microinjected into the lateral septum or dorsal hippocampus reduces immobility in the forced swim test: participation of the GABAA receptor. *Behav Pharmacol*. 2009;20:614-622.

25. Nin MS, Ferri MK, Couto-Pereira NS, et al. The effect of intra-nucleus accumbens administration of allopregnanolone on δ and γ2 GABA(A) receptor subunit mRNA expression in the hippocampus and on depressive-like and grooming behaviors in rats. *Pharmacol Biochem Behav*. 2012;103:359-366.

26. National Research Council. *Guide for the Care and Use of Laboratory Animals: A Report of the Institute of Laboratory Animal Resource Committee on the Care and Use of Laboratory Animals [NIH publication no. 85-23]*. Washington, DC: Department of Health and Human Services; 1996.

27. Norma Oficial Mexicana NOM-062-ZOO-1999. *Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio*. Ciudad de México, Mexico: Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación; 1999.

28. Thiers B.[continuously updated]. *Index herbarium: a global directory of public herbaria and associated staff*, New York Botanical Garden’s Virtual Herbarium; 2016. http://sweetgum.nybg.org/science/ih/.

29. Domínguez XA. *Phytochemical research methods*. *Ciudad de México*, MEX: Limusa; 1973.

30. Trease GE, Evans WC. *Textbook of Pharmacognosy*. 12th ed. London, England: Bailliére Tindall Press; 1983.

31. Ohadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta state of Nigeria. *Global J Pure Appl Sci*. 2001;8:203-208.

32. Cseka LJ, Kirakosyan A, Kaufman PB, Waber SL, Duke JA, Brielmann HL. *Natural Products From Plants*. Boca Raton, FL: CRC Press; 2006.

33. Lozano-Hernández R, Rodríguez-Landa JF, Hernández-Figueroa DJ, Saavedra M, Ramos-Morales FR, Cruz-Sánchez S. Antidepressant-like effects of two commercially available products of *Hypericum perforatum* in the forced swim test: a long-term study. *J Med Plant Res*. 2010;4:131-137.

34. Contreras CM, Rodríguez-Landa JF, Gutiérrez-García AG, Bernal-Morales B. The lowest effective dose of fluoxetine in the forced swim test significantly affects the firing rate of lateral septal nucleus neurons in the rat. *J Psychopharmacol*. 2001;15:231-236.

35. Reddy DS, Kulkarni SK. Role of GABA-A and mitochondrial diazepam binding inhibitor receptors in the anti-stress activity of neurosteroids in mice. *Psychopharmacology (Berl)*. 1996;128:280-292.

36. Kaluweff AV, Touhimaa P. Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). *Behav Brain Res*. 2005;160:1-10.

37. Contreras CM, Martínez-Mota L, Saavedra M. Desipramine restricts estral cycle oscillations in swimming. *Prog Neuropsychopharmacol Biol Psychiatry*. 1998;22:1121-1128.

38. Molina M, Contreras CM, Téllez-Alcántara P. *Mimosa pudica may possess antidepressant actions in the rat*. *Phytotherapy*. 1999;6:319-323.

39. Mezadri TJ, Batista GM, Portes AC, Marino-Neto J, Lino-de-Oliveira C. Repeated rat-forced swim test: reducing the number of animals to evaluate gradual effects of antidepressants. *J Neurosci Methods*. 2011;195:200-205.

40. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatment. *Nature*. 1977;266:730-732.

41. Estrada-Camarena E, Contreras CM, Saavedra M, Luna-Baltazar I, López-Rubalcava C. Participation of the lateral septal nuclei (LSN) in the antidepressant-like actions of progesterone in the forced swimming test (FST). *Behav Brain Res*. 2002;134:175-183.

42. Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. London, England: Methuen; 1959.

43. Espejo EF, Miñano FJ. Prefrontocortical dopamine depletion induces antidepressant-like effects in rats and alters the profile of desipramine during Porsolt’s test. *Neuroscience*. 1999;88:609-615.

44. Castagné V, Porsolt RD, Moser P. Use of latency to immobility improves detection of antidepressant-like activity in the behavioral despair test in the mouse. *Eur J Pharmacol*. 2009;616:128-133.

45. Nestler EJ, Gould E, Manji H, et al. Preclinical models: status of basic research in depression. *Biol Psychiatry*. 2002;52:503-528.

46. Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differently produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)*. 1995;121:66-72.

47. Martínez-Mota L, Fernández-Guasti A. Testosterone-dependent antidepressant-like effect of noradrenergic but not of serotonergic drugs. *Pharmacol Biochem Behav*. 2004;78:711-718.

48. Blier P. The pharmacology of putative early-onset antidepressant strategies. *Eur Neuropsychopharmacol*. 2003;13:57-66.

49. Opal MD, Klenotich SC, Moraes M, et al. Serotonin 2C receptor antagonists induce fast-onset antidepressant effects. *Mol Psychiatry*. 2014;19:1106-1114.

50. Marchetti C, Tafi E, Middei S, et al. Dynaptic adaptations of CA1 pyramidal neurons induced by a highly effective combinational antidepressant therapy. *Biol Psychiatry*. 2010;67:146-154.

51. Crupi R, Mazzon E, Marino A, et al. *Hypericum perforatum* treatment: effect on behaviour and neurogenesis in a chronic stress model in mice. *BMC Complement Altern Med*. 2011;11:e7.

52. Rubio FJ, Ampuero E, Sandoval R, Toledo J, Pancetti F, Wyneken U. Long-term fluoxetine treatment induces input-specific LTP and LTD impairment and structural plasticity in the CA1 hippocampal subfield. *Front Cell Neurosci*. 2013;7:66.

53. Contreras CM, Rodríguez-Landa JF, Bernal-Morales B, Gutiérrez-García AG, Saavedra M. Timing of progesterone and allopregnanolone effects in a serial forced swim test. *Salud Mental*. 2011;34:309-314.

54. Ampuero E, Rubio FJ, Falcon R, et al. Chronic fluoxetine treatment induces structural plasticity and selective changes in
glutamate receptor subunits in the rat cerebral cortex. *Neuroscience*. 2010;169:98-108.

55. Djordjevic A, Djordjevic J, Elakovic I, Adzic M, Matić G, Radić MB. Fluoxetine affects hippocampal plasticity, apoptosis and depressive-like behavior of chronically isolated rats. *Prog Neuropsychopharmacol Biol Psychiatry*. 2012;36:92-100.

56. Bitran D, Shiekh M, MeLeod M. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABA_A receptors. *J Neuroendocrinol*. 1995;7:171-177.

57. Bormann J. The “ABC” of GABA receptors. *Trends Pharmacol Sci*. 2000;21:16-19.

58. Zimmerberg B, Blaskey LG. Prenatal stress effects are partially ameliorated by prenatal administration of the neurosteroid allopregnanolone. *Pharmacol Biochem Behav*. 1998;59:819-827.

59. Molina-Hernández M, Téllez-Alcántara NP, Pérez-García J, Olivera-López JI, Jaramill MT. Anti-conflict-like actions of intralateral septal infusions of allopregnanolone in Wistar rats. *Pharmacol Biochem Behav*. 2003;75:397-404.

60. Gilad VH, Rabey JM, Elyayev Y, Gilad GM. Different effects of acute neonatal stressors and long-term postnatal handling on stress-induced changes in behavior and in ornithine decarboxylase activity of adult rats. *Brain Res Dev Brain Res*. 2000;120:255-259.

61. Rodríguez-Landa JF, Hernández-Calderón BC, Saavedra M. Anxiolytic-like effect of phytoestrogen genistein in rats with long-term absence of ovarian hormones in the black and white model. *Prog Neuropsychopharmacol Biol Psychiatry*. 2009;33:367-372.

62. Hata T, Nishimura Y, Kita T, Itoh E, Kawabata A. The abnormal open-field behavior of SART-stressed rats and effects of some drugs on it. *Jpn J Pharmacol*. 1988;48:479-490.

63. D’Aquila PS, Peana AT, Carboni V, Serra G. Exploratory behaviour and grooming alter repeated restraint and chronic mild stress: effect of desipramine. *Eur J Pharmacol*. 2000;399:43-47.

64. Drewniany E, Han J, Hancock C, et al. Rapid-onset antidepressant action of ketamine: potential revolution in understanding and future pharmacologic treatment of depression. *J Clin Pharm Ther*. 2015;40:125-130.

65. Gallegos AJ. A traditional remedy from Mexico emerges to modern times. *Contraception*, 1983;27:211-225.

66. Quijano L, Calderón JS, Gómez GF, Bautista S. Four eudesmanolides from *Montanoa frutescens*. *Phytochemistry*, 1985;24:861-862.

67. Guzmán-Durán A, Wens MA, Ponce-Monter H, Pedrón N, Gallegos AJ, Zaapatele XIII. Isolation of water soluble fractions from *Montanoa frutescens* and some biological activities. *Arch Invest Méd (Mex)*. 1988;19:157-163.

68. de Boer AG, Gaillard PJ. Drug targeting to the brain. *Ann Rev Pharmacol Toxicol*. 2007;47:323-355.

69. Medina JH, Viola H, Wolfman C, et al. Overview—flavonoids: a new family of benzodiazepine receptor ligands. *Neurochem Res*. 1997;22:419-425.

70. Johnston GA. Flavonoid nutraceuticals and ionotropic receptors for the inhibitory neurotransmitter GABA. *Neurochem Int*. 2015;89:120-125.

71. Farzaei MH, Bahramsoltani R, Rahimi R, Abbasabadi F, Abdolahi M. A systematic review of plant-derived natural compounds for anxiety disorders. *Curr Top Med Chem*. 2016;16:1924-1942.

72. Germán-Ponciano LJ, Rosas-Sánchez GU, Rivadeneyra-Domínguez E, Rodríguez-Landa JF. Advances in the preclinical study of some flavonoids as potential antidepressant agents. *Scientifica (Cairo)*. 2018;2018:2963565.

73. Marder M, Paladini AC. GABA_A-receptor ligands of flavonoid structure. *Curr Top Med Chem*. 2002;2:853-867.

74. Wang H, Hui KM, Chen Y, Xu S, Wong JT, Xue H. Structure-activity relationships of flavonoids, isolated from *Scutellaria baicalensis*, binding to benzodiazepine site of GABA(A) receptor complex. *Planta Med*. 2002;68:1059-1062.

75. Chebib M, Johnston GA. The “ABC” of GABA receptors: a brief review. *Clin Exp Pharmacol Physiol*. 1999;26:937-940.

76. Enna SJ. The GABA receptors. In: Enna SJ, Möhler H, eds. *The GABA Receptors*. Totowa, NJ: Humana Press; 2007:1-21.

77. de la Peña JB, Kim CA, Lee HL., et al. Luteolin mediates the antidepressant-like effects of *Cirsium japonicum* in mice, possibly through modulation of the GABAA receptor. *Arch Pharm Res*. 2014;37:263-269.

78. Karim N, Khan I, Ahmad N, Umar MN, Gavande N. Antidepressant, anticonvulsant and antinociceptive effects of 3'-hydroxy-6-methylflavone and 3'-hydroxy-6-methylflavone may involve GABAergic mechanism. *Pharmacol Rep*. 2017;69:1014-1020.