Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section C: Medical and Phamaceutical Biotechnology

CODEN (USA): JCBPAT Research Article

Aqueous Crude Extract of *Montanoa tomentosa* Exerts Anxiolytic-Like Effects in Female Rats with Long-Term Absence of Ovarian Hormones

Juan Francisco Rodríguez-Landa[1,*], Magdalena Guadalupe Rodríguez-Santiago[2], María de Jesus Rovirosa-Hernández[1], Francisco García-Orduña[1], Miguel Carro-Juárez[3]

[1] Instituto de Neuroetología, Universidad Veracruzana. Xalapa, Veracruz. México.
[2] Escuela de Medicina, Universidad Autónoma de Tlaxcala, Tlaxcala, Tlaxcala, México.
[3] Laboratorio de Comportamiento Reproductivo, Escuela de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tlaxcala, Tlaxcala, Tlaxcala, México

Abstract: Natural or "surgical" menopause is characterized by a reduced concentration of steroid hormones, which is associated with a major incidence of vasomotor symptoms, vaginal dryness, osteoporosis, cognitive deterioration, hot flashes, irritability, anxiety and mood swings, among others. Hormonal and pharmacological therapies have been used to treat those alterations, but in some woman produce side effects limiting their long-term use. Therefore new studies to found alternative therapies for the management of those alterations in women are needed. In the Mexican traditional medicine there are some plants as *Montanoa tomentosa* recommended to prevent and treat women´s illness, including emotional and mood swings, but specific studies on the effects produced by this plant on anxiety associated with low concentration of steroid hormones have not available. Therefore in the present study the aim was to evaluate the effect of the aqueous extract of *M. tomentosa* in rats with long-term absence of ovarian hormones induced by ovariectomy. Three concentration of *M. tomentosa* extract (12.5, 25 and 50 mg/kg) were evaluated in rats subjected to elevated plus maze and open field tests and comparisons against a control group and diazepam as reference of an anxiolytic drug were described. In the elevated plus maze only 50 mg/kg of *M. tomentosa* extract, alike to diazepam, increased the time spent and the number of entries into the open arms, respect to control group. Similar to diazepam, in the

open field test none of the evaluated treatments produced significant changes in crossing and rearing, but 50 mg/kg of *M. tomentosa* extract increase time spent in grooming behavior as compared with control group. In conclusion *M. tomentosa* extract produces an anxiolytic-like effect in rats with long-term absence of ovarian hormones. Findings partially support the traditional use of this plant in the traditional Mexican medicine to solve women's emotional alterations.

Keywords: Anxiolytic, *Montanoa tomentosa*, ovariectomy, surgical menopause, elevated plus maze.

INTRODUCTION

"Surgical menopause" occurs in women when, before natural menopause, both ovaries (bilateral oophorectomy) are removed due to any medical alteration. This physiological state is mainly characterized by a reduction of steroid hormones after eliminating ovarian function[1,2] and is associated with a major incidence of vasomotor symptoms, vaginal dryness, osteoporosis, cognitive deterioration and hot flashes, accompanied by perspiration, palpitations, irritability, anxiety and mood swings[3,4]. Hormone replacement therapy with estrogens and progestagens has been used for the management of physiological, behavioral and emotional alterations in the postmenoapusal women[5-8.] Nevertheless, this kind of therapy produces side effects that limit their long-term use particularly in women with predisposition to development any kind of cancer dependent of hormones[9]. New studies to found alternative therapies to treat the alterations associated with surgical menopause in the women are needed.

At the preclinical level, rats with long-term absence of ovarian hormones induced by ovariectomy has been proposed as an early model of "surgical menopause", which has allowed studying alterations in the bone density[10], as well as changes in sensitivity of neural receptor in specific areas of the brain[11,12] . As to the emotional and mood alterations associated with the surgical menopause, Picazo *et al.*[13] evaluated the long-term effect of ovariectomy on anxiety-like behavior in the rat. They observed that after 12-weeks post-ovariectomy, rats exhibit greater anxiety-like behavior than rats with 3-weeks post-ovariectomy, supporting the notion that rats with chronic absence of ovarian hormones induced by ovariectomy could be an useful model to understand the mood swings typical of human menopause[13], and for the screening of new substances used in their control.

Some studies using rats with long-term absence of ovarian hormones have reported the anxiolytic-like effects of some drugs. Thus, it has been shown that diazepam produces anxiolytic effects in rats with 12-weeks post-ovariectomy, which display high anxiety-like behavior in the defensive burying test[13]. Similarly, in rats with 12-weeks post-ovariectomy some doses of the phytoestrogen genistein reduce anxiety-like behavior in the light-dark box, similar to diazepam[14, 15]. Accordingly, long-term ovariectomy in rats may be a useful tool to explore potential anxiolytic-like effects of several substances including those from medicinal plants such as Cihuapatli (*Montanoa tomentosa*), which could be used to prevent and treat alterations associated with "surgical menopause."

In the ancient Mexican traditional medicine several plants including *M. tomentosa* have been used for the management of anxiety and mood disorders. Main descriptions of this plant as a traditional remedy are contained in the *Badianus Codex* or *Libellus de Medicinalibus Indorum Herbis*[16] where botanical

determinants, traditional recipes and prescriptions are provided. In addition, in the *Historia General de las Cosas de Nueva España* (*Florentine Codex*) and in the *Historia Natural de la Nueva España*, *M. tomentosa* extract was described as a remedy to facilitate parturition, also during the puerperium, as a contraceptive agent and to solve emotional and mood disorders[17]. Recent studies on *M. tomentosa* extracts have shown that the crude extract of this plant can cross the blood-brain barrier and exerts their effects at the central nervous system[18]. The aqueous extracts of *M. tomentosa*[43] and other *Montanoa* species (i.e. *M. frutescens* and *M.* grandiflora)[18,19] were reported to produce anxiolytic-like effects similar to diazepam in male and female Wistar rats, through actions on the $GABA_A$ receptors. Nonetheless, although some anxiolytic-like effects of *M. tomentosa* extract has been identified, the effects of this plant in rats with long-term absence of ovarian hormones remains unexplored. In the present study, we hypothesized that *M. tomentosa* extract produces anxiolytic-like effect in rats with long-term absence of ovarian hormones, similarly to diazepam. Therefore, the aim of the present study was to evaluate the effect of *M. tomentosa* extract on anxiety-like behavior in rats with 12-week post-ovariectomy subjected to the elevated plus maze and open field tests.

MATERIAL AND METHODS

Animals: Forty ovariectomized adult female Wistar rats weighing between 200–250 g at the beginning of the experiments were included. Rats were housed in Plexiglass cages (four rats per cage), with a 12/12 h light/dark cycle (light on at 7:00 AM), at an average temperature of 25ºC (± 1ºC) and *ad libitum* access to purified water and food. All the experimental procedures were carried out according to the Guide for Care and Use of Laboratory Animals published by the National Institute of Health (National Research Council, Publication No. 85-23, revised in 1996)[20], and the Norma Oficial Mexicana para el cuidado y uso de animales de laboratorio (NOM-062-ZOO-1999)[21]. The general protocol received authorization (No. MVZ-189-12) from Ethical Internal Committee of the Veterinary School (Universidad Autónoma de Tlaxcala, México).

Surgery: The ovariectomy was performed through an abdominal ventral incision under general anesthesia according previous studies[14,15]. After the surgery and to assure the long-term absence of ovarian hormones, rats were returned to the housing facilities for twelve weeks. After this period, rats were randomly assigned to each of the experimental groups and subjected to treatments and behavioral tests.

Groups and treatments: Rats with 12-weeks post-ovariectomy were assigned to five independent groups (n= 8 in each group): a vehicle group received 1 mL/kg of purified water, three additional groups received different dose of *M. tomentosa* extract (12.5, 25 and 50 mg/kg, respectively) and one last group received diazepam (2 mg/kg, Valium® 10, Productos Roche Químicos e Farmaceuticos, Brasil) as reference of anxiolytic drug. All of the treatments were administered in a single dosage by oral route, except diazepam that was administered by i.p. route, in an equivalent volume of 2 mL/kg. The preparation of the aqueous extract of *M. tomentosa* and schedule of treatment utilized in this study was based on the findings of previous studies[22]. In brief, *M. tomentosa* was collected in its habitat in Tlaxcala during October of 2012 and were authenticated by personal from the Universidad Autónoma de Tlaxcala Herbarium, where voucher is preserved (Serial Numbers: MT UATX10). Leaves of *M. tomentosa* were collected and dried during twenty days to prepare the aqueous crude extract; which was prepared 40 min previous to its administration to avoid any alteration of the natural bioactive compounds content in the extract. Similarly, diazepam dosage was selected from previous studies in which 2 mg/kg produces anxiolytic-like effects in rats with 12-weeks post-ovariectomy[14, 15].

Behavioral tests: Thirty minutes after the corresponding treatment, rats were individually evaluated in the elevated plus maze (5 min) and subsequently in the open field test (5 min). The elevated plus maze was constructed of wood and situated in an illuminated room (40 lux). The apparatus consisted of two opposite open and closed arms set in a plus configuration. The dimensions of the open arms were 50 cm length × 10 cm width, and the closed arms were 50 cm length × 10 cm width × 40 cm height. The entire maze was elevated 50 cm above the floor. A digital video camera (Sony, DCR-SR42, 40× optical zoom, Carl Zeiss lens) was installed above of the apparatus to record the rats' activity. To evaluate the effects of the treatments, rats were placed in the center of the maze, facing an open arm. The evaluated variables were (*i*) the time spent in the open arms, (*ii*) the number of entries into the open arms, (*iii*) and the percentage of open arm entries ([open entries] / [total entries] × 100). These variables were selected based on previous studies, in which these measures were shown to be reliable indicators of experimental anxiety[23, 24].

After elevated plus-maze, rats were evaluated in the open field test to identify hypoactivity, hyperactivity, or no changes associated with treatments, which could interfere with the interpretation of behavioral activity of rats in the elevated plus maze. An opaque plexiglass cage (44 × 33 cm) with walls 20-cm in height was used. The floor was divided into 12 squares (11 × 11 cm). A video camera was installed above the cage to record the activity of the rat. At the onset of the test, rats were gently placed in one of the corners of the cage, and the following variables were measured: (*i*) the number of squares crossed (crossing) by the rat, (*ii*) the time in seconds spent in rearing, and (*iii*) the time in seconds spent in self-grooming[25, 26]. After each test session, apparatus were carefully cleaned with a 10% ethanol solution to remove the scents of previously evaluated rat. Five minutes elapsed between each test to allow the odors and cleaning solutions to dissipate. Later, two independent observers measured the behavioral variables in the video recorded sessions from elevated plus maze and open field test until reaching at least 95% agreement in the measurements.

Statistical analysis: The data were analyzed by one-way ANOVA to independent groups and Student-Newman-Keuls *post-hoc* test when P values reached ≤ 0.05. Results are presented as mean ± standard error.

RESULTS AND DISCUSION

Elevated plus maze: The one-way ANOVA showed significant differences in the time spent in the open arms according to treatments ($F_{4,35} = 28.146$, $p < 0.001$). The *post hoc* test showed that, similar to diazepam, rats treated with 50 mg/kg of the *M. tomentosa* extract spent more time in the open arms respect to the vehicle group (Figure 1a). In the number of entries into the open arms, the statistical analysis showed significant differences among treatments ($F_{4,35} = 8.136$, $p < 0.001$) and the *post hoc* test revealed that 25 and 50 mg/kg of *M. tomentosa* extract significantly increased this variable respect to vehicle group, similar to diazepam (Figure 1b). Finally, the analysis of percentage of entries into the open arms did not reveal significant differences among treatments ($F_{4,35} = 2.233$, $p = 0.085$): vehicle, 38.12 ± 5.63 %; *M. tomentosa* 12.5 mg/kg, 31.93 ± 8.87 %; *M. tomentosa* 25 mg/kg, 29.37 ± 3.13 %; *M. tomentosa* 50 mg/kg, 34.75 ± 1.50 %; and diazepam, 45.55 ± 4.52 %.

The elevated plus maze is a well-accepted and validated behavioral model for detecting the effectiveness of anxiolytic drugs[24, 27, 28]. In this animal model, rats or mice that display anxiety-like behavior usually show a reduction of open arm exploration, reflected by a decrease in the time spent into, and in the number and percentage of entries into the open arms, as occurred in the present study in rats with long-term absence of ovarian hormones treated with vehicle. On the other hand, animals treated with clinically effective anxiolytic

drugs as diazepam[29-31], some neurosteroids with anxiolytic-like potency as progesterone and allopregnanolone[32-34] and some extracts from plants with reputed anxiolytic properties, exhibit increases in the total time spent in the open arms and in the number and percentage of entries to this arms[35,36] which together is considered an anxiolytic-like effect at the experimental level. In the present study we found that 50 mg/kg of the *M. tomentosa* extract decreased anxiety-like behavior in the elevated plus maze in female rats with long-term absence of ovarian hormones, when the concentrations of steroid hormones are lower and anxiety-like behavior increases[13]. Therefore, present data on the effects produced by 50 mg/kg of *M. tomentosa* extract, similar to diazepam, support an anxiolytic-like effect in Wistar rats with long-term absence of ovarian hormones.

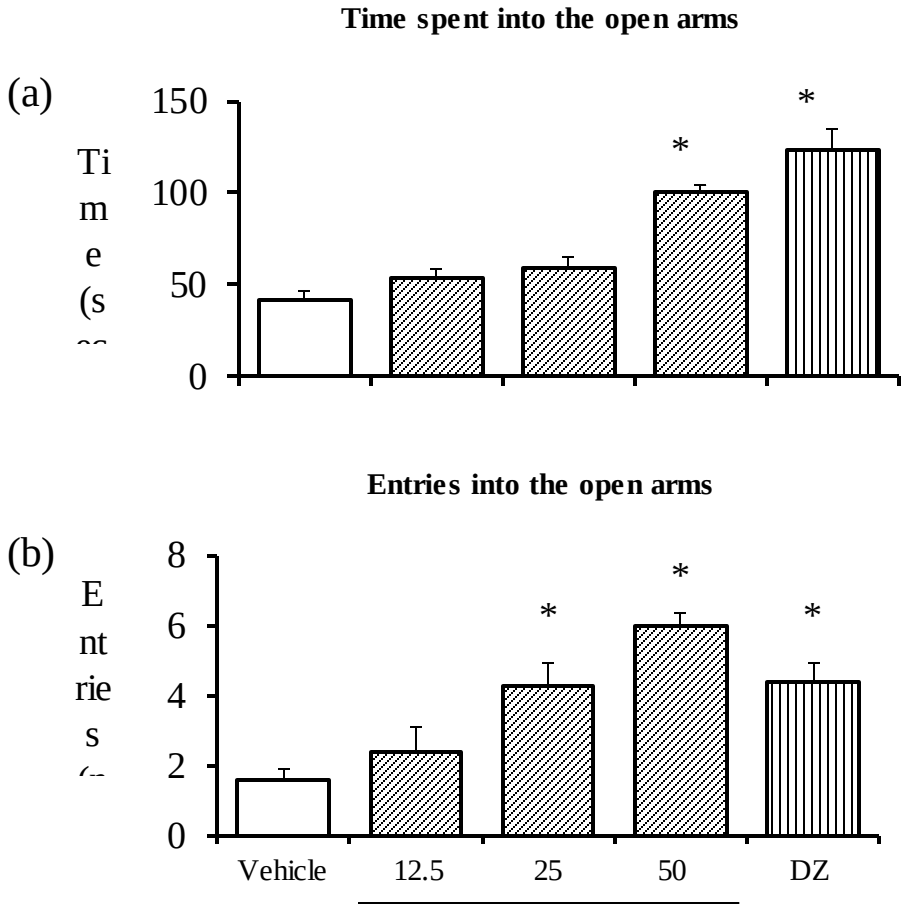

Figure 1: Elevated plus maze. 50 mg/kg of *M. tomentosa* extract increase time spent into the open arms (a), and 25 and 50 mg/kg of *M. tomentosa* extract increase the number of entries into the open arms (b), in bot cases similar to diazepam. * $p < 0.05$ versus vehicle group, One-way ANOVA, Student-Newman-Keuls. *post hoc* test.

Open field test: The one-way ANOVA did not show statistically significant differences in the number of crossings ($F_{4,35}$ = 2.323, p = 0.870) nor in the time spent in rearing ($F_{4,35}$ = 0.586, p = 0.675) according treatments. Nevertheless, significant differences ($F_{4,35}$ = 5.789, p = 0.001) were detected in time spent in grooming, among treatments. The *post hoc* test showed that only rats treated with 50 mg/kg of *M. tomentosa*

extract or with diazepam, significantly increased the time spent in grooming, respect to the vehicle group (Table1).

Table 1: Number of crossing, rearing, and grooming in the open field test.

Treatment	Crossing (n)	Rearing (sec)	Grooming (sec)
Vehicle	36.75 ± 2.54	24.35 ± 4.07	22.87 ± 6.80
M. tomentosa 12.5 mg/kg	30.01 ± 3.19	28.58 ± 5.60	27.75 ± 4.15
M. tomentosa 25 mg/kg	40.10 ± 5.26	26.63 ± 3.07	31.18 ± 5.12
M. tomentosa 50 mg/kg	41.25 ± 5.36	30.17 ± 5.55	44.23 ± 2.45 *
Diazepam	28.37 ± 4.96	33.83 ± 4.69	42.33 ± 2.35 *

Values are expressed as mean ± standard error from each variable. *p < 0.05, versus vehicle group. One-way ANOVA, Student-Newman-Keuls *post hoc* test.

In the present study, the open field test was conducted after the elevated plus maze test, allowing us to identify possible changes in general motor activity (i.e. hypoactivity, hyperactivity or not changes) associated with the treatments that may influence performance in the elevated plus maze. For instance, in the rat higher doses of diazepam (4 mg/kg) produce hypoactivity in the open field test (i.e. reduced crossing and rearing) without an anxiolytic-like effect in the elevated plus maze, while lower doses (2 mg/kg) produces anxiolytic-like effects without significant changes in crossing or rearing[20], a typical effect of anxiolytic drugs. Present data showed that in the open field test, similar to the anxiolytic dose of diazepam, 50 mg/kg of *M. tomentosa* did not produce significant modifications in crossing and rearing and therefore, we suggest that the effect of these doses of the plant extract may be considered as anxiolytic. Accordingly, it has been reported that in 12-weeks post-ovariectomized rats lower grooming behavior is detected[14] as compared with control rats, an effect reversed by acute administration of diazepam and some extracts of *Montanoa* genus plants[23,15,]. A similar effect of *M. tomentosa* here described on grooming behavior has also considered an additional indicator of anxiolytic-like activity at the experimental level[37, 38]. In the present study, rats treated with 50 mg/kg of *M. tomentosa* extract or with diazepam showed higher levels of grooming behavior compared with the vehicle group. Taken together, these data support the notion that acute administration of *M. tomentosa* possesses anxiolytic-like profiles in rats with long-term absence of ovarian hormones subjected to the elevated plus maze.

Although the present study did not identify the bioactive compounds or the potential action mechanism involved in the anxiolytic-like effects of the *M. tomentosa* extract, present results further support the traditional use of this plant as a potential anxiolytic agent. Therefore, additional studies should be conducted to identify the chemical compounds and parameters related to their bioavailability after the long-term administration as well as potential side effects. Nonetheless, considering some previous phytochemical studies on *M. tomentosa* extracts, it is possible to offer a partial explanation of the anxiolytic-like activity exhibited by the extract analyzed herein. Pentacyclic triterpenes and tetracyclic diterpenes (e.g., ent-kaurenoic acid, grandiflorenic acid, kauradienoic acid, zoapatanol, and montanol), sesquiterpene lactones and flavonoids are the main biologically active compounds identified in plants from *Montanoa* genus, including *M. tomentosa, M. frutescens* and *M. grandiflora* [39,40]. With respect to the anxiolytic-like effect produced by *M. tomentosa*, it is possible that terpenes or flavonoids could be involved, considering that these kinds of compounds have the capacity to target the GABAergic system through the activation of $GABA_A$ receptors. $GABA_A$ receptor is a heteropentameric structure containing several subunits with specific recognition sites to

γ-aminobutyric acid (GABA), benzodiazepines (i.e. diazepam), alcohol, barbiturates, neurosteroids such as progesterone and allopregnanolone[41] and natural chemical compounds (i.e. some members of the family of terpenoids, polyacetylenic alcohols and flavonoids) obtained from some plant extracts[4,42]. The $GABA_A$ receptor is characterized by their recognition of benzodiazepines and generally their activation increase the intracellular concentration of chloride ion hyperpolarizing the neuron, a physiological mechanism that is involved in the typical anxiolytic, hypnotic, sedative and anticonvulsant effects produced by benzodiazepines and other GABAergic agonists[43]. The $GABA_A$ receptor is the main target of clinically effective anxiolytic drugs, neurosteroids[2] and biologically active substances of some medicinal plants including flavonoids and some terpenes[44], [45, 46, 47,]. Thus, it is tempting to suggest that the biologically active compounds contained in the extract of *M. tomentosa* (i.e. kaurenoic, grandiflorenic or kauradienoic acids, among others) target, at least partially, the central GABAergic system to exert its anxiolytic-like effects in this animal model. In fact, in a previous study[19] in which the anxiolytic-like effect of the aqueous crude extract of *M. frutescens* was tested, the pre-treatment with picrotoxin, a non-competitive antagonist of the $GABA_A$ receptor, cancel the anxiolytic-like effect of the plant extract. As above mentioned, data of that study using diazepam as a reference of clinically effective anxiolytic drug support the hypotheses that *Montanoa tomentosa* may produce their anxiolytic-like effects through actions on the $GABA_A$ receptor. Nonetheless, the bioactive compounds contained in the *M. tomentosa* extract could also interact with other neurotransmitter systems (i.e. oxitocinergic, serotoninergic, dopaminergic or noradrenergic) that together could contribute to the anxiolytic-like effects of this plant.

CONCLUSION

Results of this study provide evidence that acute administration of *M. tomentosa* aqueous extract produces an anxiolytic-like effect in rats with long-term absence of ovarian hormones, similar to diazepam. Additionally, our data support the traditional use of this plant in the maintaining of emotional state in women, as it was written in antiques codex by ancient Mexican cultures.

ACKNOWLEDGMENTS

This study was partially supported by *Universidad Veracruzana* (*Cuerpo Académico UVE-CA-25* and Programa de Fortalecimiento Académico del Posgrado de Alta Calidad I010/458/2013, C-703/2013 y I010/152/2014 C-133/2014) and *Universidad Autónoma de Tlaxcala* (*Cuerpo Académico* UATX-CA-215).

REFERENCES

1. A.M. Paoletti, S. Floris, M. Mannias, M. Orru, D. Crippa, R. Orlandi, M.M. Del Zompo, G.B. Melis. Evidence that cyproterone acetate improves psychological symptoms and enhances the activity of the dopaminergic system in postmenopause. *J Clin Endocrinol Metab*, 2001, 86(2): 608-612.
2. G. Pisani, L. Facioni, F. Fiorani, G. Pisan. Psychosexual problems in menopause. *Minerva Ginecol*, 1998, 50(3): 77-81.
3. J.T. Bromberger, K.A. Matthews, L.L. Schott, S. Brockwell, N.E. Avis, H.M. Kravitz, S.A> Everson-Rose, E.B. Gold, M. Sowers, J.F. Randolph. Depressive symptoms during the menopausal transition: the Study of Women's Health Across the Nation (SWAN). *J Affect Disord*, 2007, 103(1-3): 267-272.

4. J. Nilsson, O. Sterner. Modulation of GABA (A) receptors by natural products and the development of novel synthetic ligands for the benzodiazepine binding site. *Curr Drug Targ*, 2011, 12(11): 1674-1688.
5. M.A. Freedman. Quality of life and menopause: the role of estrogen. *J Womens Health (Larchmt)*, 2002, 11(8): 703-718.
6. H.D. Nelson.. Menopause. *Lancet*, 2008, 371(9614): 760-770.
7. A.J. Rapkin, S.A. Winer. Drospirenone: a novel progestin. *Expert Opin Pharmacother*, 2007, 8(7): 989-999.
8. C.N. Soares, J.R. Poitras, J. Prouty. Effect of reproductive hormones and selective estrogen receptor modulators on mood during menopause. *Drugs Aging*, 2003, 20(2): 85-100.
9. S.E. McNagny. Prescribing hormone replacement therapy for menopause symptoms. *Ann Intern Med*., 1999, 131(8): 605-616.
10. D.N. Kalu D. The ovariectomized rat model of postmenopausal bone loss. *Bone Miner*1991, 15(3): 175-191.
11. R. Boseé, T. Di Paolo. Dopamine and $GABA_A$ receptor imbalance after ovariectomy in rats: Model of menopause. *J Psychiatr Neurosci*, 1995, 20(5): 364-371.
12. R. Bossé, T. Di Paolo. The modulation of brain dopamine and $GABA_A$ receptors by estradiol: a clue for CNS changes occurring at menopause. *Cell Mol Neurobiol*, 1996, 16(2): 199-212.
13. O. Picazo, E. Estrada-Camarena, A. Hernández-Aragón. Influence of the post-ovariectomy time frame on the experimental anxiety and the behavioural actions of some anxiolytic agents. *Eur J Pharmacol*, 2006, 530(1-2): 88-94.
14. J.F. Rodríguez-Landa, J.D. Hernández-Figueroa, B.C. Hernández-Calderón, M. Saavedra. Anxiolytic-like effect of phytoestrogen genistein in rats with long-term absence of ovarian hormones in the black and white model. *Prog Neuropsychopharmacol Biol Psychiatr*, 2009, 33(2): 367-372.
15. J.F. Rodríguez-Landa, F. Hernández-López, M. Saavedra. Involvement of estrogen receptors in the anxiolytic-like effect of phytoestrogen genistein in rats with 12-weeks postovariectomy. *Pharmacol Pharm*, 2012, 3(4): 439-446.
16. I.A. De Cruz-Badiano Codex. *Libellus de medicinalibus indorum herbis*. Ediciones del Instituto Mexicano del Seguro Social. México. 1552.
17. F. Ximenez. Quatro libros de la naturaleza y virtudes de las plantas y animales que están recevidos en el uso de medicina en la Nueva España, y la método, y corrección y preperación que para administrarllas se requiere con lo que el doctor Francisco Hernández escrivio en lengua latina. Viuda de Diego López Davalos, México. 1615.
18. M. Carro-Juárez, E. Cervantes, M. Cervantes-Méndez, G. Rodríguez-Manzo. Aphrodisiacs properties of *Montanoa tomentosa* aqueous crude extract in male rats. *Pharmacol Biochem Behav*, 2004, 78(1): 129-134.
19. M. Carro-Juárez, J.F. Rodríguez-Landa, M.L. Rodríguez-Peña, M.J. Rovirosa-Hernández, F. García-Orduña. The aqueous crude extract of *Montanoa frutescens* produces anxiolytic-like effects similarly to diazepam in Wistar rats: Involvement of $GABA_A$ receptor. *J Ethnopharmacol*, 2012, 143(2): 592-598.
20. National Research Council. Guide for the care and use of laboratory animals [publication no. 85-23]. National Academy Press. USA. 1996.

21. Norma Oficial Mexicana NOM-062-ZOO-1999. Especificaciones Técnicas para la Producción, Cuidado y Uso de los Animales de Laboratorio. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. México.
22. M. Carro-Juárez, M.A. Franco, M.L. Rodríguez-Peña. Increase of the ejaculatory potency by the systemic administration of aqueous crude extracts of cihuapatli (*Montanoa* genus) plants in spinal male rats. *J Evid Based Compl Altern Med,* 2014, 19(1): 43-50.
23. C.M. Contreras, J.F. Rodríguez-Landa, A.G. Gutiérrez-García, M.R. Mendoza-López, R.I. García-Ríos, J. Cueto-Escobedo. Anxiolytic-like effects of human amniotic fluid and its fatty acids in Wistar rats. *Behav Pharmacol,* 2011, 22(7): 655-662.
24. A.A. Walf, C.A. Frye. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Prot,* 2007, 2(2): 322-328.
25. A.V. Kalueff, P. Touhimaa. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioural stress research. *J Neurosc Meth.,* 2004, 143(2): 169-177.
26. A.V. Kalueff, P. Tuohimaa. Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). *Behav Brain Res,* 2005, 160(1): 1-10.
27. G. Griebel, R.J. Rodgers, G. Perrault, D.J. Sanger. Risk assessment behaviour: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. *Pharmacol Biochem Behav,* 1997, 57(4): 817-827.
28. G.B. Varty, C.A. Morgan, M.E. Cohen-Williams, V.L. Coffin, G.J. Carey. The gerbil elevated plus-maze: I. Behavioral characterization and pharmacological validation. *Neuropsychopharmacology,* 2002, 27(3): 357-370.
29. A. Fernández-Guasti, A. Ferreira, O. Picazo. Diazepam, but not buspirone, induces similar anxiolytic-like actions in lactating and ovariectomized Wistar rats. *Pharmacol Biochem Behav,* 2001, 70(1): 85-93
30. S. Pellow, P. Chopin, S.E. File, M. Briley. Validation of open:closed arm entries in the elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Meth,* 1985, 14(3): 149-167.
31. M.A. Wilson, P.R. Burghardt, K.A. Ford, M.B. Wilkinson, S.D. Primeaux. Anxiolytic effects of diazepam and ethanol in two behavioral models: comparison of males and females. *Pharmacol Biochem Behav,* 2004, 78(3): 445-458.
32. E. Engin, D. Treit. The anxiolytic-like effects of allopregnanolone vary as a function of intracerebral microinfusion site: the amygdala, medial prefrontal cortex, or hippocampus. *Behav Pharmacol.,* 2007, 18(5-6): 461-470.
33. C. Gómez, A. Saldivar-González, G. Delgado, R. Rodríguez. Rapid anxiolytic activity of progesterone and pregnanolone in male rats. *Pharmacol Biochem Behav,* 2002, 72(3): 543-550.
34. D.S. Reddy, B.W. O'Malley, M.A. Rogawski. Anxiolytic activity of progesterone in progesterone receptor knockout mice. *Neuropharmacology,* 2005, 48(1): 14-24.
35. V. Dutt, V.J. Dhar, A. Sharma. Antianxiety activity of *Gelsemium sempervirens*. *Pharmaceut Biol,* 2010, 48(10): 1091-1096.
36. M. Herrera-Ruiz, A. González-Carranza, A. Zamilpa, E. Jiménez-Ferrer, M. Huerta-Reyes, Navarro-V.M. García. The standardized extract of *Loeselia mexicana* possesses anxiolytic activity through the γ-amino butyric acid mechanism. *J Ethnopharmacol,* 2011, 138(2): 261-267.
37. P.S. D'Aquila, A.T. Peana, V. Carboni, G. Serra. Exploratory behaviour and grooming alter repeated restraint and chronic mild stress: effect of desipramine. *Eur J Pharmacol,* 2000, 399(1): 43-47.

38. T. Hata, Y. Nishimura, T. Kita, E. Itoh , A. Kawabata. The abnormal open-field behavior of SART-stressed rats and effects of some drugs on it. *Jp J Pharmacol,* 1988, 48(4): 479-490.
39. A.J. Gallegos. The zoapatle I. A traditional remedy from Mexico emerging to modern times. *Contraception,* 1983, 27(3): 211-225.
40. A. Guzmán-Duran, M.A. Wens, H. Ponce-Monter, N. Pedrón, A.J. Gallegos. Zoapatle: XIII. Isolation of water soluble fractions from *Montanoa frutescens* and some biological activities. *Arch Invest Méd (Mex),* 1988, 19(2): 157-163.
41. J. Bormann. The 'ABC' of GABA receptors. *Trends Pharmacol Sci.*, 2000, 21(1): 16-19.
42. F. Wang, M. Shing, Y. Huen, S.Y. Tsang, H. Xue. Neuroactive flavonoids interacting with GABAA receptor complex. *Curr Drug Targ CNS Neurol Disord,* 2005, 4(5): 575-85.
43. S.J. Enna. The GABA receptors. In: *The GABA receptors*. Enna SJ, Möhler H. Humana Press Inc., 2007, USA. 1-21.
44. R. Avallone, P. Zanoli, G. Puia, M. Kleinschnitz, P. Schreier, M. Baraldi. Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. *Biochem Pharmacol,* 2000, 59(11): 1387-1394.
45. C.A. Costa, D.O. Kohn, V.M. de Lima, A.C. Gargano, J.C. Flório, M. Costa. The GABAergic system contributes to the anxiolytic-like effect of essential oil from *Cymbopogon citratus* (lemongrass). *J Ethnopharmacol,* 2011, 137(1): 828-836.
46. O. Grundmann, J. Nakajima, S. Seo, V. Butterweck. Anti-anxiety effects of *Apocynum venetum* L. in the elevated plus maze test. *J Ethnopharmacol,* 2007, 110(3): 406-411.
47. P. Zanoli, R. Avallone, M. Baraldi. Behavioral characterization of the flavonoids apigenin and chrysin. *Fitoterapia*. 71(Suppl 1), 2000, S117-S123.

*** Corresponding author: Dr. Juan Francisco Rodríguez Landa**
[1] Instituto de Neuroetología, Universidad Veracruzana. Xalapa, Veracruz. México
juarodriguez@uv.mx