RESEARCH ARTICLE   **OPEN ACCESS**

EFFECTS OF ACUTE ADMINISTRATION OF VALERIANA OFFICINALIS EXTRACT IN WISTAR RATS SUBMITTED TO STRESS

Renan Santiago Pereira*[a], Annik Ianara de Souza Greco[b], Airton José Mendes[a,b], Wilson Roberto Malfará[c], Maricelma da Silva Soares de Souza[b], Sandra Maria Barbalho[a,b,d], Adriano CressoniAraujo[a,b], Patricia C. dos Santos Bueno[b,e]; Elen Landgraf Guiguer[a,b,d]

[a]Postgraduate Program in Structural and Functional Interactions in Rehabilitation, University of Marília (UNIMAR), Marília, Brazil; [b]Department of Biochemistry and Pharmacology, School of Medicine, University of Marília (UNIMAR), Marília, Brazil; [c]Barão de Mauá University Center, Ribeirão Preto, Brazil; [d]Department of Biochemistry and Nutrition, Faculty of Food Technology of Marília, Marília, Brazil, [e]Department of Animal Sciences, School of Veterinary Medicine, University of Marília (UNIMAR), Avenida Higino Muzzi Filho 1001, Marília 17525-902, SP, Brazil

ARTICLE INFO

ABSTRACT

Entre Anxiety disorders and post-traumatic stress disorder affect a considerable part of the population and can cause manifestations of fear and anxiety. Pharmacological treatment is based on the use of benzodiazepines and antidepressants and has limitations. In this sense, the search for effective and safer treatments is necessary. *Valeriana officinalis* is a plant widely used to treat stress, insomnia and anxiety states. Valerenic acid is a compound that have demonstrated a sedative effect through GABA-like activity.This study aimed to evaluate the behavioral effects of the acute administration of *V. officinalis* extract in male Wistar rats submitted to the modified forced swimming model. The animals were divided into 4 groups (n = 8), treated via gavage, as follows: G1 and G2 – treated with saline; G3 and G4 – treated respectively with extract of *V. officinalis* at doses of 22 and 66 mg/Kg. For stress induction, groups G2, G3 and G4 were submitted to swimming. All groups had their behavior evaluated in the elevated plus maze model. Behavior evaluation showed that swimming was adequate to induce anxiety in animals. However, at the doses used, the administration of *V. officinalis* extract was not able to attenuate the levels of anxiety.

Citation: *Renan Santiago Pereira, AnnikIanara de Souza Greco, Airton José Mendes, Wilson Roberto Malfará, Maricelma da Silva Soares de Souzab, Sandra Maria Barbalho, Adriano CressoniAraujo, Elen Landgraf Guiguer.* "Effects of acute administration of valeriana officinalis extract in wistar rats submitted to stress", *International Journal of Development Research*, 12, (04), 55056-55060.

INTRODUCTION

Stress is an increasingly frequent problem in modern society, being present in approximately 90% of the world population (Kurebayashi *et al*; 2016). It consists of a nonspecific response, in which the organism, when faced with a stressor, produces a physiological reaction accompanied by a concomitant emotional response (Chrousos, 2009; Koolhaas, 2011). This emotional response is determined in part by the perception of the imminence of the threat, or even by the anticipation of a future threat, expressed as fear and anxiety (Anderson and Adolphs, 2014; Hide *et al*., 2019). Over the past 50 years, the concept of stress has evolved significantly, along with a better understanding of the underlying neurobiology. Instead of only considering its biology relevant under unusual and threatening conditions, it is currently understood as a continuous and adaptive process of assessing the environment, and enabling the individual to anticipate and deal with future challenges (McEwen & Akil, 2020).

Anxiety is an emotional state associated with the perception of threat, characterized by feelings of apprehension, due to the expectation of potential threat (Kraeuter, Guest and Sarnay, 2019) or in response to acute or chronic exposure to stressors (Ray, Gulati and Rai, 2017) and, according to the American Psychiatric Association (APA, 2013), it is present in disorders related to stressors, including post-traumatic stress disorder (PTSD). PTSD affects 1-14% of the population, being more prevalent in adult women, and may vary according to the stage of development, occupation, risk of exposure to triggering factors (Martins-Monteverde *et al*, 2017). Symptomatic pharmacological treatment of this condition can be done with the use of selective serotonin reuptake inhibitors. However, these drugs, in addition to not being as effective, are implicated in some undesirable effects that can compromise treatment adherence (Akinnusi and El Solh, 2019; Steardo Jr, 2021). In this sense, complementary therapies, especially the use of medicinal plants in the treatment of anxiety disorders and PTSD, have been increasing in recent years (Dantas *et al*., 2017; Hou, 2020; Sarris, 2018), with emphasis on species of the genus *Valeriana*

(Schafer *et al.*, 2021; Shinjyo, Waddell and Green, 2020). The genus *Valeriana* includes several widely used species, among which *Valeriana officinalis* (*V. officinalis*) stands out (Schafer *et al.*, 2021; Shinjyo, Waddell and Green, 2020; Thomas *et al.*, 2016). This plant has been used since ancient times for various health problems. There is evidence of antispasmodic, hypnotic, sedative, anticonvulsant, anxiolytic and antidepressant effects. It has currently been used primarily for sleep and anxiety disorders (Das *et al.*, 2021; Guadagna *et al.*, 2020; Lans, 2019), and is potentially useful in the treatment of anxiety present in PTSD. For the above mentioned reasons, this study aimed to evaluate the effects of the acute administration of the dry extract of V. *officinalis* is used in the control of anxiety, being important its evaluation in animal models such as forced swimming and ECL.

METHODS

Ethics: Our study only started after the approval by the Animal Use Ethics Committee of the University of Marilia (UNIMAR) – Marilia – Sao Paulo - Brazil - protocol number 019/2019.

Plant material and clonazepan: *Valeriana* dry extract and clonazepam was obtained from the local markets in the city of Marília - Sao Paulo - Brazil. The intra-gastric administration of the dry extract was prepared in concentrations of 22mg/mL and 66mg/mL, diluted in saline solution 0.9%. As a reference of anxiolytic drug, clonazepam was used. This drug was prepared in saline solution 0.9% at a concentration of 5mg/mL.

Animals and modified forced swimming model: Male albino Wistar rats (n = 40), weighing around 180 - 220g were included. During all the experimental protocol, all the animals received rat food and water *ad libitum*. The rats were randomly separated into five groups (G1-G5) with eight animals each group. They were placed in plastic boxes (40x30x17cm; 4 animals per box) and were acclimated for ten days to the laboratory conditions with controlled temperature (20°C - 22°C), and light / dark cycle of 12/12 hours. The forced swimming test was performed according to the modified Model of Porsolt(PORSOLT *et al.*, 1978). In a pre-test the animals were submitted to swimming in a cylindrical container (50 cm high by 30 cm in diameter / 15 minutes). The water in this container (25-27°C) reached height of 30 cm. This pre-test was the first contact of animals with the stressful situation. Twenty-three hours later, the animals were treated according to the protocol explained below. After 60 minutes, animals were submitted to swimming in the same conditions as before, but only for 5 minutes. The second swimming phase was filmed to evaluate the behavioral parameters such as swimming (circular and horizontal movements), climbing (escape attempts and vertical movements), and immobility (absence of other movement to those necessary to the maintenance of the animal's head out of the water).

Behavioral test and Elevated Plus Maze: After the second swimming phase, the behavior was evaluated with the Elevated Plus Maze (EPM) (BOERNGEN-LACERDA e SOUZA-FORMIGONI, 2000; BLANCHARD *et al.*, 2001). This apparatus is constructed of wood, and is located 100 cm from the ground. It presents two open and opposed arms, and two enclosed arms. Stands with the same size of the open arms across them perpendicularly, delimiting a central area of 10 cm^2. The rats were placed in the EPM for 5 minutes with the intention of analysing the time spent and the frequency in the central area, and in the open and closed arms. The tests were performed in a separate room with no sound, and controlled temperature, light, and air exhaustion. The experimental protocol was performed during the day. The rats were filmed for 5 minutes to evaluate the frequency of entrances and the time spent in the center, and in the open and closed arms of the EPM. With these parameters, we calculated the anxiety index according to [(open arm time/300) + (open arm entries / total entries/2)], as described by Huynth *et al.* (HUYNH *et al.*, 2011).

Experimental protocol

The experimental groups treated via gavage were:

- G1 (Saline): treated with saline solution, 1mL/kg;
- G2 (Saline + swimming): treated with saline solution, 1mL/kg;
- G3 (Treated + swimming): treated with *V. officinalis* at a dose of 22mg/kg;
- G4 (Treated + swimming): treated with *V. officinalis* at a dose of 66mg/kg and

The animals in groups G2, G3 and G4 were subjected to stress through the forced swimming test described above, as follows:

- Swimming for 15 minutes (pre-test);
- Treatment (depending on the groups), 23 hours after the pre-test and
- Swimming for 5 minutes, 60 minutes after treatment.

After swimming, the animals were dried and transferred to an isolated room for behavior evaluation using the LCE test.

Statistical analysis: Statistical analysis was performed using the GraphPad Prim 5.0 software. The results were expressed as Mean ± Standard Error of the Mean (SEM) and were submitted to the normality test and later submitted to analysis of variance complemented by Tukey's test. The significancelevelconsideredwas 5% (p<0.05).

RESULTS

Behavior evaluation in the modified forced swimming model. Figure 1 shows the immobility time of the animals submitted to the swimming test. No significant differences were observed between the groups.
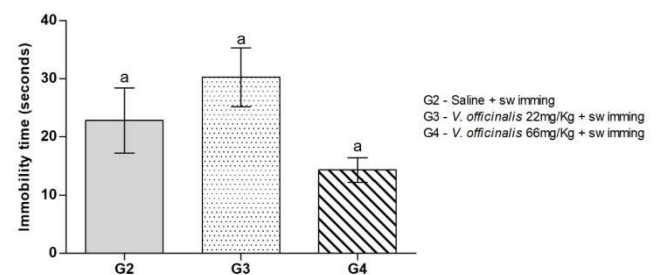

Figure 1. Effect of acute administration of *V. officinalis* extract on immobility time in the modified forced swimming model. Results expressed as Mean ± SEM. There was no statistically significant difference according to the Tukey test (p>0.05)

Figure 2 demonstrates the time spent by the animals in the open and closed arms. The results obtained show that the time spent in the open arms of group G1 (saline, without swimming) was significantly longer when compared to groups G2 (saline + swimming), G3 (V. *officinalis* 22mg/kg + swimming) and G4 (*V. officinalis* 66mg/kg + swimming), indicating a less anxious behavior in relation to the others. This fact is corroborated by the longer time spent by groups G2, G3 and G4 in the closed arms (p<0.05) in relation to G1.
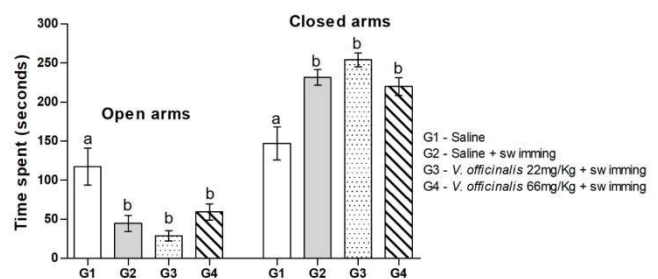

Figure 2. Effects of swimming and acute administration of *V. officinalis* extract on length of stay in open and closed arms in the elevated plus maze model. Results expressed as Mean ± SEM. Different letters for statistically different groups according to the Tukey test (p<0.05)

The number of times the animal passes through the center of the plus maze, as well as the total number of entries in the open and closed arms may be related to exploratory capacity, which in turn may be an indicator of locomotor activity, but also of anxiety. Figure 3 demonstrates that the animals that were not submitted to swimming moved more and with a greater number of entrances in the open arms.
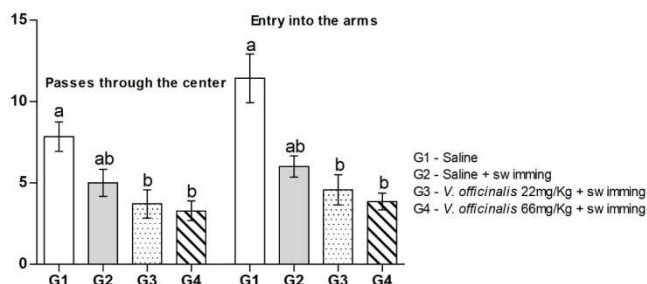

Figure 3. Effects of swimming and acute administration of V. *officinalis* extract on locomotion in the elevated plus maze model, assessed through the number of passes through the center and the total number of entries in the open and closed arms. Results expressed as Mean ± SEM. Different letters for statistically different groups according to the Tukey test (p<0.05).

It can be seen in Figure 4 that the anxiety index was statistically higher (p<0.05) for groups G2 and G3 in relation to G1, the same not being observed for G4.
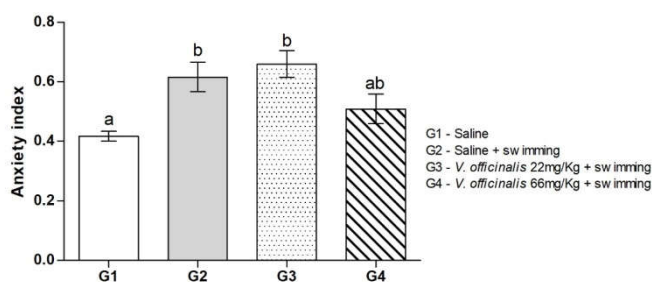

Figure 4. Effects of swimming and acute administration of V. *officinalis* extract on anxiety index. Results expressed as Mean ± SEM. Different letters for statistically different groups according to the Tukey test (p<0.05)

DISCUSSION

Animal models have been extremely important for the study of the pathophysiology of neuropsychiatric diseases as well as for the discovery of new pharmacological therapies used for these diseases (Harro, 2018). In this sense, behavioral models will not necessarily assess a specific psychiatric illness but must be able to reproduce the biological and behavioral symptoms present in them (Kraueter, Guest and Sarnyai, 2019). For the induction of fear and anxiety, symptoms present in post-traumatic stress disorders (PTSD), models can be used in which the animal is exposed to stressful or traumatic situations such as psychological (exposure to the predator or its odor), physical (underwater trauma, electric shock and physical restraint), social as "housing instability or early maternal withdrawal (Campos et al., 2013; Whitaker, Gilpin and Edwards, 2014).The model used for stress induction in this work was a modification of the swimming model described by Porsolt (1978), which originally exposed the animal to a 15 cm column of water. In the present study, the water column was increased to 30 cm, not allowing the animal's tail to rest on the bottom of the container. This makes swimming a stressor agent since the physical exercise performed by the animal as a result of the height of the water column, associated with a new environment with impossibility of escape, will provoke a set of simultaneous behavioral responses that induce the a conflicting situation (Kraueter, Guest and Sarnyai, 2019).Calil et al. (2002) described that movement time and immobility are related to the level of anxiety, since immobility would

represent a better adaptive response of the animal to a stressful situation, meaning a lower degree of anxiety. The results of the present study showed that the administration of V. *officinalis* extract was not able to significantly modify the immobility time in animals exposed to swimming, suggesting that the doses used did not present an anxiolytic effect (Figure 1). In addition to immobility in swimming as an anxiety parameter, studies have shown that the elevated plus maze (EPM) is the gold standard for performing these assessments.

This model generates a survival conflict in the animal by exposing two distinct behaviors, with the natural tendency to explore new environments contrasting with the aversive characteristics of an open and elevated space. This conflict results in behaviors correlated with anxiety, and the increase in exploration and permanence in the open arms are indicative of a lower level of anxiety, while a greater permanence and exploration of the closed arms (avoiding the open arms) can be interpreted as greater anxiety level (Arantes et al., 2013; Campos et al., 2013; Riul and Almeida, 2020). Thus, time spent in open arms is often used as a measure of anxiety (Cruz and Carobrez, 2006), and this model is useful in the discovery and selection of new compounds with anxiolytic properties capable of minimizing this manifestation of PTSD (Campos, 2013). ; Kraueter, Guest and Sarnyai, 2019). In this work, the results obtained in the elevated plus maze showed that the animals that were not submitted to the stressor agent (G1) showed a longer stay in the open arms and less stay in the closed arms compared to the group submitted to swimming G2 (Figure 2) , which demonstrates a higher level of anxiety. Sturman, Germain and Bohacek (2018) described that stressful experiences in rodents are associated with anxiety disorder, a fact that can be observed in animal models in which those less anxious tend to explore the environment more. The present study evaluated the exploratory capacity of the animals in the LCE model using the total number of entries in the arms and the number of passes through the center as parameters. As shown in Figure 3, the total number of entries in the arms and the number of passes through the center of the ECL of the animals submitted to swimming (G2) was significantly lower (p<0.05) when compared to the control group (G1), indicating less exploratory behavior, suggesting anxious behavior. In fact, the anxiety index (Figure 4) was higher in the G2 group. However, the groups treated with V. *officinalis* both at 22mg/kg and 66mg/kg did not show a statistically significant difference in the time spent in the open and closed arms in relation to the group submitted to swimming (G2) but significant in compared to the control group (G1), as shown in Figure 2. This demonstrates that, at the doses used, the administration of this plant extract was not able to reduce the anxiety behavior in the animals. The absence of anxiolytic effect is also demonstrated by the exploratory capacity and the anxiety index (Figures 3 and 47), which showed no difference between the groups submitted to swimming treated with saline solution (G2) and with V. *officinalis* extract (G3 and G4). However, it is important to highlight that there was no significant difference in the anxiety index between groups G2 and G4, which could be indicative of anxiolytic activity at higher doses.

More than 150 constituents have been identified in *Valerian*, among which we can highlight alkaloids, flavonones, sequiterpenes such as valerenic acid, valepotriates (Nandhini et al., 2018; Savage et al., 2018) and amino acids such as aminobutyric acid (GABA), tyrosine , arginine, glutamine and hydrocarbons (Yao et al., 2007). The sedative effects of *Valerian* species are attributed to terpene alcohols called valepotriates and volatile oils (which include monoterpenes and sesquiterpenes). The active compounds present in the aqueous extract of the root are amino acids (glutamate) and valerenic acid. It has been suggested that the bioactive compounds of this plant interfere with the GABA (Gamma Amino Butyric Acid) receptor in a similar way to benzodiazepines (Guadagna et al., 2020; Lans, 2019). You et al. (2012) investigated the effects of different doses of a compound containing the species *Valerianajatamansi* in mice submitted to the LCE test and observed significant changes in the time spent in the open arms and in the percentage of entries in the open arms at the highest doses used (2.4 and 4.8mg/Kg orally). Study conducted by Becker et al. (2014) demonstrated the anxiolytic effect of *Valerian*

extract at a dose of 0.5mg/kg administered orally, in the LCE model. The animals that received extract showed prolonged times in the open arm, which shows a significant anxiolytic effect. The authors also evaluated the effect of *Valerian* extract with different proportions of valerenic acid and observed that by including acetoxy valerenic acid there was abolition of the anxiolytic action, suggesting that the anxiolytic effect of *Valerian* extract is associated with its content of valerenic acid. Murphy et al., 2010 investigated the effects of V. *officinalis* in rats that received diazepam (1mg/kg), ethanol (1ml/kg), valerenic acid (3mg/kg), V. *officinalis* root extract (3mg/kg), or valerenic acid and exogenous GABA solution (75µg/kg and 3.6µg/kg, respectively). All animals submitted to ECL showed a significant decrease in anxiety levels when treated with *Valerian* or valerenic acid compared to the control group with ethanol, suggesting that *Valeriana officinalis* is a potential alternative to treat anxiety. V. *officinalis* actions have also been investigated in clinical trials involving humans. In a double-blind, randomized, placebo-controlled clinical trial, Roh et al. (2019) investigated the effects of *Valerian* root on resting state connectivity changes. This study was conducted on sixty-four volunteers who suffered from psychological stress and were given either the plant extract (100mg) or placebo three times a day. The studied groups showed significant improvement in the clinical scale. The treated group showed a significant increase in the frontal region of the brain associated with anxiolysis, indicating that the use of V. *Officinalis* root modifies the functional brain connectivity associated with anxiety. In another clinical trial, Farah et al. (2019) evaluated the effects of V. *Officinalis* (100mg) on anxiety control during third molar extraction compared to midazolam (15mg), a benzodiazepine commonly used in dental procedures, and observed that blood pressure, heart rate, and respiratory rate were significantly higher. lower in patients who used benzodiazepines. However, the use of the plant produced the necessary comfort and relaxation without sedation and less drowsiness when compared to midazolam.

Mineo et al. (2017) investigated the effects of V. *Officinalis* in a double-blind, randomized, placebo-controlled study. Participants were assigned to receive 900 mg of 0.8% valerenic acid or placebo. The results showed that a single oral dose of valerenic acid can modulate intracortical facilitatory circuits. An advantage of using extracts or products of V. *officinalis* is the occurrence of few side effects, which are mainly related to mild and transient dizziness, sedation and withdrawal symptoms. One drawback is that these plant products need to be monitored for interactions with other sedatives, anesthetics, and hypnotics (Block, Gyllenhaal, and Mead, 2004; Lans, 2019). Contrary to the studies described above, in the present study, an anxiolytic effect of V. *Officinalis* was not observed, a fact that may be related to the dose used, to the animals or even to the concentration of its constituents in the plant extract used, since a phytochemical analysis was not performed. the same. It is important to point out that, contrary to the present study, the anxiolytic effect of V. *Officinalis* was not evaluated in animal models that use swimming to induce stress. Due to the potential anxiolytic effect of V. *officinalis* and its compounds, already demonstrated in several works, further studies should be carried out in order to evaluate this effect using swimming to induce stress, as well as doses higher than those used. used in the present work.

REFERENCES

Akinnusi M, El Solh AA (2019) Drug treatment strategies for insomnia in patients with post-traumatic stress disorder, Expert Opin on Pharmacother, 20(6):691-699.

Anderson DJ,Adolphs RA (2014) Framework for Studying emotions Across species. Cell, 157(1): 187-200.

APA (2013) - American Psychiatry Association. Diagnostic and Statistical Manual of Mental disorders - DSM-5. 5th.ed. Washington: American Psychiatric Association.

ArantesR, Tejada J, Bosco GG, Morato S, Roque AC. (2013)Mathematical methods to model rodent behavior in the elevated plus-maze. J Neurosci Methods. 222(2):141-148.

Becker A, Felgentreff F, Schroder H, Meier B, Brattstrom A. (2014) The anxiolytic effects of a *Valerian* extract is based on valerenic acid. BMC ComplementAltern Med.28:267.

Bernik M, Laranjeiras M,Corregiari F. (2003) Tratamento farmacológico do transtorno de estresse pós-traumático. Rev. Bras. Psiquiatr.25(Supl I):46-50.

Block KI, Gyllenhaal C, Mead MN. (2004) Safety and efficacy of herbal sedatives in cancer care. IntegrCancerTher.3(2):128-48.

Calil CM, Bianchi FJ, Tanno AP. (2002) Análise do significado do tempo de imobilidade em modelos experimentais de natação. RevBrasCienc Farm.38(4):479-485.

Campos AC, Fogaça MV, Aguiar DC, Guimarães FS. (2013) Animal models of anxiety disorders and stress. Rev. Bras. Psiquiatria. 35:S101–S111.

Chrousos GP. (2009) Stress and Disorders of the Stress System. Nat. Rev. Endocrinol. 5(7): 374-381.

Cruz JGP,CarobrezAP.(2006) Anxiolytic effect of spermine microinjected into the dorsal periaqueductal grey in rats. Acta. Scient. Health Sci.28(1):43-47.

Dantas LP, de Oliveira Ribeiro A, Souza LMA,GroppoFC.(2017)Effects of P*assiflora incarnata* and midazolam for control of anxiety in patients undergoing dental extraction. Med. Oral Patol. Oral Cir. Bucal. 22(1)1;95-101.

DAS G, Han-Seung S, Tundis R, Gonçalves S, Tantengco OAG, Campos MG, Acquaviva R, Malfa GA, Romano A, Robles JAH, Clores MQ, Patra J-K. (2021)Plant Species of Sub-Family *Valerian*aceae - A Review on Its Effect on the Central Nervous System. Plants. 10(5).

Davis M, Walker DL, Miles L, Grillon C. (2010) Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. Neuropsychopharmacol. 35:103–135.

de Kloet ER, Joels M, HolsboerF. (2005) Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci.6:463-475.

Dietz BM, Hajirahimkhan A, Dunlap TL, Bolton JL.(2016) Botanicals and Their Bioactive Phytochemicals for Women's Health. Pharmacol Rev.68(4):1026-1073, 2016.

Farah GJ, Ferreira GZ, Danieletto-Zanna CF, Luppi CR, Jacomacci WP. (2019) Assessment of *Valerian*a*officinalis* l. (*Valerian*) for Conscious Sedation of Patients During the Extraction of Impacted Mandibular Third Molars: A Randomized, Split-Mouth, Double-Blind, Crossover Study. J Oral Maxillofac Surg.77(9):1796.e1-1796.e8.

FiebichBL, Knorle R, Appel K, Kammler T, Weiss G. (2011) Pharmacological studies in an herbal drug combination of St. John's Wort (Hypericum perforatum) and passion flower (Passiflora incarnata): in vitro and in vivo evidence of synergy between Hypericum and Passiflora in antidepressant pharmacological models. Fitoterapia82(3):474-480.

GuadagnaS. (2020) Plant Extracts for Sleep Disturbances: A Systematic Review. Evid Based Complement Alternat Med. Abril:1-9.

HarroJ. (2018)Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. Behav Brain Res.15:81-93.

Hou W, Wang Y, Zheng P, Cui R. (2020)Effects of Ginseng on Neurological Disorders. Front Cell Neurosci. 14.

Huynth TN, Krigbaum AM, Hanna JJ, Conrad CD. (2011) Sex differences and phase of light cycle modify chronic stress effects on anxiety and depressive-like behavior. Behav. Brain Res. 222:212–222.

Hyde H, Ryan KM, Waters AM. (2019) Psychophysiological Markers of Fear and Anxiety. Curr. Psychiatry Rep. 21(7):56.

Jowett S,Karatzias T, Albert I. (2019) Multiple and Interpersonal Trauma Are Risk Factors for Both Post-Traumatic Stress Disorder and Borderline Personbality Disorder: A Systematic Review on The Traumatic Backgrounds and Clinical Characteristics of Comorbid Post-Traumatic Stress Disorder/Borderline Personality Disorder Groups Versus Single-Disorder Groups. Psychol. Psychother.352:1-18.

Koolhaas JM. (2011) Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. 35(5):1291–1301.

KraeuterAK, Guest PC,Sarnyai Z. (2019) The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. Methods Mol Biol. 1916:105-111, 2019.

KurebayashiLFS. (2016) Fitoterapia chinesa para redução de estresse, ansiedade e melhoria de qualidade de vida: ensaio clínico randomizado. Rev. Esc. Enferm. USP50(5):853-860.

LansC. (2019) Do recent research studies validate the medicinal plants used in British Columbia, Canada for pet diseases and wild animals taken into temporary care? J Ethnopharmacol.23(236):366-392.

Liu L, Liu C, Wang Y, Wang P, Li Y, Li B. (2015) Herbal Medicine for Anxiety, Depression and Insomnia. CurrNeuropharmacol,13(4):481-493, 2015.

Margis R. (2003) Comorbidade no Transtorno de Estresse Pós-Traumático: Regra ou Exceção? Rev. Bras. Psiquiatr.25:17-20

Martins-Monteverde CM, Padovan T, Juruena MF. (2017) Transtornos relacionados a traumas e a estressores. Medicina (Ribeirão Preto, Online.) Suplemento Temático: Psiquiatria I Capítulo 4, 50 (Supl. 1):37-50.

Mc Ewen BS, Akil H. (2019) Revisiting the Stress Concept: Implications for Affective Disorders. J Neurosci.40(1):12-21.

Michelan CM, Michelan LD, de Paula HMG, Katsumasa H. (2006) Imobilidade tônica e imobilidade do nado forçado em cobaias. Rev. Etol.8(2):89-95.

Mineo L, Concerto C, Patel D, Mayorga T, Paula M, Chusid E, Aguglia E, Battaglia F. (2017) *Valeriana officinalis* root extractmodulates cortical excitatorycircuits in humans. Neuropsychobiology 75(1):46-51.

Murphy K, Kubin ZJ, Shepherd JN, Ettinger RH. (2010)*Valerianaofficinalis* root extracts have potent anxiolytic effects in laboratory rats. Phytomedicine17 (8-9):674-678.

Nandhini S, Narayanan KB, Ilango K. (2018) *Valeriana officinalis*: a Review of Its Tradicional Uses, Phytochemistry and Pharmacology. *Asian J. Pharm. Clin. Res.*11(1):36-41.

Pellow S, File SE. (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. PharmacolBiochemBehav.24:525-529.

Pinto WBVR, Ko GB, Valero-Lapchik VB. (2012) Teste de labirinto em cruz elevado: aplicações e contribuições no estudo de doenças neuropsiquiátricas em modelos animais. RESBCAL. 1(1):102-120.

Porsolt RD, Anton G, Blavet N, Jalfre M. (1978) Behaviour al despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol. 47(4):379-391.

Ray A, Gulati K, Rai N. (2017) Stress, Anxiety, and Immunomodulation: A Pharmacological Analysis. Vitam Horm.103:1-25.

Riul, TR, Almeida SS. (2020) Feed restriction since lactation has reduced anxiety in adult Wistar rats. Rev Nutr.33:e.190143.

RohD, Jung JH, Yoon KH, Lee CH, Kang LY, Lee S-K, Shin K, Kim DH. (2019)*Valerian* extract alters functional brain connectivity: a randomized double blind placebo-controlled trial. Phytother Res. 33(4):939-948.

Sarris J. (2018) Herbal medicines in the treatment of psychiatric disorders: 10☐year updated review. Phytother Res. 32(7):1147-1162.

Savage K, Firth J, Stough C, Sarris J. (2018) GABA☐modulating phytomedicines for anxiety: A systematic review of preclinical and clinical evidence. Phytother. Res.32:3-18.

Schafer AM, Gilgen PM, Spirgi C, Pottert O, zuSchwabedissen HEM. (2021)Constituents of Passiflora incarnata, but Not of *Valeriana officinalis*, Interact with the Organic Anion Transporting Polypeptides (OATP) 2B1 and OATP1A2. Planta Médica, 2021.

Shinjyo N, Waddell G, Green J. (2020)*Valerian* root in treating sleep problems and associated disorders – a systematic reviews and meta-nalysis. Journal of Evidence-based integrative medicine. 25:1-31.

Sturman O, Germain PL, BohacekJ. (2018) Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. Stress 21(5):443-452.

Thomas K,CanedoJ, Perry PJ, Doroudgar S, Lopes I, Chuang HM, Bohnert K. (2016) Effects of *Valerian* on subjective sedation, field sobriety testing and driving simulator performance. Accid Anal Prev.92:240-244.

Valente NLM, Mello MF, Fiks JP. (2011) Transtorno do Estresse Pós Traumático. In: Mari JdJ, Pitta JCdN, editors. Psiquiatria por meio de casos clínicos. p. 51-66, Manole, Barueri.

Whitaker AM, Gilpin NW, Edwards S. (2014) Animal Models of Post-Traumatic Stress Disorder and Recent Neurobiological Insights. BehavPharmacol., 25:398-409.

Yao M, Ritchie HE, Brown-Woodman PD. (2007) A developmental toxicity-screening test of *Valerian*. J Ethnopharmacol.113(2):204-209.

You JS, Pend M, Shi J-L, Zheng H-Z, Liu Y, Sheng B, Guo J-Y. (2012) Evaluation of anxiolytic activity of compound *Valerian*ajatamansi Jones in mice. BMC Complement Altern Med.21:1-9.

****