



Valerian treatment during the postpartum period alters breast milk composition and impairs long-term memory in female rat offspring

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Abstract

Maternal anxiety symptoms in the perinatal period might have long-term health effects on both the mother and the developing child. Valerian is a phytotherapeutic agent that is widely used for the treatment of anxiety. This study investigated the effects of valerian treatment in postpartum rats on maternal care, toxicity, and milk composition. Postnatal development, memory, and anxiety behavior in the offspring were also assessed. Postpartum Wistar rats received the valerian (500, 1000, or 2000 mg·kg⁻¹·day⁻¹) by oral gavage. Clinical and biochemical toxicity was evaluated with commercial kits. Maternal behavior was observed daily. Milk composition was analyzed by colorimetric methods. Physical and neuromotor tests were used to analyze postnatal development. Anxiolytic activity was assessed by the elevated plus maze, and memory was evaluated by the step-down inhibitory avoidance task. Maternal toxicity and care behavior were not altered by the treatment, while only the highest dose promoted a significant increase of lactose, and the doses 1000 and 2000 mg·kg⁻¹·day⁻¹ promoted a reduction of protein contents in milk. Postnatal development was similar in all offspring. Adult offspring did not display altered anxiety behavior, while long-term memory was impaired in the female adult offspring by maternal treatment with 1000 mg·kg⁻¹·day⁻¹. These results suggested that high doses of valerian had significant effects on important maternal milk components and can cause long-term alterations of offspring memory; thus, treatment with high doses of valerian is not safe for breastfeeding Wistar rat mothers.

Key words: Anxiety; Postpartum disorders; Mental health; *Valeriana officinalis*; Lactation

Introduction

The perinatal period is complex and vulnerable. Challenges during pregnancy can cause the development of postpartum psychiatric disorders (PPDs) (1). PPDs, such as depression and anxiety, are relatively common and are described as emotional conditions with frequent anxiety symptoms and episodes of low attention span, tiredness, muscle tension, and excitement (2).

Women with psychiatric disorders during the perinatal period are at increased risk for adverse maternal and child outcomes (3). Environmental changes during the periods of enhanced plasticity (intrauterine life and/or lactation) can induce disorders in the offspring. This phenomenon is known as programming or developmental plasticity (4,5).

Mental health assessment and intervention are important components of routine perinatal care (3). Thus, drug treatment is a possible intervention to reduce postpartum anxiety and its consequences on the newborn. Complementary and alternative therapies are used more often than conventional therapies by people with self-defined

anxiety attacks and severe depression (6). Valerian, an extract of the *Valeriana officinalis* root, is one of the most popular herbal medications for insomnia and anxiety (7). Valerian contains sesquiterpenes, iridoids (valepotriates), alkaloids, free amino acids, and other chemical components (8). Valerenic acid, an essential oil, is considered the main active valerian substance that inhibits GABAase enzyme in the synaptic cleft to increase gamma-aminobutyric acid (GABA) neurotransmitter availability in the cleft and enhance its inhibitory effect on the central nervous system (CNS) (9).

The use of several extracts of *Valeriana officinalis* can lead to increased anxiolytic and antidepressant activity in rats (10). Campos et al. (11) evaluated the effects of valerian treatment on rats during pregnancy and observed no alterations in postpartum development or in adult anxiety levels in the offspring. Studies indicate that valerian has antioxidant and neuroprotective effects (12,13). The extract of *Valeriana officinalis* modulates

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brain neurotransmitters (14,15). A single oral dose of valerian extract modulates intracortical facilitatory circuits (16).

Considering that drug metabolism and excretion are limited in newborns and that the blood-brain barrier is in development, xenobiotic consumption via breastfeeding can lead to metabolite accumulation in newborns (17). Xenobiotics can lead to biological, behavioral, and developmental alterations in childhood and adulthood, which justifies the studies focusing on phytotherapeutic treatment during lactation (18).

In this context, the present study used Wistar rats to investigate the toxicity, milk composition, and maternal behavioral alterations caused by valerian treatment during lactation. Furthermore, possible effects on the physical and neuromotor development of the offspring and on long-term memory and anxiety behavior in adulthood were evaluated.

Material and Methods

Animals

The animal experiments were approved by the Ethics Committee on Animal Use of the Federal University of Juiz de Fora (protocol No. 002/2017). Seventy-five female Wistar rats (60 days) were mated with male Wistar adult rats. Animals were kept in polypropylene cages (49 × 34 × 16 cm) in ventilated cabinets (ALESCO[®], Brazil) with controlled air flow and regulated temperature (22 ± 2°C); the animals had free access to food and filtered water and were housed at a 12-h light/dark cycle.

Treatment and experimental design

The extract was obtained from a commercial powder of *Valeriana officinalis*, Florian[®] (Brazil); a physicochemical quality analysis was carried out, and the valerenic acid concentration was 0.82%. The valerenic acid was calculated by the method of Al-Majed (2006) considering the daily recommended dose for an adult human (3060 mg) and adjusting to rodent metabolism (six-fold higher than the calculated value for humans) (19), and the preparations contained aqueous extract with 20% double-distilled white glycerol (Galena[®], Brazil).

On postnatal day (PD) 2, lactating rats were randomly assigned to each of the following groups (n=15 per group): Control (1 mL of distilled water), Vehicle (1 mL of distilled water + 20% glycerol), and 3 treatment groups T-500 (valerian, 500 mg·kg⁻¹·day⁻¹), T-1000 (valerian, 1000 mg·kg⁻¹·day⁻¹), and T-2000 (valerian, 2000 mg·kg⁻¹·day⁻¹). The lowest dose (500 mg·kg⁻¹·day⁻¹) was based on the data obtained in humans recalculated for the rodent model. T-1000 and T-2000 doses were two-fold and four-fold higher than the lowest dose according to the recommendations of the reproductive toxicity trials (19). The drug was administered by oral gavage from PD 2 to PD 10. All treatments and tests were carried out between 8 and 11 am.

Animals were euthanized via diaphragmatic rupture after all *in vivo* procedures were carried out. The anesthesia protocol included a combination of 90 mg/kg of a dissociative anesthetic ketamine hydrochloride (Vetanarcol, König, Brazil) and 10 mg/kg of a muscle relaxant xylazine hydrochloride (Kensol, König). Both drugs were blended and administered together via the intraperitoneal route. Blood was extracted through cardiac puncture under general anesthesia and centrifuged (4000 g, 4°C, 10 min), and the serum was stored at -80°C.

Maternal assessment

Clinical toxicity and biochemical assays. Piloerection, diarrhea, chromodacryorrhea, and weight gain or loss were recorded during the treatment period. Food and water consumption were also registered during treatment by weighing the amount of offered food and measuring offered water in a graduated cylinder. The differences between this value and the amount remaining 24 h after food and water were evaluated (20).

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine (CRE), and serum urea nitrogen (BUN) were measured by a commercial assay kit on an automatized Cobas c111 (Roche[®], Switzerland) device.

Milk analysis. Milk samples were obtained on day 10 of lactation, which was the last day of treatment. Mothers were separated from their pups for 2 h and were injected with oxytocin (5 IU/mL sc; Eurofarm, Brazil). After 30 min, mothers were lightly anesthetized with 10 mg/kg xylazine (2%) and 90 mg/kg ketamine (5%), and milk was extracted manually from the thoracic and abdominal teats. All milk samples were analyzed for lactose, total protein, total cholesterol, and triglycerides. Lactose was estimated by a colorimetric method using picric acid. Commercial lactose (Sigma, USA) was used as a standard. Protein was estimated by a colorimetric method using bovine serum albumin (Sigma) as a standard. Total cholesterol and triglycerides were determined by enzymatic and colorimetric methods, respectively, using commercial kits (Bioclin, Brazil).

Maternal behavior. Maternal behavior was observed daily for 50 min in the housing cages of mothers and litter between PD 1 and PD 10. The time of observation was during the light phase of the light-dark cycle between 8 and 9 am. The following types of behavior were observed: nursing posture, licking and grooming, nest organization, and maintenance. For recording, “1” was used to indicate that a behavior occurred and “0” was used to indicate that a behavior did not occur. The parameters were analyzed according to Slamberová et al. (21).

Offspring assessment

Physical and motor development assessment. After offspring birth, weight gain was recorded on PD 2, 4, 6, 10, and 25. From PD 2 until the appearance of the variable,

the female and male offspring were inspected daily for physical development parameters: eye opening, ear unfolding, lanugo appearance, eruption of upper and lower incisor, descent of the testes, and vaginal opening. Neuromotor development tests, including negative geotaxis, postural response, orientation, cliff avoidance, and grasping reflex, were monitored daily. Tests were carried out daily and lasted no longer than 15 s per trial. The first day with a positive response was recorded for each animal.

Behavioral studies. On PD 60, the elevated plus maze (EPM) test and step-down inhibitory avoidance task were performed in the female and male offspring (one female and one male per litter were randomly selected).

Anxiety was evaluated using the EPM. The apparatus consisted of a cross-like structure with two opposing open arms (OA) and two closed arms surrounded by 30-cm high walls, of the same dimension (50 cm long and 10 cm high). The entry frequency and time spent in the open and closed arms were recorded for 5 min. The anxiety index was calculated according to Cohen et al. (22) as follows: Anxiety Index = $1 - \frac{[\text{Open arm time} / \text{Test duration}] + [\text{Open arms entries} / \text{Total number of entries}]}{2}$.

Long-term memory was estimated using the inhibitory avoidance test. The apparatus consisted of a 30 × 30-cm structure with 50-cm high electronic avoidance response chamber with a platform at a height of 5 cm with a diameter of 7 cm at the top surface. The platform was attached to one corner of the chamber providing protection from the electrical pulse. The chamber floor consisted of 2-mm parallel stainless steel bars spaced 0.5 cm apart.

During the training session, the animal was gently placed on the platform. When the rat stepped down from the platform and touched the grid, a 0.5 μA intensity shock was delivered for 2 s. Twenty-four hours after the training session, the rat was tested under the same conditions without electric shock. The latency of stepping down from the platform was recorded automatically with an end-point of 180 s, and the values were used to estimate the retention memory.

Statistical analysis

First, the Shapiro-Wilk test was applied to verify data normality. The data were then evaluated by one-way ANOVA followed by Dunnett's *post hoc* test. Two-way ANOVA with repeated measures was applied to certify possible alterations in body weight and food intake along the study period. The significance level of the tests was $P < 0.05$. All tests were performed using SPSS v20 (IBM, USA) and GraphPad Prism[®] 5.0 (USA) software.

Results

Effects of valerian treatment on mothers during postpartum period

No signs of clinical toxicity were observed in mothers during monitoring throughout the entire treatment period. As shown in Table 1, there were no significant differences in the serum levels of ALT, AST, urea, and creatinine between the groups.

Body weight of the mothers during the postpartum period was similar in all experimental groups and no differences were found in maternal body weight during breastfeeding (Figure 1A). Also, the differences of food intake during the postpartum period were not significant (Figure 1B).

Patterns of nursing posture, licking and grooming, nest organization, and maintenance in the mothers were similar during the treatment period indicating that all mothers used in the experiment expressed maternal behavior.

Milk composition

After the treatment, the protein content was significantly lower in the T-1000 and T-2000 groups compared to that in the control group. The content of lactose was significantly higher in the T-2000 group (44.32%) compared to that in the control group. There were no significant differences in the content of cholesterol and triglycerides in milk between the groups (Figure 2).

Offspring development

Body weight during the postnatal development of the female (Figure 1C) and male (Figure 1D) newborns was

Table 1. Plasma markers of hepatic and kidney injury of Wistar rats treated postpartum with valerian.

Parameters	Experimental groups				
	Control	Vehicle	T-500	T-1000	T-2000
AST (U/L)	89.64 ± 8.95	90.60 ± 10.89	86.31 ± 18.34	93.31 ± 12.41	86.82 ± 12.68
ALT (U/L)	70.86 ± 21.30	70.44 ± 15.40	63.49 ± 15.72	61.83 ± 9.55	59.49 ± 15.28
Urea (mg/dL)	45.67 ± 9.99	46.10 ± 10.52	46.06 ± 8.85	43.75 ± 8.47	45.59 ± 13.20
Creatinine (mg/dL)	0.29 ± 0.07	0.30 ± 0.04	0.29 ± 0.05	0.30 ± 0.05	0.31 ± 0.05

Control group: 1 mL distilled water; Vehicle group: 1 mL distilled water, 20% of glycerin; T-500: 500 mg·kg⁻¹·day⁻¹ of valerian; T-1000: 1000 mg·kg⁻¹·day⁻¹ of valerian, T-2000: 2000 mg·kg⁻¹·day⁻¹ of valerian. Data are reported as means ± SE of 15 animals per group. ALT: alanine aminotransferase (F(4, 70)=0.8709, P=0.1306), AST: serum aspartate aminotransferase (F(3, 52)=0.7095, P=0.5492), urea (F(4, 69)=0.4813, P=0.9703), and creatinine (F(4, 69)=1.399, P=0.8388). One-way ANOVA and Dunnett's test.

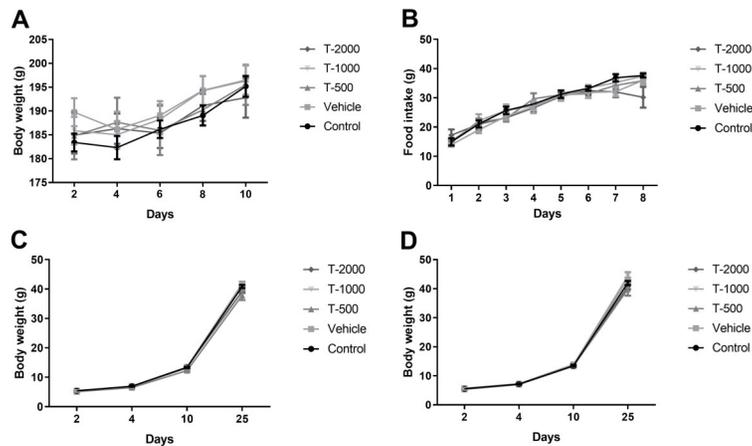


Figure 1. Effect of valerian treatment on body weight and food intake of mothers and on body weight of the offspring. Groups: Control (1 mL distilled water), vehicle (1 mL distilled water, 20% of glycerol), T-500 (500 mg·kg⁻¹·day⁻¹ of valerian), T-1000 (1000 mg·kg⁻¹·day⁻¹ of valerian), and T-2000 (2000 mg·kg⁻¹·day⁻¹ of valerian). **A**, Body weight of mothers ($F(4, 347)=1.036$, $P=0.3885$). **B**, Food intake of mothers ($F(4, 347)=8.034$, $P=0.3885$). **C**, Body weight of female offspring on postnatal days 2, 4, 10, and 25 ($F(4, 262)=6.646$, $P=0.092$). **D**, Body weight of male offspring on postnatal days 2, 4, 10, and 25 ($F(4, 254)=4.748$, $P=0.1010$). Data are reported as means \pm SE of 15 mothers/group and 4 newborns/mother. No significant difference was found among groups (2-way repeated measures ANOVA).

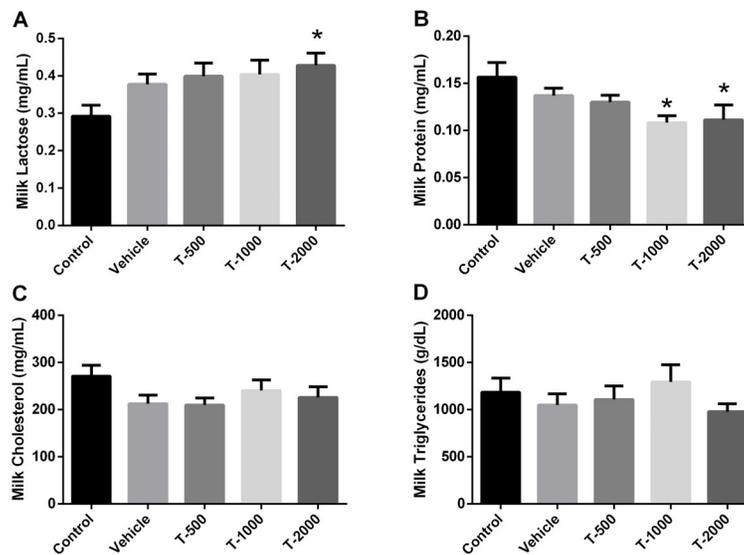


Figure 2. Effect of valerian treatment on milk composition of mothers. Values of **(A)** lactose ($F(4,75)=0.1682$, $*P=0.048$), **(B)** total protein ($F(4,71)=4.89$, $*P=0.046$), **(C)** cholesterol ($F(4,63)=4.833$, $P=0.195$), and **(D)** triglycerides ($F(4,64)=1.775$, $P=0.564$) in the control (1 mL distilled water), vehicle (1 mL distilled water, 20% of glycerol), T-500 (500 mg·kg⁻¹·day⁻¹ of valerian), T-1000 (1000 mg·kg⁻¹·day⁻¹ of valerian), and T-2000 (2000 mg·kg⁻¹·day⁻¹ of valerian) groups. $*P < 0.05$ compared to control (one-way ANOVA/Dunnett's test).

not significantly different between the groups. The days of appearance of signs indicative of physical and neuromotor parameters were similar between the experimental groups in the female and male offspring (Tables 2 and 3).

Anxiety behavior of adult offspring

The percentage of time spent in OA, percentage of number of entries into OA, and anxiety index are shown in

Figure 3A–F); the values in both the female and male offspring from valerian-treated mothers were not significantly different from those in the Control and Vehicle groups.

Long-term memory of adult offspring

The latency of descent from the platform in the female offspring (Figure 4A) was significantly shorter (by 69.49%)

Table 2. Day of appearance of the physical development signs analyzed in the groups.

Physical parameters	Gender	Experimental groups				
		Control	Vehicle	T-500	T-1000	T-2000
Lanugo	Male	3.26 ± 0.56	2.98 ± 0.48	3.04 ± 0.41	2.88 ± 0.36	2.98 ± 0.44
	Female	3.24 ± 0.54	3.10 ± 0.56	3.16 ± 0.49	2.98 ± 0.44	3.05 ± 0.61
Ear unfolding	Male	4.71 ± 0.47	4.65 ± 0.40	4.71 ± 0.66	4.53 ± 0.60	4.75 ± 0.36
	Female	4.69 ± 0.49	4.76 ± 0.35	4.70 ± 0.71	4.54 ± 0.67	4.73 ± 0.45
Hair	Male	8.28 ± 0.90	8.14 ± 0.84	8.01 ± 0.96	7.95 ± 0.44	8.31 ± 0.77
	Female	8.31 ± 0.98	8.48 ± 1.22	8.46 ± 0.90	7.98 ± 0.71	8.23 ± 0.78
Superior incisor eruption	Male	7.91 ± 0.71	8.56 ± 1.19	8.67 ± 1.06	8.41 ± 1.01	8.69 ± 1.09
	Female	7.94 ± 0.64	8.44 ± 1.00	8.66 ± 0.93	8.41 ± 1.04	8.92 ± 1.12
Inferior incisor eruption	Male	10.08 ± 0.66	10.21 ± 0.68	10.31 ± 0.85	10.03 ± 0.74	10.35 ± 0.81
	Female	10.08 ± 0.87	10.08 ± 0.75	11.45 ± 1.90	10.31 ± 0.79	10.42 ± 2.29
Eye opening	Male	16.81 ± 0.81	16.80 ± 0.58	16.96 ± 0.59	16.54 ± 0.61	16.73 ± 0.69
	Female	16.70 ± 0.78	16.62 ± 0.66	16.79 ± 0.68	16.57 ± 0.70	16.69 ± 0.53
Testis descent	Male	21.99 ± 1.20	21.73 ± 1.76	22.95 ± 1.86	22.31 ± 1.89	21.56 ± 2.09
Vaginal opening	Female	35.17 ± 2.21	34.21 ± 1.81	34.75 ± 3.31	34.71 ± 1.67	34.19 ± 2.06

Control group: 1 mL distilled water; Vehicle group: 1 mL distilled water, 20% of glycerin; T-500: 500 mg·kg⁻¹·day⁻¹ of valerian; T-1000: 1000 mg·kg⁻¹·day⁻¹ of valerian; T-2000: 2000 mg·kg⁻¹·day⁻¹ of valerian. Data are reported as means ± SE of 15 animals per group. The differences among groups were not significant (one-way ANOVA and Dunnett's test). Lanugo: Male (F(4, 70)=0.8542, P=0.2378), Female (F(4, 68)=0.5254, P=0.7174); Ear Unfolding: Male (F(4, 70)=0.3577, P=0.8029), Female (F(4, 70)=0.3524, P=0.8415); Hair: Male (F(4, 70)=1.183, P=0.6879), Female (F(4, 70)=0.7391, P=0.5921); Superior Incisor Eruption: Male (F(4, 69)=0.6426, P=0.2331), Female (F(4, 70)=0.7661, P=0.0924); Inferior Incisor Eruption: Male (F(4, 68)=0.6720, P=0.7387), Female (F(4,70)=0.8084, P=0.4354); Eye Opening: Male (F(4, 57)=0.4163, P=0.6505), Female (F(4, 58)=0.3915, P=0.9122).

Table 3. Day of appearance of the neuromotor development signs analyzed in the groups.

Reflex parameters	Gender	Experimental groups				
		Control	Vehicle	T-500	T-1000	T-2000
Grasping reflex	Male	2.06 ± 0.18	2.00 ± 0.00	2.00 ± 0.00	2.01 ± 0.06	2.01 ± 0.06
	Female	2.01 ± 0.06	2.00 ± 0.00	2.01 ± 0.06	2.03 ± 0.12	2.00 ± 0.00
Righting reflex	Male	2.15 ± 0.23	2.13 ± 0.26	2.33 ± 0.65	2.28 ± 0.46	2.31 ± 0.55
	Female	2.16 ± 0.27	2.25 ± 0.41	2.53 ± 0.65	2.36 ± 0.58	2.47 ± 0.68
Cliff avoidance	Male	8.20 ± 1.62	8.75 ± 1.70	8.86 ± 1.28	8.18 ± 1.42	8.30 ± 1.52
	Female	7.60 ± 0.93	7.67 ± 1.52	8.09 ± 1.19	8.48 ± 1.70	7.92 ± 1.06
Negative geotaxis	Male	7.05 ± 1.80	7.15 ± 1.50	6.64 ± 1.31	6.81 ± 1.34	7.30 ± 1.26
	Female	7.00 ± 1.28	7.33 ± 1.35	6.84 ± 1.19	6.81 ± 1.39	7.40 ± 1.01

Control group: 1 mL distilled water; Vehicle group: 1 mL distilled water, 20% of glycerin; T-500: 500 mg·kg⁻¹·day⁻¹ of valerian; T-1000: 1000 mg·kg⁻¹·day⁻¹ of valerian, T-2000: 2000 mg·kg⁻¹·day⁻¹ of valerian. Data are reported as means ± SE of 15 animals per group. The differences among groups were not significant (one-way ANOVA and Dunnett's test). Grasping reflex: Male (F(4, 70)=1.299, P=0.2789), Female (F(4, 70)=0.5833, P=0.6757); Righting reflex: Male (F(4, 70)=0.6120, P=0.6554), Female (F(4, 70)=0.8655, P=0.3203); Cliff avoidance: Male (F(4, 69)=0.4236, P=0.6091), Female (F(4, 68)=1.570, P=0.3605); Negative geotaxis: Male (F(4, 70)=0.8792, P=0.7442), Female (F(4, 69)=0.5593, P=0.5494).

in the T-1000 group (P < 0.01). Despite no statistical difference, the mean latency values of the other groups were: Vehicle (-21.87%), T-500 (-56.31%), and T-2000 (-42.35%).

In the male offspring, the latency of descent from the platform (Figure 4B) was: Vehicle (-1.28%), T-500 (-3.86%), T-1000 (-47.72%), and T-2000 (-17.19); however, the differences were not statistically significant (P=0.4747).

Discussion

The lack of clinical and biochemical toxicity in mothers treated with valerian in this study was in agreement with other reports in the literature on this experimental model (11). No signs of systemic toxicity were observed in mature female or male offspring after valerian treatment during pregnancy. Serum levels of AST and ALT in the mothers indicated that liver function was not affected by

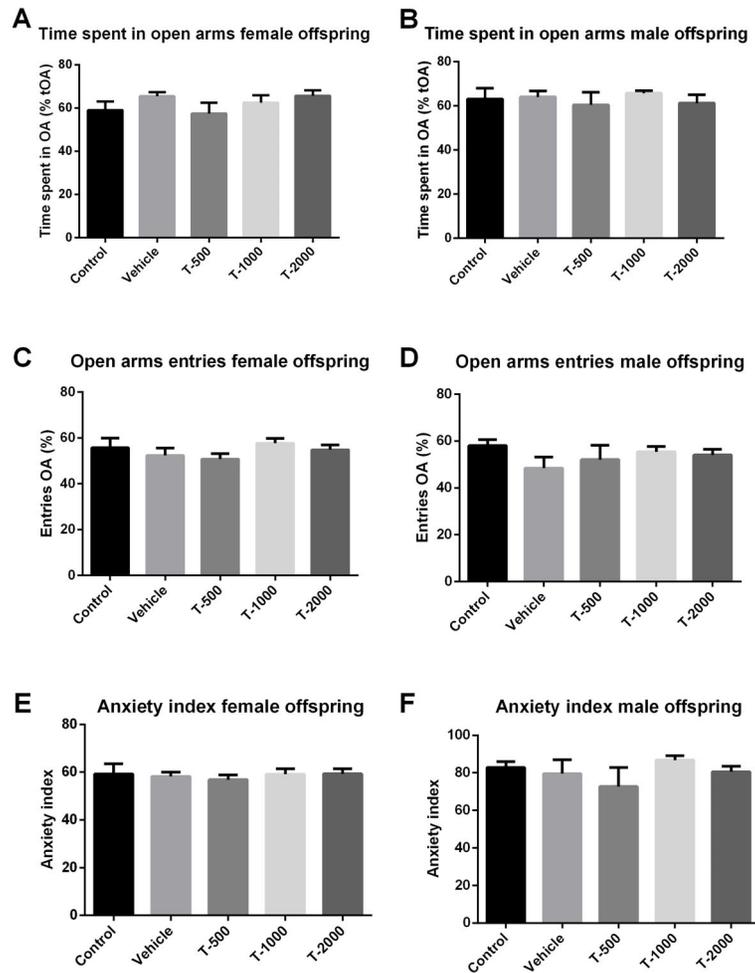


Figure 3. Anxiety-like behavior in adult offspring of mothers treated with valerian during the postpartum period. Groups: Control (1 mL distilled water), Vehicle (1 mL distilled water, 20% of glycerol), T-500 (500 mg·kg⁻¹·day⁻¹ of valerian), T-1000 (1000 mg·kg⁻¹·day⁻¹ of valerian), and T-2000 (2000 mg·kg⁻¹·day⁻¹ of valerian). **A, C, and E**, percentage of time spent in the open arms ($F(2,072, 18,65)=1.579$, $P=0.2325$), percentage of number of entries ($F(4, 53)=0.6700$, $P=0.4672$), and anxiety index ($F(4, 50)=1.357$, $P=0.9670$) of female offspring. **B, D, and F** percentage of time spent in the open arms ($F(2,031, 18,28)=0.2641$, $P=0.7740$), percentage of number of entries ($F(4, 48)=1.963$, $P=0.4912$), and anxiety index ($F(4, 41)=0.9466$, $P=0.6230$) of male offspring. Data are reported as means \pm SE. ($n=8-13$ per group). Values in the groups were normalized to those in the control group. One-way ANOVA and Dunnett's test.

valerian treatment during lactation, and creatinine and urea levels indicated normal kidney function in the treatment model.

The use of medications during lactation requires special attention because drug substances may be excreted into the milk, which is essential for newborn development, thus altering milk composition (23). In the present study, significant differences were found between the control and T-1000 and T-2000 groups (reduction in total protein) and between the control and T-2000 (increase of lactose) experimental groups, indicating that high doses of valerian significantly altered these important components. Alterations in macronutrients, such as protein and lactose, are related to altered functions in several regulatory

systems of infants (24). Protein from breast milk provides indispensable amino acids, necessary for new protein synthesis during growth, and also performs immunological, enzymatic, absorptive, and defense functions against pathogens, in addition to nutrition (24,25). In contrast to protein, the lactose content is generally more stable and is important for maintaining the osmotic pressure of breast milk (26). In this study, lactose content was increased in the T-2000 treatment, which may be related to a reduction in protein content, since lactose levels negatively correlate with protein content as lactation is established (27). The effects of increased lactose on the newborn are not known. However, since this carbohydrate accounts for about 40% of the total energy of breast milk (28) it can be

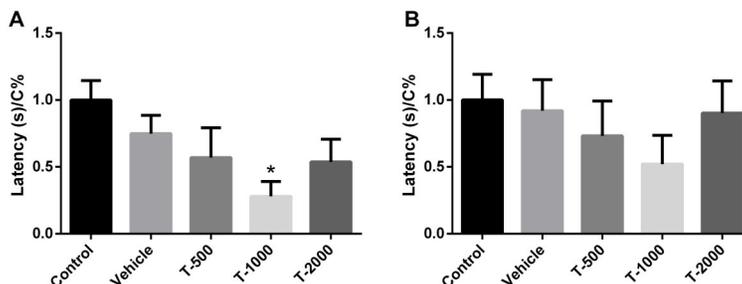


Figure 4. Long-term memory in adult offspring of mothers treated with valerian in the postpartum period. Groups: Control (1 mL distilled water), Vehicle group (1 mL distilled water, 20% of glycerol), T-500 (500 mg·kg⁻¹·day⁻¹ of valerian), T-1000 (1000 mg·kg⁻¹·day⁻¹ of valerian), and T-2000 (2000 mg·kg⁻¹·day⁻¹ of valerian). **A**, Female offspring, latency time (s) performing the step-down inhibitory avoidance task ($F(4, 5)=0.1603$, $*P < 0.01$, compared to control). **(B)** Male offspring, latency time (s) performing the step down inhibitory avoidance task ($F(4, 5)=1.172$, $P = 0.4747$). Data are reported as means \pm SE ($n=8-13$ per group). Values in the groups were normalized to those in the control group. One-way ANOVA and Dunnett's test.

assumed that the milk of the group T-2000 had a relatively higher caloric content, being an extra source of energy for offspring, but not enough to change the nutritional status. This is the first study to assess breast milk composition after maternal treatment with valerian; our results emphasized the importance of evaluating the excretion of drug substances in milk and possible alterations of milk components. Thus, further studies are needed to determine the possible relationship between these findings and the observed effects on offspring. According to the National Library of Medicine (USA) (2018) and based on our extensive literature review, there are no previous data on the safety and efficacy of valerian in nursing mothers or infants; therefore, these data may be considered the greatest strength of our study.

Behavioral patterns of mothers during lactation are essential to ensure the development of healthy offspring (29). However, the use of a substance that acts on the CNS may disrupt normal behavior (30,31). Evidence has shown that important interactions between the neonate and mother are predictive of offspring emotional behavior and neuroendocrine profiles later in life (29,32). Licking and grooming frequencies represent the main source of tactile stimulation for newborns, and a decrease in these stimuli may affect offspring brain development through reprogramming mechanisms (33,34). Licking and grooming and other maternal behaviors monitored during the entire treatment period were not statistically different between experimental groups indicating that the offspring of treated mothers received adequate developmental stimuli.

No alterations in the physical postnatal development were observed. Body weight evolution was similar in all experimental groups indicating that adequate breastfeeding occurred despite alterations of milk components, and this conclusion was in agreement with the results of maternal behavior monitoring. The physical contact between mothers and pups also ensured similar offspring development of the groups. The same considerations apply for reflex development, which is sensitive in the

postnatal period considering the process of brain maturation. Studies evaluating the consequences of benzodiazepine treatment in rats during pregnancy reported delays in the development of neonatal reflexes (35). Previous findings indicate that valerian treatment during pregnancy does not cause alterations in the physical or neuromotor development of the offspring (11).

Based on the results of the present study, anxiety behavior was not observed in the offspring of treated mothers. Female offspring of the T-1000 group submitted to step-down inhibitory avoidance test in adulthood showed significant memory impairment. A single shock stimulation is sufficient to create robust long-term memory making this task highly suitable to investigate the drug effects on memory. Apparently, maternal treatment leads to certain consequences in the offspring by influencing brain regions responsible for this type of memory, such as the hippocampus (36). Figure 4 shows a U-shaped dose-response curve, which can be related to an overcompensation in response to a disruption in homeostasis promoting different biological effects from the T-1000 treatment, commonly observed in toxicological and pharmacological studies (37). However, the T-1000 and T-2000 doses adjusted to rodent metabolism are higher compared to the doses usually used in humans, which we consider a limitation of this study. Future studies should consider different doses used in scientific studies and conduct tests to identify a dose closest to that used in humans, taking into account the specific differences between experimental models. The mechanisms mediating sex-specific effects are poorly understood; however, different phenotypes may manifest depending on the sex of the fetus or newborn (38).

Conclusions

Our study showed that high doses of valerian altered the milk composition and the memory of offspring while the lowest dose, closest to that used in humans, did not cause adverse effects and was safe with respect to the parameters evaluated. This underscores the importance

of self-medication issues and the misconception that herbal supplements are not harmful (39).

Further studies are required to determine the sex-specific responses to this treatment, identify the mechanisms related to memory impairment, and determine the consequences of changes in milk composition.

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