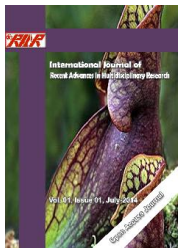

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Full Length Research Article

VALERIANA OFFICINALIS ADMINISTRATION ON PREGNANT RATS AND POSTNATAL DEVELOPMENT OF THE FEMALE OFFSPRING

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ABSTRACT

Valeriana officinalis is used as a mild sedative but should be avoided during pregnancy due to scarce data available about potential effects on the offspring. This paper evaluates physical development and anxiety in female rats whose mothers were exposed to valerian during gestation. Twenty four pregnant rats were distributed into four groups (n=6) and treated orally from the 12th to 19th day of gestation: Control (1 ml of distilled water), and three valerian-treated groups with 500, 1000 and 2000 mg/Kg/day. Four females from each mother were selected to analyzed: time of eye opening, ear unfolding, appearance of lanugo, hair, superior and inferior incisor eruption, vaginal opening; first date of righting reflex, grasping reflex, cliff avoidance, and negative geotaxis. Anxiety was evaluated by the elevated plus-maze test in offspring at age 90 days. Eruption of the inferior incisors (1000 and 2000mg/kg); appearance of lanugo and hair, and eye opening (500 mg/kg) were significantly altered in the offspring. The neuromotor reflexes and the anxiety did not differ between the groups. The physical development in the adult stage was not altered. These findings suggest that maternal treatment with valerian during gestation did not alter physical, neuromotor and anxiety of the offspring.

INTRODUCTION

Valeriana officinalis L. (Valerianaceae), or simply valerian, is a perennial flowering plant native to Europe and parts of Asia. Its use as a medicinal herb dates back to the time of ancient Greece and Rome (Hobbs, 1989) and valerian was particularly known as a sedative, anticonvulsant, migraine treatment and pain reliever (Boullata and Nace, 2000). Valerian is most commonly used to treat insominia (Tesche, 2002) and has been recommended as an alternative for hypnotic drugs (Hadley and Petry, 2003). In addition to being used for the treatment of sleep disorders (Miyasaka *et al*., 2006), valerian has also been used to treat anxiety (Miyasaka *et al*., 2006), gastrointestinal problems (to control muscle spasms and cramps), and symptons such as stress, excitability, and nervous tension (Hadley and Petry, 2003). Because there is limited information on animal and human effects, valerian is not recommended for use during gestation and lactation. The lack of long-term studies and the potential for cytotoxicity in the fetus and hepatotoxicity in the mother strengthen this concern, and do not allow a conclusion as to the safe use of valerian during pregnancy (Tesche, 2002, Taylor *et al*., 2002; Yao *et al*., 2007). Prenatal exposure to valerian has been implicated in delayed ossification in the offspring, suggesting its transit through the placenta (Tuffik *et al*., 1994); however compelling evidence for the passage of valerian through the placenta is still wanting. In contrast, an *in vivo* study showed that the administration of valerian ethanolic

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extract (2.79 g/Kg/day, p.o.) to pregnant rats between days 1-8 or 8-15 of gestation did not increase the incidence of internal and external malformation of the fetuses and did not exert any adverse effects related to fertility (Yao *et al*., 2007).The mechanism of action of valerian in general and as a mild sedative in particular has not been fully elucidated. The pharmacological effect of the valerian extract and one of its main constituents, namely valerenic acid, is believed to be mediates by modulation of the neurotransmitter gamma-aminobutyric acid (GABA) A receptor, a class of receptors on which benzodiazepines are known to act (Holzl and Godau 1989; Mennini *et al*., 1993; Benke *et al*,. 2009). It is noteworthy that alterations in juveniles in the GABA receptor may persist into adulthood. For instance, it has been demonstrated that neonatal exposure to benzodiazepines reduced the level of anxiety in adult animals (Schroeder *et al.,* 1997).

During gestation, the hematoencephalic barrier is not fully developed (Jonhson 1980), therefore drugs that pass through the placenta could be interfering with the receptors in the brain. Although it has been reported that intrauterine exposure to drugs in general may not interfere with the embryo-fetal and newborn development, the exposure may have later effects seen as health disorders in adulthood. Such concerns led to the evaluation of drug effects on animals exposed during puberty, and now special attention is being given to their effects on the offspring and their consequences in adulthood (Goodman *et al*., 2011). In a study developed by Vieira and collaborators (2013), evaluation of the behavior of Wistar rat offspring, born from mothers treated during gestation with *Hyperic perforatum* extract showed significant reduction of depression and anxiety in the offspring at 10 and 60 days postpartum, suggesting placental transit and long-term effects of hyperic extract. Previous study of our group demonstrated no alterations in the neonatal and neurobehavioral development of male rats exposed to valerian during intrauterine life (Campos *et al*., 2014). Because male and female has difference in the neuronal and behavioural process (Kelly *et al*., 1999) the objective of this study was to evaluate neonatal development and anxiety in female rats whose mothers were exposed to valerian during gestation.

MATERIALS AND METHODS

The experimental protocol followed the international guidelines outlined in the Guide for care and use of laboratory animals (NATIONAL RESEARCH COUNCIL 2003) and was approved by the Ethics Committee on Animal Experimentation of the Federal University of Juiz de Fora (protocol number 019/2011).

Valerian Extract

The dry extract of Valerian was imported by QUIMER® company (Juiz de Fora. Brazil) (registration nº 002/2009) and supplied by ORTOFARMA® Company (Juiz de Fora. Brazil), where the physicochemical quality analysis was carried out. The dry extract was suspended in deionized water and later preserved in a vacuum desiccator to avoid humidity. The dry extract was selected as the test material because it is traditionaly used by people in this form. In addition, valerian has around 150 – 200 chemical components, including alkaloids, sesquiterpenes and flavones (Carol and Linda, 1996; Marder *et al*., 2003; Shahidi; Naczk, 2004). Individual testing of components will be carried out in a future study.

Experimental Assay

Twenty four female Wistar rats (*Rattus norvegicus* Berckenhout, 1769), 90 days old and with regular estrous cycles, were obtained from the vivarirum (Registro CIAEP 01.0048.2013) of the Reproductive Biology Center at the Federal University of Juiz de Fora. Rats were housed in individually ventilated polypropylene cages (ALESCO® São Paulo. Brazil)), with hardwood chip bedding, at controlled temperature (22 ± 2º C), with a 12-hour light/dark photoperiod. The animals were fed *ad libitum* rat chow pellets NUVILAB CR1® (Nuvital Nutrients Ltda., Colombo/PR) and received water *ad libitum*. Females were placed in cages with male rats in a ratio of 2:1 for natural mating. The presence of spermatozoa in a vaginal smear indicated successful mating and was considered as day one of gestation (Beaudoin, 1985). The inseminated rats were housed individually in cages and randomly divided into four groups (n = 6): three treated and one control that received orally once daily 500 mg/Kg/day (T-500), 1000 mg/Kg/day (T-1000), 2000 mg/Kg/day (T–2000) of valerian, and distilled water, respectively. The choice of the lowest dose (500 mg/Kg) calculated for the rat was based on the dose recommended for humans while the two other doses were two and four times higher than the lowest dose (Al-majed *et al*., 2006). Treatment began on the 12th day of gestation and ended on the 19th day, corresponding to the most vulnerable period for brain development (Vorhees and Rindler, 1990).

Neonatal Development

The day of birth was considered the first postnatal day. The number of pups and the number of males and females born to each litter were registered. Afterwards, the number of animals per litter was reduced to eight animals in order to homogenize nutritional status. The litter was observed daily for detection of deaths and cannibalism. For each experimental group, four females obtained from five litters were used to evaluate the physical and reflex development of the pups. The animals were identified by specific marking criteria (ear notches) established by the vivarium of the Reproductive Biology Center. Only females were chosen as there are gender differences in the neuronal and behavioral processes (Kelly *et al*., 1999). The male rats were studied separately and the results will be presented elsewhere (Campos *et al*., 2014 article). Physical development was observed in all pups from postnatal day one. The following parameters were analyzed: date of eye opening, ear unfolding, appearance of lanugo and hair, superior and inferior incisor eruption, and vaginal opening. The day of first appearance of these features was registered and for paired structures, it was registered as the first day of appearance of both structures (Chiavegatto *et al*., 1997; Dorce *et al.*, 2009; Oliveira *et al*., 2011). Data were expressed in animal frequency per day of appearance of each physical development feature. To evaluate the reflex development, the following tests were carried out: grasping reflex (holding of a paper clip with the forelimbs), righting reflex (return to normal ventral position after lying on its back), cliff avoidance (animal movement away from the cliff), and negative geotaxis (turning 180º after being placed face-down on an inclined surface). The tests were performed daily from postnatal day one until the day of

appearance, with duration of 15 sec each (Chiavegatto *et al*., 1997; Dorce *et al.*, 2009; Oliveira *et al.*, 2011). A positive result was considered the day the animal first showed the reflex being tested.

Anxiety Test

At 90 days old, one female from each litter was randomly chosen and used in the elevated plus maze test, totaling six females for each treatment. The elevated plus maze apparatus consisted of two open arms and two closed arms (50 cm long and 10 cm wide) with walls 30 cm high, elevated 60 cm above the floor. Each arm was positioned at 90º relative to the adjacent arms and all arms were connected through a central area (10 x 10 cm), forming a plus sign. To investigate anxiety, each rat was placed at the center of the maze facing one of the open arms. The time spent (in seconds) in the open and closed arms were recorded for 5 min (Pellow *et al,* 1985). After each trial, the plus maze was carefully cleaned with 70% isopropyl alcohol. The variable used as an indicator of anxiety was the time percentage the animal spent in the open arms (% tOP): (time spent in the open arms divided by time spent in the open and closed arms) x 100. The use of % tOP instead of the measurement of time spent in the open arms alone has the advantage of taking into account an important intervening variable which is the locomotor activity of the animal (Rodgers and Dalvi, 1997).

Statistical Analysis

The data obtained from the physical and reflex tests were presented according to the frequency of pups that exhibited developmental signs in each day. The frequency was grouped in three categories, taking into account the day in which the control group showed a higher frequency of individuals displaying physical or reflex signs:

Category 1: Frequency similar to control group.
Category 2: Frequency prior to that exhibited by control group.
Category 3: Frequency posterior to (after) that exhibited by control group.

The results were analyzed using the Fisher exact test ($\alpha = 0.05$) for comparison of the control group with each treated group. Differences were considered significant when $p \leq 0.01$ (to avoid the effect of multiple comparisons on the alpha risk). The results of the elevated plus-maze test were evaluated by use of descriptive analysis presented as mean ± standard mean error. The mean values of the groups were compared using the non-parametric Kruskal-Wallis test. The level of significance considered was $\alpha = 0.05$. The tests were performed using SPSS program version 19.

RESULTS

In females, the date of appearance of the physical parameters such as vaginal opening, ear unfolding, appearance of lanugo, and eruption of the superior incisors was similar to that of the control group. However, the eruption of the inferior incisors was markedly delayed between the control group and the treated groups T-1000 (χ^2=9.91; gl=2; p=0.007) and T-2000 (χ^2=11.518; gl=2; p=0.003) (Table 1). Significant delay was also observed in the appearance of hair between T-500 (χ^2=15.99; gl=2; p < 0,001) and control animals, and in the eye opening between control and T-500 (χ^2=9.09; gl=2; p=0.01) (Table 1). The date of appearance of the reflexes did not differ among the groups (Table 2).

Elevated Plus Maze

The percentage of time spent in the open arms was not statistically different between the groups (H=2.47; p=0.48). The descriptive statistical data for all groups are shown in table 3.

DISCUSSION

The CNS is protected from entry of potential toxins through the blood-brain barrier, which is not fully developed at birth. For this reason, a given agent can be selectively toxic in that it may have no effect on the mother while being harmful to the fetus, resulting in abnormalities that affect, for instance, the development of neuronal processes (Johnson, 1980). Hence, exposure to psychopharmacs during gestation may produce alterations in development of the nervous system that could be involved in physiologic changes later in life (Kellog *et al*., 1992; Kellog *et al*., 2000; Nicosia *et al*., 2003). The evaluation of the physical development of the offspring is a relevant tool for the investigation of the toxic potential of agents administered to mothers during gestation. Despite the delay observed in the developmental parameters analyzed in this study (appearance of lanugo and hair, and eruption of the inferior incisors), none of the animals from control or treated groups exhibited morphological changes in these parameters in the adult life, indicating that valerian exerted transient adverse effects on the female offspring.

The reflex development is considered another relevant paramenter in toxicological assays as it can indicate the occurrence of deleterious effects on brain maturation (Fox, 1965). Therefore, delayed reflex responses suggest developmental alterations in the CNS which can represent a predictive factor for behavioral modifications in adulthood. The exposure to psychopharmacs that act on GABA receptors during brain develoment can induce reflex alterations such as delayed geotaxis response (Nicosia *et al*., 2003). It has been shown that the pharmacological effect of valerian extract is mediated by modulation of GABA A receptor (Benke *et al*., 2009); however the results obtained in this study do not corroborate this information as exposure to valerian at the tested doses did not alter any of the reflex responses tested when compared to control values.

During the brain development process, the interaction between the neurotransmitters and their respective receptors can influence the development of the CNS by modulating the proliferation of non-differentiated cells (Lauder, 1986; Emerit *et al*., 1992; Nguyen *et al*., 2001). GABA is a neurotransmitter known to exert inhibitory effects on the mature brain; however, during its development, GABA produces excitatory effects, which can influence early neocortical development, such as neurogenesis and synaptogenesis, that can result in futher alterations later in life (Cherubini *et al*., 1991; Owens *et al*., 1996). Benzodiazepines comprise a group of substances that enhance the effect of GABA at the GABA A receptor, resulting in sedative, hypnotic, anxiolytic, anticonvulsant and muscle relaxant properties. A study carried out by Schroeder and collaborators (1997) showed that exposure to diazepam during the neonatal stage (period in which the brain is still developing)

Table 1. Physical development in female offspring of mothers exposed to valerian aqueous extract (500, 1000, 2000 mg/Kg) or to distilled water (control) during gestation

Physical parameters	Control	T-500	T-1000	T-2000
	n=24	n=26	n=18	n=21
Ear unfolding				
Prior	29.2% (7)	23.1% (6)	38.9% (7)	33.3% (7)
Day 5	58.3% (14)	73.1% (19)	61.1% (11)	61.9% (13)
After	12.5% (3)	03.8% (1)	00.0% (0)	04.8% (1)
Eruption of inferior incisors				
Prior	12.5% (3)	00.0% (0)	00.0% (0)	33.3% (0)
Day 11	37.5% (9)	46.2% (12)	16.7% (3)	04.8% (1)
After	50.0% (12)	53.8% (14)	83.3% (15)*	95.2% (20)*
Eruption of superior incisors				
Prior	04.2% (1)	03.8% (1)	05.5% (1)	0.00% (0)
Day 9	58.3% (14)	46.2% (12)	33.3%(6)	66.7% (14)
After	37.5% (9)	50.0% (13)	61.2% (11)	22.3% (7)
Appearence of lanugo				
Prior	16.6% (4)	11.5% (3)	33.3% (6)	23.8% (5)
Day 4	66.7% (16)	34.6% (9)	38.9% (7)	61.9% (13)
After	16.7% (4)	53.9% (14)	27.7% (5)	14.3% (3)
Appearance of hair				
Prior	29.1% (7)	11.5% (3)	44.4% (8)	66.6% (14)
Day 8	66.8% (16)	34.6% (9)*	33.3% (6)	23.8% (5)
After	04.1% (1)	53.9% (14)	22.3% (4)	09.6% (2)
Eye opening				
Prior	16.6% (4)	42.3% (11)	05.6% (1)	23.8% (5)
Day 17	75.0% (18)	30.8% (8)*	66.6% (12)	47.6% (10)
After	08.4% (2)	26.9% (7)	27.8% (5)	28.6% (6)
Vaginal opening				
Prior	0.00% (0)	17.4% (4)	27.8% (5)	04.8% (1)
Day 33	42.8% (10)	08.7% (2)	44.4% (8)	57.1% (12)
After	57.2% (14)	73.9% (17)	27.8% (5)	38.1% (8)

The results are expressed in percentage followed by the corresponding number of females that presented physical development in the three categories: prior, similar day to control, after. The asterisk indicates statistical difference between control group and the respective treatment group.

Table 2. Physical development in female offspring of mothers exposed to valerian aqueous extract (500, 1000, 2000 mg/Kg) or to distilled water (control) during gestation

Reflexological parameters	Control (n=24)	T-500 (n=26)	T-1000 (n=18)	T-2000 (n=21)
Grasping reflex				
Prior	29.2% (7)	23.1% (6)	38.9% (7)	33.3% (7)
Day 2	58.3% (14)	73.1% (19)	61.1% (11)	61.9% (13)
After	12.5% (3)	03.8% (1)	00.0% (0)	04.8% (1)
Postural response				
Prior	04.2% (1)	03.8% (1)	05.5% (1)	00.0% (0)
Day 2	58.3% (14)	46.2% (12)	33.3% (6)	66.7% (14)
After	37.5% (9)	50.0% (13)	61.1% (11)	33.3% (7)
Cliff avoidance				
Prior	16.6% (4)	11.5% (3)	33.3% (6)	23.8% (5)
Day 8	66.8% (16)	34.6% (9)	38.9% (7)	61.9% (13)
After	16.6% (4)	53.9% (14)	27.8% (5)	14.3% (3)
Negative geotaxis				
Prior	45.8% (9)	73.1% (19)	61.1% (11)	57.2% (12)
Day 9	25.0%(6)	07.0% (2)	11.1% (2)	19.0% (4)
After	29.2% (7)	19.9% (5)	27.8% (5)	23.8% (5)

The results are expressed in percentage followed by the corresponding number of animals that presented physical development in the three categories: prior, similar day to control, after. None of the differences were statistically significant.

Table 3. Anxiety test carried out in female offspring of mothers exposed to valerian aqueous extract (500, 1000, 2000 mg/Kg) or to distilled water (control) during gestation

Groups	n	% (tOP)
Control	6	0.183±0.012
T-500	6	0.178±0.035
T-1000	6	0.178±0.016
T-2000	6	0.182±0020

Results expressed in mean ± standard error (median). Kruskal- Wallis test, $p \geq 0.05$. None of the differences were statistically significant. % tOP - time percentage the animal spent in the open arms

reduced the level of anxiety in the animal`s adulthood. Similar results were obtained with hyperic, which reduced anxiety in the adulthood of offspring born from mothers treated during the gestational period (Vieira *et al*., 2013). In contrast, the data obtained in this study showed that the prenatal exposure to valerian did not alter the levels of anxiety in the offspring as tested in the elevated plus maze. Considering that various subtypes of GABA A receptor showing functional and morphological differences have already been identified (Olsen and Sierghat, 2009), the difference between the information found in the literature regarding intrauterine exposure to

benzodiazepines and the obtained results regarding intrauterine exposure to valerian could be due to the binding of benzodiazepines and valerian to distinct subtypes of GABA A receptors. In conclusion, despite the significant alterations observed during the postnatal period, the findings presented in this study indicate that either valerian is not crossing the placenta or the exposure to valerian during gestation does not alter neonatal development and anxiety of the animals in adulthood.

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