Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
21(6): 1065-1076, Nov./Dec. 2011

Neurobehavioral, reflexological and physical development of Wistar rat offspring exposed to ayahuasca during pregnancy and lactation

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Abstract: Ayahuasca is a hallucinogenic beverage prepared by the decoction of plants native to the Amazon Basin region. The beverage has been used throughout the world by members of some syncretic religious movements. Despite the recent legalization of ayahuasca in Brazil for religious purposes, there is little pre-clinical and clinical information attesting to its safety, particularly in relation to the use during pregnancy. The aim of the current work was to determine the effects of perinatal exposure to ayahuasca (from the 6th day of pregnancy to the 10th day of lactation) on physical, reflexology and neurobehavioral parameters of the Wistar rat offspring. The offspring showed no statistically significant changes in the physical and reflexology parameters evaluated. However, in adult rats, perinatally exposed to ayahuasca, an increase in frequency of entries in open arms in elevated plus-maze test, a decrease in total time of interaction in social interaction test, a decrease in time of latency for the animal to start swimming and a decrease of the minimum convulsant dose induced by pentylenetetrazol were observed. In conclusion, our results showed that the use of ayahuasca by mothers during pregnancy and lactation reduced the general anxiety and social motivation of the rat offspring. Besides, it promoted a higher sensitivity for initiation and spread of seizure activity.

Article

Received 18 Nov 2010
Accepted 9 Feb 2011
Available online 23 Sep 2011

Keywords:
ayahuasca
pregnancy
rat offspring
neurobehavioral changes

ISSN 0102-695X
http://dx.doi.org/10.1590/S0102-695X2011005000170

Introduction

Ayahuasca is a beverage prepared from two Amazon native plants, *Banisteriopsis caap*i (Spruce ex Griseb.) C.V. Morton, Malpighiaceae, and *Psychotria viridis* Ruiz & Pav., Rubiaceae, which, respectively, contain the psychoactive chemicals β-carbolines and *N,N*-dimethyltryptamine (Tupper, 2008). It was initially used by indigenous peoples in shamanic rituals for spiritual purposes and in folk medicines. Its use has since expanded beyond the indigenous culture, being used in Brazilian-based syncretic religious groups, such as "União do Vegetal" and "Santo Daime", where the beverage is reported to be used to facilitate self-knowledge and introspection (Carlini, 2003; Riba et al., 2003).

In recent years, the use of ayahuasca has spread outside South America to some religious groups established in the United States and in several European countries, including Germany, Great Britain, Holland, France and Spain (Riba et al., 2003). As an increasing number of people have come into contact with this psychotropic tea, the beverage has begun to attract the attention of researchers interested in its pharmacological and toxicological aspects (Callaway, 2005; Callaway et al., 1999; Gable, 2007; Riba & Barbanoj, 2005; Riba et al., 2003).

N,N-dimethyltryptamine (DMT) is an hallucinogenic indole alkaloid, structurally related to the neurotransmitter serotonin (Callaway, 2005; Callaway et al., 1999; Gable, 2007; Riba & Barbanoj, 2005; Riba et al., 2003; Tupper, 2008). DMT has high affinity for 5-HT2A and 5-HT2C receptors in the brain and moderate to low affinity for the 5-HT1A receptor (Keiser et al., 2009). DMT is active when administered parenterally, but not orally because the gastrointestinal tract contains the enzyme monoamine oxidase (MAO), which metabolizes orally ingested DMT (Tupper, 2008). DMT is, therefore, only active in the presence of β-carbolines, such as harmine, harmaline and tetrahydroharmine, since these are potent monoamine oxidase (MAO) inhibitors (Callaway, 2005; Callaway et al., 1999; Gable, 2007; Riba & Barbanoj, 2005; Riba et al., 2003; Tupper, 2008). The synergistic interaction of these alkaloids is the basis of the psychotropic action of ayahuasca (McKenna, 2004;

Riba et al., 2003; Tupper, 2008).

The psychoactive effects of ayahuasca are qualitatively similar to those observed from drugs of the same pharmacological class, such as LSD and psilocybin (Tupper, 2008). The subjective effects of ayahuasca include visual hallucinations, synesthesia, intensification of affectivity up to ecstatic experiences, significant alterations of time and space perceptions and body image distortions, and feelings of alertness and stimulation (Callaway et al., 1999; McKenna, 2004; Tupper, 2008). Pharmacological effects in the periphery include mild increase in heart rate and blood pressure as well as elevations in the level of cortisol, growth hormone and prolactin. In some individuals episodes of nausea and vomiting occur (Callaway et al., 1999; Grob et al., 1996; Halpern et al., 2008).

Brazil is the only country in which the use of ayahuasca within religious contexts is officially recognized and protected by law (Conselho Nacional Antidrogas, 2004). This legal status is analogous to that held by the Native American Church for the use of peyote (*Lophophora williamsii*) in the United States. Ayahuasca is used during religious rituals by all members of the group, including pregnant women and children.

Currently, there are few studies on the toxicological effects of ayahuasca and no study has evaluated the effects of exposure to ayahuasca during the gestational and perinatal period in animal models. The aim of the present work was to investigate possible toxic effects in rats exposed to ayahuasca during the periods of gestation and lactation. Physical and reflex development, neurobehavioral aspects and central nervous system monoamine levels of the offspring were determined.

Materials and Methods

Plant material and extract preparation

The ayahuasca used in this study was obtained from a religious group settled in Araçoiaba da Serra city, São Paulo state, Brazil. *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton, Malpighiaceae, used in ayahuasca preparation was derived from specimens cultivated at the Santo Antônio farm in Araçoiaba da Serra, São Paulo state, Brazil (August, 2007). The species were authenticated by Dr. Maria Candida Henrique Mamede from the Botanic Institute (Department of the Environment, Government of São Paulo) and exsiccates are deposited in SPF Herbarium (Institute of Botany, University of São Paulo) under the code 181147. *Psychotria viridis* Ruiz & Pav., Rubiaceae, was derived from specimens cultivated at the Santo Antônio farm in Araçoiaba da Serra, São Paulo state, Brazil (September, 2007). The species were authenticated by Dr. Sigrid Luiza Jung-Mendaçolli from the Agronomic Institute of Campinas (Department of Agriculture and Supply, Government of São Paulo) where exsiccates are deposited under the code 48679.

The ayahuasca extract used in this experiment was prepared according to the tradition of the religious group. The process for preparation of ayahuasca is long, lasting almost one whole day, and is performed exclusively by trained members. Usually it consists of a decoction of stems of *B. caapi* and leaves of *P. viridis*. This mixture is boiled and concentrated for several hours until a member of the church confirms that the brew is ready to be used in the religious rituals.

The main active constituents found in ayahuasca delivered by the religious group were determined by a previously published chromatographic method by our group (Pires et al., 2009). Alkaloid concentrations were: *N,N*-dimethyltryptamine: 0.42 mg/mL; harmine: 1.37 mg/mL; harmaline: 0.62 mg/mL; tetrahydroharmine: 0.35 mg/ mL.

The amount of ayahuasca consumed during the religious ceremony was taken into consideration when selecting dose. A dose of approximately 100 mL is typical for a person of average body weight (70 kg) (Callaway et al., 1999; Gable, 2007). The dose given to animals was roughly equivalent to that used by humans (0.14 mL/100 g body weight) and is referred to as typical dose (TD). The final volume administered to animals was standardized to 1.0 mL/100 g, diluting ayahuasca in potable water. The dose used in this experiment was based on previous studies that assessed maternal toxicity and teratogenicity in rats exposed to ayahuasca during pregnancy. Three different doses were employed in this previous study (Oliveira et al, 2010). The lowest dose was applied in the present work.

Animals

Ninety-day old virgin adult female and adult male Wistar rats derived from the Department of Pathology (School of Veterinary Medicine, University of São Paulo), weighing approximately 200 and 300 g, respectively were utilized. Seven days before the beginning of the experiments the rats were housed in groups of five in plastic cages (40x50x20 cm) at controlled room temperature (20-25 °C) with a 12-h light-dark cycle (lights on 6:00 h). Food and water were freely available. Animals were divided randomly into control and experimental groups. The animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, USA (Guideline, 1996). The protocols for experimental studies were approved by The Faculty of Pharmaceutical Sciences Ethics Committee-USP (protocol number CEEA 93/2006).

During breeding each female rat was housed with a single breeder male in a 2:1 ratio overnight.

Mating was confirmed by the presence of spermatozoa in vaginal smears taken the following morning. On the day pregnancy was confirmed, designated as gestational day (GD) zero, the females were weighed and then housed singly. All subjects were weighed every three days to monitor health and pregnancy. Consumption of food and water were measured during treatment period.

Treatment

On gestational day 6 (GD6) to lactation day 10 (LD10) pregnant females were given doses of water (control group) or ayahuasca extract (experimental groups) by gavage at approximately the same time each day. Initial dose volume was based on GD 6 body weight and adjusted every three days throughout the treatment period.

Offspring studies

All the pregnant rats were allowed to give birth and nurture their offspring normally. No cross-festering procedure was used. Delivery day (postnatal day - PND) was defined as PND0. On PND1 all the litters were examined externally and sexed. Litters were culled to eight pups (four males and four females) in each litter. All data were analyzed treating the litter as the experimental unit.

One male and one female pup from each litter (eighteen males and eighteen females/group) were marked daily with colored felt tip pens and used for all physical and reflex developmental evaluations. The same male and female marked pups were employed for body weight accompaniment and for observation of the testis descent (considered as the scrotum purse touching the testis, observation beginning on PND 16) and vaginal opening (when the vaginal hole is visualized, observation beginning on PND 30) day appearance. They were weighed individually at PND 1, 4, 11, 17, 21, 28, 35, 42, 49, 56, 63 and 70. Once weaned (PND21), offspring were separated by gender and treatment group and maintained under the same conditions as their dams.

Physical and reflex development

The physical and reflex parameters evaluated in this study have been used in previous studies (Norton, 1989; Schwarz et al., 2003; Zbinden, 1981). In the current study physical and reflex parameters were measured daily in one male and one female from each litter and litter means were then calculated. The following physical parameters were evaluated: hair growth (beginning on PND1); pinna detachment (beginning on PND2); eruption of incisor teeth (beginning on PND7); auditive channel opening (beginning on PND10); eye opening (beginning on PND10); adult gait (when the pups walk without propping their ventral portion on the floor) (beginning on PND10); testes descent (beginning on PND18); balanopreputial separation (beginning on PND32); vaginal opening (beginning on PND25). Responses to the following reflex tests were measured: Surface righting reflex: the time taken for the pup to right itself following limb extension and release (beginning on PND1); palmar grasp reflex: the pup grasps a paper clip with forepaws when stroked (beginning on PND1 and it is recorded when this reflex disappear); negative geotaxis test: time taken for the pup to turn at least 90º after being placed faced down on a 45° incline (not exceeding 60 s per trial) (beginning on PND5); cliff avoidance: time taken for the pup to turn and back away from a platform edge (not exceeding 60 s per trial) (beginning on PND1); air righting reflex: the pups ability to turn in the air during a free fall (beginning on PND8); auditory startle reflex: time between auditory stimulus and pup response (noted as a sudden flinch or cessation of ongoing movement following) (beginning on PND10); visual placing: time taken for the pup to make visual contact with a solid object when suspended by the tail and it put its paws out toward the solid object (beginning on PND10); vibrissa placing: time taken for the pup, which is suspended by the tail and lowered so that the vibrissae make contact with a solid object (beginning on PND10).

Adult offspring studies

Open-field behavior studies

The open field test has been developed to assess the emotionality of rats (Calabrese, 2008; Pellow et al., 1985). The test involves subjecting the animal to an unknown new environment from which it is not allowed to escape due to the presence of surrounding walls. It consists of a round wooden arena (90 cm in diameter, 48-cm high walls) painted black and virtually divided by software (Ethovision® version 1.9; Noldus Information Technology, USA) into three zones: central, medium and external. At PND 70, each animal was placed individually in the center of the apparatus. Total locomotor activity (distance moved in cm), time spent (s) in locomotor activity in each of the open field zones, time in motion (s), number of movements initiated, average velocity, frequency of rearing, frequency of grooming and number of defecations (fecal pellets) were recorded over a 5 min period. Data was collected using a video camera mounted 100 cm above the arena. Data analysis was carried out using Ethovision System® software (Noldus Information Technology) installed on an IBM-compatible computer.

Elevated plus-maze test

The elevated plus-maze (EPM) test is commonly employed as an initial screening evaluation for the assessment of potential anxiolytic agents (Calabrese, 2008). In the current study the elevated plus-maze was made of wood and painted black. The structure had two open arms and two enclosed arms of equal size (50x11 cm) with 40 cm high walls. The EPM was elevated 55 cm above the floor. At PND 100, each animal was placed individually in the central square of the EPM and allowed 5 min for free exploration. Each animal was observed using the same system as described for the open field (Ethovision System® software; Noldus Information Technology). The number of entries into the open and closed arms and the time spent exploring the open and closed arms were recorded. Measurements that reflect anxiety levels in this test are: 1) percentage of entries into open arms versus closed arms; and 2) percentage of time spent in open arms versus closed arms (Pellow et al., 1985).

Social interaction studies

Social interaction has been proposed as a model to study the effects of anxiolytic drugs (File & Pellow, 1985). It may also be used to distinguish possible anti-anxiety effects of drugs from effects on motivation (Calabrese, 2008; File & Pellow, 1985). Social interaction is assessed through direct observation of pairs of rats in an arena (the same place used in tests of open field) and is measured the amount of time that two non-familiar rats interact. This model takes into account the behavior of rats in situations of anxiety, in which they interact with animals of the same species (File & Pellow, 1985; File et al., 1993).

Rats were paired based on similarity in weight (<10 g difference) and on not having had previously contact with each other. Each rat was kept isolated for five days prior to the commencement of the test. Two days before the test (PND70) each rat was placed alone in the arena for 10 min. The day before the test the pair was placed in the arena together (10 min) for habituation. On commencement of the test the rats were introduced into the arena at the same time. The following behavioral responses were timed over a 10 min period: sniffing at the partner, frequency of crawls over/under the other, frequency of partner mounting, pursuit the partner, and locomotion. Aggressive and passive behaviors were extremely rare in this test and were not quantified.

Forced swimming

The model of forced swimming, also known as behavioral despair and immobility test, predicts that rats forced to swim in a small area, after several attempts to escape, assume a posture of immobility (Porsolt et al., 1977). It is a model routinely used in the pharmaceutical industry for screening of antidepressant drugs (Borsini, 1995; Calabrese, 2008).

At PND 70, rats were placed singly in a glass cylinder containing water maintained at 20-23 °C (the cylinder measured 25 cm in diameter, 60 cm in height and contained water to a depth of 45 cm). Following an initial 15 min training session in the water, the rat was removed, dried in a heated enclosure (28 °C) before being placed in their individual cages again. The following day the rat was plunged in the cylinder for 5 min (test session). In the test session, the time of latency for the animal to start swimming and the total time of immobility were recorded.

Stereotyped behavior

Stereotyped behavior may be described as repeated movements without variation and seemingly without purpose. It is a behavior related to the progressive activation of dopamine receptors of the nigroestriatal pathway. It can be induced by various dopamine agonist drugs such as apomorphine (Setler et al. , 1976).

The stereotyped behavior in offspring was measured at PND 90. After an eight day isolation period the animals received a subcutaneous dose of 0.6 mg/kg apomorphine hydrochloride (Sigma, Milwaukee, USA). The stereotyped behavior was quantified according to the scoring system proposed by Setler et al. (1976) with scores ranging from 0 (asleep or stationary) to 6 (continuous licking and gnawing of cage grids). Observations were made at 10 min intervals for 2 h following apomorphine treatment (observation time not exceeding 10 s per animal). The observational data was used to construct time–effect curves spanning the 2 h post-treatment period.

Catalepsy

The catalepsy is characterized by both positional immobility and the difficulty to initiate voluntary movements, and is defined as failure to correct an externally imposed posture. This behavior can be obtained easily with the administration of blockers of dopamine receptors, indicating the involvement of central dopamine systems in the motor manifestations (Carlini, 1973).

Following intraperitoneal administration of haloperidol (1 mg/kg), rats (PND90) were placed in the upright position with their forepaws flattened on a horizontal bar placed 10 cm above the bench. Catalepsy was measured by timing the duration of animal immobility (total number of seconds of lack of movement) at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min following drug administration. The immobility test was carried out three times for each animal and the sum of

three immobility episodes at each time interval was used to construct the time-effect curves.

Determination of minimum convulsant dose

The convulsant activity of drugs in animal models is useful to evaluate the effects of other antiepileptic drugs. Seizures were induced by subcutaneous administration of picrotoxin (7 mg/mL, Sigma) and pentylenetetrazol (70 mg/mL, Sigma) in rats from the control and experimental groups (PND130). A continuous infusion device (Harvard Apparatus, Pumps H.94) was used to control the volume and speed of infusion (0.0136 mL/min), enabling calculation of minimum convulsant dose (MCD) for each drug.

During the administration of the convulsant drug each animal was observed in cages with glass walls measuring 90x40x50 cm. The administration of the convulsant drug was discontinued when the onset of tonic-clonic seizure was apparent. The MCD was calculated in mg/kg for each animal.

Determination of monoamine levels

The concentrations of monoamine levels and their metabolites in the animal brain were determined. At PND130, rats from control and experimental groups which had not been used for any behavior test were decapitated. Brains were dissected on dry ice and prepared as described by Felicio et al. (1996). Briefly, the striatum, prefrontal cortex and hypothalamus were weighed and stored at 80 °C until neurochemical analyses were carried out. Following sample collections, perchloric acid was added to the tissues which were then homogenized by sonication for immediate determination of monoamine levels. Dopamine (DA) and its metabolites [3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)], serotonin (5-HT) and its metabolite [5-hydroxyindolacetic acid (5-HIAA)] and norepinephrine (NOR) and its metabolite [vanilmandelic acid (VMA)] were measured by HPLC (Shimadzu, model 6A) using a C-18 column (Supelco®, Sigma), eletrochemical detector (Shimadzu, model 6A), sample injector (15 and 20 ml valve) and an integrator (Shimadzu, model 6A Chromatopac). Each sample was run for 18 min. The detection limit was 0.2 ng for all analysis.

Statistical Analysis

Results were expressed as means±SEM (standard errors). The Student´s t test was employed to analyze the parametric data of the dams and of the offspring development. The Mann-Whitney U test was employed to analyze nonparametric data. In all experiments a probability of $p<0.05$ was considered to be significant. Information about the type of statistical test chosen is described in the captions of illustrations of each test (figure or table).

Results

There were no significant differences in maternal weight gain, food intake or water ingestion between the control and experimental groups (Table 1). There were also no changes in weight of offspring (data not shown).

Table 2 shows the effects of ayahuasca on the offspring of rats exposed to ayahuasca during the gestation period. There was no significant difference between the two groups in total number of pups per litter, weight of pups measured on PND1, reflexology and physical parameters above described.

In neurobehavioral studies it was observed that ayahuasca treatment altered some parameters. In the open-field study the experimental group demonstrated a significant mean reduction in moved distance ($p=0.047$) and velocity ($p=0.033$) in the central zone (Table 3).

Table 1. Weight gain, food intake and water ingestion of from females treated with ayahuasca during gestation and lactation period (GD6 to LD10).

Parameters	Control (n=18)	Experimental (n=18)
Weight gain (g/interval)		
GD 0-7	22.6+1.1	27.6+2.5
GD 7-14	24.1+1.6	22.4+1.5
GD 14-21	75.9+3.7	72.3+3.8
GD 0-21	122.6+4.3	122.8+4.3
LD 0-7	27.8+2.1	28.1+2.5
LD 7-14	12.8+2.5	9.4+2.4
LD 7-21	3.6+2.4	7.8+2.6
Food intake (g/day)		
GD 0-7	21.7+0.6	21.9+0.3
GD 7-14	25.0+0.8	24.4+0.4
GD 14-21	26.2+0.7	26.3+0.8
LD 0-7	24.8+0.5	24.6+0.9
LD 7-14	25.1+0.8	24.9+0.4
LD 7-21	28.4+0.7	27.6+0.6
Water Ingestion (ml/day)		
GD 0-7	40.9+1.6	43.7+1.9
GD 7-14	49.4+2.3	50.3+2.1
GD 14-21	28.7+2.9	23.7+2.5
LD 0-7	41.4+1.8	42.6+2.2
LD 7-14	50.3+3.2	49.6+2.2
LD 7-21	58.9+2.6	56.6+2.6

Caption: n: number of animals in the respective groups, GD: gestation day; LD: lactation day. Data are presented as means+SEM; $p>0.05$, Student's t test.

Table 2. Reflexology and physical parameters of pups from females treated with ayahuasca during gestation and lactation period (GD6 to LD10).

Parameters	Control (n=18)	Experimental (n=18)
Total litter weigth (g)	72.4+2.7	71.3+2.5
Number of pups per litter	11.4+0.6	10.6+0.7
Weight (g) on Postnatal day 1		
Male	6.24+0.1	6.42+0.1
Female	6.13+0.1	6.32+0.2
Height (c) on Postnatal day 1		
Male	5.86+0.04	5.92+0.03
Female	5.38+0.04	5.46+0.09
Testes descent day	24.3+0.3	24.4+0.3
Balanopreputial separation day	40.4+0.4	40.2+0.5
Vaginal opening day	35.4+0.3	35.6+0.4
Pinna detachement AD	1.9+0.2	1.7+0.1
Hair growth AD	4.4+0.2	4.6+0.1
Teeth eruption AD	10.3+0.1	10.1+0.2
Eye Opening AD	13.4+0.2	13.1+0.2
Ear opening AD	14.3+0.3	14.0+0.2
Adult gait AD	13.6+0.2	13.5+0.2
Palmar grasp DD	8.2+0.3	8.2+0.4
Surface right reflex AD	1.9+0.1	2.2+0.2
Negative geotaxis AD	7.2+0.3	7.2+0.3
Air righting reflex AD	13.9+0.3	14.1+0.1
Auditory startle reflex AD	14.2+0.3	13.7+0.2
Cliff avoidance AD	6.0+0.3	5.7+0.4
Vibrissa placing AD	11.5+0.2	11.9+0.2
Visual placing AD	15.2+0.2	15.1+0.2

Caption: n: number of animals in the respective groups; AD: appearance day; DD: Disappearance day. Data are presented as means+SEM; $p>0.05$, Student's t test.

Table 3. Open-field parameters of adult offspring rats exposed to ayahuasca during gestation and lactation period (GD6 to LD10).

Parameters	Control (n=15)	Experimental (n=15)
Distance moved (cm)		
Total	1250+69	1309+72
Central zone	16.9+4.5	6.2+2.2*
Intermediate zone	19.2+4.2	14.1+4.1
Peripheral zone	1106+60	1211+58
Time (s)		
Central zone	2.3+0.5	1.3+0.5
Intermediate zone	17.4+3.6	13.2+3.4
Peripheral zone	278.7+60	284.9+3.4
Velocity (cm/s)		
Total	8.4+0.5	8.5+0.5
Central zone	12.6+2.2	6.2+1.8*
Intermediate zone	2.2+0.2	2.0+0.2
Peripheral zone	8.2+0.5	8.3+0.4
Frequency of rearing (%)	28.7+5.2	23.5+5.7
Frequency of rrooming (%)	20.3+4.4	18.3+5.0
Number of defecation	4.0+0.6	4.2+0.6

Caption: n: number of animals in the respective groups. Data are presented as means+SEM; *$p<0.05$, Student´s t test.

Figures 1A and B show the effects of ayahuasca treatment in the elevated plus-maze. In the experimental group there was an increased percentage of entries in the open arms ($p=0.041$) when compared to the control group. As expected, the contrary was observed in the closed-arms of the apparatus (data not shown). Furthermore, no differences were found in time and frequency in central zone and total arm entries.

In the social interaction study (Figure 2) pairs of rats from the experimental group presented a significant decrease in total time of interaction when compared to the control group ($p=0.0052$). Figure 3 shows the results of the forced swimming test. The experimental group showed a decrease in the time of latency to start floating when compared to the control group ($p=0.029$). However, no group differences were observed in total time of floating (immobility time).

Prenatal exposure did not alter stereotyped behavior (Figure 4). Neither the time-effect curve nor the intensity of stereotyped behavior differed significantly between the control and experimental group. Figure 5 shows the results of the catalepsy test. No alterations were observed in either group.

Figure 6 shows the results of the minimum convulsant dose induced by pentylenetetrazol and picrotoxin. The treatment with ayahuasca during pregnancy and lactation period reduced the minimum convulsant dose of pentylenetetrazol when compared with the control group ($p=0.026$). The minimum convulsant dose of picrotoxin did not cause differences between groups.

In the monoamine and metabolites study (Table 4), an increase in DA cortical levels was observed ($p=0.038$). Although this change occurred, the dopamine/metabolite ratio was not altered. The levels of DOPAC, HVA, 5-HT, 5-HIAA, NOR, and VMA did not differ significantly between control and experimental groups. No alterations were observed at central monoamine activity systems as determined by relations between metabolite and neurotransmitter.

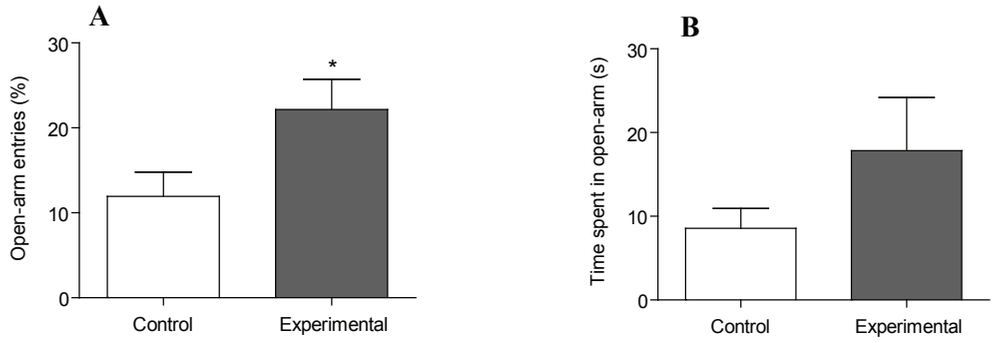

Figure 1. A. Elevated plus-maze parameters of adult offspring rats exposed to ayahuasca during gestation and lactation period (GD6 to LD10). n=10 per group. Percent of entries into the open arms during the 5 min of the test. B. Percent time spent in the open arms during the 5 min of the test. *p<0.05, Student´s t test.

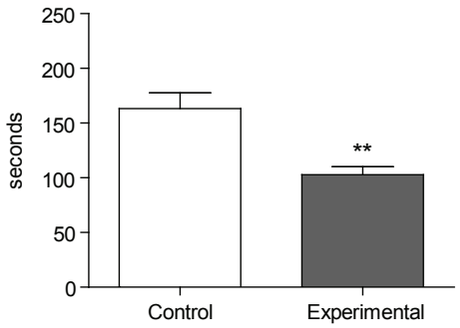

Figure 2. Social interaction test of adult offspring rats exposed to ayahuasca during gestation and lactation period (GD6 to LD10). Data are reported as mean±SEM, for n=8 pairs per group. **p<0.01, Student's t test.

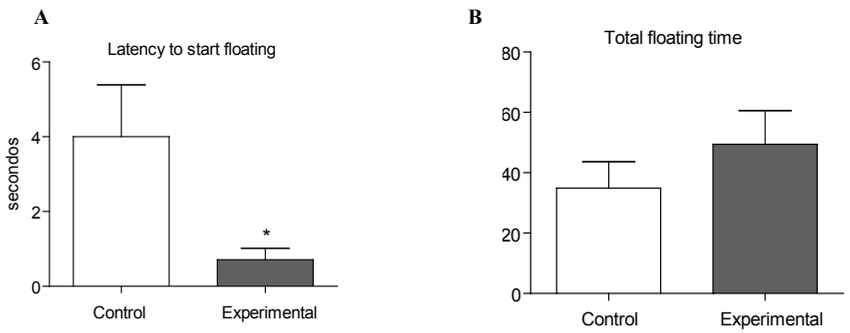

Figure 3. Forced swimming test of adult offspring rats exposed to ayahuasca during gestation and lactation period (GD6 to PND10). Data are reported as mean±SEM, for n=15 per group. A. Time of latency to start floating. B. Total floating time; *p< 0.05, Student´s t test.

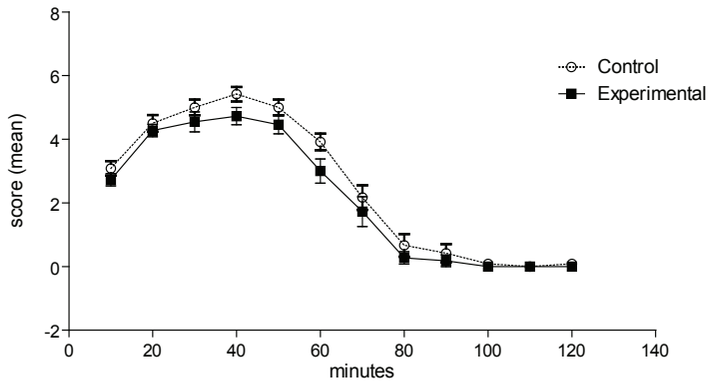

Figure 4. Time-effect curve of stereotype scores following apomorphine challenge of adult rats exposed to ayahuasca during gestation and lactation period (GD6 to LD10). n=12 per group. Mann-Whitney *U* test.

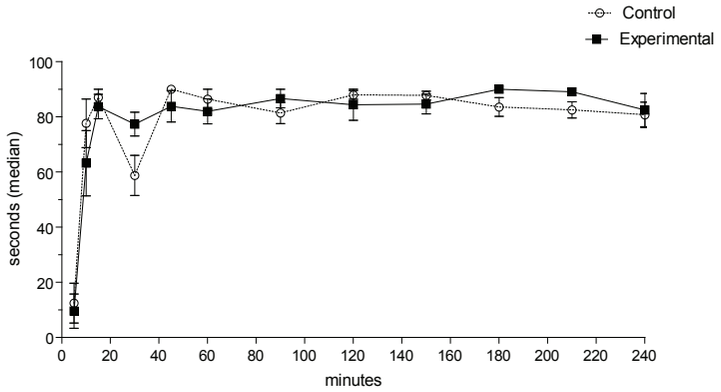

Figure 5. Sum of time of catalepsy of adult rats exposed to ayahuasca during gestation and lactation period (GD6 to LD10). n=12 per group. $p>0.05$, Mann-Whitney *U* test.

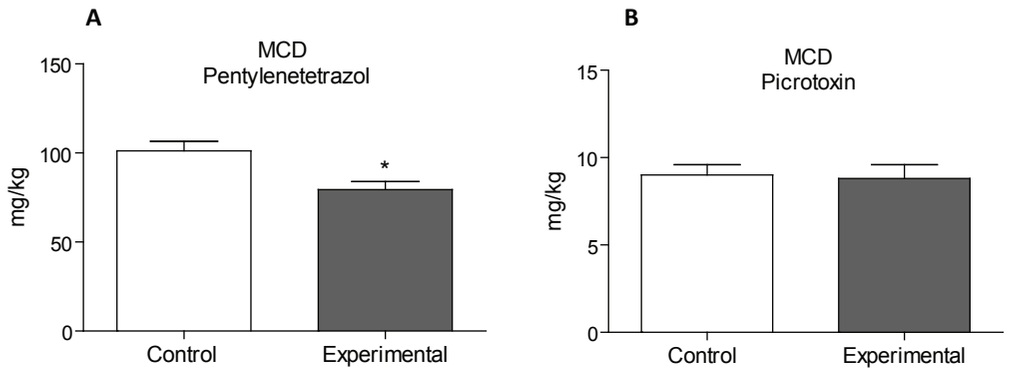

Figure 6. Miminum convulsant dose (MCD) induced by pentylenotetrazol (A) and picrotoxin (B) of adult offspring rats exposed to ayahuasca during gestation and lactation period (GD6 to LD10). n=8 pairs per group. *$p<0.05$, Mann-Whitney *U* test.

Discussion

There is a possibility that prenatal exposure to toxic agents causes postnatal alterations without causing apparent morphologic abnormalities. An agent is considered to be a toxicant to the development when it adversely affects the development of the offspring without causing severe signs of maternal toxicity, such as decreased maternal water and food consumption and/or maternal body weight gain (Farrar and Blumer, 1991; Holson et al., 2006).

In the present study, it was possible to observe that ayahuasca did not induce maternal toxicity at the evaluated conditions (dose and frequency of exposure) (Table 1). The dose was selected to study possible functional and behavioral alterations in the litter. In offspring studies, the dose of ayahuasca did not alter physical and reflexological parameters or body weight (Table 2). There were no animal deaths during the experiments.

On the other hand, neurobehavioral alterations were observed. In open field tests (Table 3) a decrease in average speed and moved distance in the central area was observed in the group whose mothers were exposed to ayahuasca during pregnancy and lactation when compared with the control group. Open field is an exploratory model that can provide information on 1) anxiety - when anxiety stimuli are sufficient to indicate danger; 2) exploration - if the anxiety stimuli are not so excessive as to inhibit the behavior of the animal; and 3) locomotor activity after periods of habituation - when the arena is no longer new (Prut & Belzung, 2003). The open field test is also considered an indicator of the emotional state of the animal (Bagdy et al., 2007; Belzung, 1999). However, the open field test was not able to show changes in anxiety, motor activity or locomotion of the animal, since although it was observed a significant reduction in distance and speed in the central zone of experimental group; no changes were observed in other areas of the arena, mainly in the peripheral zone, that could show some effect.

In the test of elevated plus-maze, an increased

Table 4. Monoamine and metabolite levels (µg/g of tissue) and metabolite/neurotransmistter ratios of adult offspring rats exposed to ayahuasca during gestation and lactation period (GD6 to LD10).

	Region	Control (n=10)	Experimental (n=10)
DA	Cortex	35.3+7.5	53.6+8*
	Striatum	199.1+44.5	261.7+39.1
	Hypothalamus	32.9+10.6	32.9+7.9
DOPAC	Cortex	17.1+3.4	23.5+2.9
	Striatum	36.6+7.5	50.0+7.7
	Hypothalamus	11.0+3.3	14.80+3.3
DOPAC/DA	Cortex	0.6+0.1	0.60+0.1
	Striatum	0.20+0.01	0.20+0.02
	Hypothalamus	0.40+0.07	0.50+0.08
HVA	Cortex	5.90+0.9	7.10+0.9
	Striatum	17.9+4.0	24.0+4.6
	Hypothalamus	3.50+1.3	4.20+1.1
HVA/DA	Cortex	0.20+0.04	0.20+0.04
	Striatum	0.10+0.01	0.10+0.01
	Hypothalamus	0.10+0.01	0.10+0.04
NOR	Cortex	38.5+3.84	45.2+5.1
	Striatum	44.9+9.7	39.6+2.9
	Hypothalamus	88.1+17.5	81.5+14.3
VMA	Cortex	269.6+17.8	308.8+26.7
	Striatum	274.6+35.8	302.7+39.5
	Hypothalamus	262.1+38.8	266.8+28.2
VMA/NOR	Cortex	7.60+0.8	7.80+1.2
	Striatum	7.10+0.9	7.60+0.8
	Hypothalamus	3.4+0.3	3.1+0.2
5-HIAA	Cortex	43.8+3.2	49.5+4.7
	Striatum	42.7+6.0	51.1+6.6
	Hypothalamus	55.7+8.7	49.0+7.3
5-HT	Cortex	80.4+5.5	95.1+8.9
	Striatum	41.5+7.5	41.4+4.6
	Hypothalamus	95.4+15.7	95.5+11.1
5-HIAA/5-HT	Cortex	0.6+0.03	0.5+0.01
	Striatum	1.4+0.5	1.3+0.1
	Hypothalamus	0.6+0.02	0.5+0.05

Caption: n: number of animals in the respective groups; DA: Dopamine; DOPAC: 3.4-dihydroxyphenylacetic acid; HVA: homovanillic acid; 5-HT: serotonin; 5-HIAA: 5-hydroxyindolacetic acid; NOR: norepinephrine; VMA: vanilmandelic acid. Data are presented as means+SEM *p<0.05, Student´s t test.

frequency of entries in open arms was observed in the group treated with ayahuasca when compared with the control group. Consequently, the frequency of closed arm entries in the experimental group decreased significantly when compared with the control group. In the social interaction test the experimental group showed a significant decrease in the period of pursuit and the total interaction time when compared with the control group indicating a lower social motivation in this group.

The elevated plus-maze and social interaction are classic tests used to evaluate anxiolytic drugs. Nowadays, the usefulness of these tests also includes understanding of the biological basis of emotion. The first intention of the elevated plus maze test is to measure anxiety. However, the most of behavioral tests have limitations. Sometimes, different models of animal behaviors can present contradictory results. Therefore, it is necessary to find other possible interpretations of the observed results, since rats display different strategies of defense while exploring the environment. It has been suggested that the social interaction and the elevated plus-maze tests measure distinct facets of anxiety (*i.e*., social anxiety in the social interaction test and generalized anxiety in the elevated plus-maze test) that may be differentially susceptible to drug treatments (Trezza et al., 2008). The elevated plus-maze may also be a good model of simple or specific phobias (Cheeta et al., 2000). Thus it can be concluded that perinatal treatment with ayahuasca promoted a reduction in general anxiety and social motivation in the rat offspring.

Serotonin exerts its influence in a number of behavioral and physiological processes in adult mammals (Cannizzaro et al., 2008; Lauder et al., 1988; Lisboa et al., 2007). Some animal researches have shown that affecting the serotoninergic system during pregnancy produce adverse neuroanatomic effects in the offspring, including reduced numbers of β-adrenergic and serotonin receptors and abnormalities in brain serotonin receptor binding. During embryogenesis, serotonin regulates axon growth, synaptogenesis, the development of γ-aminobutyric acid (GABA) and monoamine systems and is involved in cell migration (Cannizzaro et al., 2008; Lauder et al., 2000; Lisboa et al., 2007). One of the most abundant subtype receptors expressed in the mammalian brain is the 5-HT1A. Dysregulation of 5-HT1A receptors has been implicated in changes of emotional state (Cannizzaro et al., 2008). In spite of DMT has low to moderate affinity for the 5-HT1A (a receptor related to anxiolytic properties when stimulated), possibly this mechanism influenced the effects observed in the elevated plus maze test. This effect is involved in depression, anxiety and reactivity of the offspring (Lisboa et al; 2007).

In forced swim test, prenatal ayahuasca treatment reduced the time of latency to start floating without modification in total floating time. The latency to immobility is a parameter used to characterize antidepressant-like activity (Castagne et al., 2009) and also evaluates serotonergic motor behavioral response (Calabrese, 2008). Forced swimming is an inescapable stressful situation causing a relatively short escape reaction followed by floating without performing any

activity (Lazarini et al., 2001). It is possible to suggest that the reduction in latency to float observed here was the consequence of decreased motor activity.

Prenatal exposure did not alter both catalepsy and stereotyped behavior. These two tests evaluate effects on dopaminergic receptors from nigrostriatal system of rat brain. In fact, dopamine levels and its metabolites were not altered in the experimental group.

Through controlled administration of pentylenetetrazol and picrotoxin it was possible to determine the minimum convulsant dose for each animal. There was a decrease in the dose of pentylenetetrazol required to induce seizure in animals in the experimental group when compared with the control group. Perinatal treatment with ayahuasca promoted this change, indicating that these animals were more sensitive to convulsant stimuli. The mechanism of action of pentylenetetrazol is not completely elucidated. It seems to affect several neurotransmitter systems including the GABAergic, glutamatergic and dopaminergic systems (Dazzy et al., 1997; Nasser et al., 2009). Since dopamine levels were found to be altered in the cortex of the experimental group, theses results could help to explain alterations observed in the pentylenetetrazol test (determination of minimum convulsant dose). It is also known that central dopaminergic neurons could play an important role in the initiation and spread of seizure activity (Dazzy et al., 1997).

Our results did not show apparent alterations in serotonin levels. However, it is known that DMT and beta-carbolines affect the serotoninergic system. Serotonergic neurotransmission also modulates a wide variety of experimentally induced seizures and it is involved in the enhanced seizure susceptibility observed in rodents genetically prone to epilepsy. Furthermore, mutant mice lacking 5-HT1A or 5-HT2C receptors show increased seizure activity and/or lower threshold for triggering a seizure (Bagdy et al., 2007).

All the effects observed here and in a previous study performed by our group (Oliveira et al., 2010) suggest that the ayahuasca alkaloids can cross the placental barrier. In conclusion, our results showed that the use of ayahuasca by mothers during pregnancy and lactation reduced the general anxiety and social motivation of the rat offspring. Besides, it promoted a higher sensitivity for initiation and spread of seizure activity.

Acknowledgment

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo, grant no. 2006/00388-5) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for financial support. Thanks to Dr. Sigrid Luiza Jung-Mendaçolli and Dr. Maria Candida Henrique Mamede for plant identification and Prof. Dr. Jorge Camilo Flório for HPLC technical assistance. The authors also thank Kelly Ann Rees, from University of Bournemouth, United Kingdom, for the English revision of the paper.

References

Bagdy G, Kecskemeti V, Riba P, Jakus R 2007. Serotonin and epilepsy. *J Neurochem 100*: 857-873.

Belzung C 1999. Measuring exploratory behavior. In: Crusio WE, Gerlai RT. *Handbook of molecular genetic techniques for brain and behavior research - Techniques in the behavioral and neural sciences*. Amsterdam: Elsivier Science p. 739-749.

Borsini F 1995. Role of the serotonergic system in the forced swimming test. *Neurosci Biobehav R 19*: 377-395.

Calabrese EJ 2008. An assessment of anxiolytic drug screening tests: Hormetic dose responses predominate. *Crit Rev Toxicol 38*: 489-542.

Callaway JC 2005. Fast and slow metabolizers of Hoasca. *J Psychoactive Drugs 37*: 157-161.

Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, Mash DC 1999. Pharmacokinetics of hoasca alkaloids in healthy humans. *J Ethnopharmacol 65*: 243-256.

Cannizzaro C, Plescia F, Gagliano M, Cannizzaro G, Mantia G, LaBarbera M, Provenzano G, Cannizzaro E 2008. Perinatal exposure to 5-metoxytryptamine, behavioural-stress reactivity and functional response of 5-HT1A receptors in the adolescent rat. *Behav Brain Res 186*: 98-106.

Carlini EA 1973. *Farmacologia prática sem aparelhagem*. São Paulo: Editora Sarvier.

Carlini EA 2003. Plants and the central nervous system. *Pharmacol Biochem Be 75*: 501-512.

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

Cheeta S, Kenny PJ, File SE 2000. Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. *Prog Neuropsychopharmacol Biol Psychiatry 24*: 1053-1067.

Conselho Nacional Antidrogas 2004. Resolução no. 4. Diario Oficial da Uniao (DOU) da República Federativa do Brasil.

Dazzi L, Serra M, Porceddu ML, Sanna A, Chessa MF, Biggio G 1997. Enhancement of basal and pentylenetetrazol (PTZ)-stimulated dopamine release in the brain of freely moving rats by ptz-induced kindling. *Synapse 26*: 351-358.

Farrar HC, Blumer JL 1991. Fetal effects of maternal drug exposure. *Annu Rev Pharmacol 31*: 525-547.

Felicio LF, Florio JC, Sider LH, Cruz-Casallas PE, Bridges RS 1996. Reproductive experience increases striatal and hypothalamic dopamine levels in pregnant rats. *Brain Res Bull 40*: 253-256.

File SE, Pellow S 1985. The effects of triazolobenzodiazepines in 2 animal tests of anxiety and in the holeboard. *Brit J Pharmacol 86*: 729-735.

File SE, Zangrossi H, Andrews N 1993. Social-interaction and elevated plus-maze tests - changes in release and uptake of 5-HT and GABA. *Neuropharmacology 32*: 217-221.

Gable RS 2007. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. *Addiction 102*: 24-34.

Grob CS, McKenna DJ, Callaway JC, Brito GS, Neves ES, Oberlaender G, Saide OL, Labigalini E, Tacla C, Miranda CT, Strassman RJ, Boone KB 1996. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J Nerv Ment Dis 184*: 86-94.

Guideline for care and use of laboratory animals 1996. Institute of Laboratory Animal Research. Comission on Life Sciences National Research Council.

Halpern JH, Sherwood AR, Passie T, Blackwell KC, Ruttenber AJ 2008. Evidence of health and safety in American members of a religion who use a hallucinogenic sacrament. *Med Sci Monitor 14*: 15-22.

Holson JF, Nemec MD, Stump DG, Kaufman LE, Lindstrom P, Varsho BJ 2006. Significance, reliability, and interpretation of developmental and reproductive toxicity study findings. In: Hood RD. *Developmental and reproductive toxicology - A Practical approach.* 2 ed. Boca Raton: Taylor & Francis Group; p. 329-424.

Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, Jensen NH, Kuijer MB, Matos RC, Tran TB, Whaley R, Glennon RA, Hert J, Thomas KL, Edwards DD, Shoichet BK, Roth BL 2009. Predicting new molecular targets for known drugs. *Nature 462*: 175-181.

Lauder JM, Liu JP, Grayson DR 2000. In utero exposure to serotonergic drugs alters neonatal expression of 5-HT1A receptor transcripts: a quantitative RT-PCR study. *Int J Dev Neurosci 18*: 171-176.

Lauder JM, Tamir H, Sadler TW 1998. Serotonin and morphogenesis. 1. Sites of serotonin uptake and serotonin binding-protein immunoreactivity in the midgestation mouse embryo. *Development 102*: 709-720.

Lazarini CA, Florio JC, Lemonica IP, Bernardi MM 2001. Effects of prenatal exposure to deltamethrin on forced swimming behavior, motor activity, and striatal dopamine levels in male and female rats. *Neurotoxicol Teratol 23*: 665-673.

Lisboa SFS, Oliveira PE, Costa LC, Venancio EJ, Moreira EG 2007. Behavioral evaluation of male and female mice pups exposed to fluoxetine during pregnancy and lactation. *Pharmacology 80*: 49-56.

McKenna DJ 2004. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacol Therapeut 102*: 111-129.

Naseer MI, Shupeng L, Kim MO 2009. Maternal epileptic seizure induced by pentylenetetrazol: apoptotic neurodegeneration and decreased GABAB1 receptor expression in prenatal rat brain. *Mol Brain Res 2*: 20.

Norton S 1989. Methods for behavioral toxicology. In: Hayes A. *Principles and methods of toxicology.* 2 ed. New York: Raven; p. 553-571.

Oliveira CDR, Moreira CQ, De Sá LRM, Spinosa HS, Yonamine M 2010. Maternal and Developmental Toxicity of ayahuasca in Wistar Rats. *Birth Defects Res B 89*: 207-212.

Pellow S, Chopin P, File SE, Briley M 1985. Validation of open - closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Meth 14*: 149-167.

Pires APS, De Oliveira CDR, Moura S, Dorr FA, Silva WAE, Yonamine M 2009. Gas Chromatographic Analysis of dimethyltryptamine and beta-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. *Phytochem Analysis 20*: 149-153.

Porsolt RD, Lepichon M, Jalfre M 1997. Depression - new animal-model sensitive to antidepressant treatments. *Nature 266*: 730-732.

Prut L, Belzung C 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol 463*: 3-33.

Riba J, Barbanoj MJ 2005. Bringing ayahuasca to the clinical research laboratory. *J Psychoactive Drugs 37*: 219-230.

Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ 2003. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *J Pharmacol Exp Ther 306*: 73-83.

Schwarz A, Gornak SL, Bernardi MM, Dagli MLZ, Spinosa HS 2003. Effects of Ipomoea carnea aqueous fraction intake by dams during pregnancy on the physical and neurobehavioral development of rat offspring. *Neurotoxicol Teratol 25*: 615-626.

Setler P, Sarau H, McKenzie G 1976. Differential attenuation of some effects of haloperidol in rats given scopolamine. *Eur J Pharmacol 39*: 117-126.

Trezza V, Campolongo P, Cassano T, Macheda T, Dipasquale P, Carratu MR, Gaetani S, Cuomo, E 2008. Effects of perinatal exposure to delta-9-tetrahydrocannabinol on the emotional reactivity of the offspring: a longitudinal behavioral study in Wistar rats. *Psychopharmacology 198*: 529-537.

Tupper KW 2008. The globalization of ayahuasca: Harm reduction or benefit maximization? *Int J Drug Policy 19*: 297-303.

Zbinden G 1981. Experimental methods in behavioral teratology. *Arch Toxicol 48*: 69-88.

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