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Original Article

Reproductive effects of the psychoactive beverage ayahuasca in male Wistar rats after chronic exposure

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ABSTRACT

Ayahuasca is a psychoactive beverage used ancestrally by indigenous Amazonian tribes and, more recently, by Christian religions in Brazil and other countries. This study aimed to investigate the reproductive effects of this beverage in male Wistar rats after chronic exposure. The rats were treated by gavage every other day for 70 days at 0 (control), 1×, 2×, 4× and 8× the dose used in a religious ritual (12 animals per group), and animals euthanized on the 71st day. Compared to controls, there was a significant decrease in food consumption and body weight gain in rats from the 4× and 8× groups, and a significant increase in the brain and stomach relative weight at the 8× group. There was a significant increase in total serum testosterone, and a decrease in spermatic transit time and spermatic reserves in the epididymis caudae in the 4× group, but not in the highest dose group. No significant changes were found in the other reproductive endpoints (spermatozoid motility and morphology, total spermatozoid count and daily sperm production), and histology of testis and epididymis. This study identified a no-observed-adverse-effect-level for chronic and reproductive effects of ayahuasca in male Wistar rats at 2× the ritualistic dose, which corresponds in this study to 0.62 mg/kg bw *N*,*N*-dimethyltryptamine, 6.6 mg/kg bw harmine and 0.52 mg/kg bw harmaline. A potential toxic effect of ayahuasca in male rats was observed at the 4× dose, with a non-monotonic dose–response. Studies investigating the role of ayahuasca components in regulating testosterone levels are needed to better understand this action.

Introduction

Ayahuasca, which in Quechua means "wine of the souls", is a hallucinogenic plant concoction used ancestrally by Amazon indigenous groups in xamanic rituals for diagnosis, healing, and spiritual development (Grob et al., 1996; McKenna, 2004; Tupper, 2008). Since the 1930s, Christian religious communities have also used this concoction in their rituals, including Santo Daime, Barquinha and União do Vegetal (UDV) (Macrae, 2004; Tupper, 2008), and its use is legal in Brazil (CONAD, 2004). The ayahuasca religions also have centers in other countries in South America, Europe, Asian and North America (Halpern, 2004; Tupper, 2008; Labate and Feeney, 2012). Its use, however, goes beyond religious rituals, being also used for recreational purposes by people seeking its hallucinogenic effects, with a potential risk of intoxication (Dos Santos, 2013; Winstock et al., 2014). On the other hand, various studies have suggested the therapeutic action of ayahuasca, including drug addiction, anxiety and depression (Pic-Taylor et al., 2015; Dos Santos et al., 2016a; Domínguez-Clavé et al., 2016).

Ayahuasca is a concoction generally produced with *Psychotria viridis* Ruiz & Pav., Rubiaceae, bush leaves and *Banisteriopsis caapi* (Spruce ex Griseb.) Morton, Malpighiaceae, vine, both native from the Amazon. The *B. caapi* stem contains the β-carbolines alkaloids harmine, harmaline and tetrahydro-harmine, which are reversible inhibitors of mitochondrial monoamine oxidase (MAO) enzymes (McKenna et al., 1984; Harvey et al., 1998) responsible for the oxidation of neurotransmitters, such as serotonin, dopamine and noradrenalin. *P. viridis* contains *N*,*N*-dimethyltryptamine (DMT), which is also found in other plants and animals, including humans (Callaway et al., 1996). Due to its structural similarity to serotonin (5-hydroxytryptamina, 5HT), DMT binds with serotonergic receptors, mainly the 5-HT_{2A} type, producing its hallucinogenic effects (Smith et al., 1998; Halberstadt, 2015). Studies show that DMT acts as a substrate for the serotonin transporter (SERT) and for the vesicular monoamine transporter (Nagai et al., 2007; Cozzi et al., 2009; Halberstadt, 2015).

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When administered orally, DMT is rapidly degraded by the MAO present in the liver and intestine. However, in the presence of β-carbolines, it can reach the brain and becomes orally active. Therefore, the hallucinogenic effects of ayahuasca ingestion are produced by the synergic action of the active compounds present in the plant species used in its preparation (Buckholtz and Boggan, 1977; Callaway et al., 1996, 1999). The effects include alterations in affective and emotional states, thoughts, memory and body sensations, synesthesia and hallucination with alterations in the visual, olfactory and auditory senses, improved planning and inhibitory control and showed antidepressive and antiaddictive potentials (Shanon, 2003; Pires et al., 2010; Dos Santos et al., 2016b). Somatic effects may include nausea, vomiting, diarrhea, tremors, dizziness, tachycardia, mydriasis and hypertension (Callaway and Grob, 1998; Riba et al., 2003; Vives and García-albea, 2012).

A study conducted by this research group in female Wistar rats found that the acute lethal oral dose of an ayahuasca infusion prepared by the UDV was over 50× the ritual dose. This same study identified greater neuronal activity in regions of the brain rich in serotoninergic receptors, such as the amygdala, the raphe nucleus and the hippocampus, in animals exposed to a single dose of ayahuasca, equivalent to 30× the ritual dose (Pic-Taylor et al., 2015). A study conducted by Motta (2013) showed that female rats exposed daily to ayahuasca during pregnancy at doses higher than 2×X the ritual dose had reduction in reproduction rates, increase in reabsortions, lower body weight and lower relative fetus organ weights and fetus visceral malformations. Similar results were found in a previous study conducted by Oliveira et al. (2010).

Several studies have addressed the relation between psychoactive drugs and male infertility. Tests on animals show that substances such as tetra-hydrocanabinol, found in plant species of the genus *Cannabis*, reduce testosterone levels, affecting sperm production and motility, and consequently male fertility (Morgan et al., 2011; Onyije, 2012). Drugs such as alcohol, tobacco, cocaine, and androgenic anabolic steroids among others are also considered potential infertility agents (Onyije, 2012; Vignera et al., 2013; Kulkarni et al., 2014). Furthermore, Alvarenga et al. (2014) found that a single dose of ayahuasca significantly decreased sexual performance of male rats, but higher performance was observed in sleep deprived rats treated at the lowest dose (250 μg/ml). However, studies evaluating toxicity aspects regarding male reproduction in animals exposed chronically to ayahuasca have yet to be conducted.

The aim of this study is to investigate the potential toxicological effect of an ayahuasca infusion on reproduction in Wistar rats, after chronic treatment.

Materials and methods

Ayahuasca material

The ayahuasca used in this study was prepared in April, 2011 by the Núcleo Luz do Oriente of the UDV, Federal District, Brazil, and stored in a −20 °C freezer before lyophilization. The infusion was prepared with *B. caapi* collected in Águas Lindas de Goiás (15°46′17″ S, 48°14′56″ W) and the leaves of *P. viridis* collected in Sobradinho, Federal District (15°75′23″ S, 47°72′92″ W). Samples of both species were deposited in the University of Brasilia Herbarium under the references Azevedo EP149880 Brahms and Trieto B149879 Brahms, respectively. The levels of DMT, harmaline and harmine present in the ayahuasca infusion, determined prior to the experiment by GC–MS/MS (Trace Ultra coupled with a TSQ Quantum XLS Triple Quadrupole; Thermo Scientific), were 0.146 mg/ml of DMT, 0.12 mg/ml of harmaline, and 1.56 mg/ml of harmine (Pic-Taylor et al., 2015). Fig. 1 shows the GC–MS/MS total ion chromatogram of the ayahuasca provided by the UDV. Only the harmine peak can be seen, as it is present at a concentration over ten times higher than DMT and harmaline. Fig. 1 also shows the chromatograms, in selected reaction monitoring mode, of the three ayahuasca components (abundance normalized to 100% in each case). Tetrahydroharmine was not analyzed in this study. The lyophilized material was appropriately weighed according to the doses selected before treatment, considering body weight, and diluted in filtered water, maintaining a final volume of 2 ml.

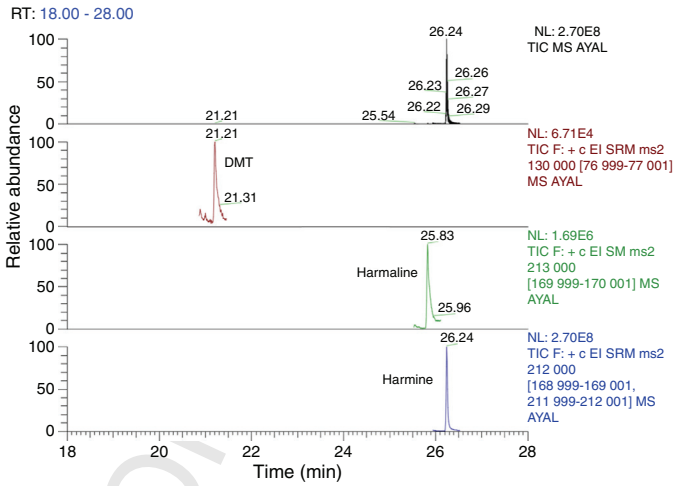

Fig. 1. GC–MS/MS total ion chromatogram of the ayahuasca sample, and the extracted ion chromatograms in selected reaction monitoring mode of DMT (*m/z* 130 → 77; RT = 21.3 min), harmaline (*m/z* 213 → 170; RT = 25.8 min) and harmine (*m/z* 212 → 169; RT = 26.2 min).

Experimental protocol

The study was conducted with 60 male rats of the species *Rattus novergicus*, Wistar lineage, provided by Granja RG (São Paulo, Brazil) aged 4 weeks, and of uniform weight (210 ± 10 g). The animals were kept at the Faculty of Health Sciences of the University of Brasilia (UnB) animal house in Alesco® polypropylene zinc bar cages on ventilated shelves. They underwent a 15-day acclimatization period before the treatment was initiated, and were maintained under controlled temperature conditions (23 ± 2 °C) and dark/light cycles of 12 h/12 h during the experiment, given commercial rodent feed (Purina®) and filtered water ad libitum. This project was approved by the Animal Use Ethics Commission of the UnB (n° 107766/2010).

Clinical assessment of the animals was performed daily, and body weight and feed consumption verified every 3 days. On the 71st day, the animals were euthanized by exposure to CO_2 and a 4 ml blood sample was immediately collected by cardiac puncture and subjected to centrifugation to collect the serum. The study was carried out according to protocol EPA/630/R-96/009/1996 (Guidelines for Reproductive Toxicity Risk Assessment).

Experimental doses

The selected doses used in the study were determined according to the ritual dose consumed during a UDV ceremony, approximately 150 ml for an individual weighing 70 kg (1×). Based on the levels found in the infusion by GC–MS/MS, the 1× dose corresponds to 3.3 mg/kg bw of harmine, 0.26 mg/kg bw of harmaline and 0.31 mg/kg bw of DMT. The animals were randomly distributed into five groups of 12: one control group that received filtered water and four treated groups that received 1×, 2×, 4× and 8× the usual dose for 70 days, by gavage. These doses coincide with

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