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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 this 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 for drug addiction, anxiety and depression (Pic-Taylor et al., 2015; Dos Santos et al., 2016a; Domínguez-Clavé et al., 2016).

Ayahuasca is 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 also 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 consumption 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 (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× 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 are still needed.

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 *Banisteriopsis caapi* (Spruce ex Griseb.) Morton, Malpighiaceae, collected in Águas Lindas de Goiás (15°46′17″ S, 48°14′56″ W) and the leaves of *Psychotria viridis* Ruiz & Pav., Rubiaceae, 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 gas chromatography-tandem mass spectrometry (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.

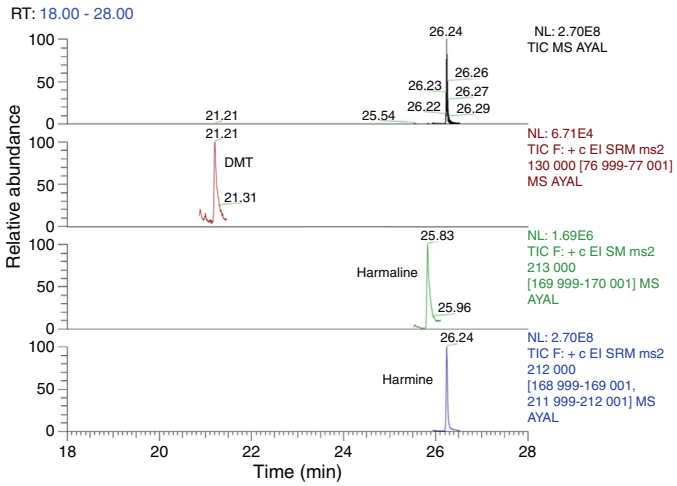

Fig. 1. GC–MS/MS total ion chromatogram (TIC) of the ayahuasca sample, and the extracted ion chromatograms in selected reaction monitoring (SRM) mode of DMT (m/z 130 → 77; RT = 21.3 min), harmaline (m/z 213 → 170; RT = 25.8 min) and harmine (m/z 212 → 169; RT = 26.2 min).

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 in ventilated shelves. They underwent a 15-day acclimatization period before the treatment was initiated, were maintained under controlled temperature conditions (23 ± 2 °C) and dark/light cycles of 12 h/12 h during the experiment, and were 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 those used in the female reproductive toxicity study conducted previously (Motta, 2013) that demonstrated that the daily ingestion of ayahuasca at doses equal or greater to 4× the usual dose is lethal for female pregnant rats after the 5th day of administration, with animals presenting signs of piloerection, convulsion, chromodacryorrhea, lordosis and cyanosis. Similar results were found in a pre-test conducted as part of the present study with male rats administered daily with 4× doses of ayahuasca. Thus, in order to ensure the survival of the animals during the treatment, the present study was conducted with administration taking place on alternating days.

Blood and organ analysis

Two ml of serum of each animal was stored at −20 °C for later serologic dosage of total testosterone, luteinizing (LH) and follicle stimulating (FSH) hormones, and biochemical analyses were performed to assess pancreatic (amylase and lipase), hepatic (total, direct, and indirect bilirubin), renal (creatinine and urea), hepatobiliary, and pancreatic (glutamic oxaloacetic transaminase, and glutamic piruvic transaminase) functions, and triacylglycerides to assess lipid metabolization. Spleen, liver, stomach, brain, heart, kidneys, testicles, epididymis, prostrate and seminal bladder were removed and washed with NaCl 0.9% saline solution, then submitted to macroscopic evaluation and weighing.

Reproductive parameters

During the necropsy, the content of the deferent duct was extracted by diffusion in 1 ml of Dulbecco's Modified Eagle Medium (GIBCO™) previously heated at 34 °C for sperm motility analysis, without surpassing the 10-min period after euthanasia. The resulting solution (100 μl) were inserted in a hemacytometer (Neubauer chamber) and readings were taken with an optic microscope (Leica Galen III), and magnified to 400×. Fifty spermatozoids were analyzed per animal, and the number of progressive (rapid or slow progression) and non-progressive (no progression or motionless) counted (Seed et al., 1996).

The right testis of each animal was perforated at one of the extremities to remove the tunica albuginea, and the parenchyma was homogenized for 1 min in Ultra-Turrax (IKA® T10 basic) in 10 ml of 0.05% Triton X-100 to determine total spermatozoid count (no. $\times 10^6$), daily sperm production, and sperm transit period (days). The right epididymis tail of each animal was cut up in a Petri dish and homogenized to determine the spermatic reserve.

Two samples of each testis and epididymis homogenates (100 μl) were analyzed in the Neubauer chamber using an optic microscope (Zeiss Primo Star; 400×). The spermatids (testis) and spermatozoids (testis and epididymis) resistant to homogenization were counted in five chamber fields and the mean of the total count reported for each animal (Strader et al., 1996).

In rats, mature spermatozoids in stages 17–19 represent 48% of seminiferous epithelium cycle length, which has a duration period of 12.75 days (Robb et al., 1978). Hence, 6.1 days is the period in the seminiferous cycle during which mature spermatozoids are present (Kempinas et al., 1998). Daily sperm production was obtained dividing the number of resistant spermatozoids in the testicle by 6.1 days (Robb et al., 1978; Blazak et al., 1985). The sperm transit time was obtained dividing the number of spermatozoids in the tail of the epididymis by the daily production of sperm (Amann et al., 1976; Robb et al., 1978).

The tail of the left epididymis of each animal was cut up in a Petri dish containing 2 ml of a phosphate-saline buffer solution (PBS; 7.4 pH) to collect the spermatozoids. The resulting solution was diluted in 5 ml of PBS and stored under refrigeration for up to 10 days for further analysis. Two slides per animal were prepared by smear and allowed to dry at room temperature, then fixed with methanol (PA grade) for 10 min and stained with 1% eosine for 45 min (Wyrobek and Bruce, 1975).

Two hundred spermatozoids of each animal were analyzed under microscope and the percentage of normal and abnormal spermatozoids (head, tail and multiple) quantified (IRDG, 2000; Sharma and Singh, 2010).

Testis and epididymis histology

The left testis of each animal was previously fixed in Bouin solution for 3 h at 4 °C, cut transversally in half and maintained for an additional 4 h at the same temperature and solution. After this period, the organs were washed in 50% ethanol (*pro analysis* grade) overnight at room temperature to remove the excess of Bouin, and maintained in 70% ethanol until the histological procedure was performed using classical techniques (dehydration, diaphanization and inclusion in paraffin). Non-consecutive 5 μm thick slices were obtained using microtome (Leica), then stained with hematoxiline and eosin (H&E). Three slides of each animal were analyzed under microscope. In each slide, ten seminiferous tubules were analyzed for the presence of complete spermatogenesis, by Johnsen's Tubular Biopsy Score (JTBS) (Johnsen's, 1970). For each animal, the final score was the mean score observed in 10 tubules of the three slides ($n = 30$) (MJTBS).

The head/body segments of the epididymis of each animal were fixed in Bouin for 7 h at 4 °C, following the same histological procedure described above. Longitudinal sections of the epididymis were analyzed to determine the sperm density, and classified as moderate hypospermia (+++), discrete hypospermia (++), and normal sperm density (+). The presence of alterations in the epididymis and testicle such as exfoliation of germ cells, tubular vacuolization, inflammatory infiltrates, granulomas, necrosis, edemas and tubules of reduced diameter, and cellular disorganization were also investigated (Lanning et al., 2002).

Statistical analysis

The statistical analysis of the sperm morphology and motility was conducted by the Kruskal–Wallis test, and the other parameters were evaluated using one-way analysis of variance (ANOVA), with *post hoc* comparisons between groups using Tukey or Dunnett (homogeneous variance) or Dunnett T3 (non-homogenous variance). The analyses were performed using IBM SPSS Statistics V.20 software, and the differences were considered statistically significant at $p \leq 0.05$.

Results

Feed consumption, body and organ weight and biochemical evaluations

The animals treated with ayahuasca at 1×, 2× and 4× doses did not show adverse clinical signs during treatment. However, animals treated with 8× doses showed evident signs of stress and constant vocalization during the gavage procedure, and piloerection and tremors after ayahuasca administration. Two animals of the 8× group died during the experiment, the first on the 16th day of treatment with signs of tremors and convulsion minutes after gavage, and the second on the 37th day due to faulty administration, with all the administered material being found in the lung.

Table 1 shows the average feed consumption, measured every 3 days, and body weight and total weight gain at the end of the

Table 1
Total weight gain, feed consumption and body weight (g) at the end of the treatment of the control and ayahuasca treated animals (mean ± standard error).

Variable	Control ($n = 12$)	1× ($n = 12$)	2× ($n = 12$)	4× ($n = 12$)	8× ($n = 10$)
Feed consumption	72.1 ± 0.7*	70.9 ± 1.4*	74.6 ± 1.1*	66.9 ± 0.7#	64.2 ± 1.0#
Final body weigh	405.4 ± 11.9*	401.1 ± 13.7*#	409.7 ± 11.4*	381.1 ± 12.3*#	355.3 ± 10.5#
Total weight gain	204.1 ± 7.9*	194.8 ± 7.6*	197.5 ± 8.9*	167.3 ± 9.2#	158.5 ± 7.5#

Different symbols between groups indicate significance at $p < 0.05$; ANOVA followed by Tukey or Dunnett.

treatment for the control and ayahuasca treated groups. A significant drop in feed consumption was observed in animals treated at 4× and 8× doses, which had a significant impact on the final body weight for the 8× group and on the total body weight gain for both groups.

Table 2 shows the relative (%) weights of the animal organs in the control and treated groups, and the absolute weights for the brain and reproductive organs. Animals of the 8× group showed an increase in the relative weight of the brain and stomach in relation to the control, however no difference was observed in the absolute brain weights. A significant decrease in the relative epididymis weight was observed in the 8× group compared with the 4× group, as well as a significant increase in the relative seminal vesicle weight compared with the 2× group. The absolute weight of the testicles was significantly lower for the animals of the 8× group in comparison with the other groups. No macroscopic alterations were observed in the evaluated organs.

The biochemical evaluations conducted to assess the hepatic, pancreatic, renal and lipid metabolizing functions did not indicate significant differences among the groups (data not shown).

Reproductive endpoints

A significant increase was observed in total testosterone of the animals treated with 4× doses of ayahuasca compared with the control group and with the 8× group (Fig. 2A). There were no significant differences in LH and FSH levels between the treated and control groups (data not shown).

The percentages of progressive (sperm motility) and morphologically normal (sperm normality) spermatozoids are shown in Fig. 3. On average, more than 75% of the spermatozoids presented normal motility (progressive), with no significant difference among the groups of the study (Fig. 3A). Although all treated groups showed lower percentages of morphologically normal spermatozoids than the control, down to 46.9% in the 4× group, this reduction was not statistically significant ($p = 0.29$) (Fig. 3B).

Table 3 shows the percentage of abnormal spermatozoids found in the animals of the experimental groups. The percentage of spermatozoids with headless tails was relatively high even in the animals of the control group, possibly as a consequence of the smearing process when preparing the slides. There was an increase in the percentage of spermatozoids with a flattened head in the animals in the 8× group in relation to the 2× group, which is probably also an artifact due to the cell osmolarity changes during the refrigerated storage period, which occurred for up to 10 days. There was an increase in the percentage of spermatozoids with a bent tail in the groups treated with 2× and 4× doses in relation to the 1× group. Overall, no significant differences in the total number of abnormalities were found between the control and treated animals. A significant reduction was observed in the sperm reserve in the epididymis tail and in the sperm transit time of the animals treated with the 4× dose in relation to the control group and the 8× group (Fig. 2B and 2C). No significant differences were observed in the total daily sperm counts in the testicle among the groups ($p = 0.55$), although the count was lower for the 8× dose in comparison with the control and the other treated groups (Fig. 2D).

No significant differences were observed in the mean JTBS score (MJTBS) among the groups, which ranged from 9.43 ± 0.12 (2× group) to 9.5 ± 0.10 (control). All animals presented score of 10 for at least one of the thirty tubules analyzed. Furthermore, no morphological changes were observed in the testis of control and treated animals. Small number of lymphocytes and macrophages were seen in the interstitial tissue of epididymis head segments of some animals from all experimental groups.

Discussion

This study showed that male rats treated with ayahuasca at 4× and 8× doses every 2 days for 70 days had lower body weight gain and lower feed consumption at the end of the treatment compared with the other groups, with significantly lower final body weight observed only in the highest dose group. The relative weights of the stomach and brain were greater in the highest dose group in relation to the control group. These results suggest chronic toxicity of ayahuasca in male rats at higher doses. A study conducted by Motta (2013) demonstrated that ayahuasca administered daily between the 6th and 21st day of gestation induced toxicity, with a significant reduction in feed consumption at 1×, 4× and 8× doses, increases in the relative weights of the stomach of the 1×, 2× and 8× groups, and dilation of the stomachs and intestines of the animals of the 8× group.

There were no significant differences observed between the relative weights of the reproductive organs of the animals in the treated groups and those of the control group, but the absolute weight of the testis of the 8× group was significantly lower than the control. No significant morphological changes were observed in the testis or epididymis of treated animals. Exposure to ayahuasca did not lead to significant changes in sperm motility and morphology, nor in the number of abnormal spermatozoids, and there were no significant alterations in daily sperm production, although the 8× group presented a reduction in this parameter.

In the testis, the MJTBS score was used to evaluate spermatogenesis according to the presence or absence of different cell types in the seminiferous epithelium (Johnsen, 1970). This study identified an average score higher than 9 (out of 10) for all groups, indicating that the spermatogenesis was completed, and that ayahuasca did not affect sperm production. In addition to spermatozoids, cells from all stages of spermatogenesis (spermatogonias, spermatocytes, round and late spermatids) were present in the testis.

The sperm densities in the epididymis head and body regions were assessed during the histological analysis in a semi-qualitative manner, and no significant differences were found among the groups. However, the sperm count in the tail of the epididymis of animals from the 4× group was significantly lower than the control and the 8× group. Indeed, Kempinas and Klinefelter (2014) pointed out that the toxic effects on sperm quantity and quality may occur in the absence of histopathological alterations in the epididymis or testicle, requiring direct verification to be detected. In addition, toxicity may occur in specific locations of the epididymis, such as in the head or tail. On the other hand, sperm density in the epididymis may reflect time-dependent events in the testis, since the spermatozoids found in the tail were released by the testicle approximately

Table 2
Relative (%) and absolute (g) organ weights of the control and ayahuasca treated animals (mean ± standard error).

	Control (n = 12)	1× (n = 12)	2× (n = 12)	4× (n = 12)	8× (n = 10)
Liver, %	3.56 ± 0.33	3.47 ± 0.20	3.43 ± 0.25	3.49 ± 0.20	3.52 ± 0.26
Spleen, %	0.20 ± 0.04	0.19 ± 0.01	0.18 ± 0.03	0.18 ± 0.02	0.18 ± 0.03
Right kidney, %	0.35 ± 0.02	0.34 ± 0.02	0.36 ± 0.04	0.35 ± 0.01	0.37 ± 0.04
Left kidney, %	0.34 ± 0.02	0.32 ± 0.02	0.34 ± 0.03	0.34 ± 0.02	0.35 ± 0.04
Stomach, %	0.46 ± 0.06*	0.50 ± 0.02*#	0.48 ± 0.03*#	0.51 ± 0.02*#	0.52 ± 0.04#
Heart, %	0.32 ± 0.03	0.32 ± 0.04	0.31 ± 0.02	0.33 ± 0.02	0.33 ± 0.03
Brain, %	0.52 ± 0.06*	0.53 ± 0.06*#	0.51 ± 0.06[a]	0.55 ± 0.04*#	0.59 ± 0.05#
Brain, g	2.10 ± 0.03	2.10 ± 0.02	2.08 ± 0.06	2.08 ± 0.03	2.07 ± 0.03
Testis, %	0.48 ± 0.06	0.47 ± 0.03	0.47 ± 0.04	0.51 ± 0.04	0.49 ± 0.04
Testis, g	1.93 ± 0.07*	1.86 ± 0.04*#	1.93 ± 0.04*	1.93 ± 0.05*	1.73 ± 0.05#
Epididymis, %	0.20 ± 0.04*#	0.21 ± 0.05*#	0.22 ± 0.07*#	0.25 ± 0.05*	0.18 ± 0.05#
Epididymis, g	0.82 ± 0.05*#	0.85 ± 0.05*#	0.92 ± 0.09*	0.93 ± 0.05*	0.65 ± 0.05#
Prostate, %	0.20 ± 0.09	0.23 ± 0.06	0.20 ± 0.06	0.23 ± 0.07	0.17 ± 0.03
Prostate, g	0.80 ± 0.11*#	0.92 ± 0.09*	0.82 ± 0.07*#	0.87 ± 0.07*#	0.59 ± 0.04#
Seminal vesicle, %	0.37 ± 0.06*	0.34 ± 0.08*#	0.29 ± 0.05#	0.34 ± 0.05*#	0.40 ± 0.09*
Seminal vesicle, g	1.48 ± 0.06*	1.32 ± 0.07*#	1.19 ± 0.05#	1.30 ± 0.05*#	1.42 ± 0.07*#

Different symbols between groups indicate significance at $p < 0.05$; ANOVA followed by Tukey or Dunnett.

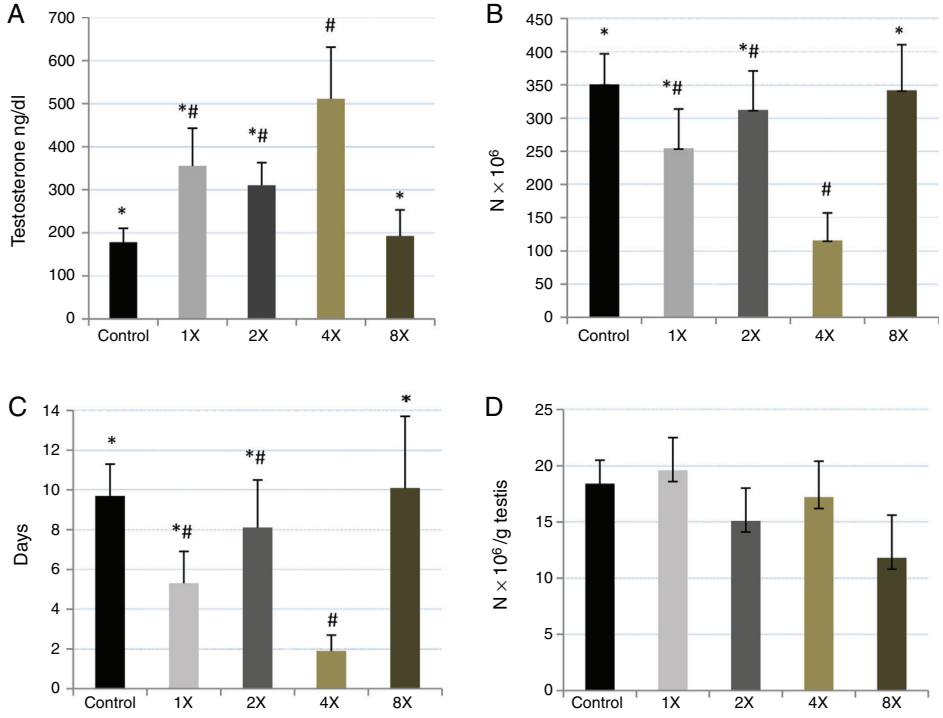

Fig. 2. Total testosterone (A); spermatic reserve in the epididymis tail (B); spermatic transit time (C); daily spermatic production (D) of the control and treated groups (n = 12 for each group, except for the 8×, n = 10) (mean ± standard error). Different symbols between groups indicate significance at $p < 0.05$; ANOVA followed by Tukey or Dunnett.

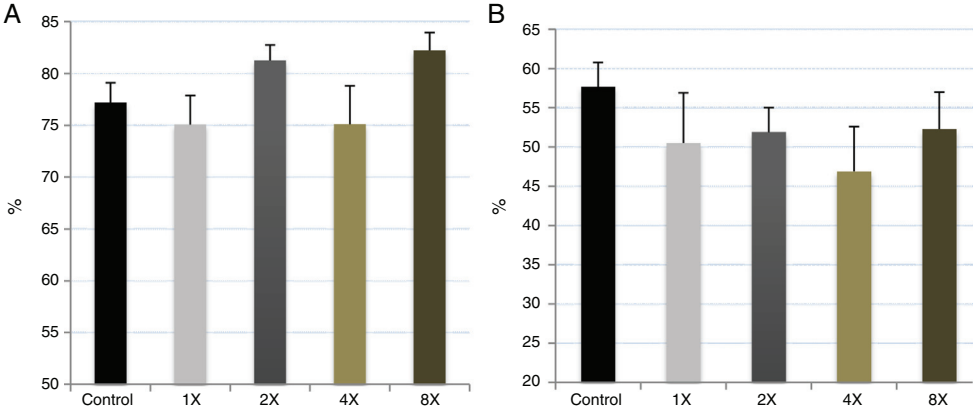

Fig. 3. Percent of moving spermatozoids (A: motility) and morphologically normal spermatozoids (B: normality) of the control and treated groups (n = 12 all groups, except for the 8×, n = 10) (mean ± standard error). Kruskal–Wallis test.

Table 3
Percentage of abnormal spermatozoids of the control and ayahuasca treated animals (mean ± standard error).

Parameter	Control (n = 12)	1× (n = 12)	2× (n = 12)	4× (n = 12)	8× (n = 10)
Headless tail	18.2 ± 3.7	28.7 ± 7.6	14.3 ± 2.3	17.2 ± 4.2	17.3 ± 1.7
Flattened head	0.3 ± 0.1*#	0.3 ± 0.1*#	0.1 ± 0.1*	0.3 ± 0.1*#	0.9 ± 0.4#
Pinhead	1.4 ± 0.5	0.7 ± 0.2	0.7 ± 0.3	1.3 ± 0.2	1.2 ± 0.5
Bent neck	1.4 ± 0.3	2.1 ± 0.7	2.0 ± 0.8	0.8 ± 0.3	0.9 ± 0.3
Bent tail	18.7 ± 3.6*#	14.6 ± 2.8*	27.3 ± 3.4#	29.1 ± 3.7#	24.2 ± 4.3*#
Coil tail	0.2 ± 0.1	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.4
Multiples abnormalities	0.0 ± 0.0	0.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1
Total	41.9 ± 3.0	49.9 ± 6.6	48.1 ± 3.1	53.1 ± 5.8	47.7 ± 4.7

Different symbols between groups indicate significance at $p < 0.05$; Kruskal–Wallis test.

2 weeks before their arrival in that region, while those found in the head were released by the testicle just a few days prior (Lanning et al., 2002).

Studies have shown that delays in sperm transport time in the epididymis do not alter the fertility of the gametes (Billups et al., 1990; Kempinas et al., 1998), but a lower transit time may affect sperm development, maturity and fertility (Klinefelter, 2002; Fernandez et al., 2008). Meistrich (1975) demonstrated that treatment of Wistar rats with estradiol derivatives together with testosterone did not affect testicular functions, but accelerated sperm transport time and consequently reduced the sperm reserve in the epididymis, similar to what was found in this study with ayahuasca. However, other authors have reported the action of toxic agents on sperm reserves in the tail of the epididymis, but no changes in sperm production (Goyal et al., 2001; Klinefelter and Suarez, 1997; Bellentani et al., 2011).

The most relevant result found in this study was the significant increase in total testosterone levels in animals treated chronically with ayahuasca at the 4× dose in comparison with the 8× group and the control, which was in parallel with a significant decrease in sperm reserve of the epididymis tail and in sperm transit time. These results indicated that the effects observed at 4× dose was not aleatory, and reflects a non-monotonic dose–response. Alvarenga et al. (2014) did not find any effect on testosterone levels of male rats after a single ayahuasca dose, but a decrease in sexual performance was observed at all doses (250–1000 μg/ml). The increase in testosterone in the 4× group may be associated to an increase in the absolute weights of the epididymis of this group, since it is an androgynous-dependent organ. Fernandez et al. (2008) found a potential relation between lower reproductive organ weights and lower testosterone plasma levels in male Wistar rats treated with diethylstilbestrol.

DMT binds strongly to the 5-HT_{2A} type serotonergic receptors, and acts as a substrate for the SERT, thus inhibiting neurotransmitter reuptake, which remain longer in the synaptic cleft (Smith et al., 1998; Nagai et al., 2007; Cozzi et al., 2009; Halberstadt, 2015). Additionally, the β-carboline alkaloids present in the ayahuasca infusion may also inhibit this reuptake (Callaway et al., 1999; Cozzi et al., 2009). McKenna (2004) stated that the regular use of ayahuasca apparently produces serotonergic alterations increasing SERT density in the brain, with an impact on the positive changes in behavior reported by ayahuasca users (Callaway et al., 1994).

The increases in testosterone levels in animals treated with ayahuasca at the 4× dose suggests an endocrine compensation mechanism, which may be responding to the increases in serotonin levels in the synaptic clefts and in serotonergic activity due to DMT. This increase was not observed at the 8× dose, indicating a non-monotonic dose–response, which is common for endocrine disruptors (Lagarde et al., 2015). This observation may be explained by the involvement of testosterone in the negative feedback mechanism, inhibiting the release of GnRH in the hypothalamus and consequently inhibiting the release of gonadotropins (LH and FSH) by the pituitary gland (Spritzer and dos Reis, 2008), although no changes in these hormones were observed in ayahuasca treated animals in the present study. Another hypothesis is that the greater serotonergic activity at the 8× dose due to higher DMT levels (Pic-Taylor et al., 2015) may be desensitizing receptors and consequently reducing the SERT density. The $5HT_{2B}$ receptors play a regulatory role in the homeostasis of synaptic serotonin levels, possibly participating in the control of SERT in the raphe nuclei neurons which, once activated, inhibit the extracellular accumulation of serotonin induced by selective serotonin reuptake inhibitors (SSRI) (Diaz et al., 2012). Furthermore, Kranz et al. (2015) showed a direct relation between increases in plasma testosterone levels induced by treatment in transsexuals and increases in the density of serotonin reuptake sites in various brain regions. Studies with animals have shown that treatment with testosterone increased the density of 5-HT_{2A} receptors in certain areas of the brain (Sumner and Fink, 1998; Mcqueen et al., 1999; Herrera-Pérez et al., 2013; Kranz et al., 2015).

This study allowed the establishment of a no-observed-adverse-effect-level of ayahuasca for male Wistar rats treated every 2 days for 70 days, at 2× the dose used at a UDV ritual, which corresponds to 0.62 mg/kg bw of DMT, 6.6 mg/kg bw of harmine, and 0.52 mg/kg bw of harmaline. The effects observed at higher doses include decreases in feed consumption and body weight gains, and increase of relative stomach and brain weights. A potential reproductive toxic effect was observed at the 4× dose, with a non-monotonic dose–response. Studies investigating the role of ayahuasca components in regulating testosterone levels, as well as of SERT in the brains of male rats are needed to better understand this toxic action.

This is the first time that the potential effect of ayahuasca on male animal reproduction parameters after chronic exposure was investigated. The results of this study are important as the worldwide use of this beverage has increased substantially in the last decades. Additionally, as the therapeutic use of ayahuasca has been considered, the safety of its use needs to be stablished.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

AFAS conducted the experiments and analyzed most of the data, performed the statistics and prepared the draft manuscript. ALSV performed the histology analysis. APT and EDC conceived, designed and coordinated the study. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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