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Effect of Ritualistic Consumption of Ayahuasca on Hepatic Function in Chronic Users

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ABSTRACT

Ayahuasca is a beverage obtained from decoctions of the liana *Banisteriopsis caapi* plus the shrub *Psychotria viridis*. This beverage contains a combination of monoamine oxidase inhibitors (harmine, harmaline, and tetrahydroharmine) and N,N-dimethyltryptamine, the main substance responsible for its visionary effect. The ritualistic use of ayahuasca is becoming a global phenomenon. Most members of ayahuasca churches consume this beverage throughout their life, and many reports have discussed the therapeutic potential of this beverage. Ayahuasca is consumed orally, and the liver, as the major organ for the metabolism and detoxification of xenobiotics absorbed from the alimentary tract, may be susceptible to injury by compounds present in the ayahuasca decoction. In this study, we evaluated biochemical parameters related to hepatic damage in the serum of 22 volunteers who consumed ayahuasca twice a month or more for at least one year. There was no significant alteration in the following parameters: alanine aminotransferase, aspartate aminotransferase, bilirubin, creatinine, urea, lactate dehydrogenase, alkaline phosphatase, and gamma glutamyl transferase. These findings indicate that chronic ayahuasca consumption in a religious context apparently does not affect hepatic function.

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Introduction

Ayahuasca is the Quechua term, common in Peru, Bolivia, Brazil, and parts of Ecuador, used to designate a decoction made from the shrub *Psychotria viridis* and the liana *Banisteriopsis caapi*. The result of decoction is a thick, brown, oily liquid. The leaves of *P. viridis* contain the alkaloid *N,N*-dimethyltryptamine (DMT), the main substance responsible for the visionary and hallucinogenic effects of ayahuasca. *Banisteriopsis caapi* contains the β-carboline alkaloids harmine, harmaline, and tetrahydroharmine that act as reversible inhibitors of type-A monoamine oxidase (MAO-A), an enzyme in the human digestive tract that inactivates DMT ingested orally (Callaway et al. 1996; Grob et al. 1996; McKenna and Towers 1984; Riba et al. 2001; Yritia et al. 2002). DMT is a tryptamine hallucinogen similar to serotonin, and its central effects result from activity at serotonergic 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors (Deliganis, Pierce, and Peroutka 1991; Fantegrossi et al. 2006; Pierce and Peroutka 1989; Smith et al. 1998). Profound changes to perception, particularly in the visual, auditory, and somatosensory systems, are induced by ayahuasca, leading to a state of significant introspection (Strassman, Qualls, and Berg 1996; Strassman et al. 1994). The hallucinogenic effects start 35–40 min after a single oral ingestion of the decoction, reach maximal intensity after 90–120 min, and end after 4 h (Grob et al. 1996; Riba et al. 2001, 2003). Nausea, vomiting, and episodes of diarrhea can also occur after ayahuasca consumption (Callaway et al. 1999; McKenna 2004).

DMT and its derivatives, as well as β-carbolines, are also endogenous substances in mammals, including humans (Airaksinen and Kari 1981; Barker, Monti, and Christian 1980). The cytochrome P450 system, MAO enzymes, and methyl transferases present in the liver, lung, brain, blood, heart, and brain spinal fluid of humans can metabolize DMT and its derivatives (McKenna and Towers 1984). Interestingly, almost 38 years ago, Checkley et al. (1979) observed that the urinary excretion of endogenous DMT was higher in individuals with liver disease than in subjects without hepatic dysfunction.

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The liver is the major organ for the metabolism and detoxification of drugs and xenobiotics absorbed from the alimentary tract, and may therefore be susceptible to injury by these compounds at any time. The hepatic oxidation and conjugation of β-carbolines play an important role in the detoxification of these alkaloids (Mulder and Hagedoorn 1974; Tweedie, Prough, and Burke 1988; Yu et al. 2003) and may protect other organs from damage by these compounds. For example, harmine and harmaline can be converted into hydroxylated intermediates such as harmol and harmalol via *O*-demethylation by cytochrome P450 isozymes.

Some in-vitro studies have shown that β-carbolines can cause cell damage. In rat isolated hepatocytes, harmine elicits concentration- and time-dependent cell death by decreasing the intracellular levels of ATP and the mitochondrial membrane potential. Harmine also depletes cellular glutathione (GSH) and increases glutathione disulfide (GSSG) and reactive oxygen species (ROS) levels, indicating oxidative stress. The other analogues, harmaline and harmol (a metabolite of harmine), are less cytotoxic than harmine (Nakagawa et al. 2010). The treatment of V79 Chinese hamster lung fibroblasts with harman and/or harmine caused DNA damage and cytotoxicity (Boeira et al. 2001). These results suggest that β-carbolines may exert their cytotoxicity via mitochondrial dysfunction, with the liver being a possible target organ.

There is ancient and extensive use of ayahuasca among Indigenous groups. However, the consumption of this psychoactive beverage has expanded beyond Indigenous cultures and has been used in the rituals of some religious syncretic movements as a means of facilitating self-knowledge and introspection (Goulart 2011; Pires, Oliveira, and Yonamine 2010). The most commonly recognized sacramental use of ayahuasca in Brazil occurs in three churches: União do Vegetal (UDV), Santo Daime, and Barquinha. The religious use of ayahuasca was recognized in Brazil as a legal practice by the Conselho Nacional de Políticas sobre Drogas (CONAD, or the National Council on Drug Policies), as stated in Resolution No. 5 (November 4, 2004), and its control has been guided by the publication of Resolution No. 1 (January 25, 2010) (Labate and Feeney 2012). In the churches indicated earlier, ayahuasca consumption can occur for at least one year or throughout life (Gable 2007), and the intermittent ingestion within a religious context occurs at frequencies of twice a month or more (Bouso et al. 2012; Grob et al. 1996).

Since most members of ayahuasca churches consume this beverage throughout their life and into old age, the safety of ayahuasca in regular users is an important issue. In addition, numerous reports have discussed the therapeutic potential of ayahuasca. Therefore, in this work, we evaluated biochemical parameters related to hepatic damage in the serum of 22 volunteers from the Center of Integral Development Luz do Vegetal, who consumed ayahuasca twice a month or more for at least one year.

Methods

Participants

After approval by the Ethics and Research Committee for research in humans of the Irmandade da Santa Casa de Misericórida de São Paulo Institutional, the volunteers were selected based on the following inclusion and exclusion criteria. The inclusion criteria were: age between 21 and 65 years, of either sex, no renal or hepatic comorbidities, and an ayahuasca consumption of twice a month for at least one year at the "Luz do Vegetal" Center of Integrated Development. The exclusion criteria were: pregnancy; the use of psychoactive substances such as alcohol, marijuana, cocaine, crack, amphetamines, and hallucinogens; and altered results in the pre-evaluation of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine, lactate dehydrogenase, alkaline phosphatase, and gamma glutamyl transferase (GGT). The volunteers drank 1.5 mL of ayahuasca/kg body weight, an amount corresponding to that consumed in the religious ritual; i.e., ~100 mL/70 kg (Callaway et al. 1999; Gable 2007).

The experimental design was that of a descriptive cross-sectional study and all participants provided informed consent after receiving clarification about the procedures involved in the study. The volunteers answered a structured questionnaire that provided socio-demographic information (age, sex, marital status, and schooling) and details of the frequency and duration of ayahuasca use.

Ayahuasca

To prepare ayahuasca, the liana *B. caapi* was carefully washed in water and pounded with wooden mallets, whereas the leaves of *P. viridis* were simply rinsed with water. The plant materials were carefully combined, boiled, and concentrated over several hours to produce approximately 100 L of beverage. The concentrations of psychoactive alkaloids detected by HPLC-DAD according to Lanaro et al. (2015) were: N, N-dimethyltryptamine 2070 mg/mL, harmaline

147.5 mg/mL, harmine 2894 mg/mL, and tetrahydroharmine 1893 mg/mL.

Biological samples

All samples were obtained from healthy individuals ≥21 years old and members of the "Luz do Vegetal" Center of Integral Development. Blood samples (3–5 mL) were collected before (T0) and at 4 h (T4), 24 h (T24), and 168 h (T168) after ayahuasca consumption. The samples were clotted at room temperature and then centrifuged at 5,000 rpm. The resulting serum was separated, stored in polyethylene tubes, and frozen at −20°C.

Determination of biochemical parameters

The biochemical parameters were determined spectrophotometrically using a Roche/Hitachi modular analyzer. The following COBAS® brand kits were used: alanine aminotransferase (ref. 11876805216), aspartate aminotransferase (ref. 11876848216), alkaline phosphatase (ref. 12173107122), total bilirubin (ref. 11822713190), creatinine (ref. 11875418216), lactate dehydrogenase (ref. 11876961216), gamma glutamyl transferase (ref. 12016958216), and urea (ref. 11982486001), according to the manufacturer's guidelines.

Statistical analysis

The sample size was calculated using a statistical power of 85% and an effect size of 27% (Cohen's f^2 method) for all variables analyzed (Button et al. 2013; Cohen 1992). For one-way ANOVA for repeated measures, the sample size calculated using the parameters indicated earlier in conjunction with G*Power 3.1.9.2 software (Franz Faul, Universitat Kiel, Germany) was 22 participants.

Additionally, in order to evaluate the possible effects of sex, age, and time of ayahuasca intake on the basal levels of biochemical parameters, we also performed a General Linear Model (GLM) for each parameter studied. In this analysis, we considered sex as a fixed factor and age and use time as co-variables.

The data were reported as the mean ± SD. One-way ANOVA for repeated measures was used to analyze the biochemical parameters and the level of significance was set at $P < 0.05$. All data analyses were done using SPSS statistics v.22 software (IBM Corp., USA).

Results

To determine the biochemical parameters, samples were collected from 22 volunteers (9 women and 13 men), aged 21 to 56 years (mean = 39.5, SD = 10.9), who had been consuming ayahuasca twice a month for 1 to 20 years (mean = 8.4, SD = 7.2).

Figures 1– 3 show the biochemical parameters evaluated in serum before (T0) and 4 h, 24 h, and 168 h after ayahuasca ingestion. Figure 1 shows that the ALT, AST, and GGT activities were below the maximum reference value, with no difference among the time intervals (before and after ayahuasca ingestion) studied in men and women. Similarly, Figure 2 shows that the lactate dehydrogenase and alkaline phosphatase activities and total bilirubin were between the maximum

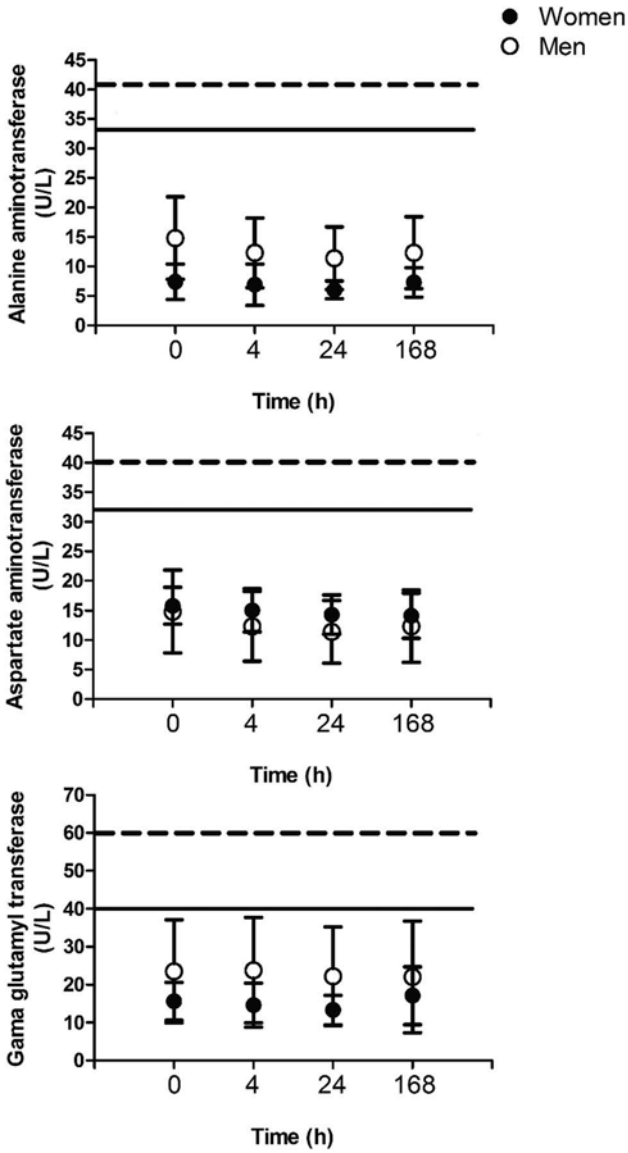

Figure 1. Serological analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT). Blood samples were collected before (T0) and 4 h, 24 h, and 168 h after the oral ingestion of ayahuasca. The points represent the mean ± SD of 9 women (black circles) and 13 men (white circles). The lines represent the reference values for men (dashed line) and women (solid line).

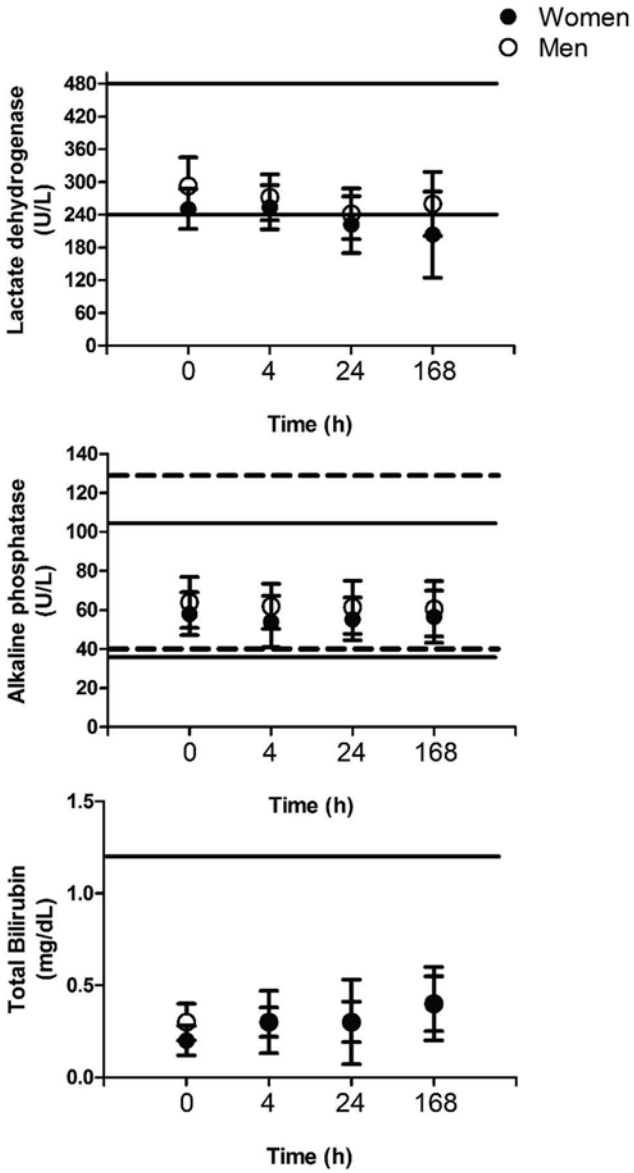

Figure 2. Serological analysis of lactate dehydrogenase, alkaline phosphatase, and total bilirubin. Blood samples were collected before (T0) and 4 h, 24 h, and 168 h after the oral ingestion of ayahuasca. The points represent the mean ± SD of 9 women (black circles) and 13 men (white circles). Lactate dehydrogenase: the solid lines represent the maximum and minimum reference values for both sexes. Alkaline phosphatase: the lines represent the maximum and minimum reference values for men (dashed line) and women (solid line). Total bilirubin: the single line represents the reference value for both sexes.

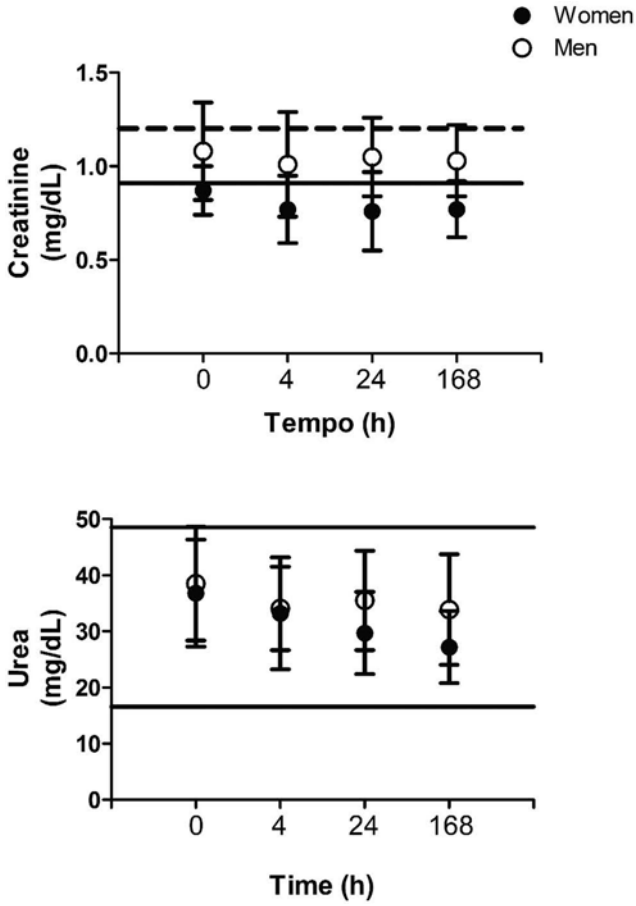

Figure 3. Serological analysis of creatinine and urea. Blood samples were collected before (T0) and 4 h, 24 h, and 168 h after the oral ingestion of ayahuasca. The points represent the mean ± SD of 9 women (black circles) and 13 men (white circles). Creatinine: the lines represent the reference values for men (dashed line) and women (solid line). Urea: the lines represent the maximum and minimum reference values for both sexes.

and minimum reference values for both sexes and there was no difference among the time intervals studied before and after ayahuasca ingestion for all parameters. Finally, Figure 3 shows that creatinine and urea were within the minimum and maximum reference values in volunteers of both sexes and that there was also no difference among the time intervals (before and after ayahuasca ingestion) studied.

Overall, considering all of the parameters analyzed (except bilirubin and urea), the average values among men were significantly higher compared to women. In addition, there were time-related differences for bilirubin (T0 to T7) and lactate dehydrogenase (T0 and T24) when all volunteers were compared (considering male and female subjects together). However, because there were no interactions among factors for all of the parameters, our results indicated that there were no differences in men or in women over time in the parameters investigated in this study (Table 1).

Considering the GLM, the effect of the variables sex, age, and time of ayahuasca intake on the basal values of the biochemical parameters was not detected. In contrast, considering ALT and total bilirubin only, the sex variable was evidenced as significant ($p < 0.05$) (data not shown).

Table 1. Statistical analysis of biochemical parameters.

Biochemical parameter	Sex effect	Time effect	Sex x time interaction
ALT	F(1,20) = 28.094, $P < 0.001$*	F(3,60) = 1.010, $P = 0.393$	F(3,60) = 0.238, $P = 0.870$
AST	F(1,20) = 26.634, $P < 0.001$*	F(3,60) = 0.812, $P = 0.491$	F(3,60) = 0.127, $P = 0.944$
Bilirubin	F(1,20) = 1.485, $P = 0.227$	F(3,60) = 3.137, $P < 0.05^{\#}$	F(3,60) = 0.417, $P = 0.741$
LD	F(1,20) = 9.197, $P < 0.01$*	F (3,60) = 3.424, $P < 0.05^{\#}$	F(3,60) = 0.671, $P = 0.572$
ALP	F(1,20) = 4.631, $P < 0.05$*	F(3,60) = 0.247, $P = 0.863$	F(3,60) = 0.077, $P = 0.972$
GGT	F(1,20) = 10.026, $P < 0.01$*	F(3,60) = 0.103, $P = 0.958$	F(3,60) = 0.163, $P = 0.921$
Creatinine	F(1,20) = 29.522, $P < 0.001$*	F(3,60) = 0.668, $P = 0.574$	F(3,60) = 0.145, $P = 0.932$
Urea	F(1,20) = 3.592, $P < 0.053$	F(3,60) = 2.148, $P = 0.101$	F(3,60) = 0.584, $P = 0.627$

One-way ANOVA for repeated measures was used to analyze the biochemical parameters. ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyl transferase, LD: lactate dehydrogenase. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ for men compared to women; $^{\#}P < 0.05$ for T0 compared to T7 (Bilirubin) and T24 (LD).

Discussion

The religious use of ayahuasca was recognized as a legal practice in Brazil and represents freedom of religious worship (Labate and Feeney 2012). In addition, apart from adults, many members of ayahuasca religious groups, including pregnant women, infants, and adolescents, consume ayahuasca in religious rituals and may continue to do so throughout life (Costa, Figueiredo, and Cazenave 2005). Regardless of the long tradition of religious use, the consumption of ayahuasca is controversial and, in 1982, the toxicological safety of ayahuasca was extensively questioned. As a consequence, ayahuasca was included in the list of prohibited substances in Brazil. Only in 1987 was the use of ayahuasca officially legalized in Brazil and, after some years, its religious use was recognized as a legal practice in Brazil (CONAD 2004).

In a systematic review of human studies based on 28 articles, Dos Santos et al. (2016) evaluated the acute, subacute, and long-term effects of ayahuasca on psychiatric symptoms, neuropsychological functioning, and neuroimaging. In this review, antidepressive and antiaddictive effects were observed. Besides that, the increased cortical thickness of the anterior cingulate cortex and cortical thinning of the posterior cingulate cortex were described in long-term ayahuasca use. This structural change could potentially affect attentional processes, self-referential thought, and internal mentation (Bouso et al. 2015). In addition, although acute use has shown damage in working memory, the authors described the enhanced positive mood and cognition, increased spirituality, and reduced impulsivity and concluded, based on the articles, that ayahuasca use seems to have low toxicity (Dos Santos et al. 2016). Likewise, a longitudinal study of long-term ayahuasca use in volunteers of two religious communities found no evidence of pathological alterations in mental health, including in the Frontal Systems Behavior Scales (Bouso et al. 2012). Subsequent reports have described the therapeutic potential of ayahuasca and its components (Domínguez-Clavé et al. 2016; Talin and Sanabria 2017). In addition, various reports have described decreased consumption of alcohol, cocaine, and other drugs of abuse in regular ayahuasca users (Brierley and Davidson 2012; Fábregas et al. 2010; Harris and Gurel 2012; Loizaga-Velder and Verres 2014; Thomas et al. 2013). The ingestion of ayahuasca in ritualistic contexts appears to have antidepressant and anxiolytic effects (Santos et al. 2007). Similarly, a significant antidepressant effect was also observed in patients with resistant depression after a single dose of ayahuasca. This effect remained for seven days after administration of ayahuasca when compared to patients receiving a placebo (Palhano-Fontes et al. 2018). Thus, the long-term consumption of ayahuasca within a religious context or a single simple dose does not appear to exert a deleterious effect on neuropsychological function.

Ayahuasca compounds have been reported to exert a neuroprotective effect, and preclinical studies have demonstrated a neuroprotective effect of harmine. In rats submitted to cerebral ischemia, harmine attenuated the cerebral infarct volume and reduced neuronal death (Sun et al. 2014). In addition, a double-blind, randomized, placebo-controlled trial study of *B. caapi*, using a single dose, revealed a significant improvement in motor function of patients with Parkinson's disease (Serrano-Dueñas, Cardozo-Pelaez, and Sánchez-Ramos 2001). An extract of *B. caapi* stem and harmine showed concentration-dependent inhibition of MAO-A in liver homogenates and, consequently, an increase in the release of dopamine from rat striatal slices (Schwarz et al. 2003). Harmine also had an anti-proliferative effect in the human hepatocellular carcinoma cell line HepG2 by inducing apoptosis through mitochondrial signaling pathways (Cao et al. 2011).

In addition to the therapeutic potential, several studies have examined the possible cytotoxicity of ayahuasca and β-carbolines (Boeira et al. 2001; Cao et al. 2011; Domínguez-Clavé et al. 2016; Nakagawa et al. 2010; Talin and Sanabria 2017). Nakagawa et al. (2010) showed that harmine decreased the viability of rat hepatocytes, accompanied by a loss of intracellular ATP, adenine nucleotides, GSH and protein thiols, and an increase in reduced glutathione (GSSH) and ROS levels. These results suggested that the cytotoxicity of harmine involved mitochondrial dysfunction and that the liver

was a potential target organ. Nakagawa et al. (2010) also noted that harmine was more toxic to hepatocytes than its metabolites harmol and harmaline.

To assess whether ayahuasca is toxic to long-time users, we examined hepatic function in volunteers who consumed ayahuasca for at least one year. AST is an enzyme that is highly expressed in the liver and heart, but is also present in brain and skeletal muscle, whereas ALT is found primarily in the liver, with lower expression in the heart and skeletal muscle. The presence of hepatic parenchymal damage could increase the release of these enzymes into the blood and consequently raise their serum levels. However, as shown here, the serum activities of AST and ALT were below the maximum reference value and there was no difference among the time intervals in men and women. Because changes in these aminotransferases can also reflect extrahepatic lesions (Lee, Kim, and Poterucha 2012; Ozer et al. 2008), we examined other markers of liver function.

Alkaline phosphatase is a membrane enzyme found in many tissues, such as bones, intestine, kidney, and especially liver (Ozer et al. 2008; Ramaiah 2007). Since an increase in serum alkaline phosphatase may also result from extrahepatic diseases, the concomitant evaluation of GGT may help to identify the possible source of increased alkaline phosphatase. In primary biliary cirrhosis or chronic cholestatic liver disease, alkaline phosphatase and GGT may be increased (Nishio et al. 2000). GGT is also present in the kidneys, pancreas, liver, spleen, heart, brain, and seminal vesicle. Elevated levels of GGT may be related to alcohol consumption or the use of drugs such as barbiturates and phenytoin (Lee, Kim, and Poterucha 2012). Our results showed that GGT was below the maximum reference value and that there was no change among the time intervals studied after ayahuasca intake in both men and women.

Lactate dehydrogenase is present in tissues such as heart, liver, skeletal muscle, kidney, and erythrocytes and is also detected in platelets and lymph nodes. The tissue levels are much higher than those found in serum, so tissue lesions can markedly increase the serum levels of this enzyme. However, since lactate dehydrogenase occurs in several tissues, an increase in the serum levels of this enzyme may represent a nonspecific finding. Alternatively, it is possible to obtain information of clinical relevance by separating lactate dehydrogenase into isoenzymatic fractions. In liver disease, an increase in lactate dehydrogenase is not as significant as that of the transaminase AST. In contrast, higher levels of lactate dehydrogenase are observed in toxic infectious hepatitis with jaundice, cirrhosis, and obstructive jaundice. Indeed, lactate dehydrogenase determinations are frequently used as part of the diagnosis for liver diseases such as acute viral hepatitis, liver metastatic cirrhosis, and carcinoma, as well as cardiopathies and kidney and lung tumors (Motta 2009; Sturk and Sanders 1990). The lactate dehydrogenase values assessed in the volunteers who ingested ayahuasca were within the minimum and maximum reference values and there were no changes among the time intervals studied in both men and women.

Total bilirubin is composed of indirect (unconjugated) and direct (conjugated) bilirubin. The heme group of hemoglobin is degraded to biliverdin by the enzyme heme-oxygenase and then into bilirubin by the enzyme biliverdin reductase (Bauer et al. 2008). Bilirubin is subsequently conjugated in the liver, secreted in bile, and eliminated in the feces. Consequently, bilirubin may be a useful parameter for assessing hepatobiliary damage such as parenchymal injury or biliary obstruction. However, bilirubin may also be increased through non-hepatic causes, such as hemolysis or muscle damage (Harris, Sasson, and Mehler 2013; Ozer et al. 2008). As shown here, the serum values of total bilirubin were below the maximum reference value at all the time intervals studied in men and women.

Since changes in hepatic function may cause a decrease in renal function, we evaluated the serum creatinine and urea levels (Davenport et al. 2017). Creatinine is a creatine residual product. The conversion of creatine to creatinine occurs in muscle tissue such that the amount of creatinine produced is dependent on muscle mass. Creatinine is completely filtered by the glomerulus and, under normal conditions, is not reabsorbed or secreted in the nephron. An increase in blood creatinine occurs when there are severe lesions of the nephrons, as in the later stages of renal disease (Martensson, Martling, and Bell 2012). The creatinine levels remained below the reference maximum values in the volunteers of this study, and there were no changes in the values at any of the time intervals after ayahuasca consumption in both men and women. Urea is synthesized in the liver from ammonia derived from protein catabolism and subsequently excreted in the urine. Low blood urea values may be related to low protein intake, food deprivation, and hepatic failure or hepatocyte injury. In addition, urea determination with creatinine may help in the diagnosis of gastrointestinal bleeding, decreased renal blood flow, or post-renal obstructions and urinary stenosis (Adeva et al. 2012; Ferguson and Waikar 2012; Magarian, Lucas, and Kumar 1992; Rypins et al. 1980). As shown here, the serum urea values remained within the maximum and minimum reference values for both sexes and there were no changes over time after ayahuasca ingestion.

In conclusion, the results of this study show that the long-term consumption of ayahuasca within a religious context apparently does not affect hepatic function in volunteers. Indeed, the indicators of hepatic function remained within the normal range, even during the acute effects of the beverage (in the first 4 h after consumption). However, in view of the small sample size used in this study (n = 22), additional studies with larger samples are needed to adequately assess the safety of ayahuasca and its potential effects on liver function.

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