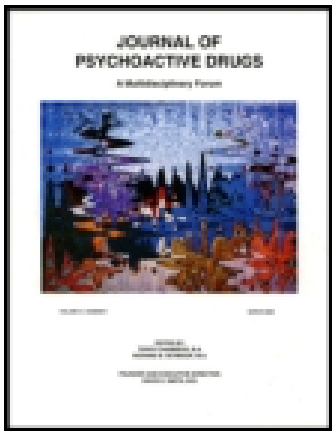
This article was downloaded by: [FU Berlin]
On: 08 May 2015, At: 16:44
Publisher: Routledge
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



CrossMark
Click for updates

Journal of Psychoactive Drugs
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ujpd20

Ritualistic Use of Ayahuasca versus Street Use of Similar Substances Seized by the Police: A Key Factor Involved in the Potential for Intoxications and Overdose?

Rafael Lanaro M.Sc.[a], Débora Bressanim de Aquino Calemi B.Sc.[b], Loraine Rezende Togni M.Sc.[b], José Luiz Costa Ph.D.[ac], Maurício Yonamine Ph.D.[d], Silvia de Oliveira Santos Cazenave Ph.D.[e] & Alessandra Linardi Ph.D.[f]

[a] Researcher, Poison Control Center, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[b] Researcher, Forensic Toxicology and Chemistry Laboratory, Criminalistic Institute of São Paulo, São Paulo, SP, Brazil

[c] Forensic Toxicologist, Forensic Toxicology and Chemistry Laboratory, Criminalistic Institute of São Paulo, São Paulo, SP, Brazil

[d] Associate Professor, Faculty of Pharmaceutical Sciences, University of São Paulo (USP), São Paulo, SP, Brazil

[e] Superintendent of Toxicology, ANVISA (Brazilian Health Surveillance Agency) Brasília, DF, Brazil

[f] Associate Professor, Department of Physiological Sciences, Santa Casa de São Paulo School of Medical Sciences, São Paulo, SP, Brazil

Published online: 07 May 2015.

To cite this article: Rafael Lanaro M.Sc., Débora Bressanim de Aquino Calemi B.Sc., Loraine Rezende Togni M.Sc., José Luiz Costa Ph.D., Maurício Yonamine Ph.D., Silvia de Oliveira Santos Cazenave Ph.D. & Alessandra Linardi Ph.D. (2015) Ritualistic Use of Ayahuasca versus Street Use of Similar Substances Seized by the Police: A Key Factor Involved in the Potential for Intoxications and Overdose?, Journal of Psychoactive Drugs, 47:2, 132-139, DOI: 10.1080/02791072.2015.1013202

To link to this article: http://dx.doi.org/10.1080/02791072.2015.1013202

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any

form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Downloaded by [FU Berlin] at 16:44 08 May 2015

Journal of Psychoactive Drugs, 47 (2), 132–139, 2015
Copyright © Taylor & Francis Group, LLC
ISSN: 0279-1072 print / 2159-9777 online
DOI: 10.1080/02791072.2015.1013202

Routledge
Taylor & Francis Group

Ritualistic Use of Ayahuasca versus Street Use of Similar Substances Seized by the Police: A Key Factor Involved in the Potential for Intoxications and Overdose?

Downloaded by [FU Berlin] at 16:44 08 May 2015

Rafael Lanaro, M.Sc.[a]; Débora Bressanim de Aquino Calemi, B.Sc.[b]; Loraine Rezende Togni, M.Sc.[b]; José Luiz Costa, Ph.D.[a,c]; Maurício Yonamine, Ph.D.[d]; Silvia de Oliveira Santos Cazenave, Ph.D.[e] & Alessandra Linardi, Ph.D.[f]

Abstract — The ritualistic use of ayahuasca is becoming a global phenomenon. 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 recreational use of similar alkaloids and *N,N*-dimethyltryptamine has increased in recent years, mainly because of their hallucinogenic effects. In the present study, the concentrations of psychoactive alkaloids in three powder samples seized by the São Paulo State Police and nine ayahuasca aqueous extracts were analyzed by HPLC-DAD in an attempt to distinguish between illicit drugs and the religious beverage. The alkaloids detected (μg/mL) in the ayahuasca aqueous extracts were *N,N*-dimethyltryptamine (402–2070.3), harmaline (27.5–181.3), harmine (294.5–2893.8), and tetrahydroharmine (849.5–2052.5), whereas, of the three powder samples, one contained only *N,N*-dimethyltryptamine (82% and 2% w/w, respectively) while the other contained only harmaline (16%, w/w) and harmine (12%, w/w). The ritualistic use of ayahuasca involves oral intake and the probability of overdose is minimized by serotonergic stimulation of vagal pathways, leading to vomiting and diarrhea. In contrast, the recreational use of *N,N*-dimethyltryptamine involves consumption mainly by smoking or inhalation, both of which markedly increase its bioavailability and the potential for intoxications.

Keywords — ayahuasca, beta-carboline alkaloids, dimethyltryptamine, forensic toxicology, HPLC-DAD, illicit drugs

[a]Researcher, Poison Control Center, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil.
[b]Researcher, Forensic Toxicology and Chemistry Laboratory, Criminalistic Institute of São Paulo, São Paulo, SP, Brazil.
[c]Forensic Toxicologist, Forensic Toxicology and Chemistry Laboratory, Criminalistic Institute of São Paulo, São Paulo, SP, Brazil.
[d]Associate Professor, Faculty of Pharmaceutical Sciences, University of São Paulo (USP), São Paulo, SP, Brazil.
[e]Superintendent of Toxicology, ANVISA (Brazilian Health Surveillance Agency) Brasília, DF, Brazil.
[f]Associate Professor, Department of Physiological Sciences, Santa Casa de São Paulo School of Medical Sciences, São Paulo, SP, Brazil.
Please address correspondence to Alessandra Linardi, Department of Physiological Sciences, Santa Casa de São Paulo School of Medical Sciences, Rua Dr. Cesário Motta Junior, 61, Vila Buarque, São Paulo, SP 01221-020, Brazil; phone: +55-11-33312008; email: alinar@uol.com.br

Downloaded by [FU Berlin] at 16:44 08 May 2015

INTRODUCTION

The Quechua term *ayahuasca* (also spelled *ayawaska*), common in Peru, Bolivia, Brazil, and parts of Ecuador, is used to refer to decoctions of the liana *Banisteriopsis caapi* plus *Psychotria viridis.* The result of decoction or boiling is a thick, brown, oily liquid. The leaves of *P. viridis* are sometimes replaced by other plants with similar principles, such as the leaves of *Diplopterys cabrerana* (Luna 2011; McKenna 2004), but the beverage is still known as ayahuasca. In this article, the term "ayahuasca" will be used to refer to the beverage from *B. caapi* plus *P. viridis*.

Banisteriopsis caapi contains the alkaloids harmine, harmaline, and tetrahydroharmine, all of which belong to the group of β-carbolines. The leaves of *P. viridis* contain the alkaloid *N,N*-dimethyltryptamine (DMT), the main substance responsible for the visionary effect or hallucinogenic aspect of ayahuasca. DMT has no significant psychoactive effect when ingested orally as it is inactivated by monoamine oxidase (MAO) present in the human digestive tract. Hence, the importance of the alkaloids present in *B. caapi* that act as reversible inhibitors of type-A monoamine oxidase (MAO-A), thereby allowing DMT to activate the central nervous system and produce its visionary effects (Yritia et al. 2002; Riba et al. 2001; Grob et al. 1996; Callaway et al. 1996; McKenna & Towers 1984). The central effects of DMT result from its agonistic properties at serotonergic 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors (Fantegrossi et al. 2006; Smith et al. 1998; Deliganis, Pierce & Peroutka 1991; Pierce & Peroutka 1989). This metabolic pathway and activation of serotonergic receptors explains why DMT exerts important central effects when smoked, injected, or inhaled (Halpern 2004).

There are extensive and ancient indigenous and mestizo traditions in the use of ayahuasca, whereas the emergence of its organized, urban, non-indigenous and religion-based use is an exclusive phenomenon of the Brazilian region (Goulart 2011; Labate 2004). The most commonly recognized sacramental use of ayahuasca occurs among members of three churches in Brazil: União do Vegetal (UDV), Santo Daime, and Barquinha. The religious use of ayahuasca was recognized as a legal practice in Brazil by the Conselho Nacional de Políticas sobre Drogas (CONAD, or National Council on Drug Policies), in Resolution no. 5 (November 4, 2004), and its control has been guided most recently by the publication of Resolution no. 1 (January 25, 2010) (Labate & Feeney 2012).

Interest in the recreational use of ayahuasca is emerging in Columbia, Peru, and Brazil. These countries now have a number of "eco-tour" operations that intentionally make an ayahuasca experience available as part of a travel package for those seeking "spiritual awakening" and/or have adventurous curiosity about the Amazon (Halpern 2004). Nevertheless, DMT, the main psychoactive compound of ayahuasca, is considered an illicit substance in every country that is a signatory to the 1971 UN Convention on Psychotropic Substances, including Brazil. Other plants, such as *Phalaris aquatica, Acacia maidenii*, or tree bark of the genus *Virola*, contain large amounts of DMT and the Internet provides readily available information on the acquisition, extraction, and use of this alkaloid (Warren, Dham-Nayyar & Alexander 2013; McKenna 2004; Collins 1990). Indeed, the Internet offers shops for online purchasing and many sites provide detailed information about psychoactive plants (Montagne 2008; Gordon, Forman & Siatkowski 2006). Moreover, the herbal products are often legal to purchase, in contrast to the acquisition of the pure active substance that, in most cases, is illegal (Björnstad et al. 2009).

DMT can produce profound changes to perception, particularly in the visual, auditory, and somatosensory systems, leading to significant introspection (Strassman et al. 1994; Strassman, Qualls & Berg 1996). There is, therefore, growing interest in the non-medical or non-religious use of DMT (Winstock, Kaar & Borschmann 2014). Since DMT is inactive orally, smoking is the preferred route of DMT consumption and produces potent psycho-spiritual effects (Strassman et al. 1994). When smoked, DMT has a very fast onset of effects, with a peak at 2–5 min and a short duration of action (20–60 min). Users report that smoked DMT leads to the rapid onset of a strong, pleasurable psychedelic experience and the inhalation route provides an easily titratable experience (Winstock, Kaar & Borschmann 2014; Haroz & Greenberg 2005).

Consumption by smoking or inhalation can increase the bioavailability of DMT and therefore the potential for intoxication. One complicating factor is that most DMT is extracted from plants (Cakic, Potkonyak & Marshall 2010) and may therefore also contain toxic hydrogen cyanide. Instructions for DMT extraction are easily found on the Internet and include the use of toxic chemicals (Winstock, Kaar & Borschmann 2014). In a previous study, approximately two-thirds of 121 DMT users (68.1%) reported concomitantly smoking DMT with other drugs such as cannabis, lysergic acid diethylamine (LSD), alcohol, psilocybin or methylenedioxymethamphetamine (MDMA; "ecstasy"), and 42% of DMT smokers reported the concomitant use of an MAO inhibitor from *B. caapi* or *Peganum harmala* (Cakic, Potkonyak & Marshall 2010). In another study of suspected cases of intoxications with psychoactive plant material, a patient with harmaline and harmine intoxication admitted ingestion of a decoction made from *P. harmala* (Syrian rue) seeds, but the urine sample also tested positive for DMT (Björnstad et al. 2009). Perhaps the patient was aware of the illegality of DMT and hence did not disclose the ingestion of another substance or plant.

The Internet provides a vast resource of plant-derived hallucinogenic compounds, including details of methods for obtaining, synthesizing, extracting, and ingesting these

Downloaded by [FU Berlin] at 16:44 08 May 2015

substances (Brierley & Davidson 2012; Deluca et al. 2012; Brush, Bird & Boyer 2004), and has been an important factor in the emerging abuse of DMT. Many of the substances involved are unfamiliar to physicians and the vast majority of users may use a combination of associated plants or their isolated components, making it difficult for authorities to accurately identify them.

In contrast, in religious rituals, ayahuasca is ingested orally, which reduces the bioavailability of DMT and other alkaloids present in the beverage. Little is known about the lethality of DMT in humans. However, based on extrapolation from animal data, the lethal dose of DMT is probably more than 20 times greater than the typical ceremonial dose ingested in ayahuasca rituals. Thus, the probability of a toxic overdose of ayahuasca in religious use is minimal (Gable 2007). In addition, ayahuasca may be beneficial in the treatment of dependence associated with alcohol, barbiturates, sedatives, cocaine, amphetamines, and solvent. The use of these drugs is lower in current ayahuasca users, who show significant abstinence from previous substance dependence when compared with socio-economically matched controls (Thomas et al. 2013; Brierley & Davidson 2012; Harris & Gurel 2012; Fábregas et al. 2010).

Ayahuasca is becoming a global phenomenon, with users coming from various traditions, such as Brazilian religious organizations, and more people are conducting rituals in their own settings; the use of ayahuasca has also been proposed in a number of therapeutic techniques (Luna 2011; Tupper 2008). Given this background, there is interest in examining the factors that can explain the different profiles of ayahuasca use in a religious context versus the recreational use of similar substances on the streets. To this end, in the present study we quantified the alkaloids in three samples seized on the streets in powder form and compared them with those of nine ayahuasca aqueous extracts obtained from a Brazilian religious organization.

METHODS

Samples

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. Since this process is performed at different times of the year (spring, summer, fall, and winter) by members of the Center of Integral Development Luz do Vegetal, we used nine samples obtained in distinct seasons. We chose to use different ayahuasca samples because we wished to compare the levels of DMT and β-carbolines in religious beverages and in the seized samples.

Three powder samples (approximately 0.5 g each) seized by the São Paulo State Police were analyzed chemically in the Forensic Toxicology and Chemistry Laboratory of the Criminalistic Institute of São Paulo.

Standard Solutions

The harmine and harmaline standards were obtained from Sigma-Aldrich (Steinheim, Germany). Tetrahydroharmine was synthesized as previously described (Callaway et al. 1996) and DMT was synthesized by a modified procedure based on the selective dimethylation method (Pires et al. 2009; Giumanini et al. 1980). Stock solutions (1.0 mg/mL) of DMT, harmine, harmaline, and tetrahydroharmine were prepared in methanol and stored at −20°C.

High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD)

The experiments were done using a Prominence HPLC system (Shimadzu, Kyoto, Japan). Chromatographic separation was done with an Atlantis T3 column (150 x 3.0 mm, 3 μm) fitted with an Atlantis T3 guard column (30 x 10 mm, 5 μm) (Waters Corporation, Milford, MA, USA) maintained at 35°C. Gradient elution was done with (A) 10 mmol/L phosphoric acid in ultrapure water (pH adjusted to 3.0 with triethylamine) and (B) acetonitrile at a flow rate of 1 mL/min. The initial gradient conditions were 40% A and 60% B held for 1 min and then a gradual change to 5% A and 95% B over the next 13 min (and maintained at these final concentrations for 5 min). The column was re-equilibrated to 60% B over 0.5 min and held at this concentration for 3 min (total gradient time = 21 min). The auto-sampler temperature was not controlled and the injection volume was 10 μL. The diode-array detector was maintained at 35°C and set to acquire spectra from 195 to 600 nm. For quantification, the chromatograms were extracted at 279 nm (DMT), 291 nm (tetrahydroharmine), 320 nm (harmine), and 375 nm (harmaline).

Sample Preparation

For ayahuasca beverage samples, the materials were homogenized by vigorous mixing with a vortex mixer. A sample aliquot of 200 μL and 800 μL of methanol were added to a 2 mL conical polypropylene tube. After vortex mixing at 1,400 rpm for 10 min, the mixture was centrifuged at 12,000 rpm for 5 min. Subsequently, 200 μL of the supernatant was collected and transferred to an auto-sampler glass vial containing 800 μL of methanol. The solution was homogenized by vortex mixing and 10 μL was injected into the HPLC-DAD system.

For the seized powder samples, an amount (10 ± 1 mg) was weighed and transferred to a volumetric flask, followed by the addition of 8 mL of methanol and vigorous mixing (1,400 rpm/10 min), before adjusting the final volume to 10 mL. After another brief mixing, the solution

Downloaded by [FU Berlin] at 16:44 08 May 2015

was centrifuged at 12,500 rpm for 5 min. One hundred microliters of supernatant were then added to 900 μL of methanol in a glass vial and mixed for 15 s. Finally, 10 μL of this mixture were analyzed by HPLC-DAD.

Method Validation

The method used in this study was validated based on international guidelines for illicit drug analysis (SWGDRUG; UNODC) with respect to limits of detection and quantification, linearity, precision, and accuracy. The limits of detection (LOD) and quantification (LOQ) represent the lowest concentration of the substance under evaluation that can be detected and quantified using a certain experimental procedure. These parameters were calculated based on the signal-to-noise ratio (S/N = 3 for LOD, S/N = 10 for LOQ). The linearity was determined with six calibrators (1 to 100 μg/mL). A calibration curve was generated by plotting the area under the curve versus concentrations. A least-square regression was used to determine the slope, intercept, and correlation coefficient.

Intra-day precision was determined by analyzing six replicates on the same day. Inter-day precision was determined by analyzing three replicate samples on three consecutive days. Precision was defined in terms of the relative standard deviation (%). The accuracy of the methods was determined based on recovery experiments that were done according to the Association of Official Analytical Chemists (AOAC).

RESULTS

Figure 1 illustrates the chromatographic profile and retention times of the DMT (3.484 min), tetrahydroharmine (7.903 min), harmaline (10.105 min), and harmine (10.658 min) standards obtained using the analytical conditions described above. The proposed method showed adequate linearity from 1 to 100 μg/mL ($r \geq 0.98$) for all analytes. The intra-day and inter-day precision and accuracy showed relative standard deviations (RSD) of <10% for all quality controls. The LOD and LOQ were calculated as the concentrations that provided signal-to-noise ratios greater than three and 10 times, respectively. The LOD and LOQ for all analytes were 0.5 μg/mL and 1.0 μg/mL, respectively.

Table 1 shows the alkaloid concentrations found in nine ayahuasca samples (A1-A9; n = 3 determinations each). The DMT concentrations ranged from 402 to 2070.3 μg/mL (mean ± SD: 823.2 ± 532.8). There was marked variation in the concentrations of β-carbolines: harmaline ranged from 27.5 to 181.3 μg/mL (121.2 ± 48.0), harmine oscillated from 294.5 to 2893.8 μg/mL (927.9 ± 784.0), and tetrahydroharmine varied from 849.5 to 2052.5 μg/mL (1541.7 ± 407.9).

Table 2 shows the concentrations of β-carbolines and DMT in samples seized on the street. Only DMT was detected in samples SS1 and SS3, whereas, in sample SS2, only the β-carbolines harmaline and harmine were identified.

FIGURE 1
Chromatographic Profile and Retention Times of the DMT (3.484 min), Tetrahydroharmine (7.903 min), Harmaline (10.105 min), and Harmine (10.659 min) Standards

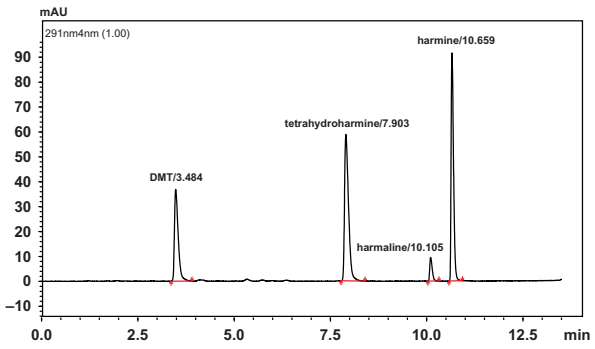


TABLE 1
Quantification of β-carbolines and DMT (μg/mL) in Ayahuasca Samples

Ayahuasca	THH	Harmaline	Harmine	DMT
A1	1768.3	130.5	477.3	402
A2	1829	181.3	581.3	324.3
A3	849.5	61.3	294.5	486
A4	1679	27.5	1245	639.3
A5	1074	127.3	773	728
A6	2052.5	160.5	860.8	948
A7	1893	147.5	2893.8	2070.3
A8	1495	132	597	678
A9	1234.8	123	628.5	1133
CV (%)	26.5	39.6	84.5	64.7

CV = coefficient of variation, DMT = *N,N*-dimethyltryptamine and THH = tetrahydroharmine.
The concentrations indicated are the mean of three determinations per sample.

DISCUSSION

In this article, a simple, fast and reliable HPLC-DAD method was developed and validated according to international guidelines. The confidence parameters for the determination of DMT, harmine, harmaline, and tetrahydroharmine in ayahuasca beverages used in a religious ceremony and a powder sample seized by the police were totally satisfactory. The LOD and LOQ

TABLE 2
Quantification of β-carbolines and DMT (mg/g) in Seized Samples

Sample	THH	Harmaline	Harmine	DMT
SS1	< LOD	< LOD	< LOD	820
SS2	< LOD	160	120	< LOD
SS3	< LOD	< LOD	< LOD	20.1

DMT = *N,N*-dimethyltryptamine, LOD = limit of detection and THH = tetrahydroharmine.
The concentrations indicated are the mean of three determinations per sample.

obtained here can be considered satisfactory when the sample preparation technique, the range of concentrations tested, and the detection system used are taken into account. The method described here can be used in forensic chemistry and research laboratories.

After the validation, we applied the method to nine ayahuasca samples obtained from the Center of Integral Development Luz do Vegetal and three samples seized by the São Paulo State Police. There was marked variation in the alkaloid (DMT, harmine, harmaline, and tetrahydroharmine) concentrations in the ayahuasca samples. This variation in alkaloid content could be explained by the different methods of preparation, as well as the different amounts/proportions of source plants used to prepare the samples. The alkaloid concentrations in ayahuasca beverages are several times greater than in the source plants from which they are prepared (McKenna 2004). In addition, alkaloid concentrations can vary considerably among plants and consequently in the ayahuasca prepared from them (McKenna, Towers & Abbott 1984). Moreover, the alkaloids' biosynthesis in the source plants may depend on the weather, the soil conditions, and the season. As a result, different ayahuasca preparations can produce psychotropic responses that vary markedly in intensity.

Our findings agree with two previous studies that reported different concentrations of alkaloids in ayahuasca. In one study, Callaway et al. (1996) observed the following concentrations of alkaloids in ayahuasca used by a Brazilian religious group: DMT–0.24 mg/mL, harmaline–0.20 mg/mL, harmine–1.70 mg/mL, and tetrahydroharmine–1.07 mg/mL. In the other study, McKenna et al. (1984) found the following concentrations in Peruvian ayahuasca: DMT–0.60 mg/mL, harmaline–0.41 mg/mL, harmine–4.67 mg/mL, and tetrahydroharmine–1.60 mg/mL.

According to Gable (2007), the lethal dose of DMT present in ayahuasca is around 20 times greater than the typical ceremonial dose ingested in rituals. In addition, the probability of a toxic overdose of ayahuasca is minimized by serotonergic stimulation of the vagus nerve that in turn induces emesis and diarrhea close to the level of an effective ayahuasca dose. Hence, the risk of overdose appears to be related primarily to the concomitant or prior use of an additional serotonergic substance (Volpi-Abadie, Kaye & Kaye 2013; Gable 2007). Indeed, most cases of poisoning with tryptamine/monoamine oxidase inhibitor (MAOI) mixtures have involved individuals who prepared their own brew and/or ingested an additional psychoactive substance (Brush, Bird & Boyer 2004; Balíková 2002; Callaway & Grob 1998).

The death of a 25-year-old male caused by amine intoxication involved 5-MeO-DMT (methoxy-*N,N*-dimethyltryptamine) in addition to DMT and β-carbolines (Sklerov et al. 2005). In this case, the subject had a tetrahydroharmine blood concentration three times higher than that found in 14 volunteers in a UDV ayahuasca study (Callaway et al. 2006; Sklerov et al. 2005). Another case reported in the literature described the death of a 71-year-old female after ingesting ayahuasca mixed with tobacco leaves. The cause of death was attributed to acute nicotine poisoning (Warren 2004). A report that described more than 30 people who were poisoned with a herbal infusion during a meditation session found that the beverage contained atropine (hyoscyamine), scopolamine (hyoscine), harmine, and other alkaloids; all patients recovered, although mechanical ventilation was required in some cases (Balíková 2002).

The use of certain drugs, such as selective serotonin reuptake inhibitors (SSRIs) and MAOIs, associated with the ingestion of ayahuasca is not advisable because these drugs increase the risk of serotonergic syndrome (Callaway & Grob 1998). Serotonergic syndrome is defined as excessive serotonergic activity in the central and peripheral nervous systems, with typical symptoms being initial euphoria followed by tremors and convulsions, loss of consciousness and, occasionally, death (Volpi-Abadie, Kaye & Kaye 2013; Ables & Nagubilli 2010). Thus, the concomitant use of serotonergic drugs and ayahuasca overactivates peripheral and central postsynaptic 5HT-1_A and 5HT-2_A receptors to induce a serotonergic syndrome (Volpi-Abadie, Kaye & Kaye 2013; Gable 2007).

Two of the street samples analyzed here were seized from a 19-year-old male at a rave party in São Paulo, who also had LSD and MDMA ("ecstasy"). The other sample was seized from a 47-year-old male in São Paulo. As reported, this sample was used at an important festival in Bahia. The user reported having smoked the drug and the effect lasted about 50 min. When smoked, the effects of DMT are very fast, with a peak between 2–5 min postingestion and duration of action of 20–60 min (Winstock, Kaar & Borschmann 2014; Haroz & Greenberg 2005).

The samples seized and analyzed by the São Paulo State Police were in powder form, which allowed their consumption by dilution with beverages or other drugs. Indeed, DMT smokers have reported the concomitant

Downloaded by [FU Berlin] at 16:44 08 May 2015

Downloaded by [FU Berlin] at 16:44 08 May 2015

use of an MAO inhibitor from *B. caapi* or *P. harmala* with other drugs such as cannabis, LSD, alcohol, psilocybin, and MDMA (Cakic, Potkonyak & Marshall 2010). Consequently, the availability of these substances on the street could lead to greater toxicity of DMT, particularly since DMT is easily found on the Internet, along with instructions for its extraction that include the use of toxic chemicals (Winstock, Kaar & Borschmann 2014; Cakic, Potkonyak & Marshall 2010; Björnstad et al. 2009).

A recent case report described a 24-year-old male from rural South Australia who was admitted to the hospital for a first-episode psychosis. He dried leaf tips, bark, and seeds from acacia and *Phalaris aquatica* plants and then ground the material into a fine powder that he added to his regular tobacco and cannabis pipe (Warren, Dham-Nayyar & Alexander 2013). The amount of DMT found in *P. aquatica* and *Acacia maidenii* is approximately 0.06% and 0.71%, respectively. A 20 mg smoked dose of pure DMT is sufficient to produce moderate effects in the central nervous system, and this dose may be achieved by smoking just 2.8 g of *A. maidenii* bark (Warren, Dham-Nayyar & Alexander 2013; Collins 1990). The amount of ayahuasca consumed in a religious ceremony is ∼100 mL (McKenna 2004; Callaway et al. 1999). In the present study, the mean DMT concentration in the ayahuasca samples was 823.2 μg/ml (or 0.823 mg/mL). Consequently, 100 mL of ayahuasca would provide 82.3 mg of DMT. Thus, other factors besides the concentration of DMT must play a role in its psychoactive effects. DMT alone is inactive after oral administration at doses up to 1,000 mg (Nichols, Oberlender & McKenna 1991). Additionally, whereas ayahuasca is ingested orally in religious rituals, the samples seized by the police can be inhaled or smoked, which would also increase the bioavailability of alkaloids and the potential for intoxication. In the case of the seized samples containing DMT, the alkaloid concentration corresponded to 820 mg/g and 20.1 mg/g (SS1 and SS3, respectively) of the dry weight. This high content allows a large amount of DMT to be ingested and its use concomitant to ayahuasca could lead to intoxication and a serotonergic syndrome.

Ayahuasca has great historical and religious importance, especially in the indigenous Amazonian cultures of several South American countries. The religious use of ayahuasca includes syncretic religious movements that seek a way of self-analysis, introspection, and social integration. CONAD Resolution no. 1 (2010) provides a set of rules, norms, and ethical principles to be followed in the use of ayahuasca, including prohibitions on commercial distribution, therapeutic uses, tourism, advertisement, and its concomitant use with illicit drugs. The Resolution also suggests that the various ayahuasca groups should form legal entities and register with CONAD. The Resolution recommends that religious entities interview and orient members or aspirants so as to prevent the administration of ayahuasca to people with mental disorders or who are under the influence of alcohol or other psychoactive substances (Labate & Feeney 2012). For this reason, it is essential to clarify the ritual consumption of ayahuasca in groups that preserve its use in a religious context, particularly since cases of poisoning and even death by other similar substances or mixed beverages can be confused with ayahuasca use.

Resolution no. 1 encourages scientific research on the therapeutic potential of ayahuasca and several studies support the therapeutic effects of ayahuasca use in a religious context (Labate & Cavnar 2014; Bouso et al. 2012). Psychiatric evaluations have shown that, in adults previously involved in alcohol abuse, there was complete abstinence after affiliation to a religious ayahuasca group (Thomas et al. 2013; Loizaga-Velder & Verres 2014). Ayahuasca has been used to treat addictions (Liester & Prickett 2012) and may be beneficial in the treatment of alcohol dependence or other drugs of abuse, such as barbiturates, sedatives, cocaine, amphetamines, and solvent (Thomas et al. 2013; Brierley & Davidson 2012; Harris & Gurel 2012; Fábregas et al. 2010). The use of ayahuasca has been associated with the remission of psychopathological conditions, with no evidence of personality or cognitive deterioration (Grob et al. 1996). The volunteers included in the latter study had consumed ayahuasca for at least 10 years. Thus, the long-term consumption of ayahuasca within a religious context does not appear to exert a deleterious effect on neuropsychological function.

Ayahuasca appears to have antidepressant properties. In an investigation into the acute effects of the ingestion of ayahuasca in members of a religious group in which measures of anxiety, panic, and hopelessness were applied, those participants who were under the effects of ayahuasca were found to have lower ratings for these symptoms compared to participants receiving placebo (Osório et al. 2011; Santos et al. 2007). In agreement with these findings, a literature search conducted in the international medical PubMed database identified 15 publications that had studied the emotional, cognitive, and physical health of ayahuasca users, with the overall conclusion being that ayahuasca was safe and may be beneficial under certain conditions (Barbosa et al. 2012). These findings stress the importance of identifying the factors that can explain the different profiles of ayahuasca use in a religious context and the recreational use of similar substances on the streets.

In conclusion, we found a wide range of alkaloid concentrations in ayahuasca aqueous extracts obtained from a Brazilian religious organization, whereas, in the powder samples seized by police on the street, the number of active compounds was much fewer. In contrast to the ritualistic use of ayahuasca, which does not appear to be harmful to its users, the consumption of illicit powder samples represents a great potential for intoxication and deleterious

psychoactive effects. Future studies should address the factors, other than alkaloid concentration, that contribute to the divergent effects of ayahuasca and powder samples in their respective users.

FUNDING

This work was supported by a grant from FAP of Santa Casa de São Paulo School of Medical Sciences.

REFERENCES

Ables, A.Z. & Nagubilli, R. 2010. Prevention, diagnosis, and management of serotonin syndrome. *American Family Physician* 81 (9): 1139–42.

Balíková, M. 2002. Collective poisoning with hallucinogenous [sic] herbal tea. *Forensic Science International* 128 (1–2): 50–2.

Barbosa, P.C.; Mizumoto, S.; Bogenschutz, M.P. & Strassman, R.J. 2012. Health status of ayahuasca users. *Drug Test Analysis* 4 (7–8): 601–9.

Björnstad, K.; Hultén, P.; Beck, O. & Helander, A. 2009. Bioanalytical and clinical evaluation of 103 suspected cases of intoxications with psychoactive plant materials. *Clinical Toxicology* 47 (6): 566–72.

Bouso, J.C.; González, D.; Fondevila, S.; Cutchet, M.; Fernández, X.; Barbosa, P.C.R.; Alcázar-Córcoles, M.Á.; Araújo, W.S.; Barbanoj, M.J.; Fábregas, J.M. & Riba, J. 2012. Personality, psychopathology, life attitudes and neuropsychological performance among ritual users of ayahuasca: A longitudinal study. *PLoS ONE* 7 (8): e42421.

Brierley, D.I. & Davidson, C. 2012. Developments in harmine pharmacology: Implications for ayahuasca use and drug-dependence treatment. *Progress in Neuropsychopharmacology & Biological Psychiatry* 39 (2): 263–72.

Brush, D.E.; Bird, S.B. & Boyer, E.W. 2004. Monoamine oxidase inhibitor poisoning resulting from internet misinformation on illicit substances. *Journal of Toxicology Clinical Toxicology* 42 (2): 191–5.

Cakic, V.; Potkonyak, J. & Marshall, A. 2010. Dimethyltryptamine (DMT): Subjective effects and patterns of use among Australian recreational users. *Drug and Alcohol Dependence* 111 (1–2): 30–7.

Callaway, J.C. & Grob, C.S. 1998. Ayahuasca preparations and serotonin reuptake inhibitors: A potential combination for severe adverse interactions. *Journal of Psychoactive Drugs* 30 (4): 367–9.

Callaway, J.C.; Grob, C.S.; McKenna, D.J.; Nichols, D.E.; Shulgin, A. & Tupper, K.W. 2006. A demand for clarity regarding a case report on the ingestion of 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) in an Ayahuasca preparation. *Journal of Analytical Toxicology* 29 (8): 406–7.

Callaway, J.C.; McKenna, D.J.; Grob, C.S.; Brito, G.S.; Raymon, L.P.; Poland, R.E.; Andrade, E.N.; Andrade, E.O. & Mash, D.C. 1999. Pharmacokinetics of Hoasca alkaloids in healthy humans. *Journal Ethnopharmacology* 65 (3): 243–56.

Callaway, J.C.; Raymon, L.P.; Hearn, W.L.; McKenna, D.J.; Grob, C.S. & Brito, G.S. 1996. Quantitation of *N,N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with Ayahuasca. *Journal of Analytical Toxicology* 20 (6): 492–7.

Collins, D. (Ed.) 1990. *Plants for Medicines: A Chemical and Pharmacological Survey of Plants in the Australia Region*. East Melbourne: CSIRO Australia.

Conselho Nacional de Políticas sobre Drogas (CONAD). Resolução no. 5 (Resolution no. 5). 2004. (Nov. 4). Diário Oficial da União. Brasília (DF).

Conselho Nacional de Políticas sobre Drogas (CONAD). Resolução no. 1 (Resolution no. 1). 2010. (Jan. 25). Diário Oficial da União. Brasília (DF).

Deliganis, A.; Pierce, P. & Peroutka, S. 1991. Differential interactions of dimethyltryptamine (DMT) with 5-HT_{1A} and 5-HT_2 receptors. *Biochemical Pharmacology* 41 (11): 1739–44.

Deluca, P.; Davey, Z.; Corazza, O.; Di Furia, L.; Farre, M.; Flesland, L.H.; Mannonen, M.; Majava, A.; Peltoniemi, T.; Pasinetti, M.; Pezzolesi, C.; Scherbaum, N.; Siemann, H.; Skutle, A.; Torrens, M.; van der Kreeft, P.; Iversen, E. & Schifano, F. 2012. Identifying emerging trends in recreational drug use: Outcomes from the Psychonaut Web Mapping Project. *Progress in Neuropsychopharmacology Biological & Psychiatry* 39 (2): 221–6.

Fábregas, J.M.; González, D.; Fondevila, S.; Cutchet, M.; Fernández, X.; Barbosa, P.C.; Alcázar-Córcoles, M.A.; Barbanoj, M.J.; Riba, J. & Bouso, J.C. 2010. Assessment of addiction severity among ritual users of ayahuasca. *Drug and Alcohol Dependence* 111 (3): 257–61

Fantegrossi, W.E.; Harrington, A.W.; Kiessel, C.L.; Eckler, J.R.; Rabin, R.A.; Winter, J.C.; Coop, A.; Rice, K.C. & Woods, J.H. 2006. Hallucinogen-like actions of 5-methoxy-*N,N*-diisopropyltryptamine in mice and rats. *Pharmacology Biochemistry Behavior* 83 (1): 122–9.

Gable, R.S. 2007. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. *Addiction* 102 (1): 24–34.

Giumanini, A.G.; Chiavari, G.; Musiani, M.M. & Rossi, P. 1980. N-Permethylation of primary and secondary aromatic-amines. *Synthesis-Stuttgart* 9: 743–6.

Gordon, S.M.; Forman, R.F. & Siatkowski, C. 2006. Knowledge and use of the internet as a source of controlled substances. *Journal of Substance Abuse Treatment* 30 (3): 271–4.

Goulart, S.L. 2011. The notion of cure in the Brazilian ayahuasca religions. In: R.G. Santos (Ed.) *The Ethnopharmacology of Ayahuasca*, pp. 23–53. Kerala, India: Transworld Research Network.

Grob, C.S.; McKenna, D.J.; Callaway, J.C.; Brito, G.S.; Neves, E.S.; Oberlaender, G.; Saide, O.L.; Labigalini, E.; Tacla, C.; Miranda, C.T.; Strassman, R.J. & Boone, K.B. 1996. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *Journal of Nervous and Mental Disease* 184 (2): 86–94.

Halpern, J.H. 2004. Hallucinogens and dissociative agents naturally growing in the United States. *Pharmacology Therapeutics* 102 (2): 131–8.

Haroz, R. & Greenberg, M.I. 2005. Emerging drugs of abuse. *Medical Clinical of North America* 89 (6): 1259–76.

Harris, R. & Gurel, L. 2012. A study of ayahuasca use in North America. *Journal of Psychoactive Drugs* 44 (3): 209–15.

Labate, B.C. 2004. A literatura brasileira sobre as religiões ayahuasqueiras. In: B.C. Labate (Ed.) *O Uso Ritual da Ayahuasca*, pp. 231–273. Campinas: Mercado de Letras Edições e Livraria.

Labate, B.C. & Cavnar, C. (Eds.). 2014. *The Therapeutic Use of Ayahuasca*. Berlin: Springer.

Labate, B.C. & Feeney, K. 2012. Ayahuasca and the process of regulation in Brazil and internationally: Implications and challenges. *International Journal of Drug Policy* 23 (2): 154–61.

Liester, M.B. & Prickett, J.I. 2012. Hypotheses regarding the mechanisms of ayahuasca in the treatment of addictions. *Journal of Psychoactive Drugs* 44 (3): 200–8.

Loizaga-Velder, A. & Verres, R. 2014. Therapeutic effects of ritual ayahuasca use in the treatment of substance dependence: Qualitative results. *Journal of Psychoactive Drugs* 46 (1): 63–72.

Luna, L.E. 2011. Indigenous and mestizo use of ayahuasca: An overview. In: R.G. Santos (Ed.) *The Ethnopharmacology of Ayahuasca*, pp. 1–21. Kerala, India: Transworld Research Network.

McKenna, D.J. 2004. Clinical investigations of the therapeutic potential of ayahuasca: Rationale and regulatory challenges. *Pharmacology Therapeutics* 102 (2): 111–9.

Downloaded by [FU Berlin] at 16:44 08 May 2015

McKenna, D.J. & Towers, G.H.N. 1984. Biochemistry and pharmacology of tryptamine and β-carboline derivatives: A mini review. *Journal of Psychoactive Drugs* 16 (4): 347–58.

McKenna, D.J.; Towers, G.H.N. & Abbott, F.S. 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants: Tryptamine and β-carboline constituents of ayahuasca. *Journal of Ethnopharmacology* 10 (2): 195–223.

Montagne, M. 2008. Drugs on the internet. I: Introduction and web sites on psychedelic drugs. *Substance Use and Misuse* 43 (1): 17–25.

Nichols, D.E.; Oberlender, R. & McKenna, D.J. 1991. Stereochemical aspects of allucinogenesis. In: R.R. Watson (Ed.) *Biochemistry and Physiology of Substance Abuse*. Boca Raton, FL: CRC Press.

Osório, F.L.; Macedo, L.R.H.; Sousa, J.P.M.; Pinto, J.P.; Quevedo, J.; Crippa, J.A.S. & Hallak, J.E.C. 2011. The therapeutic potential of harmine and ayahuasca in depression: evidence from exploratory animal and human studies. In: R.G. Santos (Ed.) *The Ethnopharmacology of Ayahuasca*, pp. 75–85. Kerala, India: Transworld Research Network.

Pierce, P. & Peroutka, S. 1989. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology* 97 (1): 118–22.

Pires, A.P.S.; De Oliveira, C.D.R.; Moura, S.; Dorr, F.A.; Silva, W.A.E. & Yonamine, M. 2009. Gas chromatographic analysis of dimethyltryptamine and beta-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. *Phytochemical Analysis* 20 (2): 149–53.

Riba, J.; Rodríguez-Fornells, A.; Urbano, G.; Morte, A.; Antonijoan, R.; Montero, M.; Callaway, J.C. & Barbanoj, M.J. 2001. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology* (Berl) 154 (1): 85–95.

Santos, R.G.; Landeira-Fernandez, J.; Strassman, R.J.; Motta, V. & Cruz, A.P.J. 2007. Effects of ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. *Journal of Ethnopharmacology* 112 (3): 507–13.

Sklerov, J.; Levine, B.; Moore, K.A.; King, T. & Fowler, D. 2005. A fatal intoxication following the ingestion of 5-methoxy-*N,N*-dimethyltryptamine in an ayahuasca preparation. *Journal of Analytical Toxicology* 29 (8): 838–41.

Smith, R.L.; Canton, H.; Barrett, R.J. & Sanders-Bush, E. 1998. Agonist properties of *N,N*-dimethyltryptamine at serotonin 5-HT_{2A} and 5-HT_{2C} receptors. *Pharmacology Biochemistry Behavior* 61 (3): 323–30.

Strassman, R.J.; Qualls, C.R. & Berg, L. 1996. Differential tolerance to biological and subjective effects of four closely spaced doses of *N,N*-dimethyltryptamine in humans. *Biological Psychiatry* 39 (9): 784–95.

Strassman, R.J.; Qualls, C.R.; Uhlenhuth, E.H. & Kellner, R. 1994. Dose-response study of *N,N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. *Archives of General Psychiatry* 51 (2): 98–108.

SWGDRUG. Scientific Working Group for the Analysis of Seized Drugs Recommendations. Available at http://www.swgdrug.org/Documents/SWGDRUGRecommendations6.pdf. Accessed April 26, 2013.

Thomas, G.; Lucas, P.; Capler, N.R.; Tupper, K.W. & Martin, G. 2013. Ayahuasca-assisted therapy for addiction: Results from a preliminary observational study in Canada. *Current Drug Abuse Reviews* 6 (1): 30–42.

Tupper, K.W. 2008. The globalization of ayahuasca: Harm reduction or benefit maximization? *International Journal of Drug Policy* 19 (4): 297–303.

UNODC. Guidance for the validation of analytical methodology and calibration of equipment used for testing of illicit drugs in seized materials and biological specimens. Available at http://www.unodc.org/documents/scientific/validation_E.pdf. Accessed April 26, 2013.

Volpi-Abadie, J.; Kaye, A.M. & Kaye, A.D. 2013. Serotonin syndrome. *The Ochsner Journal* 13 (4): 533–40.

Warren, R.J. 2004. Fatal intoxication resulting from the ingestion of ayahuasca. *Journal of Analytical Toxicology* 28 (4): 287 (abstract).

Warren, J.M.; Dham-Nayyar, P. & Alexander, J. 2013. Recreational use of naturally occurring dimethyltryptmine contributing to psychosis? *Australian & New Zealand Journal of Psychiatry* 47 (4): 398–9.

Winstock, A.; Kaar, S. & Borschmann, R. 2014. Dimethyltryptamine (DMT): Prevalence, user characteristics and abuse liability in a large global sample. *Journal of Psychopharmacology* 28 (1): 49–54.

Yritia, M.; Riba, J.; Ortuño, J.; Ramirez, A.; Castillo, A.; Alfaro, Y.; de la Torre, R. & Barbanoj, M.J. 2002. Determination of *N,N*-dimethyltryptamine and beta-carboline alkaloids in human plasma following oral administration of Ayahuasca. *Journal of Chromatography B* 779 (2): 271–81.

Downloaded by [FU Berlin] at 16:44 08 May 2015