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WAGENINGEN UNIVERSITY & RESEARCH

FRONT OFFICE FOOD AND PRODUCT SAFETY

Assessment of Shambala and a similar herbal preparation

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Subject

The TV programme Radar recently drew attention to the herbal preparation Shambala (https://radar.avrotros.nl/uitzendingen/gemist/item/vind-je-innerlijke-guru-met-kruidenmiddel-van-giel-beelen-radar-checkt/). The product was promoted by radio DJ Giel Beelen and is for sale on the Internet (Shambala herbal preparation: Caapi and Bobinsana | Superfoodies). In response to an earlier report and the Radar broadcast, the Netherlands Food and Consumer Product Safety Authority (NVWA) announced that it would be investigating Shambala. Shambala and a similar herbal preparation with comparable ingredients were sampled by the NVWA and analysed by Wageningen Food Safety Research (WFSR).

Question

Does the consumption of Shambala or the similar herbal preparation referred to above pose a risk to public health?

Yes, the use of Shambala and a similar herbal preparation with comparable ingredients does pose a risk to public health. These herbal preparations contain the β-carboline alkaloids harmine, harmaline and tetrahydroharmine. Harmful effects can occur from a single use of these herbal preparations according to the instructions on the packaging, due to the effects of these substances on the central nervous system. These effects can include pupil dilation, salivation and low blood pressure. Exposure to higher doses of β-carboline alkaloids can cause nausea and vomiting, as well as more serious effects such as hallucinations, impaired coordination, impaired vision and paralysis.
In addition, taking these products in combination with medicines, other herbal preparations, or products containing inhibitors of the monoamine oxidase (MAO) enzyme, selective serotonin reuptake inhibitors (SSRIs) or noradrenaline-dopamine reuptake inhibitors (NDRIs) can lead to harmful interactions.
With repeated use, additional effects on other organs cannot be ruled out. There are also indications of the possible genotoxicity of harmine and harmaline, but there is a lack of suitable data enabling a definitive conclusion to be drawn on this point. Finally,

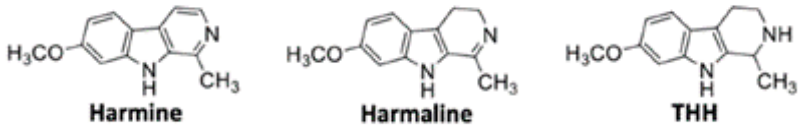
there is too little data to draw a conclusion about possible reproductive and developmental toxicity following exposure to β-carboline alkaloids.

These herbal preparations may also contain other toxic substances. In the analyses, a fourth, potentially active ingredient was detected. This substance has not yet been identified and its possible effects were not included in this assessment.

Introduction

Shambala is a herbal preparation that contains parts of two plants, Caapi (*Banisteriopsis caapi*) and Bobinsana (*Calliandra angustifolia*), and is sold with the aim of 'opening your heart and connecting with it all' (Superfoodies, 2021). The NVWA also sampled a similar herbal preparation containing both *B. caapi* and *C. angustifolia*.

B. caapi contains the alkaloids harmine, harmaline and tetrahydroharmine (THH) (Figure 1). These alkaloids are the active substances of *B. caapi* and are also known as β-carboline alkaloids or harmala alkaloids. Harmine and harmaline are inhibitors of the enzyme monoamine oxidase (MAO). THH is a serotonin reuptake inhibitor. *B. caapi* is one of the two components of the hallucinogenic substance ayahuasca, along with *Psychotria viridis* (Brito-da-costa et al., 2020). Through MAO inhibition, the β-carboline alkaloids ensure that the active, hallucinogenic substance N,N-dimethyltryptamine (DMT) from *P. viridis* is not broken down, and can therefore reach the brain. In addition, the β-carboline alkaloids themselves can also have psychotropic effects (Brito-da-costa et al., 2020; Druginfo, 2021; Natural Medicines, 2021a). Other plants, such as *Peganum harmala*, also contain β-carboline alkaloids. These substances are also present in animals, people and fungi (Brito-da-costa et al. 2020).



Little is known about the active substances in the plant *C. angustifolia*, but it is possible that this plant also contains β-carboline alkaloids (Rumlerová et al., 2021).

The NVWA took samples of Shambala and of a similar herbal preparation. These samples were analysed by WFSR for β-carboline alkaloids and synthetic MAO inhibitors. A broad screening of these products for pharmacologically active substances and contaminants was then also conducted. This assessment will investigate whether consumption of Shambala or consumption of the similar herbal preparation, poses a risk to public health based on the analytical data from WFSR.

Analysis of the herbal preparations

The codes, sample information and dosages according to the packaging/package leaflet can be found in Tables 1 and 2. It is not clear how the specified quantities should be interpreted, since it is not possible for one mL to include these quantities (in grams).

Table 1. Codes, sample information and dosage according to the Shambala packaging/package leaflet.

Product	**Shambala, 4.9 mL bottle (Superfoodies)**
Composition	*Banisteriopsis caapi* (Caapi), *Calliandra angustifolia* (Bobinsana), water, alcohol (50%). Composition per mL (+/- 20 drops) 5.7 grams Bobinsana 3.0 grams Caapi Contains: 4.9 mL (enough for 7 drops per day for 14 days) The extracts of the ingredients were produced in Switzerland. The bottles were filled and packaged in the Netherlands.
Dosage	Take 1–3 drops under the tongue three times a day for 14 days or for as long as the 'teacher plants' say.

Table 2. Codes, sample information and dosage according to the packaging/package leaflet of the similar herbal preparation

Product	**20 mL bottle**
Composition	*B. caapi* 50% 4.5 g, Bobinsana 50% 4.5 g, 20 mL Demineralised water, 15% Alcohol
Dosage	4 to 20 drops daily, or every other day. Take for one month.

The NVWA took one sample of Shambala (batch number SHAM-005) and one sample of the similar herbal preparation. These samples were analysed by WFSR. From each sample, 1.0 gram was extracted (30 min, mixing by means of mechanical shaking) with 10 mL of extraction fluid (80:20 acetonitrile:water, 1% acetic acid). This solution was diluted 200–20,000 times (in 50:50 acetonitrile:water, 0.1% acetic acid) and analysed using liquid chromatography – full scan high resolution mass spectrometry (the details are described in Biesterbos et al., 2019). In the initial analysis, the samples were screened for the presence of substances known as MAO inhibitors (both natural and synthetic), based on the theoretical exact masses of these substances. Evidence was found for the possible presence of the β-carboline alkaloids harmine, harmaline and THH in both products. Next, the analytical reference standards for these substances were acquired, and a confirmatory analysis was performed to arrive at a clear identification. The detected substances are known to be present in *B. caapi*, with harmaline and THH being reduction products of harmine. No other natural or synthetic MAO inhibitors were detected. Broad screening was also conducted for pharmacologically active substances (including hallucinogenic substances) and contaminants. This screening uncovered a possible fourth substance. This substance is still being identified, and if relevant quantified, but the information was not yet available when this assessment was being drawn up.
The extracts were measured once more for confirmation and identification purposes, along with the analytical reference standards for the three β-carboline alkaloids. Identification was made on the basis of a corresponding retention time, the exact mass of the substances ($M+H^+$), and at least one fragment ion. Quantification was based on calibration standards prepared in solvent. To understand the precision of the method, two extracts of each sample were prepared in addition to the initial extract. The average values in mg/g with the relative standard deviation (RSD) are set out as results in the table below. It was assumed that the substances were fully dissolved in the extraction fluid.

To determine the exposure of users, the analysts counted how many drops, dispensed using the dropper from the product, it took to make up 500 mg. This was then used to determine the weight of one drop.

WFSR's results are shown in Tables 3 and 4.

Table 3. Analysis results for Shambala

Mass of drops as obtained using the dropper from the product	1 drop = 0.0294 g
Substances detected and quantified:	
Harmine	10.976 mg/g product, 0.323 mg/drop (RSD = 0.8%)
Harmaline	1.845 mg/g product, 0.0543 mg/drop (RSD = 0.9%)
Tetrahydroharmine (THH)	6.184 mg/g product, 0.182 mg/drop (RSD = 1.5%)

Table 4. Analysis results for the similar herbal preparation

Mass of drops as obtained using the dropper from the product	1 drop = 0.0455 g
Substances detected and quantified:	
Harmine	2.705 mg/g product, 0.123 mg/drop (RSD = 7.8%)
Harmaline	0.258 mg/g product, 0.0117 mg/drop (RSD = 5.6%)
Tetrahydroharmine (THH)	1.929 mg/g product, 0.0877 mg/drop (RSD = 6.8%)

Exposure

Based on the analysed levels of harmine, harmaline and THH in the products, the recommended daily dosages (in drops) stated on the packaging and the measured weight per drop (Tables 1–4), the daily exposure to harmine, harmaline and THH from using Shambala or the similar herbal preparation was calculated (Table 5).

Table 5. Estimated daily exposure to β-carboline alkaloids from the recommended use of Shambala and the similar herbal preparation.

Product	**Daily dosage (in drops)**	**Exposure[1]**		
		Harmine	**Harmaline**	**THH**
Shambala	3–9	0.97–2.91 mg (14–42 µg/kg BW)	0.16–0.49 mg (2.3–7.0 µg/kg BW)	0.55–1.64 mg (7.8–23 µg/kg BW)
Similar herbal preparation	4–20	0.49–2.46 mg (7.0–35 µg/kg BW)	0.05–0.23 mg (0.7–3.3 µg/kg BW)	0.35–1.75 mg (5.0–25 µg/kg BW)

[1] The exposure per kg body weight (BW) has been calculated for a person weighing 70 kg.

Literature review approach

Searches were performed for toxicity data on the β-carboline alkaloids detected – harmine, harmaline and THH – in the Pubmed, Scopus and Embase databases. Harmine, harmaline and THH were searched for separately in Pubmed, using the MeSH terms for each substance, along with the term 'toxic*'. A similar strategy was employed for searching in Embase, using the Emtree terms for harmine, harmaline and THH. In Scopus, searches were performed for each of the three substances separately, along with the term 'toxic*'. Searches were also performed in the above databases and in a number of reference works (Natural Medicines, Hager's Encyclopaedia, and the Commission E monographs) for toxicology information on the plants *C. angustifolia* and *B. caapi*. It was also investigated whether risk assessments of these substances and/or plants were available from our sister institutes. No information was found on Shambala or the similar herbal preparation in the scientific literature or in the other sources referred to above.

Toxicity data

The available data for Shambala, the similar herbal preparation, *C. angustifolia*, *B. Caapi*, and the β-carboline alkaloids harmine, harmaline and THH, is set out below. The focus is on animal studies and human data on the toxic effects of these plants and ingredients following oral administration. Data on other administration routes and *in vitro* data was largely excluded from consideration. Information on kinetics and mechanisms of action is largely based on two recent review articles (Brita-da-costa et al., 2020; Zhang, Li & Yu, 2020).

Shambala and the similar herbal preparation

No toxicity studies and no human data were found on Shambala or the similar herbal preparation. No reports concerning the use of Shambala have been received by the National Poisons Information Centre (NVIC) (personal communication, November 2021). The websites where Shambala and the similar herbal preparation are sold list a number of contraindications and display warnings about the possible side effects of these products. Shambala can affect the ability to drive and is not intended for pregnant women or children under 16 years of age. Using Shambala in combination with medicines such as heart medication and psychotropics is also not recommended; users are advised to consult a doctor before doing so. Caution is also advised for those with psychological disorders (Superfoodies, 2021). Some of the warnings for Shambala have also been issued for the similar herbal preparation. In addition, use of this herbal preparation is discouraged while breastfeeding and for children under 18 years of age. It is also noted that *B. caapi* is a monoamine oxidase (MAO) inhibitor and should not be used in combination with foods such as smoked fish, champagne or blue cheese, or drugs such as ecstasy (XTC). Side effects are listed for both products, including headaches, a stinging sensation under the tongue and/or diarrhoea.

Calliandra angustifolia

Little is known about the plant *Calliandra angustifolia* or its active substances (Rumlerová et al., 2021). The plant is not listed in the European Food Safety Authority (EFSA) Compendium of Botanicals (EFSA, 2012) or in the reference works mentioned above. There is also little relevant information on this plant to be found in the scientific literature. The product description of a herbal preparation containing *C. angustifolia* states that this plant contains MAO inhibitors, and that it can be extremely dangerous to use the product in combination with certain foods or psychoactive substances that are not normally harmful (Indianspirit, 2021). A recent article states that *C. angustifolia* contains THH and acts as a MAO inhibitor (Rumlerová et al., 2021).

Banisteriopsis caapi and the β-carboline alkaloids harmine, harmaline and THH

Kinetics

Humans

After oral administration of 20–50 mg harmine (approximately 0.3–0.7 mg/kg BW), psychotropic effects occur within 20–30 minutes. This implies that absorption into the blood occurs following oral administration (Naranjo, 1959, cited by Brito-da-costa et al., 2020).

The absorption of β-carboline alkaloids has also been observed following oral ingestion of ayahuasca. The metabolites harmol and harmalol have been detected in plasma. The β-carboline alkaloids are primarily excreted as metabolites; only small quantities of harmine, harmaline and THH are found in urine (Brito-da-Costa et al., 2020). By way of illustration, the exposure of volunteers (n=18) to ayahuasca, corresponding to a dose of 1 mg/kg BW of harmine, 0.07 mg/kg BW of harmaline and 0.82 mg/kg BW of THH, led to maximum plasma concentrations (C_{max}) of approximately 2.5 ng/mL for harmaline and 20 ng/mL for THH. This was a lower-than-usual dose of ayahuasca, but it still resulted in subjective effects. Exposure to a higher dose of ayahuasca, corresponding to 1.4 mg/kg BW of harmine, 0.09 mg/kg BW of harmaline and 1.2 mg/kg BW of THH, led to a C_{max} of approximately 4.3 ng/mL for harmaline and 33 ng/mL for THH. Harmine was unable to be detected, but its metabolite harmol was detectable with a C_{max} of approximately 11 ng/mL at the low dose of ayahuasca and approximately 13 ng/mL at the high dose. This suggests that harmine is largely converted before reaching the bloodstream (Riba et al., 2003).

Following intravenous administration of 0.5 mg/kg BW harmine in human volunteers, the quantity in the blood decreased to 10% after 2 minutes. Four hours later, 1% of the dose was still present in the blood (Hagers Enzyklopädie). Following intravenous administration of 0.5 mg/kg BW harmine hydrochloride, 35% of the dose had been excreted within 24 hours. The majority of the excreted harmine was harmine glucuronide or harmine sulphate. Harmol constituted 0.03% of the excreted dose, while 0.06% was excreted unchanged (Hagers Enzyklopädie).

Harmine and harmaline are metabolised by various cytochrome P450 isoenzymes, with CYP1A1, CYP1A2 and CYP2D6 playing a major role. Interindividual differences can therefore occur, due to polymorphisms and/or lifestyle factors that affect the activity of these CYP isoenzymes. Harmine can be converted to harmol, a 3-hydroxyharmine analogue, a 5-hydroxyharmine analogue and a dihydroxyharmine analogue. Harmaline can be converted to harmalol, a hydroxyharmine analogue and harmine (Zhang, Li & Yu, 2020; Brito-da-Costa et al., 2020). Harmol and harmalol can subsequently become conjugated, primarily with glucuronide, before being excreted in bile or urine (Zhang, Li, and Yu 2020). THH is partially metabolised into tetrahydroharmol (7-hydroxy-tetrahydroharmine) (Brito-da-Costa et al., 2020).

In vitro experiments using human liver microsomes have found that harmine inhibits CYP3A4 and CYP2D6, with IC_{50} values (concentration when half of the maximum inhibition occurs) of 44 and 39 µM, respectively. Harmaline is a stronger inhibitor of CYP2D6 than harmine (IC_{50} = 26 µM), but a weaker inhibitor of CYP3A4 (IC_{50} > 100 µM) (Zhao et al., 2011; Zhang, Li, & Yu, 2020).

Rats

Harmine and harmaline are rapidly absorbed in rats following oral administration. After oral administration of 40 mg/kg BW harmine and harmaline, the C_{max} of harmine and harmaline was reached after 0.56 and 1.76 hours, respectively (T_{max}). The bioavailability in these rats amounted to approximately 1% for harmine and 17% for harmaline (Brito-da-costa et al., 2020). A bioavailability of approximately 3% was found after oral

administration of 20 mg/kg BW of harmine in male Sprague Dawley rats (Zhang, Li, and Yu 2020). This means that a large first-pass effect occurs with these substances, meaning that they are mainly converted in the intestines and liver before entering the bloodstream.

Following oral administration of 15, 45 and 150 mg/kg BW once per day for 4 weeks of a total alkaloid extract of *P. harmala* seeds to groups of 10 Wistar rats, harmine and harmaline were rapidly absorbed and the C_{max} was reached after 0.69–2.7 hours and 0.73–4 hours, respectively. The T_{max} and C_{max} increased with the dose. Higher C_{max} values were reached after repeated administration of these doses for 14 or 28 days. The metabolite harmol was measured in the plasma within 5 minutes for all dosages and exposure periods. The metabolite harmalol was only detected after repeated exposure. Harmine and harmaline appeared to be widely distributed around the body. The highest concentrations were found in the liver, kidneys, spleen and lungs. The levels in the organs increased with increasing doses. No accumulation occurred in the organs examined. In contrast to harmine, harmaline was also found in the brain, suggesting passage through the blood-brain barrier. The metabolite harmalol was also found in the brain (Wang et al., 2019). This research was performed in conjunction with a 28-day toxicity study, which is further described below (Wang et al., 2019).

Harmine is extensively metabolised in rats. Following intravenous administration of 5 mg/kg, 99% of the dose was detected as metabolites in the bile (73%) and urine (26%). The most common metabolites were harmol sulphate and harmol glucuronide, but unchanged harmine and harmol were also found (Hagers Enzyklopädie).

Mechanism of action
An overview of the mechanisms of action of harmine, harmaline and THH is given in the review article by Brito-da-costa et al. (2020). These substances can cause behavioural, psychological and physiological effects in humans by influencing the concentrations of monoamine neurotransmitters (such as serotonin, dopamine and noradrenaline) in the central nervous system. They can do this by influencing the metabolism of these neurotransmitters or through direct interaction with specific receptors. Harmine and harmaline are reversible, selective inhibitors of the enzyme monoamine oxidase A (MAO-A). For harmine, IC_{50} values in the low nanomolar range (2-17 nM) have been reported (Brierley & Davidson, 2012). THH inhibits serotonin reuptake and has little or no effect on MAO-A. The hallucinatory effects of these alkaloids could potentially be caused by direct binding to serotonin receptors (5-HT_{2A} or 5-HT_{2C}). These alkaloids can also cause tremors through their interaction with serotonin-binding receptors. Another important mechanism of action that has been proposed for harmine and harmaline is the promotion of dopamine efflux and potential inhibition of the dopamine transporter and dopamine reuptake (Brito-da-Costa et al., 2020).

Acute toxicity
There are almost no oral acute toxicity studies available that involve harmine, harmaline, THH or *B. caapi* extract. Wang et al. (2019) reported an oral LD_{50} (median lethal dose, dose that causes death in 50% of the animals) in mice of 118.9 mg/kg BW for harmaline and 250.3 mg/kg BW for harmine.

Wang et al. (2019) also reported that in mice, cats, rabbits and monkeys, acute action tremors (tremors that occur with muscle activity) were observed following injection with a high (but unspecified) dose of harmine and/or harmaline. In cattle, toxic effects (clonic muscle spasms, increased pulse rate and rapid breathing) were observed following intravenous administration of 9 mg/kg BW of harmine and harmaline.

Several studies involving intravenous or intraperitoneal administration are described in more detail below.

Yang et al. (2021) inferred an intravenous LD_{50} value of 26.9 mg/kg BW for harmine. The intravenous acute toxicity of harmine has been investigated in a number of experiments. Groups of 10 male Institute of Cancer Research (ICR) mice (albino mice, 6 weeks old) were exposed to a single intravenous dose of up to 250 mg/kg BW harmine hydrochloride (≥ 98% purity). The effects observed were agitation, tremors, convulsions, jumping, ataxia, opisthotonus (convulsive backward arching of the body) and death. The only dose at which no deaths occurred was 7.5 mg/kg BW. Because all deaths occurred within 30 minutes and the other symptoms had virtually disappeared within 60 minutes, the LD_{50} value was set 60 minutes after the administration of harmine. The value set was 26.9 mg/kg BW.

Another experiment used two groups of 10 male mice to investigate the effects on a number of biochemical parameters 60 minutes after intravenous exposure to 20 mg/kg BW of harmine or water. No significant alterations in the activity of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) were observed, and thus no indications of liver toxicity within 60 minutes after administration. However, creatine kinase (CK), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and creatinine (Cr) levels were statistically significantly increased in the exposed animals. An increase in the enzymes CK, CK-MB and LDH, which are found in heart muscle tissue, among other places, could be an indication that harmine can cause damage to the heart. No clear histopathological effects were observed in the heart, liver, lungs or kidneys at this length of exposure or concentration.

In a third experiment involving anaesthetised mice, after intravenous exposure to 0 (n=6), 25 (n=6), 50 (n=10) or 75 (n=6) mg/kg BW of harmine, a dose-related decrease in heart function was observed (heart rate and blood pressure). This effect was highly visible 1 minute after exposure to 25 mg/kg BW of harmine and gradually increased to 50% of the original heart rate value and 100% of the original blood pressure values within 30 minutes. At a higher concentration (50 mg/kg BW) some of the mice died; a sharp drop in blood pressure and heart rate and inversion of the QRS waves were also observed. In the mice that survived this dose, the heart rate and blood pressure returned to 40% and 70% of the original values respectively within 30 minutes and the QRS waves reverted. At 75 mg/kg BW, all of the mice died. Sharp drops in blood pressure and heart rate were observed, along with inversion of the QRS waves (Yang et al., 2021).

C_{57}BL/6 mice (9–12 weeks old) were exposed to a single intravenous dose of 5–100 mg/kg BW of harmine. After 14 days the animals were killed so the effects on the heart, liver and kidneys could be studied. The mice that had died earlier were also examined. After exposure to harmine, neurotoxic effects (tremors, muscle spasms, supination (rotation of the legs) and spasms) occurred; these effects were classified as severe. The animals that survived had tremors that lasted for 20 minutes before gradually subsiding. The next day, the animals exhibited no effects at all. No macroscopic alterations to the heart, liver or kidneys were observed. The authors inferred an LD_{50} and presented it in a graph, but this value cannot be derived from the graph (Chen et al., 2004).

Groups of female sheep (Border Leicester x Merino) were exposed to a single intraperitoneal dose of 2 mg/kg BW, 6 mg/kg BW or 18 mg/kg BW of harmine or harmaline. No effects were observed at 2 mg/kg BW. At the other doses, effects occurred for harmine within 10–12 minutes that persisted for 2.5 – 4.5 hours. For harmaline, effects occurred within 8–12 minutes and persisted for 2.5 – 7 hours. At the higher dose,

the effects began more quickly and lasted for longer. The effects observed with 6 mg/kg BW of harmine and/or harmaline included tremors, mild paralysis of the hind legs and crossing of the limbs during movement. At the highest dose, impaired consciousness, tremors, hypomobility, paralysis of the limbs and crossing of the limbs during movement were observed (Bourke et al., 1990).

Subchronic toxicity
An ethanol extract (0.7 mL solution) of *B. caapi* was administered via an oral stomach tube twice a week for a month to female Holtzman rats (5 per group). The animals were observed for motor activity, psychoactive effects, reflexes, sedation, dizziness, passivity, facial tremors, alertness, diuresis and response to touch. The animals were killed 24 hours after the final administration and the brain, liver and aorta examined under a microscope. Reduced motor activity, mild sedation, passivity and a response to touch were observed in the animals. The microscopic examination showed no alterations in the brain, but there were structural alterations, haemorrhages and mild congestion in the liver and alterations in the aorta (thickening of the wall with an increase in lipophages (macrophages containing an abundance of small lipid vacuoles) (Castro et al., 2017).

Daily doses of 0, 15, 45 or 150 mg/kg BW of a total alkaloid extract of *P. harmala* seeds were administered via stomach tube for 28 days to groups of 15 male and 15 female Wistar rats (Wang et al., 2019). The extract contained 30.57% harmaline and 27.63% harmine. The animals were observed each day for signs of toxicity and mortality. Body weight and feed intake were measured weekly. After 28 days, 10 males and 10 females from each group were killed. The other animals were killed after a four-week recovery period. Blood samples were taken before they were killed, for haematological and biochemical analysis, and urine was collected for analysis. The animals were examined macroscopically and the weight of 11 organs was determined. A number of organs (liver, heart, spleen, kidney, brain, thymus, adrenal gland, uterus, testis and epididymis) were examined microscopically. At the highest dose, in both sexes tremors began approximately 15 minutes after administration and continued for around 4 hours. The tremors stopped occurring from Day 4 onwards. In the first week, but not in the rest of the study, feed intake was reduced statistically significantly in rats from the group receiving the highest dose. There were no exposure-related effects on the haematological or urine parameters. In females in the highest dose group, various statistically significant differences were observed in biochemical parameters that point to effects on blood glucose and lipid metabolism and possible liver damage. In females and males the relative liver weight increased in the highest dose group, and in males the relative testis weight also increased (absolute organ weights were not reported). These effects disappeared after the recovery period.
Under the conditions of this study, a no-observed-adverse-effect level (NOAEL) of 45 mg/kg BW of total alkaloid extract of *P. harmala* seeds was established (Wang et al., 2019). This corresponds to 13.8 mg/kg BW of harmaline and 12.4 mg/kg BW of harmine.

Harmine hydrochloride (0, 2.5, 5, 9 or 10 mg/kg BW per day) was administered via a stomach tube for three months to groups of 3 male and 3 female CD-1 rats. After three months blood was taken and the animals were killed. The brain, heart, spleen and adrenal glands were weighed and subjected to a histopathological examination. No alterations were observed in the body weight, general condition or behaviour of the animals. At the highest dose, there was a statistically significant increase (+280%) in plasma concentration of the liver enzyme AST. At doses of 9 and 10 mg/kg BW, histopathological effects were observed in the heart, spleen and adrenal glands. The NOAEL for this study was 5 mg/kg BW per day of harmine hydrochloride (Zhanaidarova et al., 2019). This corresponds to 4.2 mg/kg BW per day of harmine.

Genotoxicity

Several genotoxicity studies have been performed with harmine and harmaline and a small number for harmol and harmalol. The results of these studies are briefly given below. The studies were not conducted in accordance with the testing guidelines of the Organisation for Economic Cooperation and Development (OECD).

In vitro

Harmine

Harmine produced positive results in a chromosome aberration test (the test was only conducted without metabolic activation) and in a comet assay (with and without metabolic activation) in V79 lung cells from Chinese hamsters (Boeira et al., 2001). Harmine also amplified the chromosome aberrations induced by ultraviolet (UV) and mitomycin C in Chinese hamster CHO K-1 cells in the presence of metabolic activation (Sasaki et al., 1992) and UVC-induced mutagenicity without metabolic activation in *E. coli* B/r WP2, but had no effect on mutagenicity without UVC (Shimoi, Kawabata & Tomita, 1992). Harmine-induced point mutations, frameshift mutations and recombinogenic effects (crossing-over and gene conversion) were observed in *Saccharomyces cervisae* during the exponential growth phase, but not during the stationary growth phase (Boeira et al., 2002). UVA irradiation led to harmine-induced formation of DNA photoproducts and micronuclei in V79 cells and bacteriophage DNA (PM2 plasmid) (Vignoni et al., 2014). In an Ames test with *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102, harmine gave a weak positive response (with metabolic activation) in strains TA97, TA98 and TA102 (Picada et al., 1997). In a different Ames test, harmine produced negative results in *S. typhimurium* strains TA98 and TA100, both with and without metabolic activation (Kummrow et al., 2019).
Harmine showed a negative response in an SOS chromosome test with *Escherichia coli* PQ37 (Picada et al., 1997), and for mutagenicity in *E. coli* B/r WP2 without UV radiation (Shimoi, Kawabata & Tomita, 1992).

Harmaline

In an Ames test with *S. typhimurium* strains TA97, TA98, TA100 and TA102, harmaline showed a weak positive response in strain TA102 with metabolic activation (Picada et al., 1997), and in a different Ames test with strains TA98 and TA100 harmaline was positive in strain TA98 without metabolic activation (Kummrow et al., 2019). Harmaline had no effect on mutagenicity in *E. coli* B/r WP2 without UV radiation (Shimoi, Kawabata & Tomita, 1992) but amplified UVC-induced mutagenicity without metabolic activation in *E. coli* B/r WP2 (Shimoi, Kawabata & Tomita, 1992).
Harmaline showed no effects in an SOS chromosome test with *E. coli* (Picada et al., 1997) or on chromosome aberrations induced by UV and mitomycin C in CHO K-1 cells in the presence of metabolic activation (Sasaki et al., 1992).

Harmol

In an Ames test with *S. typhimurium* strains TA97, TA98, TA100 and TA102, harmol showed a positive response in *S. typhimurium* strain TA97 without metabolic activation. Harmol showed a negative response in an SOS chromosome test with *E. coli* PQ37 (Picada et al., 1997).

Harmalol

Harmalol showed a negative response in both an SOS chromosome test with *E. coli* PQ37 and in an Ames test with *S. typhimurium* strains TA97, TA98, TA100 and TA102 (Picada et al., 1997).

B. caapi extract

An extract of *B. caapi* (ground, boiled and concentrated) was positive in an Ames test in *Salmonella typhimurium* strain TA98, both with (from 250 µL/plate) and without (from 750 µL/plate) metabolic activation. The extract contained 1,175 µg/mL harmine, 225 µg/mL harmaline and 1,580 µg/mL THH (Kummrow et al., 2019).

DNA intercalation has been suggested as a possible explanation for the mutagenic effect of the β-carbolines mentioned above (Taira et al., 1996).

In vivo

One *in vivo* study involving harmine has been identified (Picada et al., 1997). In a micronucleus test, Swiss Webster mice (5 males and 5 females per group) were exposed to a single intraperitoneal dose of 46 mg/kg BW of harmine (>98% purity) and killed after 24 or 48 hours so the bone marrow could be analysed. According to the authors, the dose was equal to the maximum tolerable intraperitoneal dose based on an exploratory study (data not available). The frequency of polychromatic erythrocytes (PCEs) was investigated in 1,000 erythrocytes. The frequency of PCEs with micronuclei (MNPCEs) was also established for 2,000 PCEs per animal. No bone marrow toxicity was observed for either time period (no reduction in the frequency of PCEs). There was also no observation of any increased frequency of MNPCEs (Picada et al., 1997).

Wistar rats (5 per sex per group) were given a single oral decoction of ayahuasca administered in three strengths, namely 1x, 5x and 15x the usual human dose. The usual human dose was 0.302 mg/kg BW of N,N-dimethyltryptamine (DMT), 3.35 mg/kg BW of harmine and 0.261 mg/kg BW of harmaline. The rats were killed 30 hours after administration and blood samples were taken. Genotoxicity was investigated using flow cytometry, a comet assay and a micronucleus test.

No mortality, clinically observable effects, macroscopic alterations or alterations in relative organ weights (liver, kidney, spleen, brain) were observed. There were also no significant differences in terms of haematological parameters between the treated animals and the control animals. The biochemical parameters only showed differences at the 15x strength dose. A statistically significant increase in urea and creatinine was observed, along with a decrease in the serum concentration of triglycerides.

In the comet assay (single-cell electrophoresis assay), 100 leukocyte nuclei per animal were analysed and automatically classified using software in terms of DNA damage (Class 0 (no damage) – 4 (very damaged)). There were no significant differences in relation to the DNA damage index between the treated animals and the control animals. It should be noted that the exposure and other aspects of this study do not comply with the requirements of the relevant OECD guidelines (OECD, 2016a). The guidelines specify that animals must be exposed for at least two days and that blood samples must be taken 2–6 hours after the last administration.

In the micronucleus test, 2,000 PCEs per animal were examined for the presence of immature MNPCEs. Normochromatic erythrocytes (NCEs) were also counted, and cytotoxicity was investigated using the PCEs:(PCEs+NCEs) ratio. The frequency of MNPCEs was generally low in the treated groups. However, for the 15x strength dose in both males and females, there was a statistically significant increase ($P<0.05$) in MNPCE frequency compared to the negative control group. No cytotoxicity was observed. According to the authors, this assay was performed in accordance with OECD Guideline 474 (1997). However, it should be noted that the performance of the study as a whole did not comply with the requirements of the OECD guideline (OECD, 2016b). If a single dose is administered, at least two bone marrow samples must be taken between 24 and 48 hours after administration; any deviation from these requirements must be

substantiated. In addition, the presence of immature MNPCEs must be scored for at least 4,000 erythrocytes per animal.

The flow cytometry showed a statistically significant increase in the percentage of DNA fragmentation in the bone marrow in female rats compared with the control rats at the 1x strength, but not at higher doses. This finding is not dose-related and is therefore not likely to be related to the exposure.

The authors concluded that ayahuasca is not genotoxic at 1x and 5x strengths, and established that the 5x strength (1.5 mg/kg BW of DMT) is the NOAEL (Melo Jr et al., 2016). However, no NOAEL could be established for genotoxicity, and the authors of this assessment concluded that the micronucleus test showed a positive result for ayahuasca.

Chronic toxicity and carcinogenicity
No studies were found involving *B. caapi*, harmine, harmaline or THH.

Reproductive and developmental toxicity
The available studies on harmine and harmaline are described below, along with a number of studies on ayahuasca. No studies on *B. caapi* alone were found.

Kamel et al. (1971) exposed pregnant white rats to a 2:1 mixture of harmine and harmine hydrochloride in a daily subcutaneous dose of 155 mg/kg BW for five days. The first day of the pregnancy was defined as the day on which the vaginal plug was observed. One group of pregnant rats (n=24) received this mixture on Days 7 to 12 of the pregnancy. The other group (n=24) received the mixture on Days 14 to 19 of the pregnancy. In total, 12 pregnant control animals were given no treatment. Forty-eight hours after the final administration the animals were killed and examined. Of the rats treated from Day 7 to Day 12, 6 rats were not pregnant. Foetal resorption had occurred in 7 rats, a further 7 rats had bleeding in the placenta and foetal remnants, and 4 rats still had living foetuses. Of the rats treated from Day 14 to Day 19, 6 were not pregnant. Foetal resorption had occurred in 3 rats, 8 rats had bleeding in the placenta and foetal remnants, and 7 rats still had living foetuses. Of the total of 12 control animals, 8 were pregnant and 4 were not. According to the authors, the alkaloids probably had an effect on blood flow in the uterus and placenta, as a result of vasoconstriction due to hyperserotonaemia from repeated administration of these alkaloids (Kamel et al., 1971).

Pregnant white mice received daily subcutaneous injections of 0.4 mg harmaline hydrochloride during Days 1–6, 4–7 or 6–11 of the pregnancy (n=13–14 per group). The first day of the pregnancy was defined as the day on which the vaginal plug was observed. The autopsy was performed on Day 14. At the lowest dose, 8 to 9 mice per treatment group had a normal pregnancy, remnants of a placenta and foetuses were visible in 2 mice and 14 mice showed no signs of pregnancy. There was also a group of mice (n=8) that received 0.8 mg of harmaline hydrochloride per day from Day 11 to Day 16. At the time of the autopsy on Day 18, 5 mice had a normal pregnancy and 3 mice showed no signs of pregnancy. There were no significant differences compared with the control group (no details about the control group were given) for either dose (Poulson & Robson, 1963).

Pregnant Sprague-Dawley rats (22 days pregnant; 2–5 rats per group) were exposed to a subcutaneous dose of 10 mg/kg BW of harmaline 2 or 4 hours before the foetuses were removed. The brains of the foetuses were examined for concentrations of serotonin and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA). Serotonin levels increased to 143% of the levels in the control animals 2 hours after injection and remained around that level (~145%) 4 hours after injection ($P<0.005$). Brain concentrations of 5-HIAA decreased to 65% of the concentrations in the control animals 2 hours after injection

(P<0.1), and remained at that level 4 hours after injection (P<0.05). Harmaline also had an effect on dopamine (145–158% of the control; P<0.01 – P<0.001), the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC; ~83%; P<0.1) and the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG; 136–169% of the control; P<0.10 – P<0.005) in the foetal brains. The authors stated that the results were consistent with MAO inhibition (Okonmah et al., 1988).

Oral administration of a decoction of ayahuasca (with *B. caapi*) in various strengths (0, 1x, 2x, 4x and 8x the usual human dose for a 70 kg person) on alternate days for 70 days in Wistar rats (n=12 per group) showed a statistically significant increase in testosterone levels and a statistically significant decrease in the number of sperm cells and in the sperm transit time (measure of the duration of maturation of sperm cells in the epididymis; a lower sperm transit time can have an effect on fertility) at the 4x strength dose. There was also a statistically significant lower feed intake, lower body weight and lower total weight gain at the end of the study in the 4x and 8x groups. The authors ascribed the increase in testosterone to DMT. Based on an analysis of the ayahuasca decoction, the dose that was 1x the usual human dose contained 3.3 mg/kg BW of harmine, 0.26 mg/kg BW of harmaline and 0.31 mg/kg BW of DMT. THH was not measured in this study. The authors inferred a NOAEL of 2x the usual human dose (Santos et al., 2017).

Groups of Wistar rats received an ayahuasca extract on Days 6–20 of pregnancy, administered through a stomach tube in doses of 0, 1x, 5x and 10x the usual human dose (Oliveira et al., 2010). The extract contained 0.42 mg/mL of DMT, 1.37 mg/mL of harmine, 0.62 mg/mL of harmaline and 0.35 mg/mL of THH. The rats were killed on Day 20 and the mothers and foetuses were examined. At the highest dose, maternal toxicity was observed (a statistically significant lower feed intake and lower increase in body weight, and a higher relative liver weight). There was a dose-dependent reduction in foetal body weight, and the reduction was statistically significant in the highest dose group (3.45 g versus 3.83 g in the control group). Abnormalities were observed in the organs and skeletons of the pups, in both the control group and the exposed groups. There was a dose-related increase in the incidence of dilated lateral ventricles and third ventricle in the brains. The authors considered this a malformation because both severity and incidence increased with the dose. There was also a dose-related increase in the incidence of dilated renal pelvis. The authors did not consider this a malformation, but rather a variation, since it also occurred in the control animals, and would probably not have had a harmful effect on the health of the animals. An examination of the skeletons showed a significantly increased percentage of foetuses with incomplete ossification of the hyoid bone, incomplete ossification of the nasal bone, asymmetrically-shaped sternebrae (the bones that form the sternum) and an extra cervical rib. This study showed that ayahuasca extract can lead to maternal toxicity and embryo toxicity and teratogenicity in rats (Oliveira et al., 2010).

Groups of Wistar rats received an ayahuasca extract on Days 6–20 of pregnancy, administered through a stomach tube in doses of 8x the average usual human dose (adjusted for body weight) (Da Motta et al., 2018). The extract contained 0.141 mg/mL of DMT, 1.56 mg/mL of harmine and 0.122 mg/mL of harmaline. The extract was not analysed for THH. The 1x dose corresponded to 343 mg dry matter from ayahuasca/kg BW/day, 0.30 mg/kg BW/day of DMT, 3.34 mg/kg BW/day of harmine and 0.26 mg/kg BW/day of harmaline. On Day 21 of pregnancy the animals were killed and the mothers and pups were examined. Among the animals exposed to the 4x and 8x strength doses some of the mother animals died (44% and 52%, respectively), while the remaining mother animals in these groups exhibited kidney damage. Dead foetuses were found in 2/14 of the animals still alive on Day 21 in the 4x strength group. In the 8x strength group, this was the case for 3/12 animals (these animals had lost their entire litter). By way of comparison, in the control group (n=25) there was one animal with several dead

foetuses. Effects (a decreased number of living neurons in the hippocampus) were also visible in the brains of the rats exposed to 2x, 4x and 8x strength doses. Reprotoxic effects were also seen at non-lethal doses. At the 2x strength dose there was a statistically significant increase in early resorptions and a statistically significant decrease in litter size. At non-lethal doses, abnormalities were observed in the soft tissues and skeletons of the pups. The authors inferred a NOAEL for maternal toxicity of 1x the average usual human dose. This is because a decrease in the number of living neurons in the hippocampus was observed in the mother animals at the 2x strength dose. At the 1x strength dose only a small increase occurred in skeletal and soft tissue variations, so the authors deemed this dose to be the NOAEL for developmental toxicity as well (Da Motta et al., 2018). The authors of this FO assessment consider this dose to be the LOAEL, since the increase in skeletal and tissue variations was statistically significant and of a comparable magnitude to the increase seen at the higher doses.

Neurotoxicity

A single intravenous exposure to ≥5 mg/kg BW of harmine in mice and intraperitoneal exposure to ≥2 mg/kg BW in sheep led to neurotoxic effects, such as tremors and paralysis (Chen et al., 2004; Yang et al., 2021; Bourke et al., 1990).

A single intraperitoneal exposure to ≥1 mg/kg BW of harmaline in mice and rats led to neurotoxic effects such as a reduced ability to learn, increased memory loss and tremors (studies include Welsh, 1988; Lutes et al., 1988; O'Hearn & Oliver, 1993; Stanford & Fowler, 1998; Morcuende et al., 2001; Wang & Fowler, 2001; Miwa et al., 2006; Handforth, 2012; Moura et al., 2016; Nasehi et al., 2016, Nasehi et al., 2017). The inferior olivary nucleus is involved in the occurrence of these effects (studies include Welsh, 1988; Lutes et al., 1988; O'Hearn & Oliver, 1993; Stanford & Fowler, 1998).

In a study by Stocco et al. (2020), FVB/N wild-type mice and genetically-modified mice with human CYP2D6 received a single intraperitoneal dose of 5.0, 7.5 and 10 mg/kg BW of harmine. Each dose group consisted of 6 to 8 wild-type mice and 6 to 8 genetically-modified mice. Body temperature was measured 7 times within 90 minutes of exposure. The mice were also examined to see if tremors were occurring, 7 times within 80 minutes of exposure. For both types of mice, the reduction in body temperature was significantly greater with increasing doses of harmine ($p<0.0001$). An increase in tremors was also observed with increasing doses for the wild-type mice ($p<0.001$) and the mice with human CYP2D6 ($p<0.05$). Intracerebroventricular administration of the CYP2D6 inhibitor propanolol 4 hours before exposure to harmine led to an increase in harmine-induced hypothermia and tremors in the genetically-modified mice and an increase in hypothermia in the wild-type mice. The administration of propanolol 24 hours before exposure only led to an increase in hypothermia in the genetically-modified mice. The authors said this suggests that the effectiveness of human CYP2D6 influences neurotoxicity, and that interindividual differences in CYP2D6 expression could contribute to the variability in susceptibility to neurotoxicity from harmine.

Human data

Only a small number of older publications are available in which the effects of harmine, harmaline and THH in humans following oral administration are investigated and/or reported.

Following the ingestion by humans of 20–50 mg of harmine (approximately 0.3–0.7 mg/kg BW) psychotropic effects occur after 20–30 minutes, with a peak around 30 minutes to 1 hour. The effects last for 6–8 hours. Harmine caused pupil dilation, salivation, lacrimation, hyperthermia, hyperglycaemia, low blood pressure and other, unspecified effects. Harmine has also been said to be capable of causing aggressive

behaviour and psychedelic and emotional effects in humans (Naranjo, 1959, cited in Brito-da-costa et al., 2020 and Simao et al, 2019). Although not explicitly described, this probably occurred after ingestion of a single dose.

For harmaline, Naranjo (1967) reported that an oral dose > 4 mg/kg BW or an intravenous dose > 1 mg/kg BW caused psychedelic effects and hallucinations. The author stated that the effects of harmaline are twice as strong as those of harmine, and it is possible, based on very limited observations, that they are also three times as strong as THH. A single volunteer who orally ingested 300 mg (corresponding to 4.3 mg/kg BW for a 70 kg person) of THH indicated that this concentration induced the same subjective effect as the oral ingestion of 100 mg of harmaline. The effects of harmaline began to arise approximately one hour after oral ingestion, and are almost instantaneous after intravenous administration. Subjective effects that have been reported included nausea and vomiting, dizziness, paresthesia (disruption of sense perception), pressure in the head, chest discomfort and various types of visions. These subjective effects are based on the responses of 30 volunteers who received an oral or intravenous dose of harmaline hydrochloride under standard conditions. No further details of this study were described (Naranjo 1967).

Hofmann (1963) reported that oral doses of 300–400 mg harmine resulted in psychological symptoms with autonomic effects such as nausea and ataxia (impaired muscle coordination due to the effect on the brain). Another part of the document discusses an oral active dose of 100–400 mg for harmine/harmaline.

Pennes and Hoch (1957) stated that no hallucinogenic effects occurred in mental patients following oral doses of harmine of less than 960 mg. However, other symptoms were found at doses of 300–400 mg (Pennes and Hoch, 1957).

De Smet (1985) exposed himself twice to 0.5 mg/kg BW of harmine, both orally (dissolved in water) and nasally. No psychoactive or physical effects were noted from either route. Nor could harmine be detected in plasma 15–240 minutes after exposure via either route.

Several cases of poisoning with β-carboline alkaloids are described in the literature. Some of these cases are briefly described below. This is not an exhaustive summary.

The review article (Brito-da-costa et al., 2020) states that the first symptoms following ingestion of an overdose of harmaline are nausea and vomiting, followed by alterations in mental state and other neurological effects. Effects that occur at higher doses (not specified) are tremors, convulsions, respiratory paralysis, hypothermia, suppression of the central nervous system, impaired vision, delirium, impaired coordination, paralysis and sometimes hallucinations (Brito-da-costa et al. 2020). There is also evidence that harmine can have psychological and behaviour-altering effects (Brierley and Davidson 2012).

The effects following oral ingestion of various doses of *B. caapi*, ranging from 25 to 60 drops each time, by 6 individuals, are described in a very brief article (Cardenas, 1923). The reported effects included (visual) hallucinations, drowsiness, anxiety, headaches, tightness of the chest, stomach cramps and tinnitus. Not all individuals reported experiencing these side effects at equivalent doses.

Cases of poisoning with β-carboline alkaloids are related to oral ingestion of *P. harmala*. The effects that occurred in these poisoning cases included vomiting, blurred vision, loss of consciousness, drowsiness, visual hallucinations, tremors, ataxia, bradycardia,

agitation, impaired coordination, tinnitus, and liver and renal failure. Admission to hospital was necessary in all cases (Brush, Bird & Boyer, 2004; Frison et al., 2008; Moshiri et al., 2013; Berdai et al., 2014; Mohammadi et al., 2016; Von Fabeck, de Haro & Simon, 2020). The ingested dose was not always known. A number of cases involved a home-made decoction of *P. harmala* seeds dissolved in water. Other cases concerned ingestion of the seeds themselves, in 50 g doses (Moshiri et al., 2013; Mohammadi et al., 2016).

Interactions
For information about possible interactions, Natural Medicines and the *Farmacotherapeutisch Kompas* were consulted.

From *in vitro* studies and animal testing, it is known that harmine and harmaline are strong inhibitors of the MAO-A enzyme (and weak inhibitors of MAO-B). Accordingly, interactions could occur with substances that are converted by MAO. One known example is tyramine (Natural Medicines, 2021b). Tyramine is a sympathomimetic agent (it stimulates the action of the sympathetic nervous system) and is found in protein-rich foods. The quantity of tyramine can increase when products mature over a long period of time (such as mature cheese). When MAO-A is inhibited, it can result in higher concentrations of tyramine in the blood, which in turn results in the release of adrenaline or noradrenaline. Both of these substances induce an increase in blood pressure, which could lead to a hypertensive crisis. The symptoms of a hypertensive crisis are an acute, throbbing headache (at the back of the head), tachycardia, flushes, stiff neck, nausea, vomiting and photophobia. It can also lead to intracranial haemorrhaging, which can be fatal (*Farmacotherapeutisch Kompas*, 2021a).
The inhibition of MAO-A results in increased concentrations in the brain of monoamine neurotransmitters, such as serotonin, noradrenaline and dopamine. An interaction could therefore also occur with other substances that increase monoamines, via a different mechanism of action.
Examples include interactions with medicines such as selective serotonin reuptake inhibitors (SSRIs, such as paroxetine and sertraline) and noradrenaline-dopamine reuptake inhibitors (NDRIs, such as methylphenidate). Other examples include nutritional supplements and herbal preparations that increase serotonin levels, such as *Argyreia nervosa*, L-tryptophan and St John's wort. Potential effects include serotonin syndrome and a hypertensive crisis (*Farmacotherapeutisch Kompas*, 2021a, b; Natural Medicines, 2021b).

Substances metabolised by the same CYP450 enzyme could also cause interaction if competition for conversion arises. This would result in increased plasma concentrations of the β-carboline alkaloids and/or of the substance with which they are competing. Furthermore, *in vitro* studies suggest that harmine and harmaline have an inhibitory effect on the enzymes CYP2D6 and CYP3A4. This could theoretically result in a decrease in the conversion of other substances by CYP2D6 and CYP3A4, leading to an increase in plasma levels of these substances (Natural Medicines, 2021b).

Animal testing suggests that, following intravenous administration, harmine strengthens the effect of acetylcholine or increases acetylcholine levels. Theoretically, combined use with anticholinergic substances could reduce the effectiveness of one of the substances. On the other hand, the combined use of cholinergic substances could lead to an additive effect and an increased likelihood of cholinergic side effects (Natural Medicines, 2021b).

In laboratory animals, an increase in the intensity and duration of harmaline-induced tremors was observed when caffeine was also administered (Natural Medicines, 2021b).

Liver toxicity was observed following ingestion of high doses of β-carboline alkaloids. Theoretically, use in combination with other hepatotoxic substances (either medicines or supplements) could increase the likelihood of liver damage (Natural Medicines, 2021b).

Assessment

Shambala and the similar herbal preparation contain the β-carboline alkaloids harmine, harmaline and THH. A fourth, possibly active substance was also detected in these products, but this substance has not yet been identified. The possible effects of this fourth substance are not included in this assessment.

Following ingestion via the mouth, the β-carboline alkaloids are well absorbed, rapidly converted by the body into harmol and harmalol, among other substances, and well distributed across the various tissues and organs. The substances do not accumulate in the body. Harmaline and harmalol can pass the blood-brain barrier, and, based on the results of reproductive toxicity studies, harmaline can also pass through the placenta. This has not been investigated for harmine.

The β-carboline alkaloids can affect the concentrations of neurotransmitters (such as serotonin, dopamine and noradrenaline) in the central nervous system. By doing so, they can cause behavioural, psychological and physiological effects. They do this by influencing the conversion of these neurotransmitters or through direct interaction with specific receptors. Harmine and harmaline are reversible, selective inhibitors of the enzyme MAO-A. THH inhibits serotonin reuptake and has little or no effect on MAO-A.

The inhibition of MAO-A and the serotonin reuptake result in increased concentrations in the brain of monoamine neurotransmitters, such as serotonin, noradrenaline and dopamine. Accordingly, in combination with other substances that increase the concentrations of monoamines, Shambala and the similar herbal preparation could potentially result in harmful interactions. These substances include medicines, herbal preparations and other products that contain MAO inhibitors, SSRIs or NDRIs.

Several animal studies are available that investigate these β-carboline alkaloids, but only a small number of oral studies. These animal studies were not conducted in accordance with the OECD testing guidelines.

Oral LD_{50} values of 118.9 mg/kg BW for harmaline and 250.3 mg/kg BW for harmine were reported in mice.

A range of effects arose from short-term, repeated exposure. In a 28-day study involving a total alkaloid extract of *P. harmala* seeds, a NOAEL of 45 mg/kg BW was established, corresponding to 13.8 mg/kg BW of harmaline and 12.4 mg/kg BW of harmine. At higher doses tremors occurred, along with effects on several biochemical parameters and an increase in relative liver and testes weights. These effects were reversible after a 4-week recovery period. In a limited 3-month study involving harmine hydrochloride, a NOAEL of 4.2 mg/kg BW per day was found for harmine. At higher doses histopathological alterations were observed in the heart, spleen and adrenal glands, along with a significant increase in plasma concentrations of the liver enzyme AST.

Harmine, harmaline and a *B. caapi* extract produced positive results in a number of Ames tests (bacterial reverse mutation assays). Positive results were also obtained with harmine in chromosome aberration tests and a comet assay (single-cell electrophoresis assay) in mammal cells. Variable results were observed in other genotoxicity tests in yeasts and bacteria. *In vitro* indications of genotoxicity were also discovered for harmol, but not for harmalol. One possible explanation for the mutagenic effect is DNA

intercalation, which has been demonstrated *in vitro*. No indications of genotoxicity were found in an *in vivo* micronucleus test involving harmine. An ayahuasca extract produced positive results in an *in vivo* micronucleus test and negative results in a comet assay and a flow cytometry assay. Because the test was conducted on an ayahuasca extract, the effects cannot unequivocally be ascribed to a single substance. In summary, the available studies indicate that β-carboline alkaloids could be genotoxic. In the absence of properly-conducted, preferably *in vivo* studies and clear results, no definitive conclusion can be drawn about the genotoxicity of harmine and harmaline.

In reproductive toxicity studies involving harmine and harmaline, in which pregnant rats were subcutaneously exposed to 155 mg/kg BW of a 2:1 mixture of harmine and harmaline, harmful effects on reproduction were observed, whereas this was not the case for pregnant rats that were subcutaneously exposed to 0.4 mg harmaline hydrochloride. It should be noted that this route of exposure is not relevant for the use of Shambala or the similar herbal preparation. In three oral reproductive toxicity studies involving ayahuasca extract, effects were observed in the parents and/or pups. Because these studies were conducted with ayahuasca extract, it is not known whether these effects can be ascribed to the β-carboline alkaloids, and thus whether they are relevant to the use of Shambala or the similar herbal preparation. Nor can any definitive conclusion be drawn on the possible effects of β-carboline alkaloids on reproduction and offspring.

There are a number of old (<1985) publications describing studies into the effects of harmine, harmaline and THH in humans. These studies state that harmine and/or harmaline induce effects following single oral doses of approximately 0.3 mg/kg BW. The effects mentioned for this dose of harmine are pupil dilation, salivation, lacrimation, hyperthermia, hyperglycaemia and low blood pressure. It is indicated that the effects of harmaline are twice as strong as those of harmine, and it is possible, based on very limited observations, that they are three times as strong as THH. It is also reported that oral doses of harmaline of 4 mg/kg BW and above lead to psychedelic effects and hallucinations. It should be noted that the study design and descriptions are extremely limited.
Various cases of poisoning are also described in the literature. The first symptoms following ingestion of an overdose of harmaline are nausea and vomiting, followed by alterations in mental state and other neurological effects. More serious symptoms such as hallucinations, impaired coordination, impaired vision and paralysis can also occur. There is also evidence that harmine can have psychological and behaviour-altering effects.

For the risk assessment for acute exposure we used human data, and assumed an effect dose of 0.3 mg/kg BW for the sum of harmine and harmaline. Based on the measured levels, the total exposure to harmine and harmaline from a single daily dose of Shambala is 0.049 mg/kg BW; from a single daily dose of the similar herbal preparation, it is 0.038 mg/kg BW. The margin of exposure in relation to the effect dose of 0.3 mg/kg BW in humans is a factor of 6–8. The margin of exposure should be at least 30 to take account of the uncertainties involved in using a LOAEL instead of a NOAEL (factor 3) as well as interindividual differences (factor 10). In addition, THH could contribute to the effects from the use of Shambala and the similar herbal preparation. Accordingly, harmful effects could occur from a single use of Shambala or the similar herbal preparation, due to the effects of the β-carboline alkaloids on the central nervous system.

No human data is available on the effects of repeated exposure to β-carboline alkaloids. To give some indication, exposure to harmine and harmaline from Shambala (0.049 mg/kg BW/day) and the similar herbal preparation (0.038 mg/kg BW) (Table 5) was compared with the lowest NOAEL from oral, short-term animal studies involving harmine and/or harmaline (4.2 mg/kg BW per day for harmine). The margin of exposure was a factor of 86–110. This is around the factor of 100 that is necessary to take account of

inter-species (factor 10) and interindividual (factor 10) differences. As well as the effects on the central nervous system that can occur from a single use, additional effects on other organs with repeated use can therefore not be entirely excluded.

It should be noted that no studies have been conducted involving Shambala or the similar herbal preparation, and that very little is known about *C. angustifolia*. It is possible, therefore, that the extracts in these products contain other substances that might be toxic. In the analysis, a fourth, potentially active ingredient was detected. This substance has not yet been identified and its possible effects were therefore not included in this assessment.

Conclusion

Yes, the use of Shambala and a similar herbal preparation with comparable ingredients poses a risk to public health. These herbal preparations contain the β-carboline alkaloids harmine, harmaline and tetrahydroharmine. Harmful effects can occur from a single use of these herbal preparations according to the instructions on the packaging, due to the effects of these substances on the central nervous system. These effects can include pupil dilation, salivation and low blood pressure. Exposure to higher doses of β-carboline alkaloids can cause nausea and vomiting, as well as more serious effects such as hallucinations, impaired coordination, impaired vision and paralysis. In addition, taking these products in combination with medicines, other herbal preparations or products containing MAO-inhibitors, SSRIs or NDRIs could lead to harmful interactions. With repeated use, additional effects on other organs cannot be ruled out. There are also indications of the possible genotoxicity of harmine and harmaline, but there is a lack of suitable data enabling a definitive conclusion to be drawn on this point. Finally, there is too little data to draw a conclusion about possible reproductive and developmental toxicity following exposure to β-carboline alkaloids.

These herbal preparations may also contain other toxic substances. In the analyses, a fourth, potentially active ingredient was detected. This substance has not yet been identified and its possible effects were not included in this assessment.

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