

Effects of the Natural β -Carboline Alkaloid Harmine, a Main Constituent of Ayahuasca, in Memory and in the Hippocampus: A Systematic Literature Review of Preclinical Studies

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ABSTRACT

Harmine is a natural β -carboline alkaloid found in several botanical species, such as the *Banisteriopsis caapi* vine used in the preparation of the hallucinogenic beverage ayahuasca and the seeds of Syrian rue (*Peganum harmala*). Preclinical studies suggest that harmine may have neuroprotective and cognitive-enhancing effects, and retrospective/observational investigations of the mental health of long-term ayahuasca users suggest that prolonged use of this harmine-rich hallucinogen is associated with better neuropsychological functioning. Thus, in order to better investigate these possibilities, we performed a systematic literature review of preclinical studies analyzing the effects of harmine on hippocampal neurons and in memory-related behavioral tasks in animal models. We found two studies involving hippocampal cell cultures and nine studies using animal models. Harmine administration was associated with neuroprotective effects such as reduced excitotoxicity, inflammation, and oxidative stress, and increased brain-derived neurotrophic factor (BDNF) levels. Harmine also improved memory/learning in several animal models. These effects seem to be mediated by monoamine oxidase or acetylcholinesterase inhibition, upregulation of glutamate transporters, decreases in reactive oxygen species, increases in neurotrophic factors, and anti-inflammatory effects. The neuroprotective and cognitive-enhancing effects of harmine should be further investigated in both preclinical and human studies.

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The natural β -carboline alkaloid harmine (and the related compounds tetrahydroharmine (THH) and harmaline) is found in several plants around the world (Moloudizargari et al. 2013; Ott 1994; Patel et al. 2012), but is more widely known as the main constituent of the stalks of the *Banisteriopsis caapi* vine, the principal ingredient—together with the dimethyltryptamine (DMT)-rich leaves of *Psychotria viridis* or *Diplopterys cabrerana*—in the preparation of the Amazonian botanical hallucinogen ayahuasca (McKenna and Riba 2015; Ott 1994; Schultes 1986; Schultes and Hofmann 1992).

Harmine (and related alkaloids) is also found in the seeds and roots of Syrian rue (*Peganum harmala*), a medicinal plant used in North Africa, the Middle East, and Central Asia (Moloudizargari et al. 2013; Patel et al. 2012). In fact, harmine and harmaline were first isolated from *P. harmala* seeds and roots in the 1840s (Moloudizargari et al. 2013; Ott 1994; Patel et al. 2012). Traditional uses of *P. harmala* suggest that the plant

(especially the seeds) has antihypertensive, antispasmodic, emetic, antitumor, expectorant, antimicrobial, antipyretic, antidiabetic, antiinflammatory, antiparkinson, analgesic, anxiolytic, and antidepressive properties, among others (Moloudizargari et al. 2013; Patel et al. 2012).

In the last few decades, *P. harmala* seeds have also been recognized as a main alternative source of harmine/ β -carbolines used in the preparation of ayahuasca analogues (Ott 1994).

Pharmacology and therapeutic potentials

Many of the previously mentioned therapeutic effects of *P. harmala* seem to be produced by harmine, which has vasorelaxant, antiinflammatory, analgesic, antimicrobial, antioxidative, antiparkinson, antitumor, antiaddictive, and antidepressive properties (Dos Santos et al. 2016a; Moloudizargari et al. 2013; Nunes et al. 2016; Patel et al. 2012). The pharmacological mechanisms involved in these

effects are not well-understood, and seem to involve several potential molecular targets such as monoamine oxidase (MAO), benzodiazepine/gamma-aminobutyric acid type A (GABA_A) receptors, serotonin 5-HT_{2A/C} receptors, glutamate transporter 1 (GLT-1), imidazoline I_{1/2} receptors, reactive oxygen species (ROS), and neurotrophic factors, among others (Dos Santos et al. 2016a; Glennon et al. 2000; Grella et al. 1998; Moloudizargari et al. 2013; Nunes et al. 2016; Patel et al. 2012).

Interestingly, in the late 1920s and early 1930s, harmine was used to treat symptoms of Parkinson's disease, such as muscular rigidity, depression, memory deficits, apathy, phobias, fatigue, and attention problems (Costa and Faria 1936; Sanchez-Ramos 1991). More recently, the potential therapeutic use of *B. caapi* extract in Parkinson's disease has been described in preclinical studies (Samoylenko et al. 2010; Schwarz et al. 2003), and a double-blind, randomized, placebo-controlled trial reported that *B. caapi* extracts improved motor function in Parkinson's patients (Serrano-Dueñas, Cardozo-Pelaez, and Sánchez-Ramos 2001). However, *B. caapi* has other constituents, including other β -carbolines and different compounds, so the role of harmine should be better investigated.

Regarding other potential therapeutic effects in the central nervous system (CNS), recent preclinical studies showed that harmine induces antidepressive effects (Dos Santos et al. 2016a), and an open-label study reported that a single dose of the harmine-rich botanical hallucinogen ayahuasca was associated with fast-acting and enduring antidepressive effects in patients with treatment-resistant major depressive disorder (Osório et al. 2015; Sanches et al. 2016). Moreover, preclinical and preliminary studies in humans suggest that ayahuasca also has antiaddictive potentials (Dos Santos et al. 2016b; Nunes et al. 2016). However, it is not clear if the antidepressive and antiaddictive properties of ayahuasca are related to the pharmacological properties of harmine (and related β -carbolines such as THH and harmaline) or DMT, or a combination of them (Dos Santos et al. 2016a, 2016b). More basic and clinical studies are needed to elucidate the neurochemical mechanisms underlying these effects.

Possible neuroprotective effects: Preclinical evidence

Other therapeutic potentials of harmine in the CNS include its neuroprotective effects. Lee et al. (2000) showed that harmine attenuated brain damage in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the 1-methyl-4-phenylpyridinium (MPP⁺)-induced mitochondrial damage, and also

inhibited dopamine-induced mitochondrial damage and PC12 cell death. Kim et al. (2001) reported that harmine attenuated dopamine- and 6-hydroxydopamine-induced brain mitochondrial and synaptosomal damage and PC12 cell death. The effects of harmine in these studies are apparently mediated by a scavenging action on ROS, MAO inhibition, and thiol oxidation. More recently, Li et al. (2011) showed that harmine increased glutamate transporter (GLT-1 and GLAST) protein expression and glutamate uptake activity in astroglial cells and in a mice model of amyotrophic lateral sclerosis, and Sun et al. (2014) reported that harmine reduced cerebral infarct volume, neuron death, and astrocyte activation, and increased GLT-1 expression in a rat model of global cerebral ischemia.

Regarding administration of harmine-rich plants, Biradar, Joshi, and Tarak (2013) showed that administration of *P. harmala* seed extract to mice reduced sodium nitrite- and ethanol-induced memory impairment, and these effects were associated with acetylcholinesterase (AChE) inhibition, increases in glutathione levels, and decreases in the formation of thiobarbituric acid reactive species (TBARS) in the brain, as well as reduced frontotemporal cortex DNA fragmentation. In a recent study, Favaro et al. (2015) reported that long-term (30 days) administration of ayahuasca to rats did not produce significant effects, neither in anxiety-like behavior nor in spatial memory, but improved emotional learning/fear conditioning. Both spatial and emotional memory are hippocampal-dependent processes, suggesting that this brain region could be involved in the effects of ayahuasca on learning. However, since both *P. harmala* seeds and ayahuasca have other β -carbolines and also different compounds (such as DMT in ayahuasca) in their composition, it is not clear if the observed effects are mediated by harmine, other substances, or the combination of them.

Observations from human studies

It is also not clear if the preclinical evidence of neuroprotective potentials of harmine can be translated to humans. Acute ayahuasca intake is associated with deficits in working memory and improved inhibitory control in both occasional and long-term users (Bouso et al. 2013). Regarding strategic planning, deficits were observed only among occasional users, and the performance of the volunteers in this cognitive domain was inversely correlated with lifetime ayahuasca use. However, observational studies suggest that long-term adult users of ayahuasca show improved learning/memory and executive functions when compared to non-using control groups (Bouso et al. 2015; Bouso et al. 2012; Grob et al. 1996),

suggesting that prolonged ingestion of this harmine-rich preparation could improve some cognitive functions. Regarding adolescent ayahuasca users, their neuropsychological functioning was overall similar to a non-using control group, with lower scores appearing only in a memory test (Doering-Silveira et al. 2005).

Study objective

Considering (1) the potential neuroprotective effects of harmine as reported in preclinical studies; (2) the possible cognitive-enhancing effects of ayahuasca in some cognitive domains, as reported in experimental and observational studies; and (3) the anecdotal reports of improved memory/learning among ayahuasca users, expressed in a remarkable ability of remembering plant combinations, ritual elements, myths and stories, and in music composition and execution (Goulart 2011; Labate and Pacheco 2010; Labate, Rose, and Dos Santos 2009; Luna 2011), we conducted a systematic literature review of preclinical studies assessing the effects of harmine on memory/learning and in the hippocampus, a brain region associated with these cognitive functions.

Material and methods

The data for this systematic review were obtained according to the systematic reviews and meta-analysis guidelines from the PRISMA group (Moher et al. 2010).

Data acquisition

We attempted to identify all preclinical studies available for review until 29 July 2016 in which the effects of harmine on memory and in the hippocampus were analyzed.

Search strategy

Electronic searches were performed using the PubMed (1 January 1966–29 July 2016), LILACS (1 January 1982–29 July 2016) and SciELO (1 January 1998–29 July 2016) database. The following key words were used: *harmine* AND *memory* OR *learning* OR *hippocampus*. References were retrieved by searching the aforementioned electronic databases and handsearching of reference lists of the identified literature. All studies published in English up to 29 July 2016 were included.

Eligibility criteria

The following inclusion and exclusion criteria were established prior to the literature search:

Article type

Only complete articles reporting original investigations published in peer-reviewed scientific journals were included. Reviews, qualitative studies, case reports, books and book chapters, abstracts, letters, conference abstracts, comments, and editorials were excluded.

Study design

The review included (1) studies in hippocampal cultured cells assessing the effects of harmine; and (2) animal models analyzing the effects of this alkaloid in memory/learning.

Participants/sample

Hippocampal cells and rodents (rat or mouse).

Interventions

All studies evaluating the effects of harmine in memory/learning and in the hippocampus were included.

Comparisons

The main comparators considered were saline and several psychoactive drugs (ethanol, piracetam, imipramine, scopolamine, donepezil).

Outcomes. Studies investigating the effects of harmine on biochemical (hippocampal cell) and memory/learning behavioral (animal studies) parameters were included.

Data extraction

All studies were screened independently by the two authors, with discrepancies resolved by mutual consensus. Names of authors, year of publication, study type (cell culture or animal model), type of intervention (acute or chronic treatment), and type of outcome measure/response criteria (biochemical or behavioral parameters) were recorded for all included articles. The sample was divided into acute or chronic studies in (1) cell culture or (2) animal models for the sake of clarity and to facilitate interpretation of results.

Results

Study selection

A flow diagram illustrating the different phases of the systematic review is presented in Figure 1.

The literature search yielded 59 separate references that were reviewed for abstract screening. Following this first pass, 10 potentially relevant references were identified (Abelaira et al. 2013; Fortunato et al. 2010a, 2010b, 2009; Göckler et al. 2009; He et al. 2015; Mennenga et al. 2015; Moura et al. 2006; Réus et al. 2010; Zhong, Tao, and

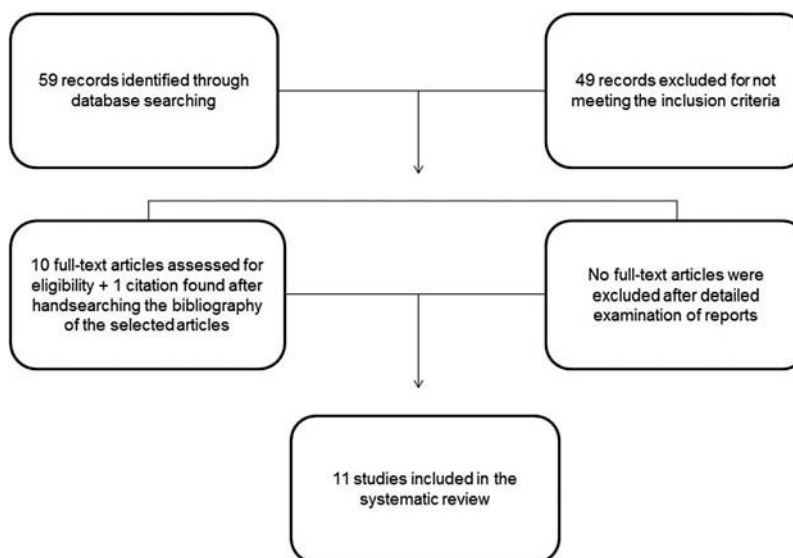


Figure 1. Flow diagram illustrating the different phases of the systematic review.

Yang 2015). An additional citation was added after handsearching the bibliography of the selected citations (Maher and Davis 1996). Full-text reports of these 11 citations were obtained for a more detailed evaluation. Following detailed examination of the reports, all 11 citations were included. Selected publications comprised two studies using hippocampal cultured cells (Göckler et al. 2009; Maher and Davis 1996) and nine studies using animal/rodent models (Abelaira et al. 2013; Fortunato et al. 2010a, 2010b, 2009; He et al. 2015; Mennenga et al. 2015; Moura et al. 2006; Réus et al. 2010; Zhong, Tao, and Yang 2015). The main information of the studies included in the review is presented in Table 1.

Studies in cultured cells

In a study assessing glutamate toxicity in mouse hippocampal cells and in cultures of rat cortical neurons, Maher and Davis (1996) reported that incubation of hippocampal cells for 8 h with harmine (50 μ M) in the presence of glutamate (5 mM) significantly ($P < 0.0001$) reduced glutamate-induced cell death 24 h later compared to the untreated cells. Moreover, cortical neurons exposed to glutamate (5 mM) and harmine (100 μ M) for 24 h were also significantly ($P < 0.0001$) protected from glutamate toxicity.

Göckler et al. (2009) reported that harmine (1.6 μ M for three days) significantly ($P = 0.021$) decreased the number of neurites in culture mouse hippocampal neurons, and this effect was mediated by the inhibitory effect of harmine in the DYRK1A, a protein implicated in normal neuronal development and that is apparently involved in abnormal brain development and memory/

learning deficits in Alzheimer's and Parkinson's disease and in Down syndrome.

Animal studies

Studies of acute administration

Moura et al. (2006) reported that intra-peritoneal (i.p.) administration of harmine (1, 2.5, and 5 mg/kg) to mice in an object recognition task was associated with significant ($P < 0.001$ for all doses) increases in the time to explore a novel object in the short-term memory test compared to the control group, suggesting enhancement of short-term memory. Only harmaline administration was associated with improved long-term memory.

Fortunato et al. (2009) reported that administration of harmine (10 and 15 mg/kg, i.p.) and of the antidepressant imipramine (20 and 30 mg/kg, i.p.) to rats was associated with significant ($P < 0.05$) reductions in immobility time and increases in climbing and swimming time during the forced swim test (FST) compared to the control group. These results suggest antidepressant effects. Neither drug affected locomotor activity as measured by the open-field test (OFT). Moreover, only the higher dose of harmine (15 mg/kg) significantly ($P < 0.05$) increased brain-derived neurotrophic factor (BDNF) levels in the rat hippocampus.

Réus et al. (2010) reported that administration of harmine (5, 10, and 15 mg/kg, i.p.) and imipramine (20 and 30 mg/kg, i.p.) to rats significantly ($P < 0.05$) decreased lipid peroxidation/TBARS formation in the prefrontal cortex and hippocampus compared to the control group. Imipramine (all doses) and harmine (5 and 15 mg/kg in prefrontal cortex, 15 mg/kg in

Table 1. Studies describing the effects of harmine on memory/learning and in the hippocampus.

References	Species	Measures	Treatment	Main results
Maier & Davis 1996	Mouse hippocampal cells	Glutamate-induced cell death	50 μ M, cell culture	Significantly ($P < 0.0001$) reduced glutamate-induced cell death
Moura et al. 2006	Mouse	Object recognition task (STM, LTM)	1, 2.5, and 5 mg/kg, i.p.	Significant ($P < 0.001$ for all doses) improvement of STM
Fortunato et al. 2009	Rat	Hippocampal BDNF levels	15 mg/kg, i.p.	Significant ($P < 0.05$) increases in BDNF levels
Göckler et al. 2009	Mouse hippocampal cells	Neurogenesis	1.6 μ M, cell culture	Significant ($P = 0.021$) decreases in the number of neurites
Fortunato et al. 2010a	Rat	Hippocampal BDNF levels	15 mg/kg/day for 7 days, i.p.	Reverted increases in BDNF levels induced by the CMS model
Fortunato et al. 2010b	Rat	Hippocampal BDNF levels	10 and 15 mg/kg/day for 14 days, i.p.	Significant ($P < 0.05$) increases in BDNF levels
Réus et al. 2010	Rat	Lipid peroxidation, protein carbonylation, catalase and SOD activity	5, 10, and 15 mg/kg, i.p. (single dose and daily for 14 days)	Significant ($P < 0.05$) reductions in hippocampal lipid peroxidation, protein carbonylation, and increases in catalase and SOD activity after acute and chronic treatment
Abelaira et al. 2013	Rat	Habituation (OFT), hippocampal CSA	15 mg/kg/day for 7 days, i.p.	Lack of significant effects (OFT, CSA)
He et al. 2015	Mice	Spatial memory (MM), AChE activity, mRNA and protein expression of Egr-1, c-Jun and c-Fos	20 mg/kg (single dose and for 2 and 10 weeks), oral/gavage	Significant ($P < 0.05$) improvement memory in MM, decreases in cortical AChE activity, and increases in mRNA expression of Egr-1, c-Jun and c-Fos, and in Egr-1 protein levels, in hippocampus and cortex
Mennenga et al. 2015	Rat	Short-term memory (spatial working and recent memory, DMS), spatial memory (MM)	1 and 5 mg, s.c.	Significant ($P < 0.05$) reduction in total errors in the DMS
Zhong, Tao, and Yang 2015	Rat	Spatial memory (MM), hippocampal GLT-1, caspase 3, IL-1 β and TNF- α expression, and neuronal survival	30 mg/kg/day for 5 days, i.p.	Significant ($P < 0.05$) improvement in MM, increases in GLT-1 expression and neuronal survival rate, and decreases in caspase 3, IL-1 β and TNF- α expression

AChE, acetylcholinesterase; BDNF, brain-derived neurotrophic factor; CMS, chronic mild stress; CSA, citrate synthase activity; DMS, delayed-match-to-sample asymmetrical 3-choice water maze task; GLT-1, glutamate transporter 1; IL-1 β interleukin 1 β ; i.p., intra-peritoneal; LTM, long-term memory; MM, Morris water maze test; OFT, open-field test; s.c., subcutaneous; SOD, superoxide dismutase; STM, short-term memory; TBI, traumatic brain injury; TNF- α , tumor necrosis factor α .

hippocampus) also significantly ($P < 0.05$) decreased protein carbonilation in the prefrontal cortex and hippocampus. Imipramine (10 and 30 mg/kg) and harmine (5 mg/kg) also significantly ($P < 0.05$) increased catalase activity in the prefrontal cortex, while all imipramine doses and the 5 and 10 mg/kg harmine doses significantly ($P < 0.05$) increased catalase activity in hippocampus. The 20 mg/kg imipramine dose and the 5 and 15 mg/kg harmine doses significantly ($P < 0.05$) increased superoxide dismutase (SOD) activity in the prefrontal cortex, and only the 5 mg/kg harmine dose significantly ($P < 0.05$) increased SOD activity in the hippocampus. All of these results suggest that harmine has antioxidant properties.

He et al. (2015) showed that harmine (20 mg/kg, oral/gavage) significantly ($P < 0.05$) increased mice hippocampal and cortical mRNA expressions of Egr-1, c-Jun, and c-Fos, early response genes/transcription factors associated with memory/learning processes. However, only Egr-1 protein levels were significantly ($P < 0.05$) increased by harmine in the hippocampus and cortex.

Mennenga et al. (2015) reported that harmine (1 and 5 mg, subcutaneous injections) administration to old (17-month-old) rats was associated with significant ($P < 0.05$) reductions in total errors in the delayed-match-to-sample asymmetrical 3-choice water maze task, suggesting improved short-term memory (spatial working and recent memory). No effect of harmine was observed on spatial reference memory, as measured by the Morris water maze test (MM). These results were observed only after excluding animals from the 5 mg dose group that could not perform a swim task due to clear but transient motor impairments.

Studies of chronic administration

Fortunato et al. (2010a) reported that chronic administration of harmine (15 mg/kg/day for 7 days, i.p.) to rats significantly ($P < 0.05$) reduced anhedonia (sweet food consumption) and reverted adrenal gland hypertrophy and increases in adrenocorticotrophic hormone (ACTH) and hippocampal BDNF levels induced by the chronic mild stress model compared to the control group.

Again using the FST, Fortunato et al. (2010b) showed that chronic (14 days) administration of harmine (5, 10, and 15 mg/kg/day) and imipramine (20 and 30 mg/kg/day) to rats significantly ($P < 0.05$ for all doses) reduced immobility and increased swimming time, and harmine (5 and 10 mg/kg) and imipramine (10 and 30 mg/kg/day) also increased climbing time compared to the control group. Furthermore, harmine

and imipramine did not affect locomotor activity, and only the higher doses of harmine (10 and 15 mg/kg) significantly ($P < 0.05$ for both doses) increased BDNF levels in the rat hippocampus.

Réus et al. (2010) reported that chronic (14 days) administration of harmine (5, 10, and 15 mg/kg, i.p.) and imipramine (10 and 30 mg/kg, i.p.) to rats significantly ($P < 0.05$) decreased lipid peroxidation/TBARS formation in the prefrontal cortex and hippocampus compared to the control group. Only the higher dose of harmine (15 mg/kg) and the 20 mg/kg dose of imipramine (only in hippocampus) significantly ($P < 0.05$) decreased protein carbonilation in the prefrontal cortex and hippocampus, and only the 20 mg/kg imipramine dose significantly ($P < 0.05$) increased catalase activity in the prefrontal cortex. Only the higher imipramine dose (30 mg/kg) significantly ($P < 0.05$) increased SOD activity in the prefrontal cortex, and only the 5 mg/kg harmine dose significantly ($P < 0.05$) increased SOD activity in the hippocampus. Similar to the results with acute harmine administration, these results suggest that chronic administration of harmine may induce antioxidant effects.

Abelaira et al. (2013) reported that, compared to the control group, chronic administration of harmine (15 mg/kg/day for 7 days, i.p.) significantly ($P < 0.05$) reduced anhedonia (sweet food consumption) and citrate synthase activity (a measure of energy metabolism) in the prefrontal cortex of rats exposed to the chronic mild stress model. Neither chronic stress nor harmine altered memory/habituation (analyzed with the OFT) or citrate synthase activity in the hippocampus and striatum.

He et al. (2015) performed a study to assess the effects of harmine on memory in mice using the MM task, which assesses spatial memory/learning, and several neurochemical measures. Harmine (20 mg/kg) and the cholinesterase inhibitor donepezil (3 mg/kg) were orally administered by gavage for 2 and 10 weeks. Acute harmine and donepezil administration after two weeks of harmine and donepezil treatment significantly ($P < 0.05$) shortened escape latency and path length in the MM test after scopolamine (1 mg/kg, i.p.) administration, suggesting improved memory. Acute harmine administration also improved memory 24 h after scopolamine administration. After 10 weeks of harmine and donepezil treatment, only acute harmine administration significantly ($P < 0.05$) shortened path length in the MM test in APP/PS1 transgenic mice, an animal model of Alzheimer's disease. Chronic harmine administration significantly ($P < 0.05$) inhibited AchE activity in the cerebral cortex of mice that received scopolamine and in APP/PS1 transgenic mice (after 2 and 10 weeks,

respectively). Harmine and donepezil did not reduce the increases in A β proteins observed in the hippocampus of mice treated with scopolamine and in APP/PS1 transgenic mice.

Using a rat model of traumatic brain injury (TBI), Zhong, Tao, and Yang (2015) showed that chronic (five days) administration of harmine (30 mg/kg/day, i.p.) was associated with significant ($P < 0.05$) reductions in cerebral edema (brain water content), learning (spatial memory, MM test), and motor function (neurologic severity score) improvement, and increases in GLT-1 protein expression and decreases in caspase 3 and inflammatory cytokine expression (interleukin (IL)-1 β and tumor necrosis factor (TNF)- α) in the hippocampus 1, 3, and 5 days following TBI, compared to the control group. Harmine administration also significantly increased neuronal survival rate in the hippocampus at 24 h.

Discussion

This systematic literature review of preclinical studies assessing the effects of harmine on biochemical and behavioral parameters associated with memory/learning suggests that this natural alkaloid improves memory/learning-related deficits and has neuroprotective effects. In rodents, harmine improved memory in several animal models: object recognition task (short-term memory) (Moura et al. 2006), MM test (spatial memory) (He et al. 2015; Zhong, Tao, and Yang 2015), and delayed-match-to-sample asymmetrical 3-choice water maze task (Mennenga et al. 2015). Moreover, harmine also improved several biochemical parameters related to memory/learning in rodent hippocampus and in hippocampal cell cultures, such as reduced glutamate toxicity (Maher and Davis 1996), increased/normalized BDNF levels (Fortunato et al. 2010a, 2010b, 2009), reduced lipid peroxidation and protein carbonilation and increased catalase and SOD activity (Réus et al. 2010), increased expression of Egr-1, c-Jun and c-Fos mRNA and of Egr-1 protein levels (He et al. 2015), and increased GLT-1 expression and neuronal survival rate and decreased caspase 3, IL-1 β and TNF- α expression (Zhong, Tao, and Yang 2015).

The mechanisms of action associated with these effects are not completely understood, but appear to involve MAO and AchE inhibition, GLT-1 upregulation, decreases in ROS, increases in BDNF, and anti-inflammatory effects. Previous studies showed that the neuroprotective effects of harmine in a mice model of amyotrophic lateral sclerosis (Li et al. 2011) and in a rat model of global cerebral ischemia (Sun et al. 2014) are mediated by increased GLT-1 expression, which

corroborates the results of Zhong, Tao, and Yang (2015). Previous studies (Kim et al. 2001; Lee et al. 2000) also reported that the neuroprotective effects of harmine could be related to a scavenging action on ROS, MAO inhibition, and thiol oxidation, which is in line with the findings from Réus et al. (2010).

Moreover, although *P. harmala* seeds have other chemical constituents apart from harmine, an extract of these seeds reduced sodium nitrite- and ethanol-induced memory impairment and this was associated with AchE inhibition, increased glutathione levels, and decreased formation of TBARS in the brain, which is similar to the results of Réus et al. (2010) and He et al. (2015). Other mechanisms seem to involve increased expression of transcription factors associated with memory/learning processes (Egr-1, c-Jun, and c-Fos) and decreased expression of inflammatory cytokines (IL-1 β and TNF- α) (Zhong, Tao, and Yang 2015). Furthermore, harmine could also induce neuroprotective effects by DYRK1A inhibition (Frost et al. 2011; Göckler et al. 2009).

It is unknown if these effects in cell cultures and animal models will be replicated in humans. For instance, it is still unclear if orally ingested harmine in ayahuasca or *P. harmala* preparations reaches the brain and produces psychoactive or hallucinogenic effects. Preclinical studies show that β -carbolines such as harmine have affinity for 5-HT_{2A/2C} receptors (Glennon et al. 2000; Grella et al. 1998), and pharmacokinetic studies on mice show that harmine rapidly penetrates the brain, and is also quickly eliminated (He et al. 2015; Li et al. 2011). In line with these findings, a study investigating the kinetics of [¹¹C]-harmine binding to MAO-A in the human brain after intravenous administration and using positron emission tomography (PET) reported that the fraction of unmetabolized [¹¹C]-harmine in plasma decreased rapidly throughout the study: from 90% at 5 minutes to 11% at 90 minutes (Ginovart et al. 2006). However, human studies about the possible psychoactive properties of harmine are inconclusive, with reports describing hallucinogenic effects (Naranjo 1959; Pennes and Hoch 1957; Shulgin and Shulgin 1997), sedative-like effects (Ott 1999, 1994), or lack of psychoactive effects (Slotkin, Distefano, and Au 1970). Interestingly, a recent study involving ayahuasca administration to healthy volunteers showed that the β -carbolines (including harmine) were associated with specific electroencephalographic (EEG) alterations, suggesting central/psychoactive effects (Schenberg et al. 2015). Therefore, more studies in humans are needed to better assess the pharmacodynamics and pharmacokinetics of harmine after oral administration, either alone or in ayahuasca and *P. harmala* preparations.

Indeed, one of the limitations of the present review is the focus exclusively on preclinical studies, since there is a lack of human studies involving the administration of harmine. Studies with harmine-rich botanical preparations can give us only some idea of the effects of this compound, and are problematic for several factors, including (1) the presence of several different compounds related or not to the β -carbolines; (2) the lack of knowledge about the doses of the compounds present in these preparations; (3) possible pharmacokinetic or pharmacodynamic interactions among these compounds; (4) the retrospective and observational nature of studies of long-term ayahuasca users, which limits the possibility of attributing these effects to ayahuasca; and (5) the possibility of a selection bias in these studies, considering the participation of people that are already adapted to ayahuasca, limiting the generalization of the findings.

Even taking into account these factors, the present review suggests that harmine may improve memory/learning in several animal models and may have neuroprotective properties. Considering the need for improved treatments for neurodegenerative disorders and the associated cognitive decline, further studies exploring the therapeutic properties of this natural alkaloid are warranted.

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