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β-Carboline alkaloids in *Peganum harmala* and inhibition of human monoamine oxidase (MAO)

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ABSTRACT

Peganum harmala L. is a multipurpose medicinal plant increasingly used for psychoactive recreational purposes (*Ayahuasca* analog). Harmaline, harmine, harmalol, harmol and tetrahydroharmine were identified and quantified as the main β-carboline alkaloids in *P. harmala* extracts. Seeds and roots contained the highest levels of alkaloids with low levels in stems and leaves, and absence in flowers. Harmine and harmaline accumulated in dry seeds at 4.3% and 5.6% (w/w), respectively, harmalol at 0.6%, and tetrahydroharmine at 0.1% (w/w). Roots contained harmine and harmol with 2.0% and 1.4% (w/w), respectively. Seed extracts were potent reversible and competitive inhibitors of human monoamine oxidase (MAO-A) with an IC_{50} of 27 μg/l whereas root extracts strongly inhibited MAO-A with an IC_{50} of 159 μg/l. In contrast, they were poor inhibitors of MAO-B. Inhibition of MAO-A by seed extracts was quantitatively attributed to harmaline and harmine whereas inhibition by root extracts came from harmine with no additional interferences. Stems and leaves extracts were poor inhibitors of MAO. The potent inhibition of MAO-A by seed and root extracts of *P. harmala* containing β-carbolines should contribute to the psychopharmacological and toxicological effects of this plant and could be the basis for its purported antidepressant actions.

1. Introduction

β-Carbolines are naturally-occurring alkaloids that exhibit a wide range of psychopharmacological effects because of their binding to benzodiazepine, imidazoline, serotonin and opiate receptors as well as MAO inhibition (Adell et al., 1996; Airaksinen and Kari, 1981; Herraiz and Chaparro, 2005, 2006a,b; Herraiz et al., 2008; Husbands et al., 2001; Miralles et al., 2005; Parker et al., 2004; Pimpinella and Palmery, 1995). In nature, β-carboline alkaloids are reported to occur in a number of plants, including *Banisteriopsis caapi* (*Malpighiaceae*) and *Peganum harmala* L. (*Zygophyllaceae*), which extracts exhibit psychoactive actions mediated and/or potentiated by these compounds (Callaway et al., 2005). *B. caapi* is a constituent of Ayahuasca, a hallucinogenic beverage, ingested in rituals by the Amazonian tribes (Callaway et al., 2005; McKenna, 2004). *P. harmala* (Syrian rue, harmal, harmel) is a perennial herbaceous plant native to arid parts of North Africa, Mediterranean Sea, Middle East, Pakistan and India, and introduced and naturalized in parts of the Southwest USA, and a few areas of South Africa and Australia. *P. harmala* is traditionally and commonly used for medicinal and psychoactive purposes since ancient times. Their seeds are known to possess hypothermic and hallucinogenic properties, and it is used as a medical remedy, incense, spice or condiment with abortifacient, narcotic, aphrodisiac, stimulant, sedative, emmenagogue, and emetic properties, and employed for the treatment of syphilis, fever, hysteria, malaria, neuralgia, parkinsonism, rheumatism, colic, asthma and eye complaints (Abdelfattah et al., 1995; Astulla et al., 2008; Berrougui et al., 2006; Elbahri and Chemli, 1991; Farouk et al., 2008; Im et al., 2009; Monsef et al., 2004; Shahverdi et al., 2008). *P. harmala* extracts are also being currently investigated as fungicidal, bactericidal and antitumor agents (Lamchouri et al., 1999; Sobhani et al., 2002; Song et al., 2004).

Overdose ingestion of *P. harmala* for medicinal use or as a recreational psychoactive product can be poison and several cases of toxicity have been already reported. It produces paralysis, euphoria, convulsions, hallucinations, digestive problems (nausea, vomiting), hypothermia and bradycardia (Ben Salah et al., 1986a; Elbahri and Chemli, 1991; Frison et al., 2009; Mahmoudian et al., 2002). Nowadays, internet access represents a new mechanism and information to the use of illicit substances (Boyer et al., 2001; Brush et al., 2004; Frison et al., 2009; Martins et al., 2008). There exist

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already numerous websites providing information or misinformation on *P. harmala* and *B. caapi*, potentially leading to an increase in their unsafe use. Indeed, urban people imitate the chamans and prepare Ayahuasca (yajé) imitations with *P. harmala* and other plants (http://www.imaginaria.org/a_yage.htm). Those preparations exhibit powerful psychopharmacological effects and could affect MAO enzymes producing hypertensive crisis in consumers who ingest foods containing vasoactive amines, and particularly tyramine (Brush et al., 2004).

Some of the pharmacological effects of *P. harmala* and *B. caapi* extracts could result from the interaction of β-carboline alkaloids with monoamine oxidase (MAO) enzymes. The activity of Ayahuasca preparation depends on a synergistic interaction between active β-carboline alkaloids in *B. caapi*, which are MAO-A inhibitors, and *N*,*N*-dimethyltryptamine (DMT) from *Psychotria viridis*, a short-acting psychoactive agent, that is slowly metabolized in presence of MAO-inhibitors (Callaway et al., 1999; McKenna, 2004; McKenna et al., 1998). MAO is a mitochondrial enzyme that catalyzes the oxidative deamination of biogenic amines and neurotransmitters. It appears as two isozymes, MAO-A and B, distinguished by substrate and inhibitor selectivities. MAO plays an important role in the central nervous system and peripheral organs (Shih et al., 1999; Youdim et al., 2006). Inhibitors of this enzyme are useful as antidepressants (MAO-A inhibitors) and neuroprotectants (MAO-B inhibitors) (Ben-Shlomo and Bhatia, 2004; Fowler et al., 2003; Herraiz and Chaparro, 2005; Herraiz et al., 2009; Yamada and Yasuhara, 2004; Youdim et al., 2006). Recent results suggest that β-carboline alkaloids may exhibit antidepressant effects (Aricioglu and Altunbas, 2003; Farzin and Mansouri, 2006), probably linked to its inhibitory actions on MAO (Herraiz and Chaparro, 2005, 2006b).

Although the occurrence of β-carbolines in *P. harmala* is known, previous analytical reports are scarce or limited, and the relative distribution and content of alkaloids in the plant are controversial or not well established (Hemmateenejad et al., 2006; Kartal et al., 2003; Pulpati et al., 2008). The present research was aimed to determine the occurrence of β-carbolines in extracts of *P. harmala* L., by studying and characterizing the presence of these compounds in different parts of the plant, and subsequently investigate their biological activity regarding MAO inhibition. As a result, it is found that seed and root extracts of *P. harmala* highly inhibited MAO-A. β-Carbolines occurred in seeds (harmine and harmaline) and roots (harmine) in a very high proportion, and these alkaloids highly contributed to MAO-A inhibition with apparently no additional interferences. Inhibition of MAO-A may likely contribute to the psychopharmacological and perhaps toxicological effects associated with the ingestion of seed or root extracts of *P. harmala*.

2. Material and methods

P. harmala L. (*Zygophyllaceae*) plants were collected in Toledo (Spain) from May to December (five plants each time), and the different parts: leaves, stems, flowers, roots, green capsules (fruits), and dry seeds (dark brown to black colour) conveniently separated, grinded, and homogenized for further studies regarding isolation, identification and quantification of β-carbolines and for assessing the MAO-inhibitory properties. Recombinant human monoamine oxidase A and B were obtained from Gentest BD Biosciences (Woburn, MA, USA). Enzymes were expressed in insect cells from MAO-A and MAO-B cDNA using a baculovirus expression system and were prepared as membrane protein fractions. Kynuramine, 4-hydroxyquinoline, harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole), harmaline (7-methoxy-1-methyl-3,4-dihydro-β-carboline), harmol (7-hydroxy-1-methyl-9*H*-pyrido[3,4-*b*]indole), harmalol (7-hydroxy-1-methyl-3,4-dihydro-β-carboline), norharman and harman were purchased from Sigma Chemical Co. (MO, USA). Tetrahydroharmine (7-methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline) was synthesized through a Pictet–Spengler reaction from 6-methoxytryptamine (Sigma) and acetaldehyde. HPLC grade acetonitrile, methanol and dimethyl sulfoxide (DMSO) were from Scharlau (Spain) and dichloromethane from Merck (Germany).

2.1. Isolation of β-carbolines from P. harmala

Seeds, roots, flowers, leaves, stems, or fruits (capsules) were grinded and aliquots (0.2–0.5 g) homogenized in 20 ml, 0.6 M $HClO_4$ + methanol (1:1) using an ultraturrax homogenizer and centrifuged (10,000*g*, 10 min). This operation was repeated twice with the residue. The extracts (60 ml) were conveniently diluted to analyze β-carbolines by HPLC, and also to carry out further studies on MAO inhibition. β-Carbolines were also isolated from the extracts by repeated injection into RP-HPLC and collection of the corresponding peaks of harmalol, harmol, harmaline and harmine at the exit of the column and detectors. These compounds were conveniently diluted and used for MAO-inhibition assays.

2.2. Monoamine oxidase (MAO-A and B) assay and inhibition

MAO enzyme assays were performed as elsewhere (Herraiz and Chaparro, 2005, 2006a,b). Briefly, membrane protein fractions containing MAO-A or MAO-B were diluted to the desired concentrations in 100 mM potassium phosphate buffer (pH 7.4). A 0.2 ml reaction mixture containing 0.01 mg/ml protein and 0.25 mM kynuramine in 100 mM potassium phosphate (pH 7.4) was incubated at 37 °C for 40 min. After incubation the reaction was stopped by the addition of 2 N NaOH (75 μl), followed by the addition of 70% $HClO_4$ (25 μl), and the sample centrifuged (10,000*g*) for 10 min. The supernatant (20 μl) was injected into the HPLC and the deamination product of kynuramine (*i.e.* 4-hydroxyquinoline) formed during the enzymatic reaction (Herraiz and Chaparro, 2006a) determined by RP-HPLC-diode array detection at 320 nm. A response curve of area versus concentration was constructed to calculate the concentration of 4-hydroxyquinoline.

To perform inhibition assays, aliquots of plant extracts and homogenates prepared as above, or instead the isolated fractions containing β-carbolines, were conveniently diluted (up to 1/10,000 in seed extracts) in buffer phosphate containing 1% DMSO, and added to reaction mixtures containing kynuramine (0.25 mM) and MAO enzyme (A or B) (0.01 mg/ml membrane protein) in 100 mM potassium phosphate buffer (pH 7.4), as above. Reversibility was determined by incubating MAO-A with seed extract, centrifugation and subsequently measuring the activity recovered and compared with controls incubated without plant extracts. MAO kinetic and mechanism of inhibition was assessed by analyzing the corresponding Michaelis–Menten curves and double reciprocal Lineweaver–Burk plots obtained at different concentrations of kynuramine and inhibitors. The concentrations that produce 50% enzyme inhibition (IC_{50} values) were calculated by adjusting the experimental data (% inhibition vs. concentration of inhibitor into the assays) to non-linear regression curves. All enzymatic assays were carried out at least in duplicate.

2.3. RP-HPLC analysis and quantitation of β-carbolines in P. harmala

The analysis of kynuramine deamination product, 4-hydroxyquinoline, as well as β-carbolines and tetrahydro-β-carbolines was performed by RP-HPLC with UV diode array and fluorescence detection using a HPLC 1050 (Hewlett Packard) with a 1100 diode array detector (DAD) and a 1046A-fluorescence detector. A 150 mm × 3.9 mm i.d., 4 μm, Nova-pak C18 column (Waters, Milford, MA, USA) was used for chromatographic separation. Chromatographic conditions were: 50 mM ammonium phosphate buffer (pH 3) (buffer A) and 20% of A in acetonitrile (buffer B). The gradient was programmed from 0% (100% A) to 32% B in 8 min, and 90% B at 15 min. The flow rate was 1 ml/min, the column temperature was 40 °C and the injection volume was 20 μl. Absorbance detection was set at 320 nm (analysis of 4-hydroxyquinoline). Detection of the β-carbolines harmol and harmine was carried out at 254 nm, harmalol and harmaline at 360 nm and tetrahydroharmine at 280 nm. Concentration of β-carbolines in *P. harmala* extracts was determined from calibration curves of the response at the corresponding wavelength versus concentration of each standard compound. Identification of compounds was done by UV, fluorescence and mass spectrometry.

2.4. Identification of β-carbolines in P. harmala by HPLC-ESI-mass spectrometry

Identification of the β-carboline alkaloids from *P. harmala* was made in plant extracts obtained as mentioned above, that were analyzed on a 150 × 2.1 mm i.d. Zorbax SB-C18, 5 μm, column (Agilent Technologies) by using a series 1100 HPLC-MSD (Hewlett–Packard) (electrospray-positive ion mode). Eluent A: acetic acid (0.5%); B: acetic acid (0.5%) in acetonitrile; 80% B in 30 min, flow rate: 0.25 ml/min; *T*: 40 °C; mass range: 50–700 amu and cone voltage: 100 V. Mass spectrometric identification of 4-hydroxiquinoline in MAO assays was carried out as previously (Herraiz and Chaparro, 2006a).

3. Results

Extracts of *P. harmala* were analyzed and five major β-carboline alkaloids (Fig. 1) were identified by mass spectrometry-electrospray and UV–VIS (DAD), as harmaline (*m*/*z* at 215 $(M+H)^+$, UV_{max} at *ca.* 375 nm), harmine (*m*/*z* at 213 $(M+H)^+$, UV_{max} at *ca.* 245 and