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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

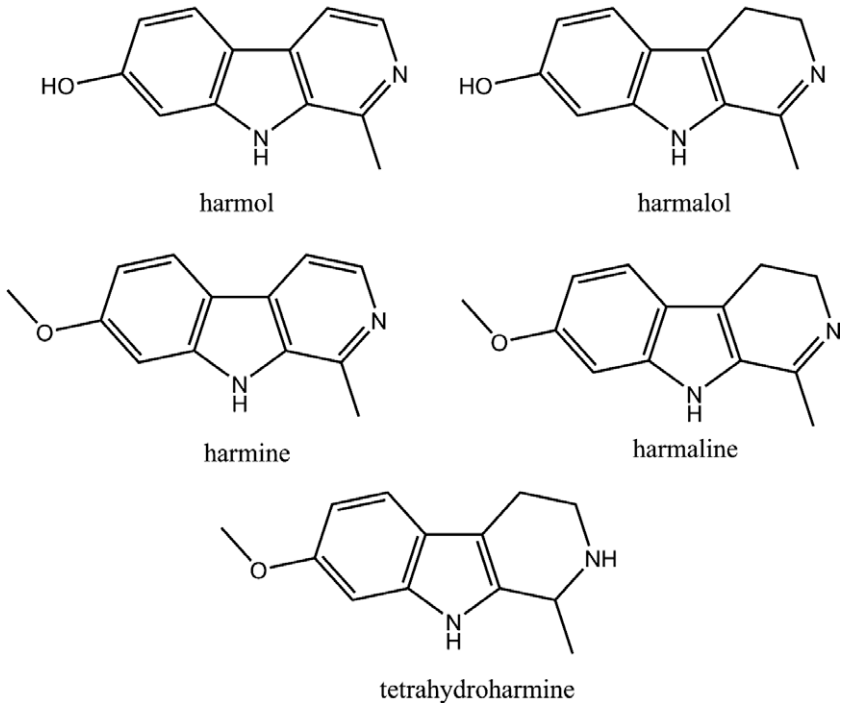

Fig. 1. Structures of β-carboline alkaloids identified and quantified in different parts of the plant *P. harmala*.

322 nm), harmalol (*m*/*z* at 201 $(M+H)^+$, UV_{max} at *ca.* 375 nm), harmol (*m*/*z* at 199 $(M+H)^+$, UV_{max} at *ca.* 245 and 322 nm) and tetrahydroharmine (*m*/*z* at 217, 200 and 188, UV_{max} at *ca.* 265 and 295 nm). After an exhaustive extraction, harmaline and harmine were the main alkaloids found in the plant followed by harmol and harmalol (Fig. 2). The qualitative and quantitative profiles of β-carbolines highly varied depending on each part of the plant (Table 1). The flowers had no appreciable presence of β-carbolines, stems had low levels of harmol and harmine and leaves only contained harmine in noticeable amount. In contrast, a very high proportion of alkaloids were found in dried seeds and roots (Fig. 2). Seeds mainly contained harmaline and harmine that reached a very high amount of 5.6% and 4.3% (w/w) respectively, harmalol at 0.6% (w/w) and tetrahydroharmine at 0.11% (w/w). A different pattern was found in roots that contained harmine and harmol as the main alkaloids reaching up to a 2.1% and 1.4% (w/w), respectively, and much lower levels of harmalol. While harmaline exclusively accumulated in seeds and fruits, harmine and harmol were more widespread in different parts of *P. harmala*, with harmol mainly occurring in roots. Whole green fruits (capsules) were also analyzed and their amount of harmine and harmaline was nine-times lower than that in dry seeds, indicating that β-carbolines highly increased during development, ripening and drying process of fruits and seeds.

Seeds and roots extracts of *P. harmala* containing a high level of β-carbolines might exhibit biological activity. Acidic–methanolic

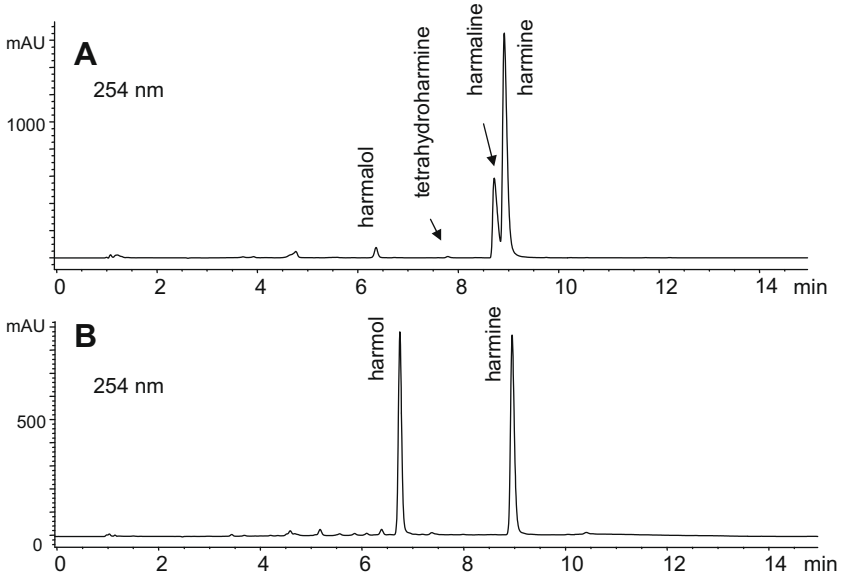

Fig. 2. Chromatographic analysis (RP-HPLC) (254 nm) of harmala alkaloids identified by mass spectrometry and UV–VIS in *P. harmala* seed (A) and root extracts (B).

Table 1
Concentration of β-carboline alkaloids (mg/g ± SEM) determined by RP-HPLC in different parts of *Peganum harmala*.

Plant	Harmalol	Harmol	Harmaline	Harmine	Tetrahydroharmine
Dry seeds	6.03 ± 0.39	0.03 ± 0.007	56.0 ± 2.28	43.2 ± 2.0	1.1 ± 0.2
Green fruits	4.17 ± 1.14	–	4.55 ± 0.02	6.14 ± 1.2	–
Capsule walls	–	–	0.54 ± 0.09	0.77 ± 0.1	–
Stems	0.09 ± 0.09	0.10 ± 0.09	–	2.02 ± 0.32	–
Leaves	–	–	–	1.04 ± 0.06	–
Flowers	–	–	–	–	–
Roots	2.03 ± 0.43	14.1 ± 2.2	–	20.68 ± 2.5	–

Different parts of the plants collected from May to December were analyzed in the next few days (before two weeks) after harvest as mentioned in material and methods. Flowers (without the ovary that was removed) and green fruits (capsules) were collected in May; roots were collected in November; dry seeds, stems, leaves and walls in October. Undetectable or very low amount (less than 0.01 mg/g) (–).

extracts of dried seeds were conveniently diluted and assayed for MAO inhibition. They strongly inhibited human MAO-A with a calculated IC_{50} value of 27.6 ± 1.3 μg/l of seed extracts (Fig. 3A). Subsequently, the corresponding identified β-carbolines in the seed extract (*i.e.* harmalol, harmine and harmaline) were isolated by preparative RP-HPLC and after the corresponding dilution, used for inhibition of MAO-A. Those β-carbolines were responsible for the inhibition of MAO-A by *P. harmala* seed extracts with a high percentage of the inhibition (around 87%) coming from the sum of harmine and harmaline with harmalol accounting for a 7.6% (Fig. 3B). The roots of *P. harmala* also contained a high concentration of the β-carbolines harmine and harmol. Then, after convenient dilution, root extracts were used for MAO-A inhibition and they also potently inhibited this enzyme with a calculated IC_{50} of 159.3 ± 17.5 μg/l of root extract into the assays (Fig. 4A). The isolation of harmine from the root extract by preparative RP-HPLC showed that the inhibition of human MAO-A produced by root extracts of *P. harmala* was due to presence of this β-carboline (Fig. 4B). On the other hand, stems and leaves extracts of *P. harmala* were also assessed as MAO-A inhibitors and results showed a much lower inhibition when compared with that of root and seed extracts (a 50% and 40% MAO inhibition at 2.5 mg/l extracts into the assays for stems and leaves, respectively). This poor inhibition is in good agreement with their low content of harmine (see Table 1).

Kinetics studies showed that inhibition of human MAO-A by seed extracts of *P. harmala* was competitive (Fig. 5). Inhibition was reversible as demonstrated by the recovery of the activity of MAO-A, following incubation with seed extracts of the plant compared with the corresponding control (results not shown). On the other hand, in order to assess the selectivity of the inhibition,

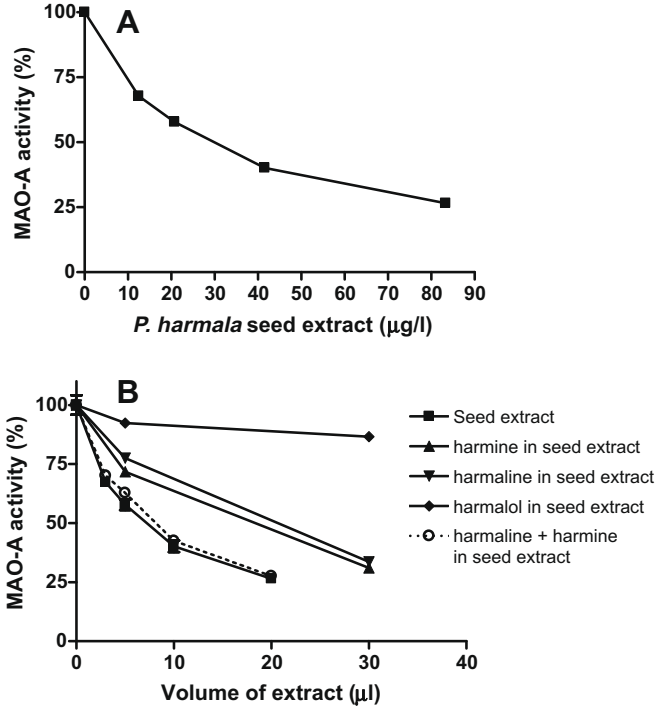

Fig. 3. (A) Inhibition of human MAO-A by *P. harmala* soluble seed extracts obtained in acidic-methanol and conveniently diluted (1/10,000) in phosphate buffer-DMSO 1% to the concentration (μg/l) included into the assays. (B) Inhibition of human MAO-A by *P. harmala* seed extract and by those β-carbolines isolated from *P. harmala* seed extract. For this, 0.5 g of seeds were homogenized in perchloric-methanol (20 ml ×3) and the soluble extract used for preparative HPLC isolation of β-carbolines. A final dilution (1/10,000) in phosphate buffer-1% DMSO from the initial extract solution was used in both the isolated β-carboline fractions and the whole seed extracts, and used for comparative inhibition of MAO-A. The concentration of soluble seed extract was 8.33×10^{-7} mg/μl. Results are at least from duplicate assays.

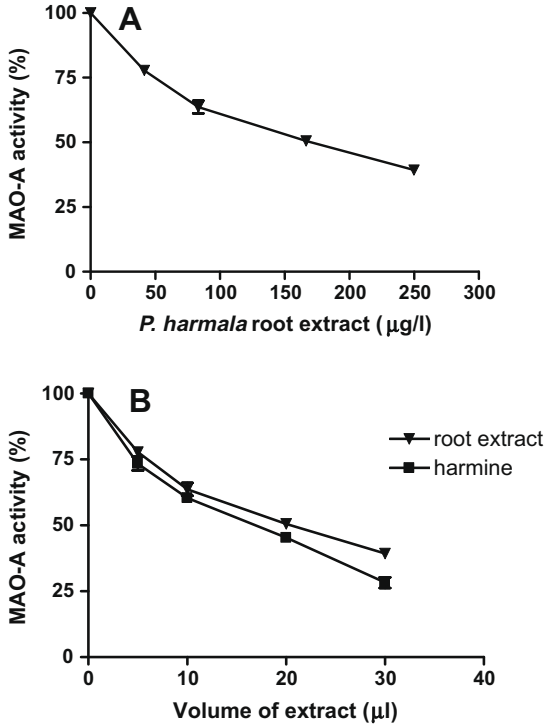

Fig. 4. (A) Inhibition of human MAO-A by *P. harmala* soluble root extracts obtained in acidic-methanol and conveniently diluted (1/5000) in phosphate buffer-1% DMSO to the concentration (μg/l) included into the assays. (B) Inhibition of human MAO-A by a soluble extract (1.66×10^{-6} mg/μl) obtained from roots of *P. harmala* and comparative inhibition produced from the corresponding harmine isolated from the same extract by preparative RP-HPLC. Results are at least from duplicate assays.

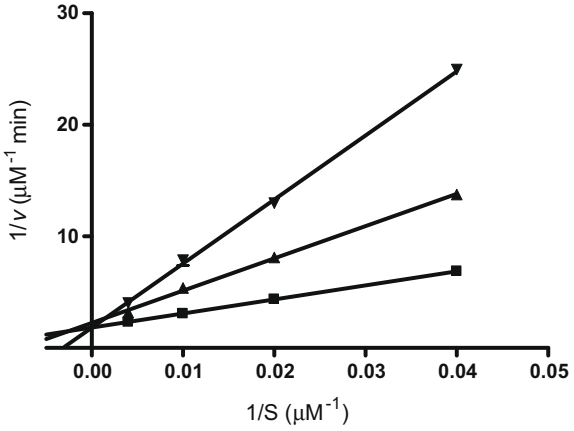

Fig. 5. Double reciprocal curves corresponding to inhibition kinetics of human MAO-A by seed extracts of *P. harmala* (■, 0 μg/l, ▲, 10 μg/l and ▼, 20 μg/l of extract). Enzymatic activity was assessed by determining reaction velocity (v) as the formation of 4-hydroxyquinoline produced (μM) into the assays per min in function of the concentration of substrate (kynuramine). Results are at least from duplicate assays.

studies were performed on human MAO-B isozyme. Seed extracts did not exhibit significant inhibition of this isoform (a calculated IC_{50} of 416 mg/l of seed extract) when compared with MAO-A. Then, seeds extracts selectively inhibited MAO-A over MAO-B.

Then, seed and root extracts of *P. harmala* strongly inhibited human MAO-A and contained harmaline, harmine, harmalol and harmol that are responsible for this biological activity. The inhibition on human MAO-A of authentic standards of these compounds was subsequently determined in the same assay and gave IC_{50} experimental values of 11.8, 8.7, 480 and 352 nM, respectively. These values are in general good agreement with inhibitory results of the same compounds and their corresponding concentrations determined in the extracts of the plant.

4. Discussion

P. harmala L. (Syrian rue, harmal, harmel) is claimed from ancient times as an important medicinal plant and a multipurpose herbal remedy (Abdelfattah et al., 1995; Astulla et al., 2008; Berrougui et al., 2006; Elbahri and Chemli, 1991; Farouk et al., 2008; Harsh and Nag, 1984; Im et al., 2009; Monsef et al., 2004; Shahverdi et al., 2008). *P. harmala* extracts are also used for recreational purposes and as a central nervous system stimulant (Kusmenoglu, 1996) (http://www.imaginaria.org/a_yage.htm). β-Carboline alkaloids appear to be the main pharmacological and toxicological active principles in *P. harmala*. As shown here, β-carbolines seemed to accumulate in seeds and roots whereas minor amounts appeared in leaves and stems and absence in flowers. The concentration of β-carbolines in seeds and roots was very high, with harmine and harmaline reaching up to a 10% (w/w) (Table 1). The molecular profile of alkaloids highly varied in the plant with harmine and harmol predominating in roots and harmine and harmaline in seeds. Interestingly, harmaline occurred almost exclusively in seeds whereas harmol was mostly present in roots with traces found in stems. Harmalol also occurred in fruits and seeds. Minor amounts of tetrahydroharmine appeared in seeds but neither harman (1-methyl-β-carboline) nor norharman (β-carboline) were detected. The concentration of β-carbolines found in seeds was higher than that reported previously (Hemmateenejad et al., 2006; Kartal et al., 2003; Pulpati et al., 2008), and also differed in the qualitative profile (Kartal et al., 2003). These differences and discrepancies could be explained by differences in plant processing or analysis and the actual plants used, including geographical origin, and the state of development of the plant (Kartal et al., 2003). In this regard, in this study a three step acidic–methanolic extraction was needed for an exhaustive extraction of alkaloids from seeds (>97%). The content of β-carboline alkaloids may also vary with the period of development of seeds. Thus, the fruits that were more ripe and mature and the dry seeds contained the highest level of the β-carbolines harmine and harmaline.

The seeds and roots of *P. harmala* are a good source of naturally-occurring bioactive β-carbolines. These compounds are known to bind to several receptors in the human body and brain, such as benzodiazepine, serotonin, opioid, and imidazoline and also to interact with enzymes such as cytochrome P450 and MAO (Adell et al., 1996; Airaksinen and Kari, 1981; Herraiz and Chaparro, 2005, 2006a; Herraiz et al., 2008; Husbands et al., 2001; Kim et al., 1997; Miralles et al., 2005; Parker et al., 2004; Pimpinella and Palmery, 1995; Rommelspacher et al., 1994; Song et al., 2004). β-Carbolines easily cross the blood–brain barrier altering the concentration of neurotransmitters, and exert a variety of neurophysiological, and toxicological effects including effects on body temperature, convulsion, antidepressant actions, vascular relaxation, platelet antiaggregation and effects on drug withdrawal and appetite (Adell et al., 1996; Airaksinen and Kari, 1981; Aricioglu and Altunbas, 2003; Boeira et al., 2002; Farzin and Mansouri, 2006; Fekkes and Bode, 1993; Herraiz and Chaparro, 2005, 2006a; Herraiz et al., 2008; Husbands et al., 2001; Louis et al., 2002; Miralles et al., 2005; Östergren et al., 2004; Parker et al., 2004; Pimpinella and Palmery, 1995; Tsuchiya et al., 1999). Some of these effects, including antidepressant actions may be mediated through interaction with MAO enzymes. MAO is involved in the metabolism and regulation of neurotransmitter levels. In the gastrointestinal tract and circulatory system, MAO may serve a protective function by regulating the levels of vasoactive dietary amines. These biological implications are of high pharmacological interest and MAO-A inhibitors are useful as antidepressants although their use is restricted because of the possibility of hypertensive crisis produced when the patients consume tyramine-containing foods (the so-called "cheese effect") (Youdim and Weinstock, 2004). Moreover, the oxidation of biogenic amines by MAO results in the production of toxic hydrogen peroxide, ammonia, and aldehydes that represent risk factors for cell oxidative injury (Cohen and Kesler, 1999). Then, the use of MAO-inhibiting substances may result in biological protection against toxicants and oxidative stress (Herraiz et al., 2009).

P. harmala extracts obtained from seeds and roots showed a potent and selective inhibition on MAO-A. Two β-carbolines, harmaline and harmine, were responsible for the inhibition in seeds, whereas harmine was the inhibitory substance in roots. Kinetic studies indicated a competitive and reversible type of inhibition with seed extracts showing six times higher inhibition than roots. These results strongly suggest that some of the pharmacological and toxicological effects of *P. harmala* extracts derive from the presence of β-carbolines and their remarkable inhibitory effects on MAO-A. Indeed, β-carbolines increase the brain levels of serotonin and other neurotransmitters (McKenna, 2004; McKenna et al., 1998) through MAO-A inhibition, suggesting a further interest of the use of seeds and roots extracts of *P. harmala* as potential antidepressants.

Owing to its chemical and pharmacological characteristics, *P. harmala* imitates *B. caapi* employed in the ethnomedical and magico-religious practices of indigenous Amazonian tribes to prepare Ayahuasca (Frison et al., 2009; Ott, 1999; Pieroni et al., 2005). β-Carboline alkaloids occur in both plants and their psychopharmacological actions are currently the basis for their use as illicit products and hallucinogenic substances. Nevertheless, the notion that β-carbolines are hallucinogenic by themselves is controversial

(Callaway et al., 2005; McKenna, 2004). In Ayahuasca, β-carbolines prolong the half-life of dimethyltryptamine (DMT), a hallucinogenic substance, whereas tetrahydroharmine may produce additional effects on the uptake of serotonin (Callaway et al., 2005; McKenna, 2004). In *P. harmala*, tetrahydroharmine appeared as a minor substance compared with harmine and harmaline whereas DMT was not reported. However, the seeds of *P. harmala* contained a higher level of alkaloids (up to 10%, w/w) compared with *B. caapi* employed in Ayahuasca (up to 2%, w/w) (McKenna et al., 1998), and consequently, an enhanced psychopharmacological effect of *P. harmala* seed extracts may be anticipated. On the other hand, compared with hoasca tea, in which harmaline is a minor compound and harmine and tetrahydroharmine are the major substances (Callaway, 2005; Callaway et al., 1999, 2005), harmaline was the most abundant species in the seeds of *P. harmala* with a concentration comparable to harmine. Then, seed extracts of *P. harmala* may exhibit different psychobiological/toxicological effects than those of *B. caapi* and Ayahuasca since harmaline and harmine can also exhibit distinct pharmacological and toxicological profiles.

P. harmala plant is thought to be toxic and severe intoxications often occur in domestic animals with digestive and nervous syndromes after consumption of sub-lethal amounts of the plant (Ben Salah et al., 1986b; Frison et al., 2009). The intoxicated animals appear in a narcotic stage interrupted by occasional short period of excitement, and abortion is frequent (Harsh and Nag, 1984). There are also a few reports on the toxic effects as well as cases of overdose and intoxication in humans (Ben Salah et al., 1986b; Elbahri and Chemli, 1991; Frison et al., 2009; Mahmoudian et al., 2002). The patients showed signs of intoxication few minutes after taken the extracts of the plant with hallucinations and neuro-sensorial syndromes, bradycardia and GI disturbances such as nausea and vomiting (Frison et al., 2009). The signs and symptoms of intoxication relieved in a few hours and the patients left the hospital in good health (Ben Salah et al., 1986b; Mahmoudian et al., 2002). Currently, many internet sites contain recommendations and information (Boyer et al., 2001; Brush et al., 2004) about the use of *P. harmala* as a recreational and hallucinogenic product, being a concern about a possible occurrence of severe intoxications. In some sites, 3 g of seeds is recommended as a standard and starting dose (http://leda.lycaeum.org/?ID=360), and higher doses are reported to exhibit potent psychoactive effects. From this work, 3 g of seeds will mean 130 mg of harmine and 168 mg of harmaline. These doses might exceed the amount needed to induce MAO inhibition. Harmine and harmaline are thought to be psychoactive in oral administration above 8 and 4 mg/kg (http://leda.lycaeum.org/?ID=134). Then, these doses could be reached with the ingestion of a few grams of seeds of *P. harmala*. Furthermore, because of its potent effect on MAO, the ingestion of *P. harmala* might result in the interaction with the metabolism of dietary biogenic amines, and particularly tyramine, resulting in hypertensive crisis as a secondary effect. Then, precautions should be always taken avoiding foods containing tyramine or other dietary amines. Nevertheless, results obtained here indicate that MAO inhibition by seeds extract is reversible and highly selective for MAO-A over MAO-B, which is the main isozyme involved in the metabolism of tyramine. These facts could lower the possibility of hypertensive crisis. On the other hand, harmala alkaloids are metabolized in the liver and extrahepatic tissues with the participation of the cytochrome P450 2D6 to harmalol and harmol (Yu et al., 2003a,b), which are much less potent inhibitors of MAO. As this P450 is polymorphic and there exist poor and extensive metabolizers, the pharmacological and toxicological effects of *P. harmala* extracts might be highly affected by individual genetic polymorphism (Herraiz et al., 2006, 2008; Wu et al., 2009; Yu et al., 2003a,b). Finally, *P. harmala* might produce toxicological effects because of occurrence of quinazoline alkaloids such as peganine (Frison et al., 2009).

In conclusion, seed and root extracts of *P. harmala* were potent inhibitors of MAO-A with β-carbolines being the key contributors to this inhibition, and apparently without additional interferences. β-Carbolines abounded in seeds (harmaline and harmine) and roots (harmine and harmol) of the plant, accounting for up to 10% w/w and 3.6% w/w, respectively. These results confirm that β-carbolines are likely responsible for some of the pharmacological and toxicological effects of this plant, including inhibition of MAO-A and potential antidepressant actions. These results also suggest that controlled extracts of this plant might be useful as pharmacological, nutraceutical or herbal agents producing selective and reversible MAO-A inhibition with potential application as antidepressants.

Conflict of interest

The authors declare that there are no conflicts of interest.

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