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Inhibition of myeloperoxidase activity by the alkaloids of *Peganum harmala* L. (Zygophyllaceae)

Sihem Bensalem [a,b,*], Jalal Soubhye [c,**], Iyas Aldib [c], Lamine Bournine [a], Anh Tho Nguyen [b], Michel Vanhaeverbeek [d], Alexandre Rousseau [d], Karim Zouaoui Boudjeltia [d], Ahmad Sarakbi [e], Jean Michel Kauffmann [e], Jean Nève [c], Martine Prévost [f], Caroline Stévigny [b], Fadila Maiza-Benabdesselam [a], Fatiha Bedjou [a], Pierre Van Antwerpen [c,g,1], Pierre Duez [b,1]

[a] *Laboratoire Biotechnologie Végétales et Ethnobotanique, Faculté des Sciences de la Nature et de la Vie, Université Abderrahmane Mira de Bejaia, 06000 Bejaia, Algérie*
[b] *Laboratoire de Pharmacognosie, Bromatologie et Nutrition Humaine, Université Libre de Bruxelles (ULB), 1050 Bruxelles, Belgique*
[c] *Laboratoire de Chimie Pharmaceutique Organique, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), 1050 Bruxelles, Belgique*
[d] *Laboratoire de Médicine Expérimentale, CHU Charleroi, A. Vesale Hospital, Université Libre de Bruxelles (ULB), Montigny-le-Tilleul, Belgique*
[e] *Laboratoire de Chimie Analytique Instrumentale et Bioélectrochimie, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), 1050 Bruxelles, Belgique*
[f] *Laboratoire de Structure et Fonction des Membranes Biologiques, Université Libre de Bruxelles (ULB), 1050 Bruxelles, Belgique*
[g] *Plateforme Analytique, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), 1050 Bruxelles, Belgique*

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ABSTRACT

Ethnopharmacological relevance: Seeds and aerial parts *of Peganum harmala* L. are widely used in Algeria as anti-inflammatory remedies. Evaluation of *Peganum harmala* total alkaloids extracts and pure β-carboline compounds as an anti-inflammatory treatment by the inhibition of an enzyme key of inflammatory, myeloperoxidase (MPO) and HPLC quantification of the alkaloids from the different parts of plant.

Materials and methods: MPO inhibition was tested using taurine chloramine test. The inhibition of LDL oxidation induced by MPO was carried out. The molecular docking analysis of *Peganum harmala* alkaloids on MPO was performed using the Glide XP docking protocol and scoring function and the redox potential of alkaloids was determined using an Epsilon potentiostat. The concentration of harmala alkaloids was determined using HPLC analysis.

Results: The HPLC profiling of the active total alkaloids indicates that β-carboline *e.g.* harmine, harmaline, harmane, harmol and harmalol are major components. As β-carbolines resemble tryptamine, of which derivatives are efficient inhibitors of MPO, the harmala alkaloids were tested for their activity on this enzyme. Total alkaloids of the seeds and of the aerial parts strongly inhibited MPO at 20 μg/mL (97 ± 5% and 43 ± 4%, respectively) whereas, at the same concentration, those of the roots showed very low inhibition (15 ± 6%). Harmine, harmaline and harmane demonstrated a significant inhibition of MPO at IC_{50} of 0.26, 0.08 and 0.72 μM respectively. These alkaloids exerted a similar inhibition effects on MPO-induced LDL oxidation. Molecular docking analysis of *Peganum harmala* alkaloids on MPO showed that all active *Peganum harmala* alkaloids have a high affinity on the active site of MPO (predicted free energies of binding up to −3.1 kcal/mol). Measurement of redox potentials versus the normal hydrogen electrode clearly differentiated (i) the high MPO inhibitory activity of harmine, harmaline and harmane (+1014, 1014 and 1003 mV, respectively); and (ii) the low activity of harmalol and harmol (+629/778 and 532/644 mV, respectively). A reverse phase HPLC method has been developed to determine simultaneously five alkaloids of *Peganum harmala*. Seeds contained all five β-carboline derivatives with the main active alkaloids, harmaline and harmine, being up to 3.8% and 2.9%, respectively. Up to 3.2% of harmine was determined in the roots. The four β-carboline derivatives, harmine, harmaline, harmane and harmalol were identified in the aerial parts. The highest inhibitory effect observed in seeds and the moderate

* Corresponding author at: Laboratoire Biotechnologie Végétales et Ethnobotanique, Faculté des Sciences de la Nature et de la Vie, Université Abderrahmane Mira de Bejaia, 06000 Bejaia, Algeria. Tel.: +213 5 50296286.
** Corresponding author.
E-mail addresses: bensalemsihem06@yahoo.fr, sihem.bensalem@univ-bejaia.dz (S. Bensalem), jalal.soubhye@ulb.ac.be (J. Soubhye).
[1] P. Duez and P. V. Antwerpen contributed equally to this paper.

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effect of aerial parts could be explained by their harmine and harmaline content. In contrast, the very weak inhibition of the root extract, despite the presence of harmine, may tentatively be explained by the high concentration of harmol which can reduce Compound II of MPO to the native form.
Conclusion: The inhibition of MPO by *Peganum harmala* β-carboline alkaloids, herein reported for the first time, may explain the anti-inflammatory effect traditionally attributed to its herbal medicine.

1. Introduction

The heme enzyme myeloperoxidase (MPO, 1.11.2.2) plays a critical role in innate immunity system. It is very important for the optimal antimicrobial effect of neutrophils. This enzyme is packed in azurophilic granules of the neutrophil. It is released in the phagosome upon the phagocytosis producing the very potent oxidative compound hypochlorous acid (HOCl) starting from chloric ions Cl^- and hydrogen peroxide H_2O_2. HOCl oxidizes the vital members of pathogens (Klebanoff, 2005; Arnhold and Flemmig, 2010). However, MPO is considered as sward with two edges, in some cases it can be secreted outside the neutrophils causing oxidative damages to the host tissues (Nicholls and Hazen, 2009), promoting a series of inflammatory syndromes such as Alzheimer disease (Green et al., 2004), Parkinson disease (Choi et al., 2005; Hirsch and Hunot, 2009), renal damages (Anders et al., 1999; Scheuer et al., 2000; Malle et al., 2003; Ohtani et al., 2007; Madhusudhana Rao and Anand, 2011), rheumatoid arthritis (Goto et al., 2005), cancer (Ames, 1989; Esterbauer et al., 1990; Rojas et al., 2001; Henderson et al., 2003; Roman et al., 2008), and atherosclerosis (Klebanoff, 2005).

It is well documented that the key role of MPO in atherosclerosis is the oxidation of apolipoprotein B100 (Apo B100), the essential protein in low density lipoprotein LDLs (Itabe, 2009). Oxidation of high density lipoprotein HDLs by MPO products has also been reported (Zheng et al., 2004). MPO is also implicated in the decreasing of nitric oxide bioavailability that leads to endothelial dysfunction (Eiserich et al., 2002) and in the activation of protease cascades that are linked to plaque vulnerability (Fu et al., 2001). Because of these deleterious effects of MPO, it is important to find inhibitors of this enzyme which can inhibit the activity of MPO outside neutrophils.

Many natural compounds were reported as MPO inhibitors, including flavonoids (Regasini et al., 2008; Shiba et al., 2008), polyphenols (Kostálová et al., 2001; Sies et al., 2005; Han et al., 2007; Antonio et al., 2011) and melatonin (Kleszczynski et al., 2011). Most of them are competitive substrates for MPO, through preventing the production of HOCl and other hypohalides (Regasini et al., 2008). In fact, several authors demonstrated the inhibition of MPO by plants, such as *Hamamelis virginiana* L. (Díaz-González et al., 2012), *Hibiscus acetosella* Welw. ex Hiern, *Pteridium aquilinum* L. Kuhn (Pabuçcuoğlu et al., 2003; Zeraik et al., 2011; Tsumbu et al., 2012) and *Hypericum* sp. (Pabuçcuoğlu et al., 2003).

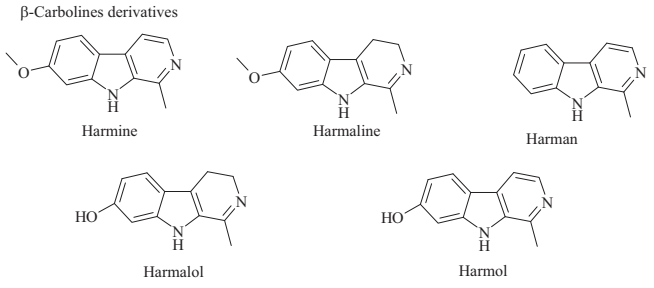

Fig. 1. The structures of *Peganum harmala* alkaloids.

Most of these plants contain polyphenols and flavonoids, shown to inhibit MPO at micromolar concentrations (Franck et al., 2006; Meotti et al., 2008; Regasini et al., 2008; Zeraik et al., 2011).

Peganum harmala (Zygophyllaceae) is a plant distributed in a large area of the world. In Algeria, the plant spontaneously grows in semi-arid and pre-desert regions and a poultice of seeds or aerial parts has been widely used in traditional medicine to treat inflammatory diseases (Baba-aissa, 2000). It has been reported that this plant largely used in Spanish traditional medicine as anti-inflammatory agents (Bremner et al., 2009).

Peganum harmala is rich in alkaloids of β-carbolines derivatives (harmine, harmaline, Harman, harmol and harmalol) (Fig. 1) (Lamchouri et al., 2000; Faskhutdinov et al., 2001; Kartal et al., 2003). These alkaloids have a broad spectrum of potent therapeutical activities such as anticancer (Lamchouri et al., 2000), analgesic (Farouk et al., 2008), hypothermic (Abdel-Fattah et al., 1995), antinociceptive (Monsef et al., 2004), antibacterial, antiviral (Prashanth and John, 1999; Astulla et al., 2008) and hallucinogen effects (Mahmoudian et al., 2002). Harmine inhibits the production of interleukin-6 (IL-6) in RAW264 cells and induces transcription of TNF-α; it attenuate the inducible NO synthase in a dose dependent manner (Zhao et al., 2012). The ethyl acetate and total plant extract has been reported to be anti-inflammatory by inhibiting the activity of TNF-α (10 μg/mL) (Bremner et al., 2009).

Previous studies reported the quantification of *Peganum harmala* compounds by spectrophotometry (Hemmateenejad et al., 2006), high-performance liquid chromatography (HPLC) (Kartal et al., 2003), gas chromatography (GC), GC/mass spectrometry (GC/MS) (Zayed and Wink, 2005), high voltage ionophoresis thin layer chromatography densitometry (TLC) and high performance TLC (HPTLC) (Sobhani et al., 2002; Pulpati et al., 2008). However, the very near structures of *Peganum harmala* alkaloids make the separation quite difficult.

In the present study, the total alkaloids were extracted from Algerian *Peganum harmala*, separated and quantified by HPLC. The structures of major alkaloids, β-carbolines, are similar to those of tryptamine derivatives which are good inhibitors of MPO (Soubhye et al., 2010) which led us to investigate the effect of these alkaloids on MPO *in vitro* and by virtual docking studies. This will allow drawing up a preliminary structure–activity relationship and understanding the mechanisms of this inhibition. The obtaining data can be a starting point to develop anti-inflammatory drugs targeting MPO.

2. Material and methods

2.1. Chemicals and reagents

All solvents and sorbents were from Merck, Darmstadt, Germany. All solutions were prepared with water purified in a Milli-Q system (Millipore, Bedford, MA). Harmine (98%), harmaline (90%) harmane (98%) and harmol (98%) were bought from Sigma-Aldrich and harmalol (90%) from Extrasynthese. Harmaline was purified by silicagel 60 column chromatography (CC) using CH_2Cl_2/MeOH/NH_4OH (100/20/0.5) as mobile phase. The fractions were combined

from their HPLC profiles, evaporated under vacumn and pure harmaline (98%) was obtained. Harmalol 90% was purified on C_{18} CC by stepwise gradient elution with mixtures of MeOH and aqueous solution with 0.1% formic acid (2%, 4%, 6%, 8%, 10%, 12%, 14% and 16% MeOH). The fractions were combined from their similar HPLC profiles, evaporated under vacuum and pure harmalol (98%) was obtained.

Each MPO solution is characterized by its protein concentration (mg/mL), its activity (U/mL), and its specific activity (U/mg).

The protein concentration was measured by the Lowry assay, for both MPO and LDL (Van Antwerpen et al., 2005).

2.2. Phythochemical studies

2.2.1. Plant material

Peganum harmala aerial part, seeds and roots were harvested in Sétif (Algeria) in June 2011. A voucher specimen was deposited at the Herbarium of the Botanical Garden of Meise, Belgium (BR0000013496968). The plant material was washed and dried in a ventilated room (40 °C) and then ground to a fine powder.

2.2.2. Extraction of total alkaloids

The extraction was performed using the method described by Kartal et al. (2003) with some modifications. Briefly, the plant material (20 g) was extracted three times with 200 mL ethanol (80%, v/v) for 30 min in an ultrasonic bath. The extracts were combined, filtered, concentrated under vacuum, treated with HCl (2% v/v) and 100 mL petroleum ether to remove apolar impurities. The aqueous layer was brought to pH 9 with ammonia and extracted three times with chloroform (20 mL). The organic layer was evaporated under vacuum to obtain total alkaloids for each part of the plant (aerial parts, seeds and roots) (Kartal et al., 2003).

2.2.3. Quantitative HPLC analysis of Peganum harmala alkaloids

2.2.3.1. Chromatographic conditions. The chromatographic separation was performed on a HPLC Kontron System consisting of a HPLC Pump 422, a Degasys DG-1300 (Uniflows), a HPLC Autosampler 465, a column oven (Spark Holland, Mistral) and a HPLC UV-detector 430. The compounds were eluted on a Luna 5 μ phenyl hexyl column (250 × 4.60 mm) (Phenomenex, Utrecht, Netherlands) using a gradient of 0.1% formic acid in water and CH_3CN–CH_3OH (1:1, v/v). The gradient was as follows: equilibration time 15 min at 0% B; 0 min, 0% B; 5 min, 10% B; 10 min, 25% B; 15 min, 50% B; 17 min, 100% B; at a flow rate of 1.5 mL/min at 40 °C. The UV-detector was set at 254 nm and 20 μl samples were injected.

2.2.3.2. Preparation of standard solutions. Five stock solutions (1 mg/mL) were prepared and diluted in 0.1% HCOOH as follows: harmine and harmol (10–80 μg/mL), harmaline (25–250 μg/mL), harmane (10–70 μg/mL) and harmalol (25–300 μg/mL). Linear curve fitting was applied with determination coefficients (R^2) typically higher than 0.98.

2.2.3.3. Sample preparation. Two hundred milligrams of plant material were macerated three times with methanol: formic acid (99: 1) for 1 h and centrifuged (1500*g*; 10 min). The supernatant was evaporated under vacuum and taken up in 2 mL of 0.1% formic acid. The solution was sonicated and centrifuged (1500*g*; 10 min), filtered through Whatman 0.45 μm Filter.

2.3. Pharmacological studies

2.3.1. Assessment of MPO inhibition

Recombinant MPO was prepared as described by Moguilevsky et al. (1991). Each batch solution is characterized by its protein concentration (milligrams per milliliter), its activity (units per milliliter) was determined according to Hewson and Hager (1979). HOCl, which is produced by MPO/H_2O_2/Cl^- system, quantitatively transforms taurine into taurine chloramine that can be, in turn, determined by 5-thio-2-nitrobenzoic acid (TNB). The reaction mixture contained the following reagents in a final volume of 200 μL: pH 7.4 phosphate buffer (10 mM PO_4^{3-}/300 mM NaCl), taurine (15 mM), solutions of *Peganum harmala* raw alkaloid extracts (20 μg/mL) or *Peganum harmala* molecules (0.05, 0.2, 0.5, 1, 2, 5 and 10 μM) and a fixed amount of the recombinant MPO (6 μL of MPO batch solution diluted 2.5 times with water, 40 nM). When necessary, the volume was adjusted with water. This mixture was incubated at 37 °C and the reaction initiated with 10 μL of H_2O_2 (100 μM). After 5 min, the reaction was stopped by the addition of 10 μL of catalase (8 U/μL). To determine the amount of taurine chloramines produced, 50 μL of 1.35 mM solution of TNB were added and the volume adjusted to 300 μL with water. The absorbances were measured at 412 nm with a microplate reader. To remove the influence of HOCl scavenging carried out by the compounds, the same procedure of MPO inhibition assay was done but with adding 6 μL of HOCl (60 μM) instead of the solution of MPO and H_2O_2. The difference of the absorbance between the two tests was considered and the IC_{50} values were determined using standard procedures, taking into account the absence of hydrogen peroxide as 100% inhibition and the absence of inhibitors as 0% inhibition (Van Antwerpen et al., 2008). To study the scavenging effect on H_2O_2, the same test was done by addition of 10 μL of H_2O_2 (100 μM) in the absence of MPO, the same concentrations of the tested alkaloids were added. Percentage values of MPO inhibition were determined for 7 concentrations of inhibitors (0.05, 0.2, 0.5, 1, 2, 5 and 10 μM). The data were obtained from three independent tests.

2.3.2. Determination of inhibition of LDL oxidation

2.3.2.1. Preparation of LDL. Human plasma served for the isolation of LDL by ultracentrifugation according to Havel et al. (1955). Before oxidation, the LDL fraction ($1.019 < d < 1.067$ g/mL) was desalted by two consecutive passages through PD10 gel-filtration columns (Amersham Biosciences, The Netherlands) using PBS buffer. The different steps were carried out in the dark (Havel et al., 1955).

2.3.2.2. Inhibition of LDL oxidation. LDL oxidation was carried out at 37 °C in a final volume of 500 μL. The reaction mixture contained the following reagents with their final concentrations: pH 7.2, PBS buffer, MPO (1 μg/mL), LDL (1000 μg/mL), 2 μL 1 N HCl (4 mM), *Peganum harmala* compounds at different concentrations and H_2O_2 (100 μM). The reaction was stopped after 5 min by cooling the tubes in ice. The assay was performed as described by Moguilevsky et al. (2004) in a NUNC maxisorp plate (VWR, Zaventem, Belgium): 200 ng/well of LDL was coated overnight at 4 °C in a sodium bicarbonate pH 9.8 buffer (100 μL). Afterward, the plate was washed with Tris-buffered saline (TBS 80) buffer and then saturated during 1 h at 37 °C with the PBS buffer containing 1% BSA (150 μL/well). After washing the wells twice with the TBS 80 buffer, the monoclonal antibody Mab AG9 (200 ng/well) obtained according to a standard protocol and as previously described was added as a diluted solution in PBS buffer with 0.5% BSA and 0.1% of Polysorbate 20. After incubation for 1 h at 37 °C, the plate was washed four times with the TBS 80 buffer and

a 3000 times diluted solution of IgG antimouse alkaline phosphatase (Promega, Leiden, The Netherlands) in the same buffer was added (100 μL/well). The wells were washed again four times and a revelation solution (150 μL/well) containing 5 mg of para-nitrophenyl phosphate in 5 mL of diethanolamine buffer was added for 30 min at room temperature. The reaction was stopped with 60 μL/well of 3 N NaOH solution. The absorbances were measured at 405 nm with a background correction at 655 nm with a Bio-Rad photometer for a 96-well plate (Bio-Rad laboratories, CA, USA). Results were expressed as IC_{50} (μM) (Van Antwerpen et al., 2005).

2.3.3. Docking experiments

Docking studies were based on the X-ray structure of human myeloperoxidase complexed to cyanide and thiocyanate (PDB code: 1DNW) (Blair-Johnson et al., 2001; Friesner et al., 2004). The PDB was prepared using the Protein Preparation Wizard protocol in the Schrodinger software graphical user interface Maestro. The X-ray water and CN^- and SCN^- molecules were removed from the active site. The ligand input files were prepared according to the following procedure. The initial 3D structures of the ligands were generated using Corina (Sadowski and Gasteiger, 1993), and the ligand partial charges were ascribed using the OPLS force-field as performed by Glide (Friesner et al., 2004). As part of an effective 3D ligand structure preparation process, the Epik program was used to predict pKa values and generate protonation states of all ligands (Shelley et al., 2007). Docking was performed using the Glide XP docking protocol and scoring function which approximates a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using a series of hierarchical filters (www.schrodinger.com).

2.3.4. Determination of redox potential

The linear cyclic voltammetry measurements were achieved in a conventional three-electrode cell at 25 ± 2 °C, using an Epsilon potentiostat (BASinc. West Lafayette. USA). The working electrode was a glassy carbon disk polished with 0.05 mm alumina (Metkron) before each run. The auxiliary electrode was a platinum wire. The reference electrode was Ag/AgCl (3 M NaCl). Harmala alkaloids were dissolved in phosphate buffer (0.1 M, pH = 7.4) and diluted by the same buffer until 10^{-4} M. Cyclic voltammograms were obtained by a single cycle performed at a scan rate of 100 mV s^{-1}. Concerning scan rate studies, the scan rate varied between −200 and 1000 mV s^{-1}. E' values were obtained by converting redox potentials from Ag/AgCl, 3 M NaCl to normal hydrogen electrode N.H.E by adding +198 mV. The standard redox potentials of our alkaloids are obtained according to Nernst equation as following $E' = E^{\circ\prime} + RT/nF(\ln(a_{ox}/a_{red}))$, in our compounds the value of $RT/nF(\ln(a_{ox}/a_{red}))$ is very low that means $E' \approx E^{\circ\prime}$ (Garrels and Christ, 1990). In our experiment we determined E' according to hydrogen electrode and we did not determined $E^{\circ\prime}$ because the values of the last one are close to the values of E'. The abbreviation of Nernst equation: E' is the reduction potential, $E^{\circ\prime}$ is the standard redox potential, R is the universal gas constant, T is the absolute temperature, F is the Faraday constant and n is the number of moles of electrons transferred in the reaction.

2.3.5. Statistical analysis

SigmaStat® software (SPSS, 3.0) was used for the analysis. Data are presented as mean ± SD and were evaluated by one-way ANOVA, with Bonferroni *post-hoc* test. When appropriate, an ANOVA on Rank with Dunn's *post-hoc* test was used.

3. Results

3.1. Phytochemical analysis

The different parts of *Peganum harmala* were analyzed by HPLC (Fig. 2) and the compounds were identified by comparison of their retention time (t_r) on HPLC and Rf on TLC chromatography. Five β-carboline alkaloids – harmine, harmaline, harmane, harmol and harmalol – were found and their t_r values were determined (Table 1). Harmine, harmaline and harmol were found to be the major alkaloids in *Peganum harmala*.

The results indicate that the seeds contain harmaline and harmine as major alkaloids (3.8% and 2.93%, respectively), followed by harmalol (0.12%). It contained a low quantity of harmane and harmol. We found that the root extract contains very high proportion of harmol (2.9%) and harmine (3.15%) and considerable quantity of harmalol (0.65%), while we noticed the absence of harmaline and harmane. The aerial parts including leaves, stems and flowers contain harmine and harmaline at the same quantity (0.05%) followed by harmalol and harmane (0.026% and 0.011%, respectively).

3.2. Assessment of MPO inhibition by the extracts

Total alkaloids were tested by taurine chloramine test to assess their capacity to inhibit MPO. High to mild activities were found for these extracts; at 20 μg/mL, the total alkaloids inhibited MPO by 43 ± 4% for aerial parts, 97 ± 5% for seeds and 15 ± 6% for roots ($n = 3$).

3.3. Determination of MPO inhibition by Peganum harmala alkaloids

The pure alkaloids harmine, harmaline, harmalol, harmane and harmol were tested by taurine chloramine assay for their capacity to inhibit MPO (Table 2). While no scavenging effect of HOCl and H_2O_2 were observed (data not shown); harmaline was shown to have the best activity inhibiting MPO in the nanomolar range ($IC_{50} = 80$ nM); it was found to be even more active than 5-fluorotryptamine ($IC_{50} = 200$ nM, see supporting information). The compounds with a methoxy group on the indole ring are more active than those without polar groups. Like 5-hydroxy-tryptamine, the alkaloids with an OH group on the indole ring have no activity towards MPO. Therefore, the highest inhibitory effect observed in seeds and the moderate effect of aerial parts (Table 1) could be explained by their harmine and harmaline content. Despite the high concentration of harmine in roots, this part of plant was found to have low activity in the inhibition of MPO.

3.4. Assessment of the inhibition of MPO-induced LDL oxidation

The MPO-dependent oxidation of LDL is an important event by which MPO contributes to the development of atherosclerosis. This oxidation process starts with LDL binding MPO which modifies some properties of the enzyme, notably modulating the behavior of its inhibitors. Thus the inhibition effects of *Peganum harmala* alkaloids were determined on the MPO-dependent LDL oxidation. No significant changes in activities were found between MPO inhibition (Table 2) and LDL oxidation inhibition (Table 3). In both tests, harmaline has the highest inhibitory activity with IC_{50} of 80 nM and 70 nM, respectively; the compounds that are not active towards MPO were also not be able to inhibit the MPO-dependent LDL oxidation.

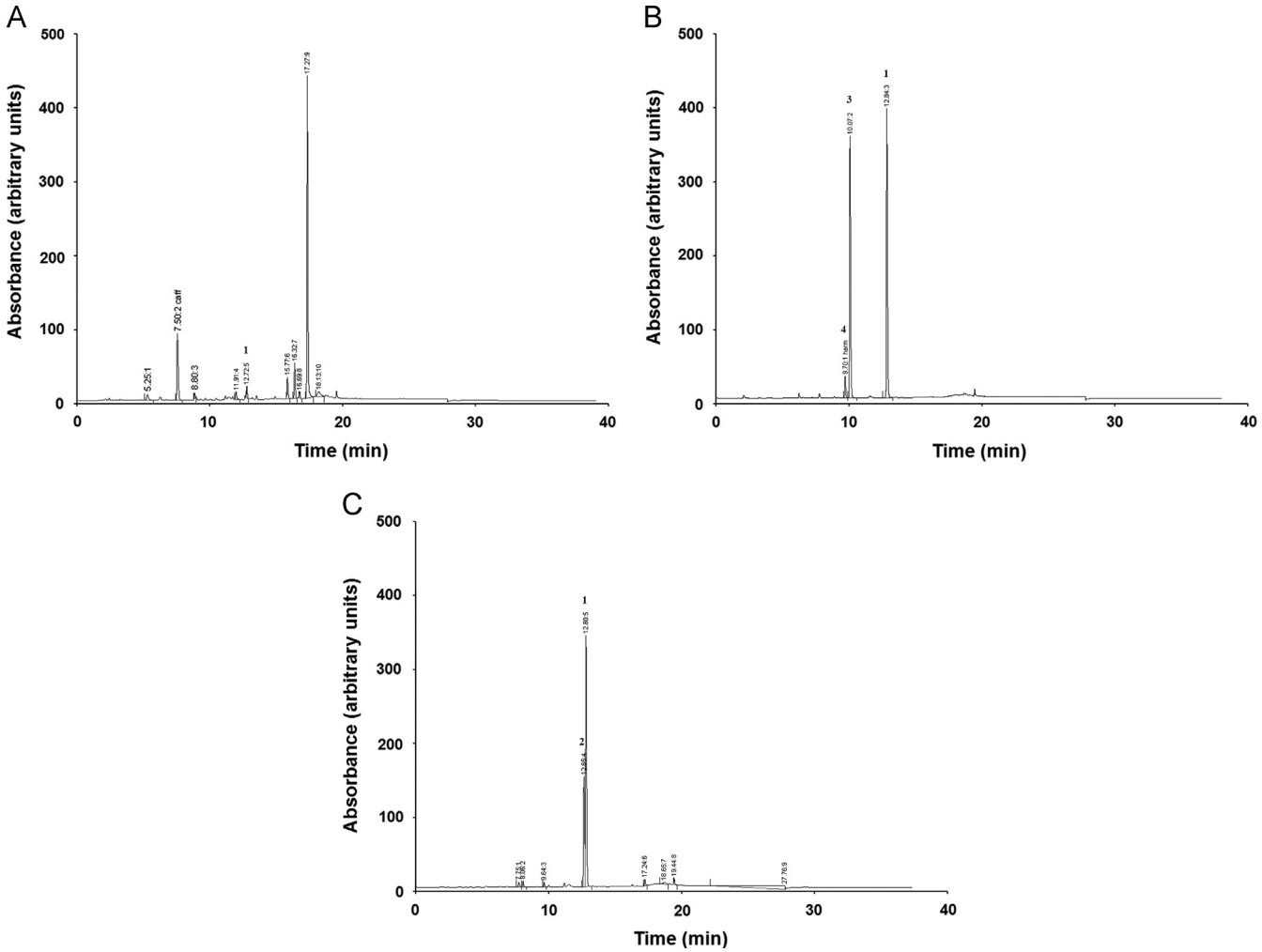

Fig. 2. HPLC chromatograms of *Peganum harmala* methanolic extracts (A) aerial parts extract, (B) Root extract and (C) seed extract. Peak identification: Harmine (1), harmaline (2), harmol (3), harmalol (4).

Table 1
Alkaloids levels (%) in different parts of *Peganum harmala* and inhibition of MPO by total alkaloids.

	Concentration of alkaloids (mean ± SD; $n=3$)			Retention time (min)
	Seeds	Roots	Aerial parts	
Total alkaloids	5	1.75	1.86	
Harmine	2.93 ± 0.01	3.2 ± 0.2	0.055 ± 0.005	12.8
Harmaline	3.8 ± 0.2	n.d.	0.05 ± 0.01	12.7
Harmane	0.029 ± 0.002	n.d.	0.011 ± 0.001	11.7
Harmol	0.020 ± 0.002	2.9 ± 0.2	n.d.	10.2
Harmalol	0.120 ± 0.007	0.65 ± 0.08	0.026 ± 0.004	9.7
% of MPO inhibition by total alkaloids at 20 μg/mL	97 ± 5	15 ± 6	43 ± 4	

n.d.—not detectable.

3.5. Docking experiments

To study the interaction between the alkaloids and the enzyme, *Peganum harmala* alkaloids were compared by docking experiments with tryptamine derivatives, known as MPO inhibitors with low IC_{50}. Docking of 5-fluorotryptamine showed stacking pose of the indole 6-membered ring onto the pyrrole ring D of the heme. This pose featured one salt bridge with Glu102 and one hydrogen bond with Thr100 (Soubhye et al., 2010). All of β-carboline alkaloids tested also showed stacking poses with hydrogen bonding between the nitrogen atom of the indole and the heme propionate. Beside this bond the compounds with methoxy moieties (harmine and harmaline) featured additional hydrogen bond between the oxygen atom of the compound and Gln91 (Fig. 3A and B). A hydrogen bond was found between nitrogen atom of dihydropyridine of harmaline and Glu102 (Fig. 3B). The hydroxyl group in harmol featured two hydrogen bonds with His95 and Gln91 (Fig. 3C), whereas, the hydroxyl group of harmalol, formed only a hydrogen bond with His95 (Fig. 3D). A hydrogen bond was also found between the nitrogen of harmalol dihydropyridine and Glu102. In the case of

harmane no additional hydrogen bound was observed due to the absence of functional group on indole ring.

Free energy score (ΔG score) values (Table 2) confirm that harmalol and harmaline, the compounds with oxy group on the indole ring and dihydropyridine, have the highest affinity with ΔG score of −6.9 and −6.1 kcal/mole respectively. All of the tested molecules predicted affinity more or equal to 5-fluorotryptamine (Table 2). The aromatic nitrogen seems to reduce the affinity and the ΔG score values of harmaline and harmalol are significantly higher than harmine and harmol. The absence of protonable group on harmane reduces its affinity towards MPO.

Table 2
Inhibition of MPO by *Peganum harmala* alkaloids (IC_{50} in the taurine chloramine test) and predicted free energies of binding obtained from docking experiments.

Alkaloid	IC_{50} (μM; $n=3$)	ΔG (kcal/mol)
5-Fluorotryptamine	0.20 ± 0.03	−4.8
Harmine	0.26 ± 0.03	−4.4
Harmaline	0.08 ± 0.01[b]	−6.1
Harmalol	Not active[a]	−5.1
Harman	0.72 ± 0.04[b]	−3.1
Harmol	Not active[a]	−6.9

[a] Highest tested concentration 10 μM;
[b] IC_{50} are significantly different from 5-fluorotryptamine ($p<0.001$, Bonferroni's test).

Table 3
IC_{50} values for the inhibition of the oxidation of LDL carried out by MPO/Cl^-/H_2O_2, redox potential values according to normal hydrogen electrode.

Compound	IC_{50} for the inhibition of the LDL oxidation (μM)	Redox potential (mV)
Harmine	0.12 ± 0.05	1114
Harmaline	0.07 ± 0.02	1014
Harmalol	Not active[a]	629 and 778
Harmane	0.52 ± 0.03	1003
Harmol	Not active[a]	532 and 644

[a] Highest tested concentration, 5 μM.

3.6. Determination of redox potentials

Tryptamine derivatives are reversible inhibitors that interact with the enzymatic activity cycle. The native MPO Por (Fe^{III}) is activated by H_2O_2 through a $2e^-$ oxidation process to give the

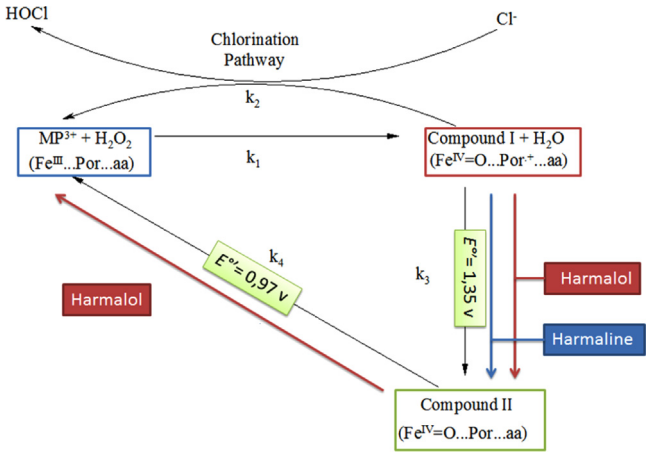

Fig. 4. The chlorination and peroxidase cycles of MPO. Reaction k1: ferric MPO is oxidized by hydrogen peroxide to compound I. Reaction k2: compound I is directly reduced back to the resting state by halides, thereby releasing hypohalous acid. Reaction k3: compound I is reduced to compound II by a one-electron donor, all the alkaloids are thought to be able to reduce Compound I to Compound II. Reaction k4: compound II is reduced to native state, only the inactive alkaloids can easily reduce Compound II to the native enzyme. Reactions k1 and k2 constitute the chlorination cycle. Reactions k1, k3, and k4 constitute the peroxidase cycle.

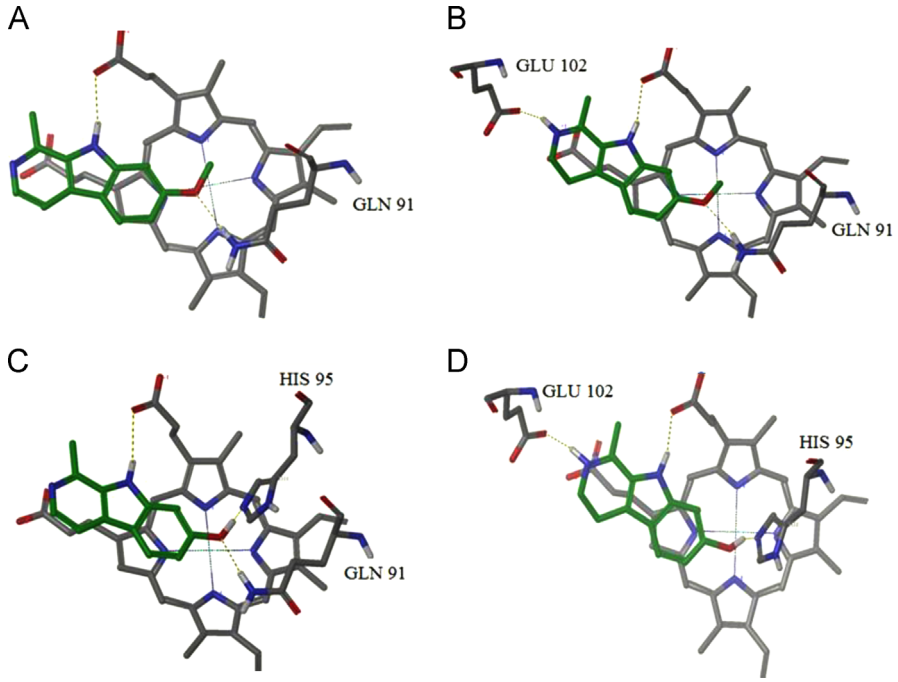

Fig. 3. (A) The interaction of harmine with MPO active site residues. Stacking pose is observed with ring D of the heme, two hydrogen bonds formed with Gln91 and heme propionate. (B) Docking poses of harmaline illustrating the stacking with pyrrole D of the heme, the ionic interaction with Glu102 and hydrogen bond with propionate. (C) Staking pose of harmol onto D ring of heme. The hydroxyl group forms two hydrogen bonds with Gln91 and His95, the nitrogen atom of indole forms hydrogen bond with propionate. (D) Docking pose of harmalol indicating stacking with heme ring D, two hydrogen bonds between hydroxyl group of the compound and His 95 and between nitrogen of indole and Gln91. Salt Bridge is also observed between nitrogen atom of dihydropyridine and Glu102.

active form Compound I $Por^{+\bullet}$ (Fe^{VI}). This radical from Compound I can be reduced to the native form via $2e^-$ in the presence of Cl^- to yield the powerful oxidative species HOCl (*i.e.* "chlorination cycle"). Compound I can also be reduced by $1e^-$ donors to Compound II Por (Fe^{VI}). This form of MPO can be reduced to the native form upon reaction with another electron donor (*i.e.* "peroxidase cycle"). It must be noticed that Compound II is considered as an inactive form that cannot oxidize Cl^- to HOCl (Fig. 4) (Jantschko et al., 2005).

Because the activity of MPO depends on redox reactions, it is important to compare the redox potentials of tested alkaloids with those of MPO. The E'° values of MPO redox couples have been reported as 1160 mV (Compound I/ MPO Por (Fe^{III})), 1350 mV (Compound I/Compound II) and 970 mV (Compound II/ MPO Por (Fe^{III})) (Jantschko et al., 2005). Table 3 shows that all the active alkaloids (harmine, harmaline and harmane) have almost the same redox potentials, slightly above 1000 mV. The phenol groups of the inactive alkaloids harmol and harmalol imply lower redox potentials. As a consequence, they can easily reduce MPO Compound II to the native MPO preventing the accumulation of the inactive form of MPO and keeping MPO in chlorination cycles.

4. Discussion

The total alkaloids of seeds present the most potent inhibitory activity on MPO (97% inhibition at 20 μg/mL); their high concentration of the strong inhibitors harmine (2.9%, w/w) and harmaline (3.8%, w/w) may explain this activity. Although the roots have high content in harmine (3.2%, w/w), their alkaloids show a modest inhibition of MPO (15% inhibition at 20 μg/mL); this may be explained by the concomitant presence of harmol (2.9%, w/w) probably through its redox capabilities. Harmol may be able to easily reduce both MPO Compound I and Compound II preventing the accumulation of Compound II the inactive form of MPO. Aerial parts inhibited MPO by 43% (20 μg/mL); this effect may be due to harmine, harmaline and harmane with a partial reduction of activity by the inactive harmalol. This high activity of *Peganum harmala* seeds and aerial parts towards MPO is promising like previously tested herbal extracts and phytochemicals; for example, *Passiflora edulis* Sims and *Passiflora alata* Curtis extracts were inhibitor at 1 mg/mL; *Manihot esculenta* Crantz, *Abelmoschus esculentus* (L.) Moench, *Hibiscus acetosella* Welw. ex Hiern, *Hypericum* sp and *Pteridium aquilinum* (L.) Kuhn at microgram level (Pabuçcuoğlu et al., 2003; Zeraik et al., 2011; Tsumbu et al., 2012); curcumin, resveratrol, kaempferol, quercetin, myricitrin and myrcetin at 100 μM (Franck et al., 2006; Meotti et al., 2008; Regasini et al., 2008; Zeraik et al., 2011). Interestingly, this inhibition of the native enzyme is not affected by the LDL-bound enzyme. Upon LDL-binding (i) there is narrowing of the entrance tunnel leading to the active site, which prevents access to voluminous inhibitors; (ii) there is an increase in the lipophilicity of the enzyme that modifies the activity of some inhibitors. Clearly the *Peganum harmala* inhibitors are not affected by this binding.

Docking experiments indicate that all the *Peganum harmala* alkaloids, including the non-active harmol and harmalol, have high affinity for MPO. As for tryptamine derivatives, the residue Glu102 plays a critical role in binding the molecules to the MPO active site. There is however no clear correlation between G score values of the inhibitors toward MPO and their inhibition activity, indicating that other factor(s) contribute to the inhibition of MPO.

Based on data obtained with tryptamine derivatives (Jantschko et al., 2005), it has been suggested that an efficient MPO inhibitor needs to present a redox potential inferior to the couple Compound I/Compound II (1350 mV) so it can easily reduce Compound I to form Compound II which cannot react with halogen ions, shifting the chlorination cycle to the peroxidation cycle; simultaneously, the inhibitor must have a redox potential higher than the couple Compound II/ MPO Por (Fe^{III}) (970 mV) to prevent the reduction of Compound II to the native form and block further redox cycles (Jantschko et al., 2005); the kinetic aspects of these reactions are also important for this inhibition of MPO. The measured redox potential values (Table 3) indicate that the inhibitory alkaloids have an oxidation potential suited to these redox requirements. By contrast, the inactive harmol and harmalol have very low redox potential values (< 800 mV), indicating that these molecules can reduce both Compound I and Compound II in a very fast rate, preventing the accumulation of Compound II and the blockage of redox cycles. This explains why harmol and harmalol do not inhibit the enzyme and prevent inhibition by the other β-carbolines despite their predicted efficient interactions with the enzyme.

5. Conclusion

This study demonstrates for the first time an anti-inflammatory activity of *Peganum harmala* alkaloids through the inhibition of myeloperoxidase. Total alkaloids of seeds are particularly active, followed by total alkaloids of aerial part. The β-carbolines harmaline, harmine and harmane are key contributors to this inhibition. Given the psycho-activity and toxicity of the alkaloids, their administration to patients may be problematic. The extracts and alkaloids of *Peganum harmala* should be reserved to external use, as is the case in Algerian traditional medicine. These compounds would however be an excellent starting point for pharmacomodulation to develop suitable anti-inflammatory drugs. It is interesting to note that the 3 active β-carbolines are highly lipophilic and can easily penetrate the blood brain barrier. This explains their psycho-active properties but may also be an important factor to protect CNS tissues from MPO-induced damages.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2014.03.070.

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