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Vasorelaxant effects of harmine and harmaline extracted from *Peganum harmala* L. seed's in isolated rat aorta

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Abstract

The present work describes the mechanisms involved in the vasorelaxant effect of harmine and harmaline. These alkaloids induce in a dose-dependent manner the relaxation in the aorta precontracted with noradrenaline or KCl. However, the removal of endothelium or pre-treatment of intact aortic ring with L-NAME (inhibitor of NOSe synthetase) or with indomethacin (non-specific inhibitor of cyclo-oxygenase), reduces significantly the vasorelaxant response of harmaline but not harmine. According to their IC_{50} values, prazosin (inhibitor of α-adrenorecepteors) reduces the vasorelaxant effect only of harmaline, whereas, pre-treatment with IBMX (non-specific inhibitor of phosphodiesterase) affects both the harmaline and harmine-responses. Inhibitions of L-type voltage-dependent Ca^{2+} channels (VOCs) in endothelium-intact aortic rings with diltiazem depress the relaxation evoked by harmaline as well as by harmine. Pre-treatment with harmaline or harmine (3, 10 or 30 μM) shifted the phenylephrine-induced dose response curves to the right and the maximum response was attenuated indicating that the antagonist effect of both alkaloids on α_1-adrenorecepteors was non-competitive. These two alkaloids also exert an antioxidant activity by scavenging the free radical generated by DPPH. Therefore, the present results suggest that the vasorelaxant effect of harmaline but not harmine is related to its action on the prostacyclin pathway and on the endothelial cells to release NO. However, both alkaloids can act as blockers VOCs, as inhibitors of phosphodiesterase resulting in an increase of the second messenger (cAMP and cGMP) levels and finally reduce the levels of free radicals in tissues.

Keywords: *Peganum harmala* L.; Harmaline; Harmine; Rat aorta; Vasorelaxant response; Antioxidant; Endothelium

1. Introduction

Peganum harmala L. (Zygophyllaceae), known as *harmel*, grows spontaneously in uncultivated and rocky areas from Mediterranean region (semiarid region) [1]. *Peganum harmala* is used in traditional medicine and is rich in alkaloids that have a wide spectrum of pharmacological actions in various areas. These include antispasmodic, antipyretic [2,3], anticancerous [4], central nervous system effects [5], hallucinogenesis [6], central monoamine oxidase inhibition [7], binding to various receptors including 5-HT and the benzodiazepine-binding receptors [8], platelet aggregation inhibitory [9] and immunomodulatory effects [10]. Ethnopharmacological observations have reported the hypotensive effects of *Peganum harmala* [1], also Aarons et al. [11], have demonstrated that harmala alkaloids include systemic arterial blood pressure reduction. However, the cellular and molecular mechanisms by which these alkaloids exert their actions remains unclear even though recent studies have elucidated in part the mechanism of action related to the vasorelaxant effects of synthetic β-carboline harmala-alkaloids [12,13].

We recently reported the vasorelaxant effect of a methanolic extract from seeds of *Peganum harmala* (MEP); our results suggested that the vasodilatory effect of this extract is endothelium-independent and is related to the inhibition of cyclic-AMP phosphodiesterase [14]. The present study

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extends the exploration of the vasorelaxant-mechanism of harmala-alkaloids and compares the mechanism-pathways between natural compounds extracted from the plants and their synthetic form as proposed by Shi et al. [13].

2. Material and methods

2.1. Chemical reagents and drugs

Acetylcholine chloride, noradrenaline (bitartrate salt), phenylephrine hydrochloride, diltiazem, sodium nitroprusside, N^{ω}-nitro-L-argenine methyl ester (L-NAME) and indomethacin were purchased from Sigma Chemical Co. (St. Louis, USA) and dissolved in distilled water. Prazosin (Pfizer), IBMX (3-isobutyl,1-methylxanthine), Sigma Chemical Co. (St. Louis, USA), were dissolved in dimethylsulfoxide (DMSO). The final DMSO concentrations (0.001%) did not significantly affect the results. Ascorbic acid (10^{-4} M) was added to fresh noradrenaline solutions to prevent possible oxidation.

2.2. Plant

Peganum harmala L. (Zygophyllaceae) fresh seeds were collected from the Atlas region of Morocco, in May 2000 and botanically identified by the botanical section of U.F.R: Naturals Products, Faculty of Medicine and Pharmacy (Rabat), where a specimen is preserved (number PH-00052).

2.3. Extraction of natural compound

Fresh and powdered seeds were prepared as previously described (Berrougui et al. [14]). The natural compounds (harmaline and harmine) were extracted from the last fraction (methanolic extract) as fellow: briefly, methanolic extract was eluted on a silica gel column initially with pure chloroform ($CHCl_3$), and then increasing amounts of methanol (CH_3OH). All fractions obtained from this silica gel column were subjected to thin layer chromatography (TLC) examination using $CHCl_3$–MeOH (9:1). Same fractions were pooled and the harmine and harmaline were washed successively in $CHCl_3$ and MeOH, crystallised and then analysed by TLC, ^{1}H NMR, ^{13}C NMR (Nuclear Magnetic Resonance) and mass spectrometry.

2.4. Animals

Wistar rats weighing 100–120 g, were purchased from Harlan Ibérica (Barcelona, Spain). All experiments were performed according to guidelines for the ethical treatment of animals of the European Union (86/609/EEC). All rats were fed with standard rat chow (Panlab SRL, Barcelona, Spain) and maintained in a temperature-controlled room ($24 \pm 2\,^{\circ}C$) with $60 \pm 20\%$ relative humidity, a 12 h light–dark cycle and with free access to standard rat chow and drinking water. All experiments were performed on 12–14-week-old rats. The animals were sacrificed by cervical dislocation and the aorta was rapidly dissected.

2.5. Aortic ring preparation

The descending thoracic aorta was placed in a modified Krebs-Henseleit solution (PSS) containing (mM), NaCl (118), KCl (4.75), $NaHCO_3$ (25), $MgSO_4$ (1.2), $CaCl_2$ (1.8), KH_2PO_4 (1.2) and glucose (11). After excess fat and connective tissues were removed, the aortas were cut into 2–3-mm rings. Aortic rings were mounted under a basal tension of 2 g in 20 ml organ baths containing PSS as previously describe (Herrera-Gonzalez et al., 1996) and attached to an isometric transducer (harvard UF-1), the signal was recorded by powerlab® data acquisition system (AD-Instruments). The tissue bath was maintained at 37 °C and bubbled with a 95% O_2–5% CO_2 gas mixture. In some experiments, the endothelium was mechanically removed by gently rubbing the inner surface of the rings. The absence of endothelium E(−) was then verified by addition of acetylcholine (ACh 10^{-6} M) in aortic rings previously contracted by phenylephrine (Phe 10^{-6} M). Each preparation was allowed to equilibrate for at least 60-min prior to initiation of experimental procedures, and during this period the incubation media was changed every 20 min [15].

After equilibration, the following experiments were performed:

To determine whether harmine and harmaline could relax an existing contraction, aortic rings were contracted by a single sub-maximal concentration of noradrenaline (NA 10^{-6} M) or KCl (80 mM). When the contractile response to either agonist was stable, harmine or harmaline were added in progressively increasing cumulative concentrations (1–300 μM) at 20-min intervals (time interval necessary to done the maximal effect of extract: plate of action). Harmaline was dissolved in the PSS, whereas, harmine was in the DMSO with final DMSO-concentrations (0.001%) that did not significantly affect the results. The results were expressed as a percentage of the maximal control agonist-induced response.

The involvement of the endothelium-related vasorelaxation in the harmine and harmaline-induced relaxation was examined in intact aortic ring pre-treated with nitric oxide synthase-inhibitor, L-NAME (30 μM) or with non-selective cyclooxygenase inhibitor, indomethacin (10 μM) [13].

To investigate the effects of these alkaloids on the endothelium-independent relaxation, endothelium-denuded aortic preparations E(−) were incubated for 30 min with harmine or harmaline (10^{-5} M) prior to the precontraction with phenylephrine (10^{-6} M), and when the contraction was stabilised, cumulative concentrations of sodium nitropruside (10^{-10} to 10^{-7} M, nitric oxide donor) were added.

In another experiment, intact aorta was treated with an α-adrenoreceptors inhibitor, prazosin (10^{-8} M) or a non-specific phosphodiesterase-inhibitor, IBMX (10^{-4} M). Cumulative concentrations of harmine and harmaline were added following the stabilisation phase of KCl-induced contraction.

The effect of alkaloids on the VOCs was studied in the aortic ring previously treated during 20 mn with a voltage-dependent Ca^{2+}-channels inhibitor, diltiazem (10^{-6} M). Preparation ring was then contracted by noradrenaline followed by the adding of cumulative concentration of harmine or harmaline were added.

In the aim to explore the involvement of α_1-adrenoceptors on the vasorelaxant effect of alkaloids, three concentrations of harmine and harmaline (3, 10 and 30 μM) were added 20 min before addition of cumulative concentrations of phenylephrine-induced contraction (10^{-9} to 10^{-5} M). Results are expressed as the percentage of the maximum contractile tension to phenylephrine before and after harmine or harmaline addition.

The net relaxation induced by extract was expressed as percent decrease in the tension existing at the moment before drugs administration (100% = return to baseline) [9].

2.6. Free radical scavenging activity

Determination of the free radical scavenging activity of harmine and harmaline was conducted using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH)-test as already described by Mensor et al. [16]. Briefly, DPPH solution dissolved in ethanol was added to harmine and harmaline at different concentrations (10 μM) and allowed to react at room temperature. After 30 min, the absorbency was measured at 517 nm. DPPH plus ethanol was used as a negative control and DPPH plus Vitamin E was used as a positive control. Antioxidant activity was calculated using the following formula:

$$AA\% = 100 - \left\{ \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \right\}$$

2.7. Statistical analysis

Results were expressed as a percentage changes from the initial pre-contraction level and as mean ± S.E.M. for the number (n) of determinations obtained from different animals. Analysis of variance (ANOVA) followed by Tukey's Multiple Comparisons tests were used for statistical analysis. $P < 0.05$ values were considered to present a significant difference. Dose-response slopes were analysed to give the concentration of compound producing a 50% inhibition of the maximal contractile response (IC_{50}) using a non-linear regression analysis.

3. Results

3.1. Chemistry

TLC analyses of compounds revealed two spots under UV light (data not shown). Retention factors (R_f) values of the isolated harmine (0.62) and harmaline (0.36) compared well with the reported values 0.64 and 0.35, respectively [17]. The results of ^{1}H NMR and ^{13}C NMR are illustrated in Table 1 and the chemical structure of both alkaloids is reported in Fig. 1.

3.2. Effect on KCl and noradrenaline induced contractions

Addition of high KCl or noradrenaline (NA) produced a contractile response, which averaged (1.98 ± 0.10 and 2.42 ±

Table 1
^{1}H- and ^{13}C NMR data of harmaline and harmine in $CDCL_3$ and DMSO, respectively

Harmaline (200 MHz $CDCl_3$)				Harmine (200 MHz DMSO)			
H	δ (ppm)	Multiplicity	J (Hz)	H	δ (ppm)	Multiplicity	J (Hz)
NH	11.54	s		NH	11.42	s	
H-8	7.04	d	1.9	H-8	7.00	d	2.1
H-6	6.80	dd	1.9/8.9	H-6	6.83	dd	2.1/8.6
H-5	7.42	d	8.9	H-5	8.04	d	8.6
H-4	3.14	m		H-4	7.79	d	5.3
H-3	3.88	m		H-3	8.14	d	5.3
OCH_3	3.83	s		OCH_3	3.86	s	
CH_3	2.99	s		CH_3	2.71	s	

No. C	Harmaline (200 MHz, $CDCl_3$)	Harmine (200 MHz, DMSO)
1	161.889	14.934
2	–	–
3	41.839	137.762
4	19.908	111.947
5	122.212	122.625
6	115.754	109.068
7	164.575	160.074
8	94.049	94.57
9	–	–
10	125.029	134.534
11	119.038	114.833
12	125.549	127.206
13	144.201	141.274
CH_3-1	19.05	20.344
CH_3O-7	55.704	55.319

d = doublet, dd = doublet doublet, m = multiplet, s = singulet.

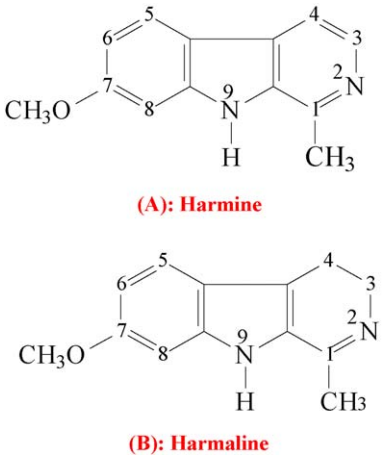

Fig. 1. Chemical structures of harmala alkaloids. Harmine (A) and harmaline (B).

0.16 g, $n = 6$; respectively). Fig. 2A and B shows that harmine and harmaline (10^{-6} to 3×10^{-4} M) produce a concentration-dependent inhibition of the contractile response induced by both stimulatory agents (KCl or NA). However, in KCl induced contraction, the relaxation produced by harmine was endothelium-independent, whereas, the effect of harmaline was significantly decreased in the endothelium denuded aortic ring [IC_{50}: E(+): 32.80 ± 1.17 μM versus E(−): 143 ± 1.23 μM, $P < 0.001$]. This observation was reversed when the contraction was induced by NA, because based on the IC_{50} values the relaxant effect of harmine but not harmaline was partially dependent on the endothelium [IC_{50}: E(+): 3.73 ± 1.20 μM versus E(−): 10.10 ± 1.36 μM, $P < 0.05$]. In addition, in KCl E(+)-induced contraction, the relaxation related to harmaline incubation was more potent than that with harmine (harmaline-IC_{50} was 2.7-fold greater than harmine-IC_{50}).

In the both cases, with intact or denuded endothelium-ring, the relaxant effect of harmine and harmaline was higher in (NA) than in (KCl) induced contraction.

3.3. Effect on pre-treatment with L-NAME and indomethacin

In the order to analyse the involvement of endothelial factors, concentration-response curves of (harmine and hamaline) were constructed in the absence or in the presence of the L-NAME or indomethacin in the E(+)-KCl induced contraction. The pre-treatment with L-NAME curves are shifted to the right showing that cumulative concentration of harmaline reduced significantly the relaxation (Fig. 3B. IC_{50}: 89.21 ± 1.33 (+L-NAME) versus 32.8 ± 1.17 (control), $P < 0.001$). In cases of indomethacin, the curves are not shifted right, however, the IC_{50} values led suggest a significant reduction in the vasorelaxation (IC_{50}: 43.3 ± 1.23 versus 32.8 ± 1.17, $P < 0.01$). These results support the fact than harmaline-induced relaxation was endothelium dependent as mentioned above. However, under the same treatment, the vasorelaxation induced by cumulative doses of harmine in endothelium intact preparation, was unaffected by pre-treatment of L-NAME (IC_{50}: 85.9 ± 1.38 (+L-NAME) versus 88.92 ± 1.4 (control), $P > 0.05$), whereas, pre-incubation with indomethacin enhanced this vasorelaxant effect (IC_{50}: 28 ± 1.15 (+Indomethacin) versus 88.92 ± 1.4 (control), $P < 0.001$) (Table 2 and Fig. 3A).

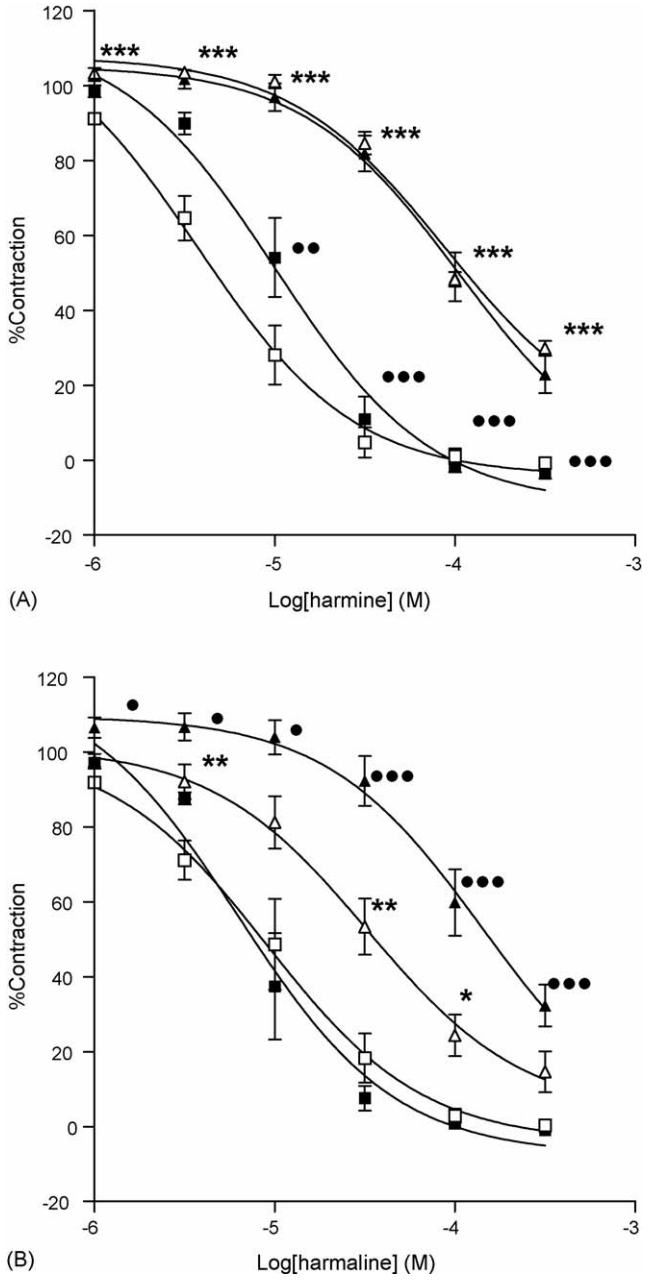

Fig. 2. Vasorelaxant effects of harmine (A) and harmaline (B) on KCl and NA-induced contractions in isolated endothelium-intact E(+) and denuded E(−) rat aortic preparation. (□) NA E(+), (■) NA E(−), (△) KCl E(+), (▲) KCl E(−). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. KCl E(+) vs. NA E(+). $^{\bullet}P < 0.05$, $^{\bullet\bullet}P < 0.01$, $^{\bullet\bullet\bullet}P < 0.001$. NA E(−) vs. KCl E(−). Results are expressed as the means ± S.E.M. of five to six independent experiments in aorta preparation from five to six rats.

3.4. Effects of pre-treatment with prazosin and IBMX

Results obtained following prazosin or IBMX incubation show that prazosin decreases significantly the relaxant effect of

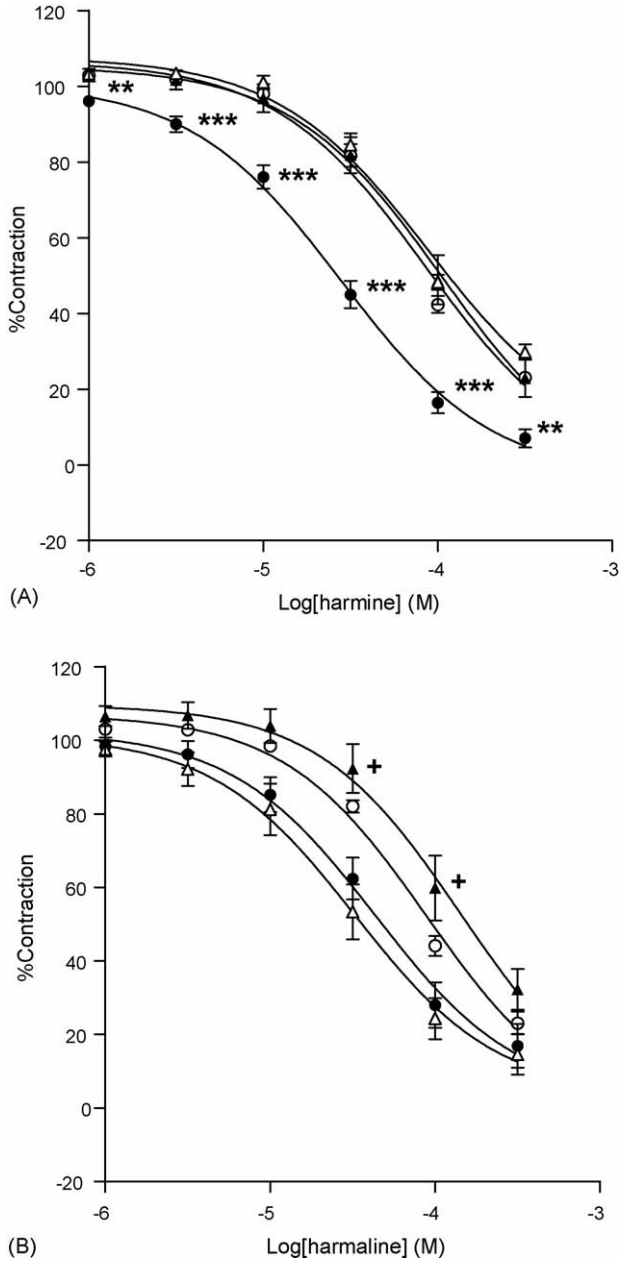

Fig. 3. Vasorelaxant effects of harmine (A) and harmaline (B) on KCl-induced contractions in isolated endothelium-intact E(+) and denuded E(−) rat aortic preparation in the presence or not of Indomethacin or L-NAME. (△) KCl E(+) alone, (◯) KCl E(+) in the presence of the L-NAME or (●) KCl E(+) indomethacin; (▲) KCl E(−) alone. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. INDO vs. KCl E(+) control. $^{+}P<0.05$. L-NAME vs. KCl E(+) control. Results are expressed as the means ± S.E.M. of five to six independent experiments in aorta preparation from five to six rats.

harmaline but not harmine, whereas, IBMX reduces this effect in both harmine and harmaline induced relaxation (Table 2).

3.5. Effects of harmine or harmaline on endothelium-independent relaxation

Sodium nitroprusside (SNP) assay was used in order to study if harmine and harmaline exert an effect on the cGMP activation. Addition of cumulative concentration of Sodium nitroprusside (10^{-10} to 10^{-7} M) to the aortic endothelium-denuded preparation evoked a concentration-dependent inhibition of the phenylephrine-induced contraction (IC_{50}: 2.673 ± 1.06 nM, control). The results show that pre-treatment of tissues with 10 μM of harmine or harmaline did not affect significantly the nitroprusside-induced relaxation (IC_{50}: (harmine: 1.79 ± 1.07 nM); (harmaline: 3.27 ± 1.11 nM) versus (control: 2.673 ± 1.06 nM), $P>0.05$).

Table 2
IC_{50} values of (harmine and harmaline) to inhibit the contractions induced by KCl (80 mM), in intact-endothelium aortic rings

Drugs	IC50 value (μM)	
	Harmine	Harmaline
KCl E(+)	88.92 ± 1.4	32.8 ± 1.17$^{###}$
Indomethacin	28 ± 1.15***	43.3 ± 1.23**
L-NAME	85.9 ± 1.38	89.21 ± 1.33***
IBMX	356.1 ± 1.04***	224.9 ± 1.36***
Prazosin	76.07 ± 1.10	68.25 ± 1.25***

Experiments were carried out in the absence or presence of IBMX (10^{-4} M), prazosin (10^{-8} M), indomethacin (10 μM), and L-NAME (3×10^{-5} M). Data are the mean ± S.E.M. ($n=5$–6) of IC_{50} (the concentration to produce a 50% maximal relaxation) values. $^{**}P<0.01$, $^{***}P<0.001$ vs. KCl E(+) control; $^{###}P<0.001$, harmine vs. harmaline.

3.6. Effects of pre-treatment with diltiazem

To investigate a possible relation between the relaxation caused by the two alkaloids and inhibition of the L-type voltage-dependent Ca^{2+} channels (VOCs), E(+)-aortic rings were incubated in the presence of diltiazem before depolarisation by NA (1 μM). Results show that diltiazem significantly reduces the relaxation evoked by harmine (IC_{50}: 10.74 ± 1.19 (+Diltiazem) versus 3.73 ± 1.20 (control), $P<0.01$) and harmaline (IC_{50}: 90.23 (+Diltiazem) ± 1.19 versus 9.03 ± 1.16 (control), $P<0.001$) (Fig. 4).

3.7. Effect on phenylephrine contractions

To determine whether these two compounds act on the α_1-adrenoceptors of vascular smooth muscles, concentration response curves of phenylephrine (10^{-9} to 10^{-5} M) were established in E-(+) aorta preparations pre-treated with various concentrations of harmine or harmaline (3, 10 or 30 μM). The maximum responses of the cumulative concentration response curves to PE were concentration dependently depressed by two alkaloids indicating a non-competitive antagonist effect on vascular α_1-adrenoceptors (Fig. 5A and B).

3.8. Free radical scavenging test

To investigate the possible free radical scavenging activity of harmine and harmaline, these compounds (10 μM) were incubated for 30-min with DPPH. This incubation induced a significant increase in the free radical scavenging capacity. It can be seen that the potency of free radical scavenging activity was higher with harmaline than harmine. However, Vitamin E

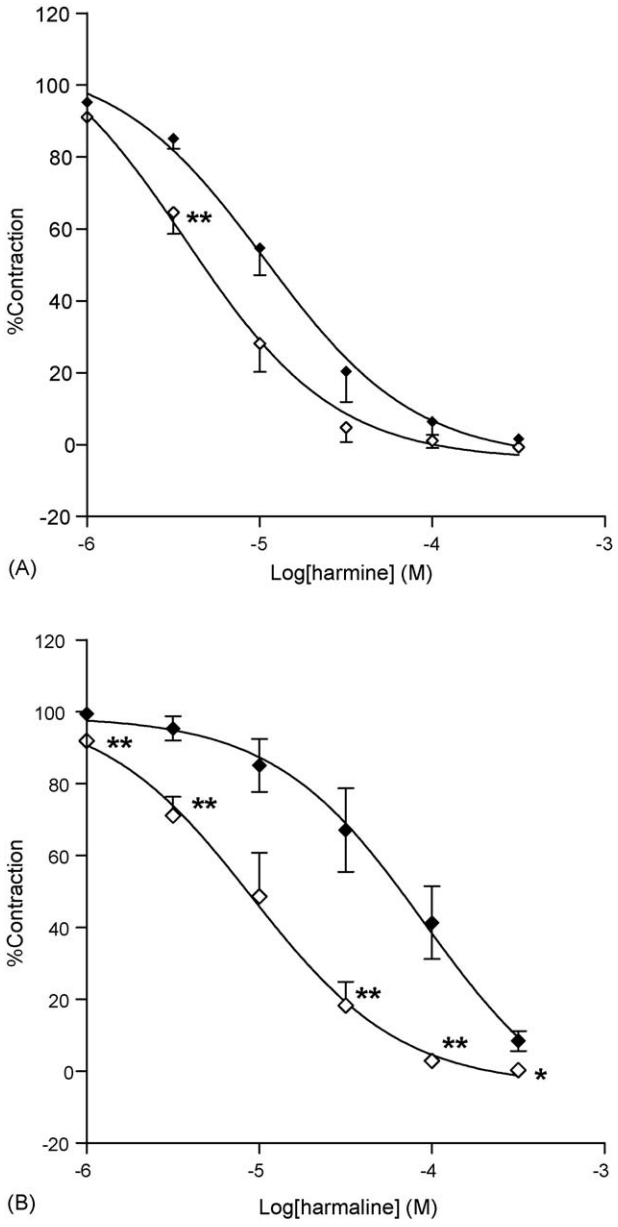

Fig. 4. Vasorelaxant effects of harmine (A) and harmaline (B) on NA-induced contraction in isolated endothelium intact E(+) in the presence or not of diltiazem. (◊) NA E(+), (♦) NA E(+) in the presence of diltiazem. $^{*}P<0.05$, $^{**}P<0.01$. Results are expressed as the means ± S.E.M. of five to six independent experiments in aorta preparation from five to six rats.

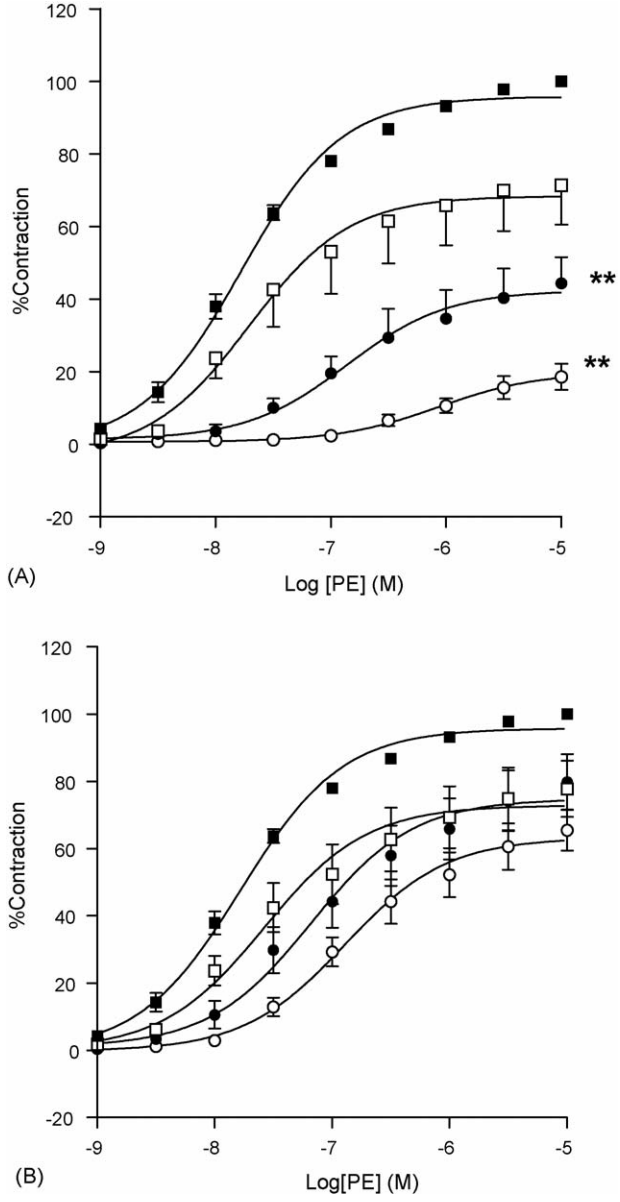

Fig. 5. Effects of harmine (A) and harmaline (B) (3, 10 or 30 μM) on the concentration response curves of phenylephrine in endothelium intact preparations. (■) Control, (□) 3 μM, (●) 10 μM, (○) 30 μM of harmine or harmaline. $^{**}P<0.01$ vs. control. Results are expressed as the means ± S.E.M. of five to six independent experiments in aorta preparation from five to six rats.

Table 3
Radical scavenging of DPPH by harmine and harmaline (10 μM) compared to control as indicated by decrease in absorbance at 517 nm after 30 mn of incubation

	Free radical scavenging activity (%)
Control	6.23 ± 0.650
Harmine	13.42 ± 1.20**
Harmaline	36.11 ± 2.14***
Vitamin E	76.11 ± 3.56***

Values are the mean ± S.E.M. of a minimum of three independent experiments. $^{**}P<0.01$, $^{***}P<0.001$ vs. control.

(positive control) also has a strong capacity to scavenge the free radical generating-DPPH (Table 3). The concentration used in this assay was selected on the basis of our previous work, showing that the range of 10 μM represents the IC_{50} of harmine and harmaline cytotoxic activity assayed on various human tumour cell lines [18].

4. Discussion

This study shows that the vasorelaxant effects of harmine and harmaline act on both endothelial and vascular smooth muscles cells. The contraction generated by noradrenaline in the rat aorta is less dependent upon Ca^{2+} influx through voltage-

operated Ca^{2+} channels, as indicated by its partial resistance to organic Ca^{2+} channels blokers. However, several reports have shown that noradrenaline contraction is the complex result of the mobilisation of both intracellular and extracellular Ca^{2+} and the Ca^{2+} sensitisation of the contractile machinery [23]. Indeed, NA-induced contraction is characterised by the phasic component to depend on calcium release from intracellular stores while the tonic component depends on calcium flux and activation of several proteins such as protein Kinase C (PKC).

Harmine and harmaline inhibited this contraction in a dose dependent manner. However, the endothelium removal partially attenuates the relaxant effects of these alkaloids, suggesting that in addition to the effect of alkaloids on the vascular smooth muscles cells, harmine and harmaline might also induce an endothelium dependent relaxation.

To gain more insight in the implication of endothelium in the alkaloids-induced relaxation, L-NAME and indomethacin have been used respectively to block the NO and prostacyclins-production pathway. Our results show that IC_{50} values of harmaline increase significantly by 2.72-fold in the presence of L-NAME and by 1.32-fold in the presence of indomethacin, indicating that NO and (PGI_2) are involved in the vasorelaxant effects of harmaline. However, in the case of harmine, it appears that its vasorelaxant mechanism is endothelium independent since no changes has been observed with L-NAME pre-treatment and furthermore the vasorelaxant of harmine was enhanced in the presence of indomethacin. These observations are in part in agreement with those reported by Shi et al., since they reported that NO but not endothelium-derived hyperpolarization factor or prostacyclin is involved in the vasorelaxant effect of harmine and harmaline.

To investigate the endothelium independent factors, endothelium-intact aortic rings were pre-treatment with IBMX (non-specific inhibitor of phosphodiesterases (PDE)) [19]. The results shown that in the presence of IBMX, the IC_{50} value of harmine and harmaline were increased by respectively four- and six-fold in comparison with the control. These data indicate that the vasorelaxant effect of these two alkaloids involve also their inhibitory effect on the PDE-pathway and thus consequently induce an increase of intracellular cAMP and cGMP levels. In deed, cAMP activates the phosphorylation of myosin light chain kinase, decreasing its affinity for calcium and consequently blocking smooth muscle contraction [20]. Moreover, cGMP activates the protein-kinase G and the phosphorylation of phospholambans (sarcoplasmic reticulum-ATPase associated protein), inducing a decrease in the intracellular-calcium availability [21]. However, of harmine or harmaline (10 μM) had no effect on the endothelium-independent relaxation by sodium nitroprusside. This result is in agreement with our previous finding [14] and led to suggest that the effect of the both alkaloids may be related to an inhibition of the PDE specific to cAMP more than to cGMP.

The action of both compounds on α_1-adrenoceptor was studied using prazosin (specific antagonist) [22]. The relaxation produced by harmaline but not harmine was significantly attenuated in the presence of prazosin. However, it has been recently reported that synthetic harmine, harmaline and harmalol have a similar affinity for vascular α_1-adrenoceptor in rat aorta [13].

The maximum response to phenylephrine was attenuated by the presence of harmine and harmaline, indicating that the antagonistic effects of these two alkaloids on vascular smooth muscle cell α_1-adrenoceptors was non-competitive. This finding is consistent with that obtained with the methanolic extract from seeds of *Peganum harmala* [14]. The inhibitory effect of harmaline on the contractile response was attenuated in the presence of diltiazem (calcium antagonist) indicating that the vasorelaxant effect of harmaline could be partly attributed to blockage of VOCs [23]. The same observations have also been reported by Splettstoesser et al. [24].

We evaluated the anti-radical activity of the compounds by using a stable free radical, DPPH in a homogenous ethanolic solution. DPPH is a weak hydrogen abstractor and is considered a good kinetic model for peroxyl $ROO^{\bullet}$ radicals [25,26]. We conclude that alkaloids (harmaline > harmine) are able to scavenge the free radicals generated by DPPH. This effect supports the hypothesis that reduction of oxidative stress improves the relaxation response. In fact it has been demonstrated that treatment with Vitamin E [27] abolishes the release of the superoxide anion and consequently promotes the endothelial response. Moreover, we have previously demonstrated that incubation of tissue with antioxidant reduce the oxidant status and enhance the vasorelaxant response [28,29]. Similarly, we conclude that the antioxidant properties of harmine and harmaline might also be implicated as a secondary factor in the improvement of their relaxant effect on aortic ring preparation.

Related to their structure-activity relationship, we suggest that the difference in the relaxant potency between harmine and harmaline might be related to the change of carboline to dihydro-β-carboline (harmine versus harmaline).

Taken together, these results suggest that harmaline and harmine exerts their vasodilatory effects by inhibiting the PDE and by enhancing the prostacyclin-induced relaxation pathway. In addition, the both alkaloids exert a α_1-adrenoceptors competitive antagonism. Moreover, harmaline also act as modulator of NO-induced relaxation via and exerts an inhibitory effect on the VOCs channels. Free radical scavenger activity of harmine and harmaline can also indirectly contribute to the improvement of their vasorelaxant effects.

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