**Artemisia herba-alba** Asso relaxes the rat aorta through activation of NO/cGMP pathway and \( \text{K}_{\text{ATP}} \) channels

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**Abstract**

*Artemisia herba-alba* Asso (Compositae) is used in oriental Morocco to treat diabetes and arterial hypertension. The present work evaluated the vasorelaxant effect of *Artemisia herba-alba* aqueous extract (AHAE) in isolated rat aorta and the mechanism underlying this effect. In endothelium-containing aorta preparations, AHAE (10\(^{-3}\), 10\(^{-2}\), 10\(^{-1}\), 1 and 2 mg/mL) relaxed the contraction elicited by noradrenaline in a concentration-dependent manner. This effect is dependent upon integrity of the vascular endothelium as it was fully abolished in endothelium-denuded preparations. The vasorelaxant effect of AHAE (2 mg/mL) was also inhibited by NG-nitro-L-arginine methyl-ester (100 \( \mu \text{M} \)), methylene blue (10 \( \mu \text{M} \)) or 1H-[1,2,4] oxadiazolo [4,3-a] quinoxaline-1-one (50 \( \mu \text{M} \)) but not by 10 \( \mu \text{M} \) atropine. This effect remained unchanged by tetraethylammonium (5 mM) or indomethacin (10 \( \mu \text{M} \)) whereas it was significantly attenuated by glibenclamide (10 \( \mu \text{M} \)). These results suggest that AHAE produces an endothelium-dependent relaxation of the isolated rat aorta, an effect that seems mainly mediated through stimulation of the endothelial nitric oxide synthase by mechanisms other than activation of muscarinic receptors. Activation of ATP-dependent potassium channels partly contributes in the mediation of AHAE-induced endothelium-dependent relaxation.

Key words: *Artemisia herba-alba*, compositae, rat isolated aorta, NO-dependent vasorelaxation, \( \text{K}_{\text{ATP}} \) channels

**Introduction**

Morocco is characterized by a climatic diversity which is favorable for growth and development of more than 4,200 species of plants divided into 130 families and 940 genus (Bellakhdar et al., 2004).
1997). The Moroccan population has used plants since time immemorial to treat various types of diseases (Bellakhder et al., 1997). In the last decade, some studies have been performed in different areas of Morocco in order to describe the main medicinal plants used in the cardiovascular system pharmacopoeia (Ziyyat et al., 1997; Jouad et al., 2001; Eddouks et al., 2002; Tahraoui et al., 2007). In oriental Morocco Artemisia herba-alba Asso (Asteraceae or Compositae) is one of the commonly used plants to treat diabetes and arterial hypertension (Ziyyat et al., 1997).

This plant is a medicinal and aromatic dwarf shrub that grows wild in the steppes of arid areas of North Africa, Spain and the Middle East. In Morocco, this species is widespread in the oriental high plains, the high Moulouya, southern piedmonts of Atlas and the versant of Souss. In our region (oriental), the meat of sheep of Béni-Guil nourished by Artemisia is very famous for its flavor and its high-quality particularly for the roasted sheep (Méchoui). The plant is used in folk medicine as anthelmintic, poison antidote and emmenagogue (Bellakhder et al., 1997). Several experimental studies have shown antimicrobial (Yashphe et al., 1979; Benouda et al., 1988), hypoglycemiant (Al-Waili, 1986; Al-shamony et al., 1994; Tastekin et al., 2006), fungicide (Saleh et al., 2006), antileishmania (Hatimi et al., 2001), antioxidant (Abid et al., 2007; Al-Mustapha et al., 2008), and neurological (Salah et al., 2005) effects of Artemisia. Recently, it has been shown that Artemisia herba alba aqueous extract (AHAE) possesses antihypertensive activity in spontaneously hypertensive rats through a mechanism involving at least in part, an increase in urine and electrolyte output (Zeggwagh et al., 2008).

The present study was undertaken using rat isolated thoracic aorta preparations to assess whether hypotensive action of AHAE could result, at least in part, from its vasodilator effects directly upon vascular smooth muscle. In addition, putative mechanism underlying these effects has been addressed.

**Materials and Methods**

*Collection of the plant and preparation of aqueous extract*

Artemisia herba-alba was collected in oriental Morocco after the flowering period in April 2005 near Tourirt city (Morocco). Taxonomic identification was performed by Professor B. Haloui from our department, where a voucher specimen has been deposited (collection ZL15). 50 g of small pieces of the aerial part of the dried and sliced plant was infused into 500 ml of boiled distilled water during 30 min. After decantation and filtration, the filtrate was again dried in evaporator at 50°C to give a crude residue. The yield of extraction is 24.4% of the dried plant. The AHAE was then prepared at different concentrations in physiological solution.

*Preparation of aorta and experimental device*

Male and female Wistar rats weighing 250–300 g were obtained from our local colonies. They were kept under conditions of constant temperature (22 ± 2°C) with a standard 12-h light:12-h dark cycle and free access to food and water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85–23, revised 1996; see http://grants.nih.gov/grants/olaw/olaw.htm).

Animals were anesthetized with sodium pentobarbital (50 mg/kg of body weight, i.p.) and then
the thoracic aorta was removed carefully in cold physiological salt solution (PSS). After the removal of adhering fatty and connective tissues, an aortic ring (about 2–3 mm in length) was suspended between two stainless steel hooks in a 10 ml water-jacked bath containing PSS of the following composition (mM): NaCl 119, KCl 4.7, CaCl$_2$ 1.6, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.2, NaHCO$_3$ 25, glucose 11. The tissue bath solution with pH 7.4 was maintained at 37°C and gassed with 95% O$_2$ + 5% CO$_2$. The isometric contraction was recorded via a force-displacement transducer (EMKA Technologies, Paris, France) connected to a polygraph (Leybold-Heraeus, Austria). A tension of 1 g was initially applied to the ring which was equilibrated in the medium for 30 min. Before each experiment, vasoconstriction was induced by 1 $\mu$M noradrenaline (NA) in normal PSS and when the steady contraction was reached, 100 $\mu$M carbachol (CCh, a cholinesterase resistant analogue acetylcholine) was added to induce endothelium-dependent relaxation. This step was necessary to verify the endothelium integrity. When the relaxation induced by CCh was less than 50%, the experiment was interrupted and another ring was mounted. Twenty min after the NA/CCh step, three series of experiments have been performed as follows:

**Series 1.**

In order to assess the effects of AHAE on NA-induced contraction, aortic ring preparations with intact endothelium were exposed to cumulative increasing concentrations of AHAE (10$^{-3}$, 10$^{-2}$, 10$^{-1}$, 1 and 2 mg/mL) during 5-min, period once a sustained contraction elicited by a submaximal concentration (1 $\mu$M) of NA was established.

**Series 2.**

In order to investigate whether AHAE-induced relaxation is dependent upon the integrity of the vascular endothelium, vascular responses to AHAE (10$^{-3}$, 10$^{-2}$, 10$^{-1}$ and 1 mg/mL) were determined in endothelium-denuded rings precontracted by NA (1 $\mu$M). The endothelium was removed immediately after dissection by gentle rubbing of the aortic lumen with plastic tubing. Each isolated aortic preparation without intact endothelium was challenged at the beginning of the experiment with 100 $\mu$M of CCh. The absence of CCh-induced vasorelaxant effects was taken as evidence that the preparation was effectively stripped of endothelium. Finally, 1 $\mu$M of sodium nitroprusside (SNP, an NO-donor) was added to produce endothelium-independent relaxation.

**Series 3.**

This series of experiments were carried out to assess the mechanism underlying the vasorelaxant effects of AHAE in endothelium-containing aorta preparations. For this purpose, maximal relaxation induced by AHAE (2 mg/mL) alone was determined in aortic preparations precontracted by NA (1 $\mu$M). Thereafter, the organ bath was rinsed two times with fresh PSS and the same protocol was repeated after pre-treatment of the ring with 10 $\mu$M atropine (ATR, a non-selective muscarinic receptor antagonist), 100 $\mu$M N$^{\text{G}}$-nitro-L-arginine methyl-ester (L-NNAME, an inhibitor of NO synthase), 10 $\mu$M methylene blue (MB, a non-selective inhibitor of NO synthase, see Ziyyat *et al.*, 2002), 50 $\mu$M 1H-[1,2,4] oxadiazolo [4,3-a] quinoxaline-1-one (ODQ, a specific inhibitor of the soluble guanylyl cyclase), 5 mM tetraethylammonium (TEA, an inhibitor of voltage-dependent potassium channels), 10 $\mu$M glibenclamide (an inhibitor of ATP-dependent potassium channels) or 10 $\mu$M indomethacin (an inhibitor of cyclooxygenase). When the vasorelaxant effect of AHAE was blocked by a given pretreatment, 1 $\mu$M SNP was added to the bath to produce an endothelium-independent relaxation.
Chemicals
The following drugs NA, L-NAME, carbamylcholine chloride (carbachol), TEA, indomethacin were purchased from Sigma Chemical. ATR was obtained from Labosi, MB from Grraw, SNP from Farco chemical, ODQ from Alexis Biochemicals and glibenclamide (Daonil®) from Aventis labo. All compounds were dissolved in distilled water except ODQ which was dissolved in DMSO.

Statistical analysis
Data from male and female rats were pooled and are presented as mean ± SEM. They were expressed as percentage of relaxation of NA-precontracted aorta (compared to a maximal contraction induced by NA). Data obtained were analyzed using unpaired Student’s t-test and one-way analysis of variance (ANOVA) test. Probability of less than 5% ($P<0.05$) was considered significant.

Results
In endothelium-containing preparations, the $\alpha_1$-adrenergic agonist NA (1 $\mu$M) induced a contraction which developed a tension of 2.01 ± 0.27 g (n=30). CCh (100 $\mu$M) relaxed the precontracted aortic ring preparations by 77.0 ± 3.2% (n=30, Fig. 1A). Cumulative concentrations (10$^{-3}$, 10$^{-2}$, 10$^{-1}$, 1 and 2 mg/mL, n=8) of AHAE produced a concentration-dependent ($P<0.05$) relaxation (Figs. 1A and 1B) with an IC$_{50}$ value of 0.912 mg/mL. Relaxant response to all concentrations used herein was completely reversible suggesting that AHAE is devoid of any toxic effect.

Both vasorelaxant actions of CCh and AHAE disappeared completely when the endothelium was removed (n=6, Fig. 1C). Removal of the endothelium did not damage the vascular muscle underneath as aortic ring preparations were relaxed with 1 $\mu$M SNP (by 76.0 ± 7.0%, n=6; Fig. 1C). To examine whether activation of muscarinic receptors was involved in the effect of AHAE, its vasorelaxant effect was examined in the presence of ATR. As shown in Fig. 2A (summary data in Table 1), ATR (10 $\mu$M, n=7) did not modify the relaxant effect of AHAE while it completely antagonized that of CCh.

The vasorelaxant effect of AHAE (2 mg/mL) was fully abolished ($P<0.001$) in the presence of 100 $\mu$M of L-NAME (n=7; Fig. 2B and Table 1) or 50 $\mu$M ODQ (n=7; Fig. 2D and Table 1) and significantly ($P<0.05$) reduced in the presence of 10 $\mu$M MB (n=6; Fig. 2C and Table 1). It should be noted that the endothelium-independent vasorelaxant effect of NO was still preserved in the presence of either L-NAME or MB, since 1 $\mu$M SNP still produced a strong relaxation of NA-precontracted aorta in the presence of 100 $\mu$M L-NAME (85.0 ± 1.8%, n=7) or 10 $\mu$M MB (60.2 ± 1.5%, n=6). However, the action of SNP was completely blocked by 50 $\mu$M ODQ (n=7). Vasorelaxant action of AHAE was almost completely abolished ($P<0.001$) by glibenclamide (n=8; Fig. 2E and Table 1) but remained unaltered by TEA (n=8; Fig. 2F and Table 1) or indomethacin (n=8; Fig. 2G and Table 1).
Discussion

Our results show that AHAE induces concentration-dependent vasorelaxant action in rat isolated aortic preparations by a mechanism including release of an endothelium-derived relaxing factor (EDRF), notably nitric oxide (NO) unrelated to endothelial muscarinic receptor activation and also involving an increased K+ efflux following the activation of ATP-sensitive K+ (K\textsubscript{ATP}). This is the first in vitro investigation showing vasorelaxant activity of AHAE in isolated rat preparations, a finding that may constitute pharmacological basis for its antihypertensive activity (Zeggwagh et al., 2008). It might have suggested that AHAE-induced vasorelaxant effects have

Fig. 1. Typical tracings showing (A) the effect of carbachol (CCh, 100 μM) and Artemisia herba-alba aqueous extract (AHAE, 2 mg/mL) on noradrenaline (NA, 1 μM) precontracted intact aorta preparations and (B) Effects of cumulative concentrations of AHAE 10^{-3}, 10^{-2}, 10^{-1} and 1 mg/mL on intact aorta and (C) Effects of CCh, AHAE on denuded rat aorta. In the last case, sodium nitroprusside (SNP, 1 μM) was added to produce an endothelium-independent relaxation. Wash indicates the moment when all drugs were washed out.
been related to its putative toxic effects. However, this hypothesis seems unlikely since all vasodilator responses to AHAE were reversible (data not shown).

The present study investigated the participation of the vascular endothelium in mediation of vasorelaxant activity of AHAE. Our data showed that, like the vasorelaxant effects of CCh, those

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**Table 1.** Mean values of the vasorelaxant effect (expressed as percent of relaxation on intact NA-precontracted aorta) of carbachol (CCh) and *Artemisia herba-alba* aqueous extract (AHAE) alone or in the presence of atropine (ATR), L-NAME, methylene blue (MB), 1H-[1,2,4] oxadiazolo [4,3-a] quinoxaline-1-one (ODQ), glibenclamide, tetraethylammonium (TEA) and indomethacin.

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<tr>
<th></th>
<th>CCh 100 μM</th>
<th>AHAE 2 mg/mL alone</th>
<th>AHAE 2 mg/mL + Atropine 10 μM</th>
<th>AHAE 2 mg/mL + L-NAME 100 μM</th>
<th>AHAE 2 mg/mL + MB 10 μM</th>
<th>AHAE 2 mg/mL + ODQ 50 μM</th>
<th>AHAE 2 mg/mL + Glibenclamide 10 μM</th>
<th>AHAE 2 mg/mL + TEA 5 mM</th>
<th>AHAE 2 mg/mL + Indomethacin 10 μM</th>
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<tr>
<td>Mean</td>
<td>84.6 ± 2.3</td>
<td>79.4 ± 3.6</td>
<td>75.4 ± 5.1</td>
<td>3.1 ± 1.5</td>
<td>14.3 ± 0.8</td>
<td>7.2 ± 0.6</td>
<td>78.4 ± 3.1</td>
<td>80.0 ± 2.6</td>
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<tr>
<td>(n)</td>
<td>(16)</td>
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Values are mean ± SEM. a: *P*<0.001 and b: *P*<0.05 by unpaired Student’s *t*-test vs AHAE alone.
evoked by AHAЕ were completely abolished by mechanical removal of the endothelium suggesting that they are mediated by NO and/or prostacyclin release. AHAЕ-induced vasodilatation seems unrelated to prostacyclin release as it was unaffected by pretreatment with indomethacin, a non-selective cyclooxygenase inhibitor. Vasorelaxant responses to AHAЕ were also abolished following NO synthase by L-NAME. This finding, however, supports the hypothesis that AHAЕ may latterly stimulate endothelial NO production and/or release. Whether AHAЕ is directly interacting with endothelial NOS (e-NOS) or with other factors, which may increase the e-NOS activity remains to be further investigated.

It is well known that NO is formed in the endothelium by the activation of the e-NOS, which uses L-arginine as substrate. Once formed, NO diffuses into the underlying vascular smooth muscle where it binds to and activates soluble guanylyl-cyclase. This enzyme catalyzes the conversion of GTP to cGMP leading to a vasorelaxation in smooth muscle cells (Rapoport et al., 1983; Knowles et al., 1992). Since in NA-precontracted aortic preparations incubated with MB, an inhibitor of guanylate cyclase (Moncada et al., 1991), the vasorelaxant effect of AHAЕ and CCh were completely abolished, we hypothesized that AHAЕ may relax vascular tissue through an interference with the NO-cGMP cellular pathway. However, MB should be used with caution since it exerts a number of other effects including inhibition of muscarinic receptors (Abi-Gerges et al., 1997) and inhibition of NO-synthase. As such, we found here that MB inhibited the effect of CCh on NA-precontracted aorta but left unchanged the relaxant effect of the NO-donor SNP. Therefore, we interpreted our results with MB as an effect due to inhibition of NO-synthase rather than inhibition of guanylyl cyclase.

Moreover, inhibition of soluble guanylyl cyclase by ODQ abolished the vasorelaxant effect of AHAЕ as well as that evoked by CCh (Lucas et al., 2000). Although magnitude of vasorelaxant effect of AHAЕ was similar to that of CCh, AHAЕ’s effect was not due to activation of muscarinic receptors since it was unaffected by ATR pretreatment. We concluded that AHAЕ produces an endothelium-dependent relaxation of the aorta through a mechanism unrelated to muscarinic receptor activation involving activation of endothelial NO-synthase and subsequent cGMP synthesis in the vascular myocytes.

Potassium channels, which play important roles in the regulation of muscle contractility and vascular tone, are implicated in the NO synthesis and release by endothelial cells (Busse et al., 2002). In many situations, the vasodilatation mediated by membrane hyperpolarization is attributed to a rise in K+ permeability (Nelson et al., 1995; Seino and Miki, 2003). Direct activation of K+ channels on arterial smooth muscle cells normally hyperpolarizes the cell membrane and thus inhibits Ca2+ influx through voltage-dependent Ca2+ channels. Several types of K+ channels are present on vascular smooth muscle, ATP-sensitive K+ (KATP), Ca2+ activated K+ channel (KCa), voltage-dependent K+ channel (KV) and inward-rectifier K+ channel (KIR), which can be blocked by glibenclamide, TEA, 4-AP and BaCl2, respectively (Standen et al., 1989; Ferrer et al., 1999). It is well known that KATP plays a crucial role in cardiovascular system, especially in antagonizing the effects of ischemia/reperfusion injury (Seino and Miki, 2003). The present study showed that AHAЕ-induced relaxation was blocked by glibenclamide, but not by TEA, suggesting that action of AHAЕ is related to activation of ATP-sensitive K+ channels.

Various secondary metabolites have been isolated from the aerial part of Artemisia herba-alba.
the most important being the sesquiterpene lactones (Ahmed et al., 1990; Boriky et al., 1996), the essential oils (Salido et al., 2004) and flavonoids. The vasodilator effect of AHAE could be attributed to a number of polyphenolic compounds such as flavonoids which are known for their cardiovascular effects such as vasodilator and antioxidant effects (Fitzpatrick et al., 1995; Middeleton et al., 2000). Work is in progress to isolate these compounds in order to assess their vascular effects in vitro and to identify the active principles responsible for the endothelium-dependent vasorelaxation induced by AHAE.

In conclusion, the vasorelaxant effects of AHAE are dependent upon the integrity of functional endothelium but unrelated to activation of endothelial muscarinic receptors. Beside the involvement of endothelial eNOS/cGMP pathway, activation of K<sub>ATP</sub> is also involved in the mediation of AHAE-induced vasodilatory effects in vascular smooth muscle. The present results support the ethno-medical application of this plant in the handling of cases of hypertension.

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References


