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Antihypertensive and endothelium-dependent vasodilator effects of aqueous extract of *Cistus ladaniferus*

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ABSTRACT

Cistus ladaniferus L. (Cistaceae) is a medicinal plant originated from the Mediterranean region which exerts different pharmacological effects. In the present study, our goal was to examine whether the plant possessed antihypertensive properties. Aqueous extract of Cistus leaves (AEC, 500 mg/kg/day) reduced systemic blood pressure (SBP) in two animal models of hypertension, the L-NAME and renovascular two kidney-one clip (2K-1C) hypertensive rats. In the former, AEC prevented the increase in SBP when co-administered with L-NAME during four weeks (164 ± 3 mm Hg in L-NAME *vs.* 146 ± 1 mm Hg in L-NAME + AEC, $p < 0.001$). In the latter, AEC reversed the increase in SBP when administered during four weeks after installation of the hypertension (146 ± 5 mm Hg with AEC *vs.* 179 ± 6 mm Hg without, $p < 0.05$). AEC treatment also reversed the endothelial dysfunction observed in both animal models of hypertension. A direct effect on cardiac and vascular tissue was also tested by examining the contractile effects of AEC in rat isolated aortic rings and Langendorff perfused hearts. AEC (10 mg/L) had no effect on left ventricular developed pressure and heart rate in isolated perfused heart. However, AEC produced a strong relaxation of pre-contracted rat aortic rings (80 ± 2% relaxation, $n = 25$). When the rings were denuded from endothelium or were incubated with 1 mM Nω-nitro-L-arginine (L-NNA), the relaxant effect of AEC was lost. We conclude that *C. ladaniferus* possesses antihypertensive properties which are mainly due to an endothelium-dependent vasodilatory action.

Introduction

Hypertension is a major risk factor that predisposes to cardiovascular disorders and is responsible for a large morbidity and mortality in patients [1,2]. The usual method for controlling hypertension is the use of long-term drug therapy. However, an ethnobotanical survey has revealed that herbs are commonly used in the treatment of hypertension in Morocco [3–5].

Cistus ladaniferus L. (Cistaceae), commonly known as rock-rose, is a medicinal plant originated from the Mediterranean region [6]. This plant exerts different pharmacological effects such as antiaggregant [7], antioxidant [8] and antispasmodic effects [9]. In the present study, our aim was to investigate whether the plant had also antihypertensive properties. For this, the effect of the aqueous extract of *C. ladaniferus* (AEC) was examined on blood pressure in two animal models of hypertension, the L-NAME and renovascular two kidney-one clip (2K-1C) hypertensive rats [10,11]. A direct effect on cardiac and vascular tissue was also tested by examining the contractile effect of AEC on isolated aortic rings and Langendorff perfused hearts from these animals.

Materials and methods

Vegetal material and plant extract preparation. The plant was collected from Oujda region in oriental Morocco. A voucher specimen (No. rab 502//63) was previously deposited in Scientific Institute of Rabat (Morocco). The aqueous extract (AEC) was obtained from the aerial part of the plant using a traditional method described in folk medicine: 50 g of dried aerial part of Cistus were extracted by decoction with 2 L of water for 30 min. The aqueous extract was obtained after filtration and evaporation to dryness in vacuo (yield: 18%).

Animals. Adult Wistar rats (200–300 g) were used in the whole study. They were purchased from house animal facility of the Faculty of Science (Oujda, Morocco) and the Faculty of Pharmacy

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(Châtenay-Malabry, France). The animal investigation conforms to the European Community guiding principles in the care and use of animals (86/609/CEE, *CE Off J* No. L358, 18 December 1986).

L-NAME hypertension model. Hypertension was inducing in adult Wistar rats by orally administration of N^G-nitro-L-arginine methyl ester (L-NAME) for four weeks. Animals were divided into three groups: a control group receiving tap water; a group receiving tap water complemented with L-NAME 30 mg/kg/day; and a group receiving tap water complemented with L-NAME 30 mg/kg/day plus AEC 500 mg/kg/day. The treatment lasted four weeks for the three groups. The systolic blood pressure (SBP) was weekly measured in all groups using the tail-cuff method.

Renovascular hypertension. A two kidney-one clip (2K-1C) model was used to induce renovascular hypertension. Wistar rats were subjected to partial clamping of the left renal artery. Weight-matched control animals (sham-operated) underwent the same procedure without occlusion of the renal artery. After six weeks, rats whose SBP was less than 150 mm Hg were discarded. The hypertensive selected rats were allocated into two groups: one group receiving tap water; one group receiving tap water complemented with AEC at 500 mg/kg/day. The treatment lasted four weeks for the two groups.

Vasorelaxant effects of the extract. Wistar rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the thoracic aorta was removed and placed in Krebs–Henseleit solution (KHS). An aortic ring of about 2–3 mm in length was suspended between two stainless steel hooks in 20-ml water-jacketed bath containing KHS of the following composition (in mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25 and glucose 11. The tissue bath solution was maintained at 37 °C and gassed with 95% O_2, 5% CO_2 (pH 7.4). The isometric contraction was recorded via a force–displacement transducer (Senso Nor, type 801) connected to a paper recorder (Leybold-Heraeus, type SE122). A tension of 1 g was initially applied to the ring which was equilibrated in the medium for 30 min. After equilibration, the aortic rings were stabilized with a near maximal contraction induced by 0.1 μM noradrenaline (NA) in normal KHS. When the steady contraction was reached, 1 μM carbachol (CCh: a muscarinic analogue) was added to the bath to assess endothelium integrity.

The relaxant effect of AEC on 0.1 μM NA pre-contracted aortic rings was examined. When contraction had reached a steady-state after about 10 min (considered as 100%, and was defined as control), AEC (10^{-3}–10^{-2} g/L) was added. The effect of AEC was evaluated as the percentage of relaxation of the NA-induced contraction. AEC was also tested on denuded aorta. The endothelium was removed mechanically by gently rubbing the lumen of the artery with a plastic tubing. The absence of CCh-induced relaxation indicated that the vessel was successfully denuded. AEC was tested also in the presence of 10 μM atropine (a muscarinic receptor antagonist) or 1 mM Nω-nitro-L-arginine (L-NNA, an inhibitor of NO-synthase). In this case, the preparations were incubated with atropine or L-NNA for 20 min prior to NA application.

Vascular reactivity. At the end of treatment of both hypertensive rats, L-NAME and 2K-1C rats, thoracic aorta from all groups were isolated for an examination of the effect of different treatments on vascular reactivity. After equilibration, aortic rings were contracted with NA (10^{-6} M). When the steady contraction was reached, the response to increasing concentrations of CCh (10^{-9}–10^{-4} M) was tested. Relaxation level was expressed as percent of the maximal NA-induced contraction.

In vitro cardiac study. Adult Wistar rats were anaesthetised with sodium pentobarbital (50 mg/kg, i.p.) and the heart was rapidly excised and the aorta cannulated for Langendorff perfusion at 36 °C using a constant flow of 12 ml/min with the following perfusate composition (in mM): NaCl, 118; KCl, 5.9; $MgSO_4$, 1.2; $CaCl_2$, 1.5; $NaHCO_3$, 25; glucose, 10; pyruvate, 2 and mannitol, 1.12. The perfusate was saturated with 95% O_2, 5% CO_2 (pH 7.4). A latex balloon was introduced into the left ventricle via the left atrium and connected to a pressure-transducer (Statham gauge Ohmeda, Bilthoven, Holland) for measurement of contractile parameters: heart rate (HR) and left ventricular pressure (LVP). The effect of AEC (10^{-3}–10^{-2} g/L) on contractile parameters was studied after a 30 min equilibration period.

Chemicals. (−)-Norepinephrine hydrochloride (Noradrenaline, NA), carbamylcholine chloride (carbachol, CCh), N^G-nitro-L-arginine methyl ester (L-NAME) and Nω-nitro-L-arginine (L-NNA) were purchased from Sigma Chemical, Atropine from Labosi, Sodium nitroprusside (SNP) from Farco Chemical.

Statistics. Results are expressed as the means ± SEM for *n* separate experiments. Within-group comparisons were performed by analysis of variance (ANOVA) test for repeated measurements followed by Bonferoni *t*-test and a difference was considered as statistically significant when *p* value was less than 0.05.

Results

Antihypertensive effect on L-NAME-induced hypertension

The effect of the aqueous extract of *C. ladaniferus* (AEC) was first examined on blood pressure in the L-NAME rat model of hyperten-

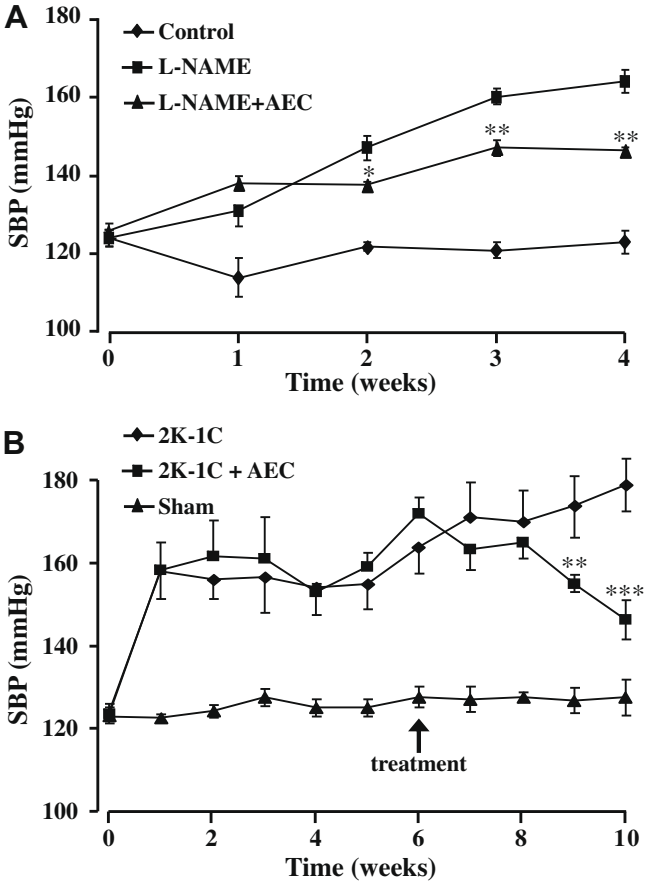

Fig. 1. Effect of chronic administration of aqueous extract of *Cistus ladaniferus* (AEC, 500 mg/kg/day) on systemic blood pressure (SBP) in two animal models of hypertension. In (A), hypertension was induced by four-week administration of L-NAME (30 mg/kg/day). Control indicates rats receiving only tap water. L-NAME + AEC indicates rats receiving simultaneously L-NAME and AEC during four weeks. In (B), hypertension was induced by partial clamping of the left renal artery (2K-1C). The results are compared with sham-operated rats receiving tap water, or with 2K-1C rats receiving AEC during four weeks after installation of the hypertension. Values are mean ± SEM (n = 6 rats for each group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* L-NAME group or 2K-1C group.

sion [11]. Mean value of SBP in control rats was 123 ± 3 mm Hg (n = 6). As shown in Fig. 1A, treatment by L-NAME (30 mg/kg/day) for four weeks increases SBP to 164 ± 3 mm Hg ($p < 0.001$). However, a concomitant treatment by L-NAME and AEC (500 mg/kg/day), significantly reduced the increase of SBP after four weeks (146 ± 1 mm Hg *vs.* L-NAME, $p < 0.001$, Fig. 1A).

Antihypertensive effect on 2K-1C renovascular hypertension

Next, the effect of AEC was examined on blood pressure in the renovascular two kidney-one clip (2K-1C) rat model of hypertension. SBP of sham-operated rats was 124 ± 2 mm Hg (n = 6) and remained constant throughout the period of investigation. However, six weeks after surgical procedure, SBP in 2K-1C rats rose to 172 ± 4 mm Hg (n = 6) reflecting an installation of hypertension. As shown in Fig. 1B, hypertensive rats receiving tap water during four additional weeks maintained a high SBP (179 ± 6 mm Hg). However, hypertensive rats receiving AEC during the same four-week period had their SBP decreased to 146 ± 5 mm Hg. Altogether, these experiments indicate that AEC possesses antihypertensive properties.

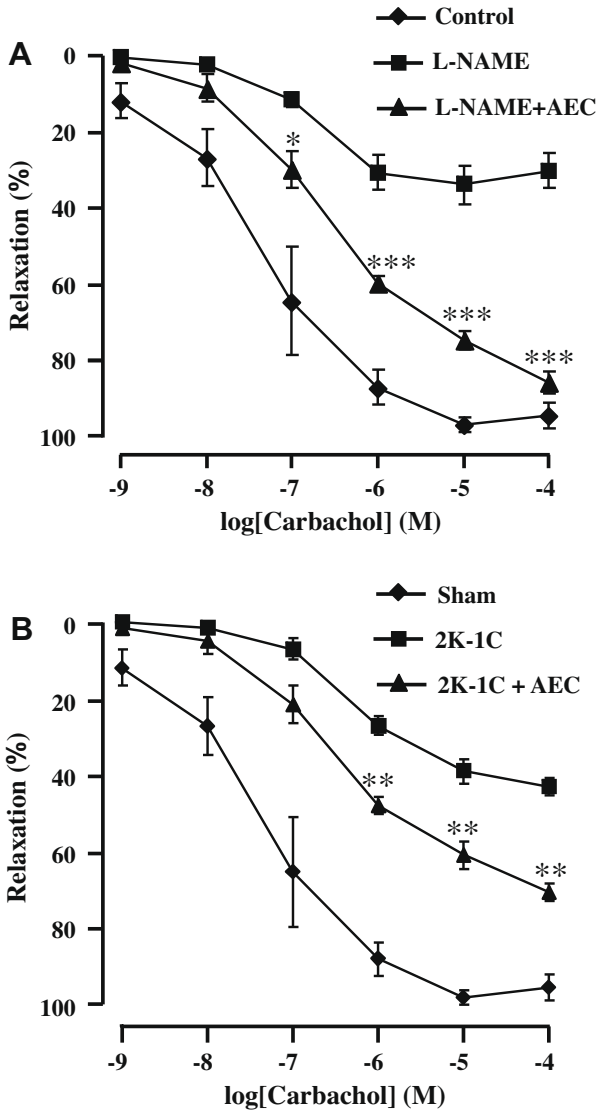

Fig. 2. Vascular reactivity measured on rings of thoracic aorta isolated after the end of treatment in the L-NAME group (A) and the 2K-1C group (B). Each symbol shows the mean ± SEM (n = 6 rats for each group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* L-NAME group or 2K-1C group.

Vascular reactivity

At the end the above treatment, vascular reactivity of aortic rings was examined for each group of animals. The relaxant response to CCh on aortic rings pre-contracted with 0.1 μM noradrenaline (NA) was used as an index of endothelial function. As shown in Fig. 2, endothelial function was altered in hypertensive animals. Indeed, the relaxant effect of CCh was strongly diminished in either L-NAME (Fig. 2A) or 2K-1C rats (Fig. 2A). However, in both hypertensive rat models, the animals that received AEC showed an improved CCh response (Fig. 2). This indicates that a treatment with AEC, either preventive as in the case of the L-NAME model or curative as with the 2K-1C model, reduced the vascular damage induced by hypertension.

Vasorelaxant effect

The next experiments were designed to evaluate whether AEC possessed direct vasorelaxant effects. Intact aortic rings developed a tension of 1.9 ± 0.1 g (n = 25) in response to 0.1 μM NA. As shown above, CCh (1 μM) added on top of NA-induced a strong relaxation (Fig. 3A) confirming the integrity of the endothelium. Interestingly, AEC (10^{-2} g/L) produced a similar relaxing effect as CCh (Fig. 3A). On average, the relaxation induced by CCh on pre-contracted intact aortic rings was 74 ± 2% (n = 22) and that of AEC was 80 ± 2% (n = 25). As shown in Fig. 3B, the relaxant effect of AEC was dose dependent: half-maximal effect was obtained at a concentration near 3×10^{-3} g/L and maximal effect was obtained around 10^{-2} g/L. To see whether the observed effect of AEC was endothelium-dependent, rings of aorta were denuded before testing the extract. Fig. 4A shows an experiment performed in a denuded aortic ring. NA still produced a strong contraction of the aorta but, as expected, CCh had no effect. Interestingly, addition of AEC (10^{-2} g/L) to the cocktail had no effect either (n = 8, Fig. 4D), while application

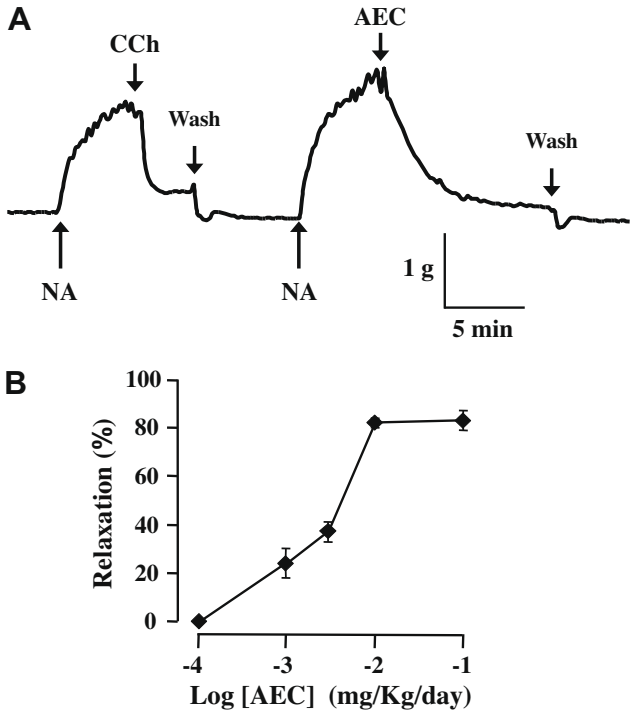

Fig. 3. Relaxant effect of AEC on aortic rings pre-contracted by noradrenaline (NA, 0.1 μM). (A) Typical experiment comparing the effect of carbachol (CCh, 1 μM) and AEC (10^{-2} g/L). (B) Concentration–response curve for the relaxant effects of AEC. Each symbol shows the mean ± SEM of 3–8 separate experiments.

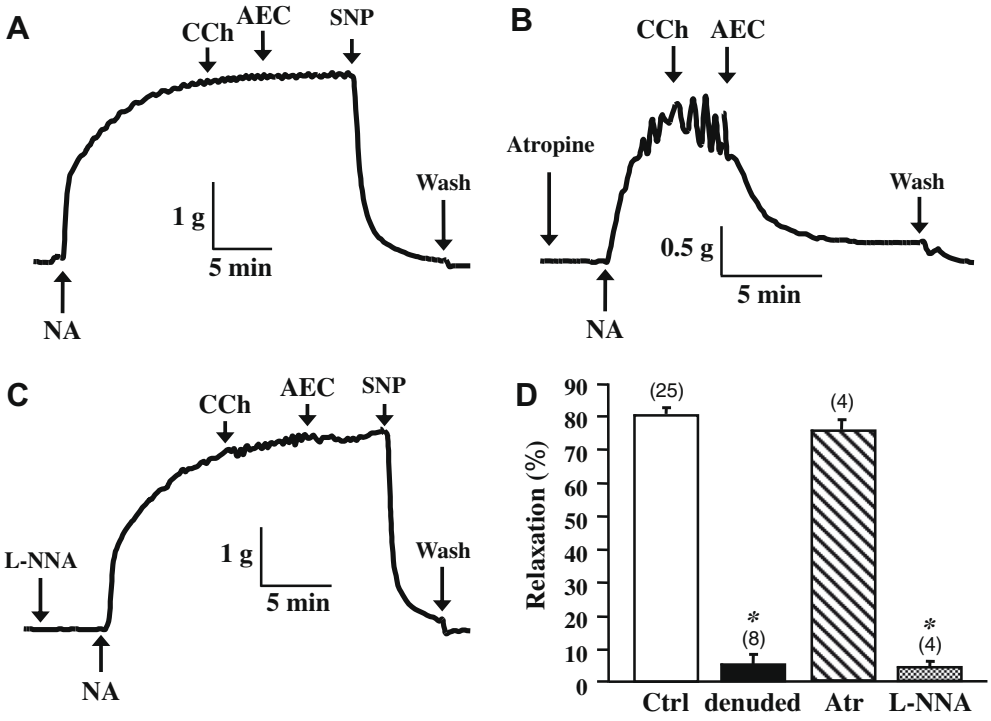

Fig. 4. Mechanisms of the relaxant effect of AEC. The aortic rings were either denuded (A), or pre-treated with either 10 μM atropine (Atr, B) or 1 mM L-NNA (C). In each case, carbachol (CCh, 1 μM), AEC (10^{-2} g/L) and/or sodium nitroprusside (SNP, 1 μM) were tested. (D) Summary data of several experiments performed as in A–C. Each bear indicates the mean ± SEM (n) of the relaxation produced by AEC in the different conditions. Ctrl: effect of AEC in intact rings. $^{*}p < 0.05$.

Table 1
Cardiac effect of aqueous extract of Cistus.

	RP (mmHg)	LVDP (mmHg)	HR (bpm)	AP (mmHg)	T_{pic} (ms)	$T_{1/2}$ (ms)
Control	27 ± 6	94 ± 7	279 ± 14	57 ± 7	43 ± 2	58 ± 2
AEC (10^{-2} g/L)	27 ± 6	87 ± 6	283 ± 15	48 ± 3	43 ± 2	55 ± 2

RP, resting pressure; LVDP, left ventricular developed pressure; HR, heart rate; AP, aortic pressure; T_{pic}, time to pic of contraction; $T_{1/2}$, time of half relaxation. Each value is the mean ± SEM of five individual experiments.

of the NO-donor sodium nitroprusside (SNP, 1 μM) strongly relaxed the muscle. This result indicates that AEC requires an intact endothelium to produce a relaxant effect.

Because the effects of AEC and CCh were similar, we examined whether the relaxant effect of AEC was due to activation of muscarinic receptors. To do this, the vasorelaxant effect of AEC was tested in the presence of atropine, a muscarinic receptor antagonist. As shown in Fig. 4B, atropine (10 μM) completely antagonized the relaxant effect of CCh ($n = 4$) but left unchanged the relaxant effect of AEC (75 ± 3% relaxation, $n = 4$, Fig. 4D). Therefore, muscarinic receptors do not mediate the effect of AEC.

To determine the contribution of endothelium-derived nitric oxide (NO) in the vasorelaxant effect of AEC, endothelium-intact preparations were pre-incubated with L-NNA, an arginine analog shown to cause a complete blockade of the NO-synthase in the same preparation [12]. In the presence of L-NNA (1 mM), the tension developed by the aortic rings was 3.5 ± 0.1 g ($n = 4$) in response to 0.1 μM NA. As shown in Fig. 4C and D, the vasorelaxant effect of AEC was totally blunted in the presence of L-NNA (3 ± 2% relaxation, $n = 4$) while that of SNP was maintained. This indicates that the relaxant effect of AEC involves activation of the NO-synthase.

Cardiac effect

The direct cardiac effect of AEC was examined on isolated and perfused rat hearts. After installation of the balloon in the LV, spontaneous cardiac contractions were recorded. Control heart rate (HR) and left ventricle developed pressure (LVDP) values were, respectively, 279 ± 14 beat/min and 94 ± 7 mm Hg. Then, after administration of AEC at 10^{-2} g/L during 10 min, these values became, respectively, 283 ± 15 beat/min and 87 ± 6 mm Hg ($n = 6$). This indicates that AEC had no direct effect on cardiac function. Values of the other cardiac parameters are summarized in Table 1. They also show no significant effect of AEC.

Discussion

In the present study, we investigated the antihypertensive effect of the aqueous extract of *C. ladaniferus*. We used two recognized models of experimental hypertension, the L-NAME and renovascular 2K-1C hypertensive rats [10,11]. We found that AEC produced a clear antihypertensive effect in both models. We also found that AEC improved vascular reactivity and induced an endothelium- and NO-dependent relaxation on vascular smooth muscle, which may partly account for its antihypertensive action.

Arterial hypertension is frequently associated with an endothelial dysfunction which is revealed by reduction of endothelium-mediated vascular relaxation in response to vasodilator agents such as acetylcholine and CCh [13]. Accordingly, we found a clear impairment of vasorelaxation in response to CCh in pre-contracted aortic rings from both L-NAME and renovascular hypertensive rats. Interestingly, a four-week treatment with AEC substantially improved endothelial function in both animal models of hypertension. In the L-NAME model, AEC showed a *preventive* action because when

administered together with L-NAME during four weeks, SBP increased less and endothelial function was less reduced. In the 2K-1C model, AEC showed a *curative* action because administration of the extract during four weeks after installation of the renovascular hypertension reversed the SBP increase and improved the endothelial function. These findings suggest that long-term AEC administration prevents the alterations of arterial vessel reactivity in chronically NO-deficient and 2K-1C hypertensive rats, most likely through re-establishment of endothelial function.

To get some insight into the mechanisms responsible for the antihypertensive effect of AEC, we investigated the effects of the extract on cardiac and vascular function in normotensive rats. While AEC did not affect basal cardiac function, it strongly relaxed thoracic aortic rings pre-contracted with NA. Moreover, the relaxant effect of AEC was endothelium-dependent and involved activation of the NO-synthase. Several medicinal plants, known to possess antihypertensive properties, were shown to involve activation of the NO pathway. These include *Allium sativm* [14], *Alpinia zerumber* [15] and *Arbutus unedo* [16]. Among these, we found that *Arbutus unedo* behaved very similarly to *C. ladaniferus*, producing a antihypertensive effect on L-NAME hypertensive rats [16] and an endothelium-dependent vasorelaxant effect involving activation of endothelial NO-synthase [17,18].

A number of chemical compounds present in the aqueous extract of *C. ladaniferus* may account for the observed pharmacological activity. These include quercetin, kaemferol, myricetin or other flavonoids witch are the major constituents of Cistus [19,20]. Interestingly, many studies have demonstrated that flavonoids decrease vascular tone and agonist-induced contraction in isolated rat arteries through stimulation of endogenous NO production from the endothelium [21]. Moreover, chronic administration of antioxidant flavonoids such as quercetin was shown to reduce blood pressure and enhance endothelium-dependent relaxation in various animal models of hypertension [22]. This vascular beneficial effect of quercetin and related bioflavonoids were suggested to derive from their protective antioxidant effects which lead to improved bioavailability of endothelium-derived NO [23–25].

In conclusion, the aqueous extract of *C. ladaniferus* decreases blood pressure in hypertensive rats, and acts both in a curative and preventive manner. This antihypertensive effect is partly due to a vasorelaxant effect which involves NO production by the endothelium. Further studies are needed to identify the chemical compound(s) involved and to examine potential beneficial effects in hypertensive patients.

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