
Vasorelaxant effect of the aqueous extract of *Ajuga iva* in rat aorta

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Abstract

The aim of the present study was to investigate the ex vivo and in vitro vascular activity of the aqueous extract of *Ajuga iva* (L.) Schreber (Labiatae) in normotensive Wistar rats. Chronic oral administration of the extract of *Ajuga iva* did not significantly affect the systolic blood pressure. In aorta isolated from *Ajuga iva*-treated rats, the contractile response to noradrenaline was depressed compared to the responses obtained in aorta from untreated rats but the endothelium-dependent relaxation evoked by acetylcholine was not affected. In vitro, *Ajuga iva* extract inhibited the contraction evoked by noradrenaline. The addition of *Ajuga iva* extract during the plateau phase of noradrenaline-evoked contraction produced a relaxation that was sensitive to *N*-nitro-l-arginine. After pre-incubation of the artery in the presence of the plant extract, vasorelaxant effect was markedly less pronounced. The endothelium-dependent relaxation induced by acetylcholine was concentration-dependently blunted in the presence of *Ajuga iva* extract in the bathing solution. This study indicates that the aqueous extract of *Ajuga iva* possesses NO-mediated and NO-independent vasorelaxing properties in vitro while only the endothelium-independent effect was observed ex vivo.

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Keywords: *Ajuga iva*; Vasodilatation; Rat aorta; NO; Endothelium

1. Introduction

*Ajuga iva* L. (family Labiatae), is one of the most commonly prescribed drug in Moroccan pharmacopoeia. It is used as an anthelmintic, against intestinal disorders (Bellakhdar et al., 1991). According to ethnobotanical data collected in oriental Morocco (Ziyyat et al., 1997), *Ajuga iva* is also alleged to possess a hypoglycaemic effect, which has been experimentally demonstrated (Hilaly and Lyoussi, 2002). Furthermore, it has been reported that some species of the genus *Ajuga* exert a broad spectrum of biological and pharmacological actions, as anti-mitotic (Takasaki et al., 1999), anti-bacterial (Chen et al., 1997), cardiotonic (Kuria and Muriuki, 1984), and antifungal (Kariba, 2001).

Nevertheless, vascular activities claimed for *Ajuga iva* have never been proved. Thus, we considered it interesting to investigate whether there is a scientific basis for the traditional use of this plant as an anti-hypertensive drug. In the present study, we examined the in vivo effect of the aqueous extract of *Ajuga iva* on the systolic blood pressure, and its ex vivo and in vitro effects on the vasomotor tone of aortic rings isolated from normotensive rats.

2. Materials and methods

2.1. Plant material and preparation of the extracts

*Ajuga iva* was collected in the septentrional Moroccan province named Tiaouate in April-May (2001). Authentic samples were identified by the department of Botany, Scientific National Institute (Rabat), where a voucher specimen was deposed (H63).

Fifty gram of dried powder of the whole plant of *Ajuga iva* was decocted with 500 ml of water. The mixture was heated and boiled under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at −20 °C and then lyophilised (FreeZone® Dry 4.5, USA), yielding 12.5 g of extract.

2.2. Experimental animals

Healthy adult Wistar rats, 250–350 g, were used in this study. All rats were maintained at a constant temperature...
KCl, 5.9; NaHCO₃, 15; MgCl₂, 1.25; CaCl₂, 1.25; glucose, a physiological solution (composition (mM): NaCl, 122; dissected and mounted in 12.5 ml organ baths filled with ±(24 °C), with a 12 h dark-light cycle and on standard chow.

2.3. In vivo study

An adaptation period of 3 days for vehicle administration and blood pressure measurement was allowed before the initiation of the treatment. Twelve Wistar rats were randomly assigned to control group (distilled water, 1 ml/100 g) and Ajuga iva-treated group (500 mg/kg). Rats were treated orally by force-feeding once a day for 8 days. During the experimental period, animals had free access to tap water and chow. Systolic blood pressure (SBP) was measured in conscious, prewarmed, restrained rats in thermostatic cages by the tail-cuff method (Physiograph Narco, Houston, TX, USA).

2.4. Ex vivo and in vitro vascular reactivity

Vascular effects of Ajuga iva were tested ex vivo and in vitro. In ex vivo experiments, aorta was isolated from rats that had received an oral daily dose of Ajuga iva (500 mg/kg) for 8 days, whereas in vitro tests were carried out with aorta isolated from untreated rats. Rats were killed by decapitation using small animal guillotine. The thoracic aorta was rapidly removed, and immersed in cold (4 °C) physiological solution. Aortic rings (±2 mm) were dissected and mounted in 12.5 ml organ baths filled with a physiological solution (composition (mM): NaCl, 122; KCl, 5.9; NaHCO₃, 15; MgCl₂, 1.25; CaCl₂, 1.25; glucose, 11) supplemented with indomethacin (10 μM), gassed with a mixture of 95% O₂-5% CO₂ and maintained at 37 °C. The rings were stretched to a resting tension of 20 mN. Isometric tension was measured as described (Morel and Godfraind, 1994).

2.5. Statistical analysis

Results are expressed as mean ± S.E.M. Contractile response to noradrenaline is expressed as mN/g tissue (wet weight). Student’s t-test or ANOVA were used to compare data and P-values < 0.05 were considered to denote statistical significance of differences (GraphPad Prism). Concentration-response curves were analysed by non-linear regression (GraphPad Prism).

3. Results

3.1. In vivo study

The treatment of the rats with Ajuga iva water extract (500 mg/kg) did not affect the body weight of the rats. Fig. 1 shows that there was no significant effect of the treatment on the SBP of normotensive Wistar rats.

3.2. Effect of Ajuga iva on the contractile responses to noradrenaline

Fig. 2A and B shows the ex vivo effect of Ajuga iva extract (500 mg/kg) on cumulative concentration-contraction curves to noradrenaline in aortic rings incubated without (Fig. 2A) and with the NO synthase inhibitor N-nitro-l-arginine (l-NNA, 100 μM) (Fig. 2B). In the absence as well as in the presence of l-NNA, the contraction curves obtained in aortas isolated from Ajuga iva-treated rats were significantly depressed compared to the curves obtained in arteries from untreated rats. In the absence of l-NNA, the maximal response obtained with 3 μM noradrenaline was reduced by 21% in rings isolated from treated compared to untreated rats (from 15.6 ± 1.3 mN/mg (control) to 12.3 ± 0.5 mN/mg (treated-rats), P < 0.05, n = 5). In rings incubated with l-NNA the response to the highest noradrenaline concentration was depressed by 30% in Ajuga iva-treated rats (from 20.8 ± 0.9 in untreated rats to 14.9 ± 1.7 mN/mg in treated rats, n = 4; P < 0.05). The shift of the contraction curves to noradrenaline induced by l-NNA was observed in aorta from untreated as from treated-rats. pD₂ (−log ED₅₀) values of noradrenaline in untreated rats were 7.79 ± 0.09 (n = 4) and 8.58 ± 0.04 (n = 5, P < 0.01), in the absence and in the presence of l-NNA, respectively. In Ajuga iva-treated rats, pD₂ values were 7.18 ± 0.16 (n = 4) and 8.27 ± 0.06 (n = 5, P < 0.01), in the absence and in the presence of l-NNA, respectively. The treatment with Ajuga iva decreased the pD₂ value of noradrenaline but the difference did not reach a statistically significant level when the artery was incubated in the presence of l-NNA (ANOVA).

Fig. 2C and D shows the in vitro effects of Ajuga iva extract on the contraction evoked by noradrenaline, after 30 min pre-incubation of aortic rings with increasing concentrations of Ajuga iva (0.12, 0.60, 1 mg/ml) in the bath. Higher concentrations of Ajuga iva (3–5 mg/ml) contracted the artery, probably because of their hypertonicity.
Fig. 2. (A), (B) Ex vivo and (C), (D) in vitro effects of *Ajuga iva* on the contractions evoked by noradrenaline in aortic rings incubated without (A), (C) and with *N*-nitro-1-arginine (l-NNA; (B), (D)). Ex vivo effect was determined in aorta isolated from rats which had received either *Ajuga iva* extract (500 mg/kg, *n* = 5) or water (*n* = 5) for 8 days orally. In vitro effect of *Ajuga iva* was determined after preincubation of aortic rings in the presence of various concentrations of *Ajuga iva* extract in the bathing solution and are expressed as a percentage of the maximum response obtained before the incubation with the plant extract. Each point is the mean from 3–5 determinations. (*) indicates significantly different responses (*P* < 0.05).

(data not shown). In the absence of l-NNA (Fig. 2C), the noradrenaline concentration-contraction curves were not significantly affected by concentrations of *Ajuga iva* extract lower than 1 mg/ml. At 1 mg/ml *Ajuga iva* extract depressed the maximal response to noradrenaline by 19 ± 4% (*n* = 4, *P* < 0.05). pD2 values of noradrenaline were not significantly changed. In the presence of l-NNA (Fig. 2D), *Ajuga iva* extract at concentration higher than 0.2 mg/ml produced a concentration-dependent depression of noradrenaline-evoked contraction without affecting the pD2 value of noradrenaline (Table 1).

In another series of experiments, the effect of *Ajuga iva* extract was tested by adding the extract into the bathing solution containing noradrenaline (0.3 μM),

<table>
<thead>
<tr>
<th><em>Ajuga iva</em> (mg/ml)</th>
<th>Without l-NNA</th>
<th>In the presence of l-NNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noradrenaline pD2</td>
<td>Maximum contraction (%)</td>
</tr>
<tr>
<td>0</td>
<td>7.34 ± 0.07</td>
<td>98.8 ± 2.5</td>
</tr>
<tr>
<td>0.12</td>
<td>7.55 ± 0.05</td>
<td>92.7 ± 3.1</td>
</tr>
<tr>
<td>0.20</td>
<td>7.43 ± 0.15</td>
<td>97.2 ± 3.5</td>
</tr>
<tr>
<td>0.60</td>
<td>7.51 ± 0.12</td>
<td>91.9 ± 2.4</td>
</tr>
<tr>
<td>1</td>
<td>7.61 ± 0.20</td>
<td>81.9 ± 2.4*</td>
</tr>
</tbody>
</table>

pD2 (−log ED50 (M)) and maximum responses were calculated by non-linear regression of experimental data. Maximum response is expressed as a percentage of the maximum contraction obtained in the first stimulation to noradrenaline. Values are means of 3 to 5 determinations.

*Significantly different from the control (*P* < 0.05, ANOVA).
when the contraction had reached a plateau. Under this condition, *Ajuga iva* extract produced a rapid and concentration-dependent relaxation of the contractile tension evoked by noradrenaline (Fig. 3A). At 0.6 mg/ml *Ajuga iva* relaxed the contraction by 79 ± 2.2% (*n* = 4). This effect was observed within 15 min after the addition of the extract into the bathing solution; it was significantly larger than the inhibition observed when the artery was pre-incubated for 30 min in the presence of *Ajuga iva* extract before inducing the contraction (Fig. 3B). Inhibition of NO synthase with l-NNA significantly depressed the relaxation evoked by *Ajuga iva* extract added during noradrenaline-contraction: under this condition, *Ajuga iva* 1 mg/ml relaxed the contraction by 16 ± 5.3% (*n* = 5), an effect similar to the inhibition of the contraction observed after pre-incubation of the artery with the extract (Fig. 3B).

### 3.3. Effect of *Ajuga iva* on the endothelium-dependent relaxation to acetylcholine

The ex vivo effect of the oral treatment with the *Ajuga iva* extract (500 mg/kg) was tested on the endothelium-dependent relaxation evoked by acetylcholine in noradrenaline-contracted artery. As shown in Fig. 4A, the relaxation evoked by acetylcholine in aortic rings isolated from *Ajuga iva*-treated rats was not markedly different from the responses observed in aorta from untreated rats. The effect of the lowest concentration of acetylcholine was slightly

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![Fig. 3. Influence of pre-incubation on the in vitro effect of *Ajuga iva* extract on noradrenaline contraction. (A) *Ajuga iva* extract was added into the bathing solution during the plateau phase of the contraction to noradrenaline (0.3 μM) in rat aortic rings in the presence (●) or in the absence (□) of l-NNA. (B) Artery rings were incubated with *Ajuga iva* extract for 30 min before the contraction was evoked by adding noradrenaline (0.3 μM) into the bathing solution in the presence (●) or in the absence (□) of l-NNA. Each value is the mean ± S.E.M. from five to seven determinations. *P* < 0.05.](image1)

![Fig. 4. (A) Ex vivo and (B) in vitro effect of *Ajuga iva* extract on the relaxation curves to acetylcholine in aorta. Ex vivo effect was determined in aortic rings isolated from control rats (*n* = 5) and from *Ajuga iva*-treated rats (500 mg/kg, *n* = 5). In vitro effect was determined in aortic rings preincubated without (control), or with various concentrations of *Ajuga iva* extract (0.040, 0.12 and 0.20 mg/ml) for 30 min. Contraction was evoked by noradrenaline (0.3 μM). Acetylcholine was added into the solution when the contraction had reached a plateau. Each point is the mean from 3–5 determinations. (♦) *P* < 0.05 vs. untreated rat or control.](image2)
increased but the maximum relaxation evoked by acetylcholine was not significantly different: it reached 64 ± 6.2% (n = 4) and 59 ± 2.5% (n = 4) of the contraction, in untreated and treated rats, respectively (P > 0.05). pD2 values of acetylcholine were not significantly different (6.81 ± 0.04, n = 4, and 7.07 ± 0.07, n = 10, in untreated and treated rats, respectively).

The in vitro effect of Ajuga iva extract on the endothelium-dependent relaxation to acetylcholine (10−6 and 10−5 M) performed in rings contracted by noradrenaline (3 × 10−6 M) was, respectively, 38 ± 4.4% (n = 3) and 24 ± 11.5% (n = 3) of the contraction while control rings relaxed by 76 ± 6% (n = 4, P < 0.05).

3.4. Effect of Ajuga iva on the contraction evoked by 100 mM KCl solution

Fig. 5 shows the in vitro effect of Ajuga iva extract on the contraction evoked by high KCl solution (100 mM) in rat aortic rings. Pre-incubation in the presence of increasing concentrations of Ajuga iva extract (0.2, 0.6 and 1 mg/ml) for 30 min before a second contraction was evoked by KCl. Contractile responses are expressed in percentage (%) of the contraction of the first run. Each value is the mean of 3 to 5 determinations.

4. Discussion

The present study showed that the aqueous extract of Ajuga iva elicited ex vivo and in vitro vasorelaxant effects in aorta isolated from Wistar rats pre-contracted by noradrenaline, but did not affect the systolic blood pressure of normotensive rats, when given by oral administration. The lack of effect of Ajuga iva treatment on the SBP of normotensive rats could be attributed to the mechanisms of regulation involved in homeostasis.

Two different modes of action can be distinguished in the vascular effects of Ajuga iva, in view of the kinetics of the effects and of their sensitivity to the NO synthase inhibitor, l-NNa. The first mode of action of Ajuga iva was dependent on the activity of the endothelial NO synthase. It could be observed when Ajuga iva extract was applied during a contraction evoked by noradrenaline in preparations with functional endothelium. Under these conditions, Ajuga iva produced a large relaxation, which was markedly reduced in the presence of l-NNa. This effect disappeared after 30 min incubation of the artery in the presence of the plant extract. Alteration of the NO-mediated relaxation after preincubation with Ajuga iva could be related to the inhibition of the endothelium-dependent vasorelaxation evoked by acetylcholine, which, in rat aorta, is mainly mediated by the activation of endothelial NO synthase and the release of NO (Egleme et al., 1984; Shimokawa et al., 1996). The other action of Ajuga iva was not dependent on NO, was of smaller amplitude and was still observed after prolonged incubation of the artery in the presence of the plant extract.

Ajuga iva produced a significant depression of noradrenaline-evoked contraction in ex vivo tests, performed after 8 days of oral treatment of the rat with the plant extract. This effect was not dependent on the release of NO since it was not depressed by l-NNa. Moreover, Ajuga iva-treatment did not affect the endothelium-dependent acetylcholine-evoked relaxation, suggesting that the treatment of the rat with the plant extract did not produce the endothelium-damaging effect observed in vitro.

KC1-evoked contractions, which result from the entry of Ca2+ through voltage-operated Ca2+ channels (Godfraind and Kaba, 1969), were not markedly depressed by Ajuga iva extract, indicating that the plant does not interact with voltage-operated Ca2+ channels.

Taken together these observations suggest that the Ajuga iva extract contains more than one vaso-active compound. One compound could be responsible for the inhibition of the contraction evoked by noradrenaline through a pathway that was not dependent on NO or prostanooids (all experiments were performed in the presence of indomethacin) in vitro as well as ex vivo. The effect of a second compound could only be identified in vitro as a transient NO-dependent relaxation. Ajuga iva could have a biphasic effect on the NO pathway in endothelial cells: the rapid stimulation could be followed by a toxic effect resulting in the inhibition of the activity...
of the NO synthase. Alternatively, the deleterious effect of 
*Ajuga iva* on the endothelial cells could be caused by yet 
another compound. Since the latter effect was not observed 
after oral administration of the extract, it should be caused 
by a compound that is either not resorbed or subjected to 
first pass metabolism.

A preliminary phytochemical analysis of the total extract 
of *Ajuga iva*, according to the Paris and Nothis’s method 
(Paris and Nothis, 1969), has revealed the presence of 
flavonoids and tannins as major compounds while saponins 
are very scarce. Flavonoids have a broad spectrum of bio-
logical and pharmacological activities including vasodila-
tory effect (Duarte et al., 1993), which make them good 
candidates for the effects observed in the present study. 
However, it cannot be ruled out that the vascular relaxation 
to *Ajuga iva* extract may be due to the presence of tannins 
since their vasorelaxant activity has been reported (Calixto 
et al., 1986). Many other classes of natural substances 
with vasodilatory effect have been identified in the genus 
*Ajuga* such as triterpenes (Ben Jannet et al., 2000; Cantrell 
et al., 1999), diterpenes and glycosides (Takasaki et al., 1999).

In conclusion, the present study demonstrates that 
*Ajuga iva* possesses different vasodilatory properties. Further 
chemical and pharmacological experiments are required to 
investigate its potential anti-hypertensive activity in hyper-
tensive rats and to identify the active principle(s) responsible 
for the vascular activity attributed to the plant in Moroccan 
pharmacopoeia.

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