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Acute hypotensive and diuretic activities of *Artemisia herba alba* aqueous extract in normal rats

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ABSTRACT

Objective: To evaluate the effect of *Artemisia herba alba (A. herba alba)* intravenous injection on cardiovascular and renal function in normal rats.

Methods: The effect of intravenous injection of *A. herba alba* extract at the different doses of 50, 100 and 200 mg/kg was investigated in normal rats. Diuresis, heart frequency and electrolytes concentrations were analyzed.

Results: Intravenous bolus injection of *A. herba alba* at the different doses of 50, 100 and 200 mg/kg produced a dose dependent reduction in arterial blood pressure ($P<0.001$). A significant reduction in heart frequency was observed after *A. herba alba* injection at the doses of 100 and 200 mg/kg ($P<0.001$). Perfusion of aqueous *A. herba alba* extract at a dose of 200 mg/(kg·h) caused a significant increase in urine output after 4 h of perfusion ($P<0.001$). In addition, a significant increase in urinary sodium and potassium excretion was observed from the first ($P<0.05$) to the fourth hour ($P<0.001$) of *A. herba alba* perfusion. Urinary chlorure excretion increased after 2 h of perfusion ($P<0.001$). However, glomerular filtration rate remained unchanged after *A. herba alba* perfusion ($P<0.05$).

Conclusions: We conclude that the aqueous *A. herba alba* extract possesses a potent acute hypotensive effect on normal rats. In addition, *A. herba alba* perfusion may affect renal function to increase urine and electrolytes excretion.

1. Introduction

Pharmacological research on the medicinal properties of phytochemicals has become mandatory to establish the claimed medicinal properties of herbs[1]. According to the World Health Organisation, more than 80% of the world's population relies on plants and plant-derived medicines for their healthcare[2]. More than 231 medicinal plants are communally used in the Moroccan pharmacopoeia. As a part of Moroccan territory, Tafilalet Region is a repository of many plants used by the local population for the treatment of cardiovascular diseases[3]. Increasing urine and electrolytes excretion may be beneficial for the treatment of hypertension[4]. Previous studies have reported the diuretic effect of several plants extract on normal and hypertensive rats[5-7]. Furthermore, the observed diuretic activity associated several cases with a reduction in arterial blood pressure[8].

Artemisia herba alba (A. herba alba) is a plant belonging to Asteraceae family. The aqueous extract of this plant is used for the treatment of diabetes and cardiovascular diseases[9]. The scientific information concerning the cardiovascular effect of aqueous *A. herba alba* extract is very limited. This study was carried out to evaluate the effect of intravenous injection of *A. herba alba* on arterial blood pressure and renal function in normal rats.

2. Materials and methods

2.1. Plant material

Specimens of *A. herba alba* were collected from the

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Tafilalet Region (semi-arid area) of Morocco in May-June 2010, and air-dried at 40 °C. The plant was previously identified and authenticated (Agronomy and Veterinary Institute, Rabat) and a voucher specimen (ME23) was deposited at the herbarium of the Faculty of Sciences and Techniques Errachidia.

2.2. Preparation of the aqueous extract

About 1 g of powdered plant mixed with 100 mL distilled water were boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm, St Quentin en Yvelines, France) to remove particulate matter. The extract was yellow with a percent yield of 14%, and its average osmolarity was 32 mOsm/kg, pH 6.3, and with a very low viscosity. The filtrate was then freeze-dried and the desired dose (mg of lyophilized aqueous extract of *A. herba alba* plant per kg body weight) was then prepared and reconstituted in physiologic saline solution just before administration.

2.3. Animals

The hypotensive and diuretic effects of aqueous *A. herba alba* extract were studied in adult male Wistar rats weighing 250-300 g and 6 weeks old were used. Animals were housed under standard environmental conditions [(23±1) °C, 55%±5% humidity and a 12 h light/dark cycle] and had free access to water and standard laboratory diet *ad libitum*.

2.4. Surgery

The rats were anaesthetized by an intraperitoneal injection of inactin at a dose of 50 mg/kg of body weight. They were then placed on a thermostated table to keep them at a constant temperature.

2.5. Experimental procedures

2.5.1. Effect of intravenous bolus injection of aqueous A. herba alba extract on mean arterial blood pressure and heart rate

Arterial blood pressure was measured from the carotid artery via heparinized polyethylene cannula PE 50 connected to a blood pressure transducer (Gould Statham transducer) allowing the monitoring of blood pressure.

The desired dose of furosemide and *A. herba alba* lyophilized aqueous extract were dissolved in a constant volume of isotonic saline solution (200 μL) and injected as a bolus injection via a catheters PE-10 inserted into the external jugular vein followed by a saline flush (200 μL). Animals were allowed to equilibrate for at least 30 min before administration of aqueous *A. herba alba* extract.

Three different groups of six rats for each group were administered with graded doses (50, 100 and 200 mg/kg) of aqueous *A. herba alba* extract intravenously; the corresponding blood pressure and heart rate were recorded immediately. A control group of 6 rats received a bolus intravenous injection (200 μL) of saline solution (0.9%). Mean arterial blood pressure is a sum of diastolic blood pressure and 1/3 pulse pressure (mmHg).

2.5.2. Renal effect of aqueous A. herba alba extract perfusion

Two catheters PE-50, one filled with physiological saline solution (NaCl), the other filled with heparinized physiological saline solution, were introduced respectively to the right jugular vein of the cardiac side and to the left jugular vein of the encephalic side. The first served to perfuse the test solutions, and the second for blood sampling. The bladder was also catheterized in order to collect urine for determination of different parameters. At the end of the experiment, the animals were sacrificed by cutting their carotids; urine and plasma were conserved at -20 °C until biochemical analysis.

The first group of six rats serving as an untreated control were treated with physiological saline solution (0.9%), whereas the second group was given a perfusion of *A. herba alba* at a dose of 200 mg/(kg·h). The third group received furosemide as a reference drug at a dose of 0.1 mg/(kg·h). The experimental protocol adopted had two phases: a control phase of 30 min when saline solution was administered and an experimental phase of 4 h for aqueous *A. herba alba* extract and furosemide perfusion. Five urine and blood samples were then taken at the start of control phase and within the experimental phase at the start, 1, 2 and 4 h of treatment. All experiments were performed in fasted rats.

2.5.3. Parameters

Blood samples were collected from the jugular vein. Urinary samples were collected from the catheterized bladder. Creatinine was evaluated by colorimetric methods, according to the manufacturer's protocol (Boerhinger, Germany) using a spectrophotometer (HITACHI Model U-2001). Sodium, potassium and chloride levels were determined in urine samples using an auto-analyser (HITACHI 911-Boehringer). Glomerular filtration rate (GFR) was evaluated by the clearance of creatinine.

2.6. Statistical analysis

Results were expressed as mean±SEM of six observations. ANOVA was used to calculate the levels of significance for comparison made within group and between groups using Graphpad Prism 4.0 software.

3. Results

3.1. Effect of intravenous injection of aqueous A. herba alba extract on arterial blood pressure

Lyophilized aqueous *A. herba alba* extract was injected intravenously at three different doses: 50, 100, 200 mg/kg to anesthetized normal rats. Figure 1 shows the arterial blood pressure evolution after intravenous injection of aqueous *A. herba alba* extract. The three doses of *A. herba alba* extract

used in this study produced a significant reduction in arterial blood pressure 2 min after intravenous injection (P<0.001). Maximal reduction was observed 2 min after the intravenous injection of 200 mg/kg of aqueous *A. herba alba* extract (P<0.001). The observed fall in arterial blood pressure disappeared progressively, causing a return to the baseline values after 10 min of intravenous injection. However, in the control group, intravenous injection of 200 μL of saline solution did not affect arterial blood pressure.

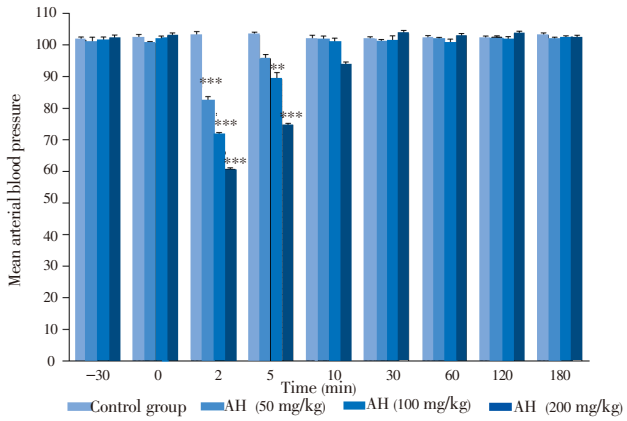

Figure 1. Effect of a bolus intravenous injection of aqueous *A. herba alba* extract (50, 100 and 200 mg/kg) on arterial blood pressure (mmHg) in anesthetized normal rats.

AH: *A. herba alba*. Data are expressed means±SEM, n=6. $^{**}P$<0.01, $^{***}P$<0.001.

3.2. Effect of intravenous injection of aqueous *A. herba alba* extract on heart rate

Intravenous injection of aqueous *A. herba alba* extract at the doses of 100 and 200 mg/kg produced a reduction in heart rate (P<0.01 and P<0.001 respectively) (Figure 2). No significant change in heart rate was observed after 5 min of *A. herba alba* intravenous injection. The intravenous injection of aqueous *A. herba alba* extract at a dose of 50 mg/kg did not affect significantly the heart rate.

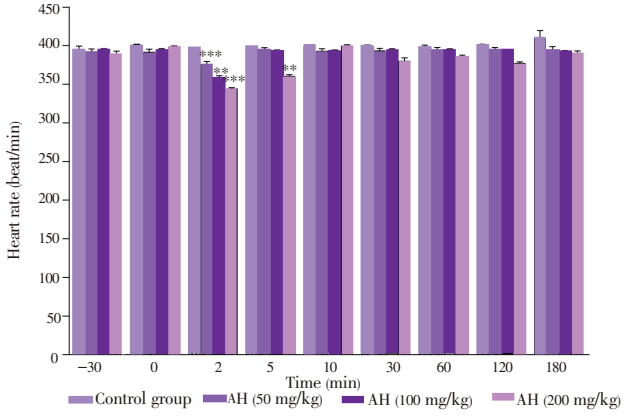

Figure 2. Effect of a bolus intravenous injection of aqueous *A. herba alba* extract (50, 100 and 200 mg/kg) on heart rate (beats/min) in anesthetized normal rats.

AH: *A. herba alba*. Data are expressed means±SEM, n=6. $^{**}P$<0.01, $^{***}P$<0.001.

3.3. Renal effect of intravenous perfusion of aqueous *A. herba alba* extract

3.3.1. Effect on urine output

Intravenous perfusion of aqueous *A. herba alba* extract at a dose of 200 mg/(kg·h) produced a significant increase in urine output 4 h after the start of perfusion. However, furosemide perfusion significantly increased the urine output after 1 h of intravenous perfusion. The increase in urine output was sustained until 4 h of perfusion. No change in the urine output was observed during 4 h of intravenous perfusion of saline solution (Figure 3).

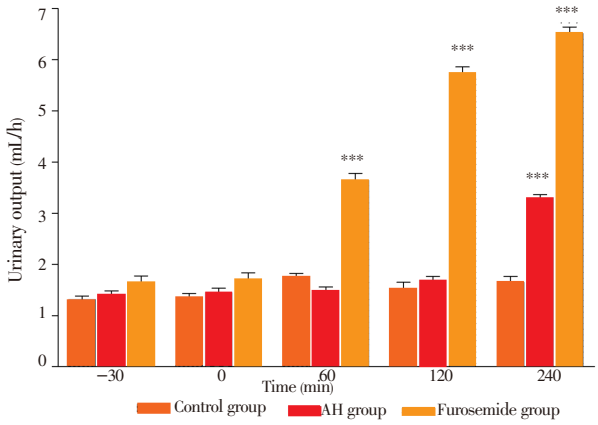

Figure 3. Effect of intravenous administration of aqueous *A. herba alba* extract (200 mg/(kg·h) on urinary excretion of water (mL/h).

AH: *A. herba alba*. Data are expressed as means±SEM, n=6. $^{***}P$<0.001 when compared to the respective control values.

3.3.2. Effect on GFR

No significant change in GFR was observed after intravenous perfusion of *A. herba alba* extract at a dose of 200 mg/(kg·h) (P>0.05). Furosemide treated group showed a significant increase in GFR after 2 h of intravenous perfusion (P<0.01). Additional increase was observed at the end of experiments (P<0.001) (Figure 4).

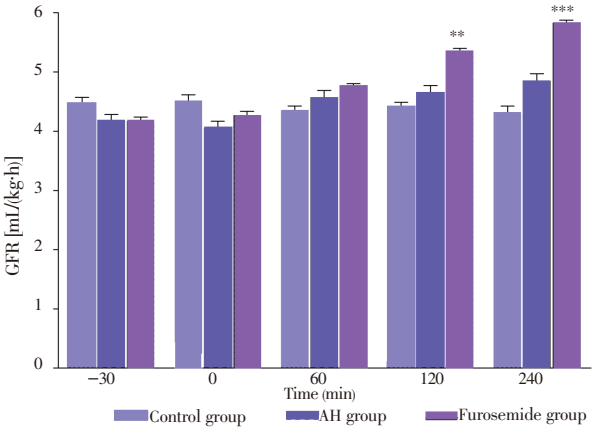

Figure 4. Effect of intravenous administration of aqueous *A. herba alba* extract [200 mg/(kg·h)] on GFR.

AH: *A. herba alba*. Data are expressed as means±SEM, n=6. $^{**}P$<0.01, $^{***}P$<0.001 when compared to the respective control values.

3.3.3. Effect on urinary sodium excretion

Figure 5 depicts changes in urinary electrolytes excretion after intravenous perfusion of aqueous *A. herba alba* extract. When administered at a dose of 200 mg/(kg·h), aqueous *A.*

herba alba extract produced a significant increase in the urinary sodium excretion after 2 h of perfusion ($P<0.001$). Additional perfusion during 1 h did not cause further increase in urinary sodium excretion. Furosemide perfusion when administered at a dose of 0.1 mg/(kg·h) produced a significant and sustained increase in urinary sodium excretion from the first to the fourth hour of perfusion.

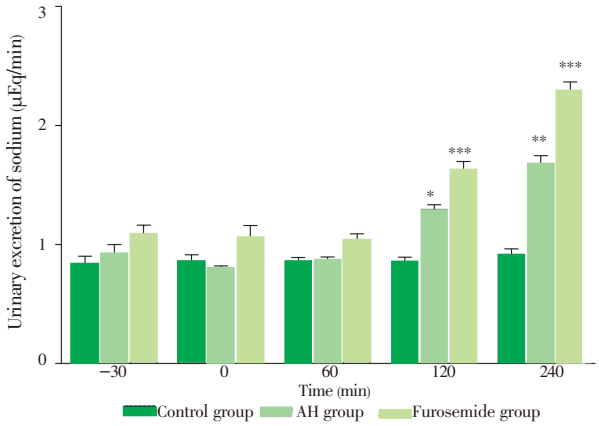

Figure 5. Effect of intravenous administration of aqueous *A. herba alba* extract [200 mg/(kg·h)] on urinary excretion of sodium.
AH: *A. herba alba*. Data are expressed as means±SEM, $n=6$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared to the respective control values.

3.3.4. Effect on urinary potassium excretion

Intravenous perfusion of aqueous *A. herba alba* extract at a dose of 200 mg/(kg·h) caused a significant increase in urinary potassium excretion after 2 h ($P<0.05$) and 3 h ($P<0.01$) of intravenous perfusion (Figure 6). In addition, furosemide perfusion produced a significant increase in urinary potassium excretion from the first hour ($P<0.05$) to the fourth hour ($P<0.001$).

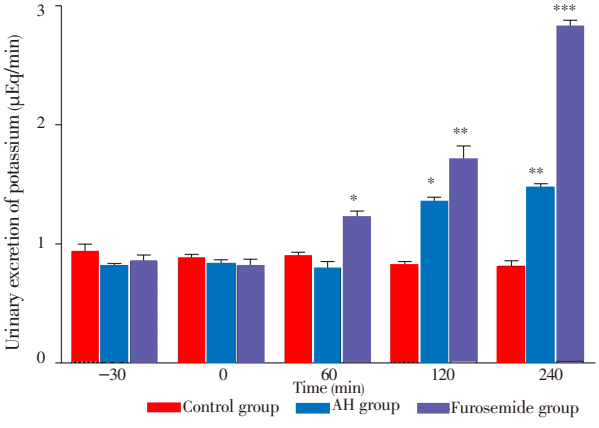

Figure 6. Effect of intravenous administration of aqueous *A. herba alba* extract [200 mg/(kg·h)] on urinary excretion of potassium.
AH: *A. herba alba*. Data are expressed as means±SEM, $n=6$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared to the respective control values.

3.3.5. Effect on urinary chlorure excretion

Figure 7 shows the evolution of urinary chlorure excretion. Intravenous injection of aqueous *A. herba alba* extract caused a significant increase 2 h after the start of perfusion ($P<0.001$). No additional increase was observed at the fourth hour of perfusion ($P<0.001$). Finally, a significant increase in urinary chlorure excretion was observed after 1 h of furosemide perfusion ($P<0.001$).

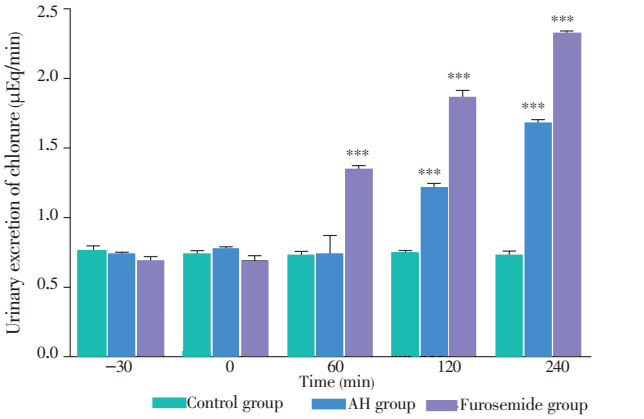

Figure 7. Effect of intravenous administration of aqueous *A. herba alba* extract [200 mg/(kg·h)] on urinary excretion of chlorure.
AH: *A. herba alba*. Data are expressed as means±SEM, $n=6$. $^{***}P<0.001$ when compared to the respective control values.

4. Discussion

A. herba alba is widely used in the treatment of various diseases[9,10]. This study was carried out to evaluate the hypotensive and diuretic effects of intravenous injection of aqueous *A. herba alba* extract on normal rats. Among the various plants, about 500 plants belong to the genus of *Artemisia*[11]. Most *Artemisia* herbs are perennials and grow in the northern hemisphere. They are commonly used for various purposes, such as medicine, food, spices and ornaments. The medicinal effects of *Artemisia* herbs are extremely diverse and these effects include cell protection from peptic ulcers, liver protection, anti-malarial, anti-tumor and anti-diabetic[9,11-13]. Several *Artemisia* herbs like *A. herba alba*, *Artemisia santonicum* and *Artemisia pallens*, have been reported to be beneficial for experimental animal or people with diabetes[9]. In the present study, aqueous *A. herba alba* extract was lyophilized and injected intravenously as a bolus at the doses of 50, 100 and 200 mg/kg of body weight and arterial blood pressure was monitored via a catheter placed in the carotid artery. In spite of some disadvantages, the method used in this study for blood pressure measurement was largely used in the screening of cardiovascular effect of drugs[14]. The intravenous injection of aqueous *A. herba alba* extract produced a dose dependent reduction in arterial blood pressure. Previous studies have established the hypotensive effect of plant extracts after intravenous injection[1,15]. Furthermore, aqueous *A. herba alba* extract produced a significant reduction in heart frequency, so, aqueous *A. herba alba* extract may reduce arterial pressure via at least a reduction of heart rate[16]. However, it is not excluded that the aqueous *A. herba alba* extract may act via the adrenergic system to reduce vascular tone[16]. The rapid unset of arterial pressure reduction leads us to postulate the probable implication of sympathic or cholinergic system in the hypotensive effect of aqueous *A. herba alba* extract. In normal rats, blood pressure is strictly controlled via a complex nervous and hormonal function[17], the observed reduction in the arterial blood pressure shows

the strong hypotensive effect of aqueous *A. herba alba* extract.

In the second part of this study, aqueous *A. herba alba* extract was administered intravenously at a dose of 200 mg/(kg·h). Urine volume and electrolytes were measured in order to evaluate the renal effect of aqueous *A. herba alba* extract. Furosemide, a diuretic agent, was used as a reference drug[18]. We demonstrate that aqueous *A. herba alba* extract increased diuresis. Furthemore, sodium, potassium and chlorure urinary excretion was increased. Previous studies have demonstrated a diuretic activity of medicinal plants[6-8]. The increase in urinary electrolytes may be probably due to the inhibition of renal Na^+-K^+ pump which would lead to a reduction in Na^+ and K^+ reabsorption inducing thus an osmotic water flow into the lumen and diuresis[6]. Plant mineral content may probably increase urinary and electrolytes excretion[15,16]. *A. herba alba* did not produce a renal artery vasodilatation since GFR was unchanged during perfusion. Previous studies have reported the increase of GFR in parallel with increased diuresis[14]. It is well established that the elevation of diuresis and electrolytes excretion may reduce arterial blood pressure[15,16]; aqueous *A. herba alba* extract may reduce blood pressure after chronic administration.

A phytochemical investigation reported the abundance of flavonoid products in *A. herba alba*[18]. Flavonoids are known to possess a beneficial cardiovascular effect and may be responsible for the observed hypotensive and diuretic effect.

We conclude that the aqueous *A. herba alba* extract possesses a dose dependent hypotensive and diuretic effect when administered intravenously in rats. Further phytochemical and toxicological studies are warranted to support the role of *A. herba alba* in the management of hypertension.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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