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Hypotensive and diuretic activities of aqueous extract of *Rauvolfia obscura* K. Schum root bark in normotensive rats

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Abstract
Rauvolfia obscura is a plant belonging to the Apocynaceae family, used in traditional medicine for its therapeutic virtues. Scientific information on the hypotensive and diuretic effects of the aqueous extract of *R. obscura* is very limited. The aim of this study was to evaluate the hypotensive and diuretic activities of the aqueous extract of *R. obscura* root barks in normotensive rats. The hypotensive effect of intravenous injection of the aqueous extract of *R. obscura* root bark at doses of 15 and 30 mg/kg was studied in normotensive rats. SAP and HR were measured by the invasive method. Diuretic activity was also assessed in rats. Cumulative excreted urine volumes over 6 h in rats were measured along with electrolyte analyses. Intravenous injection of *R. obscura* extract at doses of 15 and 30 mg/kg produced a dose-independent reduction in SAP (p<0.001). There was a significant reduction (p<0.001) in Fréquence Cardique. In addition, rats treated with *R. obscura* aqueous extract (150 and 300 mg/kg) and furosemide (40 mg/kg) had higher cumulative urine excreted volumes (EEV) over 6 h than control rats. These volumes were 53.08%, 114.14% and 166.72% respectively, compared with 50.6% in control rats. The results of electrolyte analyses (Na+, K+, Cl-) show that the extract increases natriuria, but decreases kaliuria. *R. obscura* aqueous extract has a powerful hypotensive effect in rats. It also has a weak diuretic effect in rats. It eliminates more sodium ions and spares potassium ions.

Keywords: Hypotensive, diuretic, *Rauvolfia obscura* and furosemide

Introduction
Since ancient times, medicinal plants have been a major source for the treatment of many human diseases worldwide. Thus, the demand for remedies derived from natural sources to replace synthetic therapeutic drugs and minimize their side effets and toxicity, has been steadily increasing. According to the World Health Organization (WHO), 80% of the African population still use medicinal plants to meet their health and care needs (WHO, 2000). These include diuretic plants, which are very useful when urination is reduced. In addition, these plants help increase urinary and electrolyte excretion, which is beneficial in the treatment of hypertension (Ali Zeggwagh *et al*., 2007) [3]. Previous studies have reported the diuretic effect of several plant extracts on normo and hypertensive rats (Ngolo, 2021; Sanogo *et al*., 2009) [11, 15].

However, *Rauvolfia obscura (R. obscura*) is a plant belonging to the Apocynaceae family, used in traditional medicine to treat gastrointestinal, genital-urinary and pulmonary disorders. It is also reputed for pain relief (Bouquet, 1969) [5]. Scientific studies on the hypotensive and diuretic effects of *R. obscura* aqueous extract are very limited. The present study was carried out to evaluate the hypotensive and diuretic activities of *R. obscura* root bark aqueous extract in rats.

Materials and Methods
Plant material
R. obscura root bark was used. The roots were harvested in Gamboma, 365 km north of Brazzaville, in May 2023. Identification of a leaf sample was carried out at the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) in the national herbarium department under number IEC001856 dated 9/12/96.

Animal material

Male and female wistar rats (250g to 300g) were used. They were supplied by the animal house of the Laboratoire des Sciences de la Vie et de la Terre (S.V.T) of the Ecole Normale et Supérieure (ENS) of the Marien NGOUABI University. These animals were raised under standard conditions of air temperature of around 25 °C, humidity of 50-60% and a 12-hour day/night photoperiodic cycle. These animals had free access to tap water and a standard feed in the form of patties.

Preparation of aqueous extract of *R. obscura* root bark

Fifty grams (50 g) of *R. obscura* powder were macerated in 500 ml of distilled water for 48 hours. After filtration through absorbent cotton and "Wattman" filter paper, the macerate was concentrated in a water bath thermostated at 55 °C, to obtain the brown aqueous extract. The aqueous extract obtained was stored in a flask. This aqueous extract enabled us to carry out the experiments.

Evaluation of the hypotensive effect of aqueous extract of *Rauvolfia obscura* in normotensive rats

Animal preparation

Animal anesthesia

Rats were anaesthetized by intraperitoneal injection of 15% urethane at a dose of 1.5 g/kg at a rate of 1 mL/100 g body weight (Dimo, 2003) [6].

Exposure and catheterization of the femoral vein

The anaesthetized rat was fixed in dorsal recumbency, with pins planted on its four (4) legs on a cork board. After depilating the animal at the hip, a median and longitudinal incision was made in the skin on the inner side of the hip. This revealed the femoral vein, which had a dark-red appearance. This vein is then separated from the other tissues and freed over a length of approximately 1 cm using a suture guide. A ligature is applied to the peripheral end, and a holding wire is passed under the femoral vein. After incision of the vein, a catheter filled with 10% heparinized 0.9% NaCl solution is introduced into the vein and held in place by the second ligature (Nguelefack, 2008) [12].

Carotid artery exposure and catheterization

To expose the carotid artery, the neck area was waxed, the muscles removed and the thyroid glands located. The carotid arteries, with their opalescent walls, are located on either side of the trachea. One of the carotid arteries was separated from the vagus nerve, freed for about 1 cm and clamped with a haemostatic clamp. A catheter was inserted into the artery and connected to the rest of the set-up.

Measurement of cardiovascular parameters

The device used to record cardiovascular parameters (BP and HR) consisted of a transducer, a "Biopac Student ^{LabR} " type MP 36 recorder, a computer and an elevator on which the animal was placed on a cork board. These devices were used to visualize the various recorded tracings on the computer screen.

Substance administration

All substances dissolved in physiological 0.9% NaCl solution were administered via a catheter placed in the animal's femoral vein. This administration is carried out at a rate of 0.1 mL/100 g body weight of the animal and always preceded by a period of stabilization of BP and HR of around 30 minutes (Dimo, 2003) [6].

The effects of each dose on SBP and HR were measured for one hour by the invasive method. Twelve (12) normotensive rats were divided into four (4) batches of three (3) rats and treated as follows.

Lot 1 negative control received physiological NaCl 0.9% i.v.;

Lot 2 positive control received furosemide 40mg/kg i.v;

Batches 3 and 4 received aqueous extract of *R. obscura* root bark at doses of 15 and 30 mg/kg i.v. respectively.

Evaluation of the diuretic activity of aqueous extract of *Rauvolfia obscura* root bark in rats

The diuretic effect of the aqueous extract of *R. obscura* root barks was evaluated using the method reported by Sanogo *et al*. (2009) [15]. Twelve (12) rats fasted 18 h before the experiment were divided into four (4) batches of three (3) rats each and treated orally as follows.

Lot 1 received hypertonic NaCl 1.8% water overload at 50 mL/kg bw, followed immediately by distilled water at 1 mL/100 g.

Lot 2 received hypertonic NaCl 1.8% water overload at 50 mL/kg bw, followed immediately by furosemide 40 mg/kg;

Batches 3 and 4 each received hypertonic NaCl 1.8% water overload at 50 mL/kg bw, followed immediately by aqueous extract of *R. obscura* root bark at 150 and 300 mg/kg respectively.

After treatment, each rat was placed in a metabolic cage.

Urine was collected in tubes and the following parameters were recorded: latency or time to elimination of the first drop of urine, volume of urine excreted after six (6) hours of experimentation and urinary Na^+, Cl- and K+ concentrations were determined.

Statistical analysis

Results are expressed as mean minus standard error (M±ESM). Comparisons of mean measurements between batches were made using the Student's t-test. At $p< 0.05$, differences were considered significant.

Result

Effects of aqueous extract of *R. obscura* root bark on systolic blood pressure in normotensive rats

Intravenous injection of the aqueous extract of *R. obscura* root bark at doses of 15 and 30 mg/kg produced an immediate fall in systolic blood pressure (SBP) around 5 seconds after administration. The respective decreases in SAP are - approximately 5 seconds after administration. The decreases in SAP were -27.98 ± 3.96% ($p< 0.01$) and -13.07 ± 3.78% ($p< 0.05$) respectively. These decreases were followed by a significant rise in SAP from the 5th minute for both doses of 15 and 30mg/kg, below the initial values. For the 15mg/kg dose, after the rise at the 5th minute, PAS remained below the initial value until the 60th minute or the 30 mg/kg dose, after the rise at the 10th minute followed by stabilization of PAS until the 50th minute, there was a slight, non-significant rise from thea slight, non-significant rise was observed from the 55th minute to the 60th minute (figure no. 1).

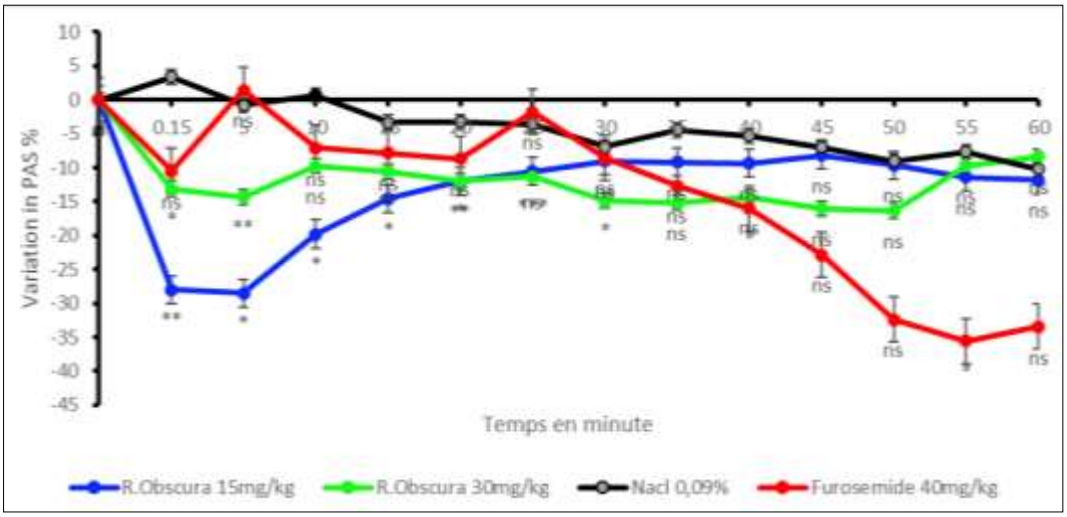

Fig 1: effects of *R. obscura* root bark aqueous extract on systolic blood pressure (SBP) in normotensive rats. Each point is mean ± MSE (n = 3). ns difference not significant; * $p< 0.05$; ** $p< 0.01$ and *** $p< 0.001$ significant difference from baseline SBP

Effects of aqueous extract of *R. obscura* root bark on heart rate in normotensive rats

Intravenous injection of aqueous extract of *R. obscura* root bark at doses of 15 and 30 mg/kg produced a significant reduction in HR of -32.82±17.35% ($p< 0.001$) and -23.61±7.69% ($p< 0.001$) respectively. This reduction was followed by a rapid rise in HR at 10 minutes for the 15 mg/kg dose and at 5 minutes for the 30 mg/kg dose. For the 15 mg/kg dose, there was a significant relapse at 15 minutes of -20.69±17.35% ($p< 0.001$), followed by a non-significant rise at 20 minutes and stabilization of HR at 50 minutes. Significant decreases from the initial value were noted at 35 and 45 minutes, followed by stabilization of HR from 50 to 60 minutes. For the 30 mg/kg dose, there was a non-significant relapse at the 10th minute of -9.92 ± 17.35% (ns), followed by an increase at the 15th minute above the initial value, which remained until the 60th minute (Figure n°2).

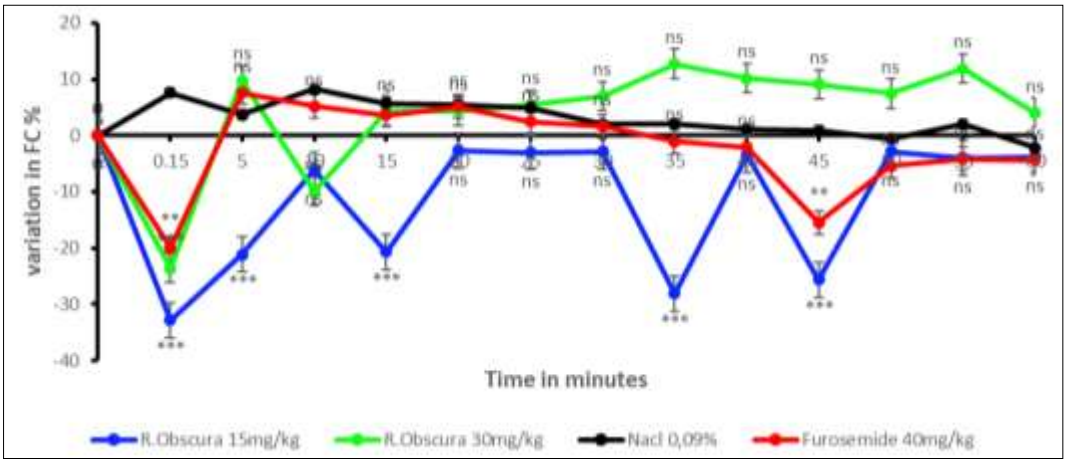

Fig 2: Effects of aqueous extract of *R. obscura* root bark on heart rate (HR) in normotensive rats. Each point is mean ± MSE (n = 3). ** $p< 0.01$ and *** $p< 0.001$ significant difference from baseline HR

Effects of *Rauvolfia obscura* aqueous extract on diuresis in rats

These results indicate that the cumulative urine excreted volume (EUV) over 6 h in rats treated with *R. obscura* aqueous extract (150 and 300 mg/kg) and furosemide (40 mg/kg) is significantly higher than in control rats. These volumes are 53.08% ($p< 0.01$); 114.14% ($p< 0.001$) and 166.72% ($p< 0.001$) respectively versus 50.6% of control rats.

At a dose of 150 mg/kg, aqueous extract of *R. obscura* induces elimination in urine from the 1st to the 6th hour, with maximum elimination at the^{3rd} hour compared with the negative control.

On the other hand, at a dose of 300 mg/kg, the aqueous extract of *R. obscura* produced maximum elimination in urine from the 1st to the 6th hour, compared with controls treated with distilled water. The action is similar to that of the furosemide assay.

On the other hand, at a dose of 300 mg/kg, the aqueous extract of *R. obscura* produced maximum elimination in urine from the 1st to the 6th hour, compared with controls treated with distilled water. The action is similar to that of the furosemide assay.

Table 1: Diuretic activity of *Rauvolfia obscura* aqueous extract in rats.

Treatments	Administered volume (ml)	Excreted volume/6h (ml)	Urinary volumetric excretion (%)	Micturition time (minutes)	Conclusion
NaCl 1,8% + distilled water (1 ml/100 g)	31,62 ±2,76	16±0,33	50,6±0,61	15±0,08	Antidiuretic
NaCl 1,8% + Furosemide (40 mg/kg)	29,99±2,74 ns	50±4,17***	166,72±0,08***	13±0,33ns	Significant activity
NaCl 1,8% + Ext aqueouse (150 mg/kg)	35,79±0,72 ns	19±0,16***	53,08±1,28**	36±0,0ns	Antidiuretic
NaCl 1,8% +Ext aqueouse (300 mg/kg)	31,54±1,77 ns	36±0,00***	114,14±10,50**	5±0,00ns	Weak activity

Each point is mean ± MSE (n = 3). ** $p< 0.01$ and *** $p< 0.001$ significant difference from negative control; ns non-significant difference

Effect of *Rauvolfia Obscura* aqueous extract on urinary electrolyte excretion in rats

The flow rates and concentrations of Na^+, K^+ and Cl^- ions in the aqueous extract of *R. obscura* roots (150 and 300 mg/kg) were not significantly different from those in the controls (Figure 3). Few variations were noted in urinary excretion of electrolytes, although the increase in natriuresis was greater than that of kaliuresis, resulting in a higher $^{Na+/K+}$ ratio ^{at} 150mg/kg compared with control rats. There was also a non-significant increase in Cl^- concentrations at doses of 150 mg/kg (308.33 mmol/L) and 300 mg/kg (288.33 mmol/L). On the other hand, administration of furosemide at a dose of 40mg/kg significantly increased diuresis compared with different doses of *R. obscura.* The results of electrolyte analyses (Na+, K+, Cl^-) show that the extract increases natriuria, but decreases kaliuria.

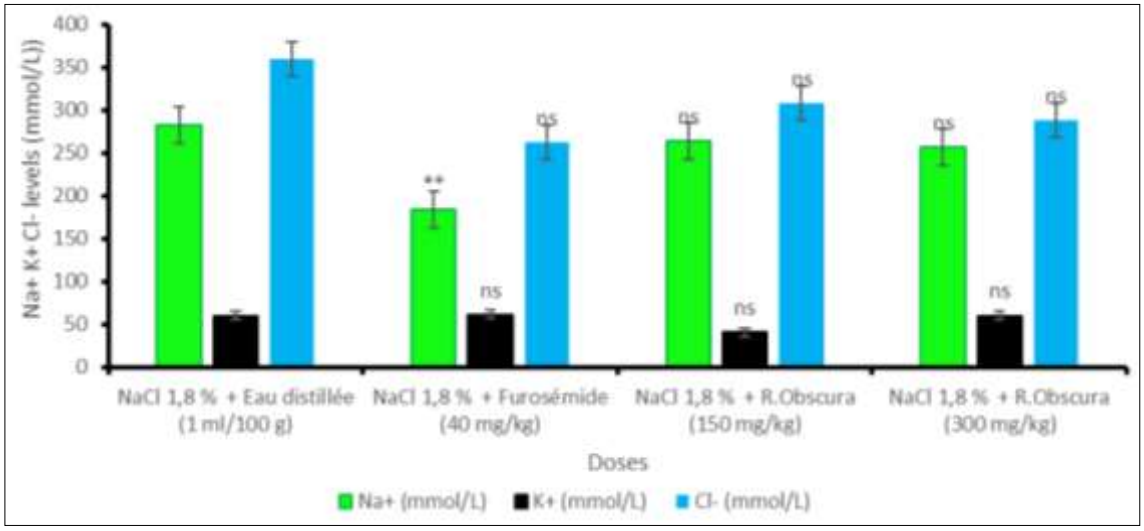

Fig 3: Effects of aqueous extract of *Rauvolfia Obscura* roots and furosemide on electrolyte excretion in rats. Each point is mean ± MSE (n = 3). ** $p< 0.01$ significant difference from negative control; ns non-significant difference

Discussion

The results of the present study show that intravenous injection of the aqueous extract of *R. obscura* root bark at doses of 15 and 30 mg/kg produces an immediate and significant fall in SAP and HR in normotensive rats. This is followed by a rise in SAP from the 5^{th} minute for both doses, followed by a second phase of decline which persists for at least one hour. Thus, the sustained fall in SAP observed at both doses in normotensive rats could well be explained by a reduction in peripheral vascular resistance.

The rapid rise in SAP observed at 5 minutes could be explained by a reflex phenomenon following the increased discharge of catecholamines, which stimulate the heart and cause vasoconstriction (Guerrero *et al*., 2001) [8].

This aqueous extract could therefore have a hypotensive effect. Similar results have been obtained with other plant extracts. These include *Lippia multiflora* (Etou ossibi, 2012) [7], *Trema orientalis* (Ngolo, 2015) [10], *B. coriacea* (Ondelé, 2015) [14] and *Bidens pilosa* (Ngelefack, 2008) [12].

In terms of heart rate, this extract produces an immediate significant drop, followed by a rise, then a relapse, then a rise again, then stabilization. This movement occurred twice with the 15mg/kg dose, and once with the 30mg/kg dose, with a rebound after stabilization from the ^{30th} minute. These may be explained by the negative chronotropic effect. The aqueous extract of *R. obscura* root bark therefore has a negative chronotropic effect, which could partly explain the hypotensive effect of this extract. Indeed, some authors have shown that the drop in HR following the administration of plant extracts would be at the origin of the fall in blood pressure (Etou Ossibi *et al.* 2012) [7]. The aqueous extract of *R. obscura* root bark has a non-dose-dependent effect. These results prompted us to propose another experiment with this extract, that of verifying its diuretic properties, as the oldest antihypertensive drugs are natriuretic diuretics.

The aim of this part of the work was to evaluate the diuretic potential of the aqueous extract of *R. obscura* root barks with furosemide in rats. Under our experimental conditions and according to the evaluation scale of Kau *et al*. (1994) [9], there was no diuretic activity of the aqueous extract at a dose of 150 mg/kg, but a weak diuretic activity at a dose of 300 mg/kg. However, the volume of urine excreted (VUE) accumulated over 6 h in rats treated with aqueous extract (300 mg/kg) and furosemide (40 mg/kg) was respectively 114.14% and 166.72% higher than in control rats (50.6%).

In addition, the aqueous extract (300 mg/kg) and furosemide promote diuresis in rats, whose urinary elimination times are shorter than in control rats. Furosemide causes increased excretion of sodium and water by inhibiting the Na+/ K+/2Cl- co-transporter responsible for sodium and water reabsorption at the ascending branch of the loop of Henlé (Amonkan, 2006) [2]. This increased elimination of sodium and water would be beneficial in the treatment of arterial hypertension and edema (Albaghdadi *et al*., 2011) [1].

It could be hypothesized that the aqueous extract acts at the distal tubule level, reducing circulating aldosterone levels, or inhibiting aldosterone.

Another hypothesis is that the aqueous extract blocks the sodium channel, as does furosemide, a potassium-sparing substance with natriuretic, diuretic and antihypertensive activity.

The results obtained with furosemide, the reference molecule, validate the model used in this study to study diuresis in situations of fluid overload.

With regard to urinary excretion of electrolytes, we note that aqueous extract has a dose-dependent diuretic activity. At a dose of 300 mg/kg, it eliminates more sodium and spares potassium, resulting in a drop in natriuria. This can be explained by the fact that diuretics, which increase the elimination of water and sodium in the urine, first reduce circulating blood volume, then secondly resistance to blood flow, leading to a drop in blood pressure (Ben Abdallah, 2015) [4]. This is because an excess of circulating sodium stimulates the sympathetic nervous system in the brain, which in turn increases the production of catecholamines, which cause vasoconstriction, resulting in resistance to blood flow (Ben Abdallah, 2015) [4].

For this reason, a blood pressure study was carried out, and it was observed that the blood pressure of normotensive rats decreased significantly after administration of the aqueous extract.

Conclusion

The results of this study show that the aqueous extract of *R. obscura* root bark has a hypotensive effect in rats. This effect could well be explained by a reduction in heart rate. It also has a weak diuretic effect in rats. Further studies will provide more information on this plant's hypotensive and diuretic mechanism of action.

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