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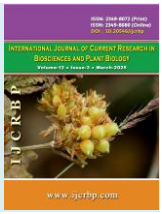

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Diuretic Activity of the Aqueous Extract of the Fruits of *Picralima nitida Stapf.* (Apocynaceae) in Wistar Rats

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Abstract

In Côte d'Ivoire, an ethnobotanical survey revealed that *Picralima nitida* is used as a diuretic in the treatment of hypertension. The objective of this work is to evaluate the effects of an aqueous extract of *Picralima nitida* (EAPn) on urinary excretion in wistar rats. To conduct this study, four groups of five rats were divided as follows: - Group 1 was the control group, where the rats received 30 ml/kg BW of distilled water ad libitum; - Groups 2, 3, and 4 received 30 ml/kg BW of *Picralima nitida* fruit aqueous extract (EAPn) at doses of 250 mg/kg BW, 400 mg/kg BW, and 500 mg/kg BW, respectively. The time to first urination was recorded, as was the urine volume collected after 24 hours from each group of rats. Sodium (Na^+), potassium (K^+), and chlorine (Cl^-) ions were determined using the Smartlyte Electrolyte Analyzer. The results showed that que EAPn administered at doses of 250, 400, and 500 mg/kg BW caused a progressive and significant increase in urinary excretion in these treated rats compared to controls. The time to first urination decreased in rats treated with *Picralima nitida* compared to control rats. Electrolyte determination showed elevated concentrations of sodium (71.61 %), potassium (71.52 %) and chlorine (62.57 %) in the urine of rats treated with EAPn at a dose of 500 mg/kg BW after 24 hours compared to the concentration of the controls.

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Introduction

Traditional medicine has always remained the most sought-after means for relieving or curing illnesses in developing countries, especially in African countries (Aké-Assi, 1991). Indeed, in most sub-Saharan African countries, traditional medicine provides 70-80 % of the population's health coverage with the same effectiveness as modern medicine (Kabena et al., 2014; Kambale et al., 2016). In Côte d'Ivoire, there are a

multitude of medicinal species, some of which specialize in the treatment of various conditions, including hypertension. An ethnobotanical survey revealed that *Picralima nitida* is used as a diuretic in the treatment of hypertension (Bickii et al., 2007). The general objective of this work is to evaluate the effects of an aqueous extract of *Picralima nitida* (EAPn) on urinary excretion in wistar rats.

Materials and Methods

Plant Materials

The plant material consisted of the fruits of *Picralima nitida*, a member of the Apocynaceae family, harvested in Daloa in western Côte d'Ivoire. This plant was identified and authenticated in the botany laboratory of Jean Lorougnon Guédé University.

Animal Materials

Mus musculus (Muridae) mice, from Swiss homogeneous parental strains, weighing between 20 g and 25 g, were used for toxicity tests. Rats, *Rattus norvegicus* (Muridae), of the Wistar strain, were used for pharmacological studies on urinary excretion in rats. These animals were raised under standard conditions of temperature, nutrition, and atmospheric pressure. They are bred in the Animal Physiology Laboratory.

At the animal facility of the Faculty of Biological and Pharmaceutical Sciences of the Félix HOUPHOUËT-BOIGNY University. They are fed with food supplied by the company IVOGRAIN® of Abidjan, Ivory Coast, and have free access to water.

Method for preparing the aqueous extract of *Picralima nitida* (Apocynaceae) fruits

To prepare the aqueous extract of *Picralima nitida*, 300 g of chopped fruits are boiled for 15 minutes in 1.5 liter of distilled water. The resulting decoction is filtered twice through absorbent cotton and then once through Wattman filter paper. The collected filtrate is dried in an oven (Thermo SCIENTIFIC VT 6060 M) at 40°C for 72 hours. The preparation of this aqueous extract is summarized in the diagram below (Figure 7). After drying, the aqueous extract of *Picralima nitida* fruits (EAPn) is presented in powder form. This product (EAPn) is used for toxicity tests and pharmacological studies on urinary excretion in rats.

Dose-response effect of *Picralima nitida* fruit aqueous extract (EAPn) on urinary excretion in rats over 24 hours

Rats weighing between 120 and 150 g were fasted for 12 hours prior to the experiment. Four groups of five rats were divided as follows:

- Group 1 was the control group, where the rats received 30 ml/kg BW of distilled water ad libitum;

- Groups 2, 3, and 4 received 30 ml/kg BW of *Picralima nitida* fruit aqueous extract (EAPn) at doses of 250 mg/kg BW, 400 mg/kg BW, and 500 mg/kg BW, respectively.

The time to first urination was recorded, as was the urine volume collected after 24 hours from each group of rats.

Comparative effects of EAPn, hydrochlorothiazide, and furosemide on urinary excretion in rats

Four groups of 5 rats were composed and distributed as follows:

- Rats in group 1 (control group) received ad libitum 30 ml/kg BW of distilled water;

- Groups 2, 3, and 4 received 30 ml/kg BW of EAPn (500 mg/kg BW), hydrochlorothiazide (25 mg/kg BW), and furosemide (20 mg/kg BW), respectively.

The urine volume collected after 24 hours from each group of rats was recorded.

Measurement of Electrolyte Parameters

After 24 hours of treatment, the collected urine was also stored in Eppendorff tubes for electrolyte determination. Sodium (Na+), potassium (K+), and chlorine (Cl-) ions were determined using the Smartlyte Electrolyte Analyzer (Diamond Diagnostics, USA).

Processing of Results

The *GraphPad InStat* computer program (San Diego, CA, USA) was used for statistical analysis of the results. Values are given as the mean followed by the standard error of the mean.

The difference between two values was determined using the Student-Newman-Keuls comparison test.

It was considered non-significant (ns) for a probability greater than 5 % ($P > 0.05$) or significant: (*) for $P < 0.05$; (**) for $P < 0.01$; (***) for $P < 0.001$.

Results and Discussion

Studies of the pharmacological effects of the aqueous extract of *Picralima nitida* (EAPn) on diuresis in rats

Dose-response effect of EAPn on urinary excretion in rats

Gavage administration of the aqueous extract of *Picralima nitida* (EAPn) (Apocynaceae) resulted in urinary excretions that varied considerably depending on the dose (Figure 1).

Indeed, EAPn administered at doses of 250, 400, and 500 mg/kg BW caused a progressive and significant increase in urinary excretion in these treated rats compared to controls.

The urinary excretion induced by 250 mg/kg BW was 16.08 ± 2.917 ml/kg after 24 hours. However, it is respectively 24.60 ± 2.08 ml/kg and 30.40 ± 3.19 ml/kg after 24 hours when rats are treated with 400 and 500 mg/kg BW.

This represents an increase of 28.02%, 95.85% and 142.04% respectively.

The urinary excretion induced by EAPn is therefore dose-dependent and reaches its maximum after 12 hours and tends to become stable until the end of the experiment (Figure 1).

Evaluation of time to first urination and urine volume after treatment of rats

Aqueous extract of *Picralima nitida* (EAPn) at a dose of 500 mg/kg BW resulted in a 34.24 % reduction in time to first urination compared to the control.

In control rats, the time to first urination was 62.5 ± 3.25 min. In contrast, in rats treated with 500 mg/kg BW of EAPn, this time was significantly reduced to 41.10 ± 3.04 min ($p<0.05$).

Similar observations were recorded with the administration of hydrochlorothiazide (25 mg/kg BW) and furosemide (20 mg/kg BW), inducing respective reductions of 42.88 % and 52.96 % in the time to first urination ($P < 0.01$).

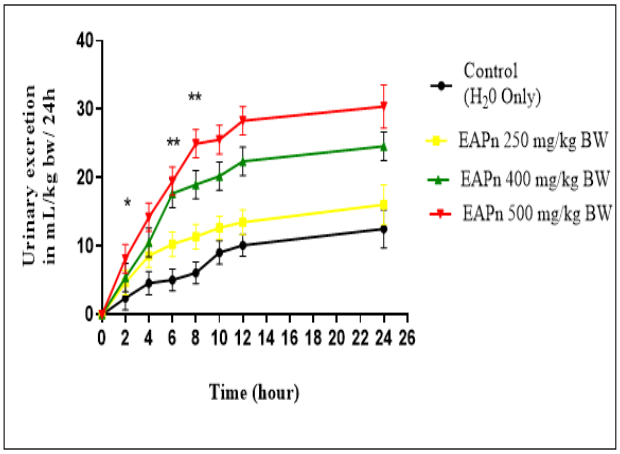

Figure.1 Dose-response effect of aqueous extract of *Picralima nitida* (EAPn) on urinary excretion in rats over 24 hours

The time to first urination decreased in rats treated with these three substances compared to control rats.

The urine volume after 24 hours in control rats was 12.56 ± 2.8 ml/kg BW/24 hours.

In rats treated with furosemide, hydrochlorothiazide, and EAPn, the urine volume was 44.25 ± 3.20; 45.36 ± 5.09 and 30.40 ± 3.19 ml/kg BW/24 hours, respectively.

This represents an increase of 252.31 %; 261.15 % and 142.04 %. Urinary volume therefore increased in the treated rats compared to the control rats. The results are summarized in the table below.

Table.1 Urinary volume excreted in 24 hours and time to first urination

	Time to first urination in minutes	**Urine volume in ml/kg BW/24 h**
Control rats (H_20)	62.5 ± 3.25	12.56 ± 2.8
Rats treated with EAPn (500 mg/kg BW)	41.07 ± 4.10*	30.40 ± 3.19*
Rats treated with hydrochlorothiazide	35.7 ± 3.40**	45.36 ± 5.09***
Rats treated with furosemide	29.4 ± 5.5**	44.25 ± 3.20***
*P<0.05, **P<0.01, ***P<0.001 vs. control.		

Studies of the comparative effects of aqueous extract of *Picralima nitida* (EAPn), furosemide and hydrochlorothiazide on urinary excretion in rats

Urine volume after 24 hours in control rats was 12.56 ± 2.8 ml/kg BW/24 hours. In rats treated with furosemide, hydrochlorothiazide and EAPn, urine volume was 44.25 ± 3.20; 45.36 ± 5.09 and 30.40 ± 3.19 ml/kg BW/24 hours, respectively. This represents an increase of 252.31 %; 261.15 % and 142.04 %. Urinary volume therefore increased in treated rats than in control rats (Figure 2).

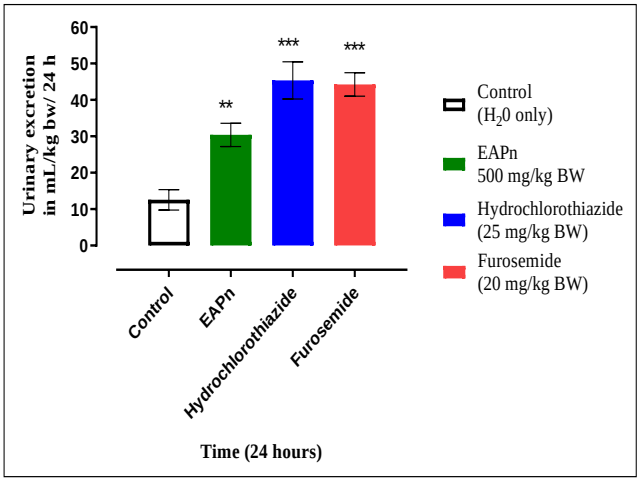

Figure.2 Comparative effects of aqueous extract of *Picralima nitida* (EAPn), hydrochlorothiazide and furosemide on urinary excretion in 24 hours

Evolution of electrolytes contained in the urine of rats treated with *Picralima nitida* (EAPn), hydrochlorothiazide and furosemide.

Electrolyte determination showed elevated concentrations of sodium, potassium and chlorine in the urine of rats treated with aqueous extract of *Picralima nitida* (EAPn) at a dose of 500 mg/kg BW after 24 hours (Table 4). These different concentrations are for sodium (71.61 %), potassium (71.52 %) and 62.57 % for chlorine compared to the concentration of the controls. Also, hydrochlorothiazide (25 mg/kg BW) causes a high urinary excretion concentrated at 72.95 % sodium, (76.84 %) potassium and 79.73 % chlorine. With furosemide (20 mg/kg BW), sodium concentrations were 81.35% sodium, 80.48% potassium, and 74.46 % chlorine compared to the electrolyte content of controls (Table 2).

Table.2 Concentration of electrolytes in urine (meq/L/24h)

	Na^+	K^+	Cl^-
Control (H_20 only)	46.5 ± 3.4	37.4 ± 2.5	51.30 ± 6.6
EAPn 500 mg/kg BW	79.80 ± 6.07**	64.15 ± 7.36**	83.4 ± 5.1**
Hydrochlorothiazide 25 mg/kg BW	80.42 ± 2.5**	66.14 ± 5.25**	92.2 ± 6.1**
Furosemide 20 mg/kg BW	84.33 ± 5.3**	67.50 ± 5.02**	89.50 ± 7.23**

Changes in the Na+/K+ ratio in the urine of rats treated with *Picralima nitida* (EAPn), hydrochlorothiazide, and furosemide

Compared to that of control rats, the Na+/K+ ratio did not vary significantly. It was around 1.24 for each test substance.

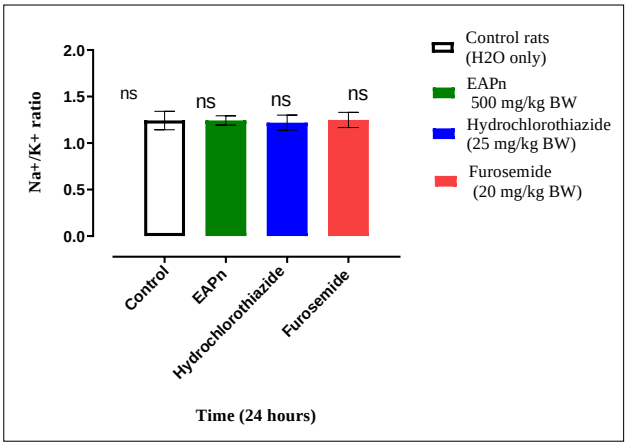

Figure.3 Evolution of the sodium/potassium ratio in the urine excreted from rats treated with the aqueous extract of *Picralima nitida* (EAPn), hydrochlorothiazide and furosemide after 24 hours.

The study of the pharmacological effects of the aqueous extract of *Picralima nitida* on urinary excretion revealed that the time to first urination decreased in rats treated with these three substances compared to control rats. Then, urinary volume increased in rats treated with EAPn, furosemide, and hydrochlorothiazide compared to control rats. Also, urinary excretion increased with increasing doses of EAPn. It is therefore a dose-dependent increase that reaches its maximum after approximately 12 hours. Electrolyte determination showed elevated concentrations of sodium, potassium, and chlorine in the urine of rats treated with EAPn,

hydrochlorothiazide, and furosemide in virtually the same proportions. Furosemide inhibits sodium reabsorption at the loop of Henle by blocking NKCC (Na+/K+/2Cl- cotransporter) at the ascending limb of the loop of Henle, resulting in increased urinary excretion of Na+, K+, Cl-, Mg2+, and Ca2+. The natriuretic effect is significant and short-lived (Bia and Defronzo, 1981).

Hydrochlorothiazide directly inhibits NaCl reabsorption by competing with the Cl site of the cotransporter. They indirectly stimulate calcium reabsorption (increased proximal tubular reabsorption parallel to that of Na). Their effect is weak; they allow an excretion of 5 to 10% of filtered sodium (Russel, 2000).

Compared to that of control rats, the Na+/K+ ratio does not vary significantly in rats tested with EAPn, furosemide, and hydrochlorothiazide. This means that aldosterone secretion is not affected by the tests.

Indeed, it is known that aldosterone stimulation is stimulated by low plasma Na+ concentration and high k+ concentration (Amar et al., 2010). While its inhibition follows the opposite conditions, i.e. high natremia and low kalemia (Okusa et al., 1992; Payne and Forbush, 1994). Then, EAPn would not be an antialdosterone. These are drugs that aim to counter the action of aldosterone by blocking it at the level of the renal tubule.

They will therefore result in the elimination of sodium and water and will be said to be potassium-sparing because, unlike other diuretics, they prevent potassium from being eliminated in the urine (Andreas, 2006).

At the end of this investigation, pharmacological studies show that the urine volume after 24 hours in control rats is 12.56 ± 2.8 ml/kg BW/24h. However, when rats are treated with furosemide, hydrochlorothiazide and EAPn, the urine volume is respectively 44.25 ± 3.20; 45.36 ± 5.09 and 30.40 ± 3.19 ml/kg BW/24h. It is clear that the urine volume increases in the treated rats compared to the control rats.

Electrolyte assay showed high concentrations of sodium (71.61 %), potassium (71.52 %) and chlorine (62.57 %) in the urine of rats treated with aqueous extract of *Picralima nitida* fruits (EAPn) at a dose of 500 mg/kg BW after 24 hours. Based on the results obtained, EAPn is therefore a diuretic substance. The observed pharmacological properties of the extract justify its use as a diuretic in the treatment of high blood pressure (HBP).

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