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Comparative Effects of *Ficus exasperata* Aqueous Leaf Extract and Furosemide on Urinary Excretion in DOCA-salt Hypertensive Rat

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The prevalence of hypertension is increasing in many parts of the world. Management of this disease requires the use of diuretics, ACE inhibitors and β-blockers. In many pharmacopoeias, the leaves of *Ficus exasperata* are used to treat hypertension. The hypotensive effects of leaves of this species have been shown. The purpose of this study was to evaluate the diuretic effect of *F. exasperata* aqueous leaf extract (FEFIX) comparing to those of furosemide in salt hypertensive rats treated with deoxycorticosterone acetate (DOCA). Animals treated with DOCA were divided into three groups which received intraperitoneally NaCl (9‰, saline solution), FEFIX (100 mg kg^{-1} b.wt.) and furosemide (10 mg kg^{-1} b.wt.), respectively. Urine output was collected for 24 h. At the end of the experiment, the blood was collected and sampled. FEFIX and furosemide increased urinary volume (EUV) to 153.32±6.89 and 105.71±9.37%, respectively. *F. exasperata* aqueous leaf extract and furosemide increased the urinary excretion of electrolytes (Na^+, Cl^- and Ca^{2+}), urea and creatinine. However, excretions induced by the plant extract were greater than those induced by furosemide. FEFIX and furosemide decreased the rate of plasma electrolytes with a decrease in serum sodium greater for this extract. The studied extract decreased urea and plasma creatinine like furosemide. These results showed that the diuretic effects of *F. exasperata* aqueous leaf extract were similar to those of furosemide in hypertension due to salt overload. However, the diuretic effects of this plant extract were superior to those of furosemide.

Key words: Urinary excretion, electrolyte, *Ficus exasperata*, furosemide, hypertension

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INTRODUCTION

Hypertension is an elevation in blood pressure leading to a systolic blood pressure greater than or equal to 140 mm Hg and a diastolic blood pressure greater than or equal to 90 mm Hg (Erdine *et al*., 2006). It is currently a public health problem worldwide due to its frequency and risk of cardiovascular and renal diseases which are attached. Kearney *et al*. (2005) indicate a prevalence of 1.6 billion hypertensive subjects in 2025. Indeed, the prevalence of this disease increase in the world in general and developing countries in particular (Whitworth *et al*., 2003, Kearney *et al*., 2004). Generally, the treatment of this disease requires the use of diuretics, ACE inhibitors and beta-blockers (Armario and Waeber, 2013). So many people in developing countries employ several herbals to treat this pathology (Abrogoua *et al*., 2012; Anwar *et al*., 2007). *Ficus exasperata* Vahl. 1805 (Moraceae) is one of the plants used in this field. Its leaves are often found in many beverages antihypertensive preparations. Previous studies showed that *F. exasperata* aqueous leaf extract decreased the blood pressure in a dose-dependent manner (Ayinde *et al*., 2007; Amonkan *et al*., 2010; Adewole *et al*., 2011). According to this disease etiology, the sodium intake *via* a diet is involved in the onset of the disease. In addition, the control of sodium balance is necessary in hypertensive subjects. Therefore reducing sodium intake and promote renal excretion of sodium are necessary to reduce the blood pressure (Forman *et al*., 2007; Karppanen and Mervaala, 2006). Thus, the aim of this work was to evaluate the diuretic effect of *F. exasperata* aqueous leaf extract comparing to those of furosemide in the case of hypertension induced by salt overload.

MATERIALS AND METHODS

Ethics: Experimental procedures and protocols used in this study were approved by Ethical Committee of Health Sciences, University Felix Houphouet-Boigny. These guidelines were in accordance with the internationally accepted principles for laboratory animals' use and care (NRC, 1996; Mosihuzzaman and Choudhary, 2008).

***F. exasperata* Aqueous leaf extract:** Fresh leaves of *F. exasperata* Vahl. 1805 (Moraceae) were collected in a forest of the Southern region of Côte d'Ivoire (Region des Lagunes). This plant was authenticated by a Botany expert, Prof. Ake-Assi Laurent of the "Centre National de Floristique", UFR-Biosciences, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire. *Ficus exasperata* aqueous leaf extract (FEFIX) preparation was previously described (Amonkan *et al*., 2010 and 2013). Fresh leaves of *F. exasperata* were washed and dried in an oven at a temperature of 40±2°C. They were pulverized to obtain a fine powder which was left to macerate in n-hexane at a rate of 10 g of powder in 100 mL of n-hexane for 24 h. After filtration, the residue was collected and dried to be subjected to further maceration in distilled water at a rate of 5 g per 100 mL of solvent. The filtrate was then collected and dried using a rotavapor (Buchi, France). A powder of *F. exasperata* aqueous leaf extract (FEFIX) was obtained with a yield of 14.27±3.26 %. FEFIX was stored at 4°C until experiments.

Animals: Male Wistar rats weighing 200-250 g were used for these experiments. From Pasteur Institute, Abidjan (Côte d'Ivoire), the animals were acclimatized in plexiglass cages for 14 days before experimentation. They were maintained at a temperature of 25±2°C with dark and light cycle (12/12 h). They have free access to standard dry pellet diet and water *ad libitum*. Animals were treated with deoxycorticosterone acetate (DOCA) for 4 weeks. They received DOCA subcutaneously twice a week (25 mg kg^{-1} b.wt.). They were normally fed with a drink of NaCl 1% and KCl 0.2% *ad libitum* (Fournie-Zaluski *et al*., 2004; Bodineau *et al*., 2008). After 4 weeks of treatment, the animals become hypertensive and exhibit hemodynamic parameters and kidney similar to those observed in the spontaneously hypertensive rat (Johnson *et al*., 2004). The day before the experiment, all animals were fasted overnight. At the end of the experiment, the animals were anesthetized with ether and blood rats were sampled from the inferior vena cava.

Evaluation of the diuretic: The day of the experiment, the animals were divided into three groups of six rats and placed individually in metabolic cages. Fluid overload was conducted at 50 mL kg^{-1} and the animals received immediately following substances according to the group: saline (NaCl 9‰, control) FEFIX (100 mg kg^{-1} b.wt.) and furosemide (10 mg kg^{-1} b.wt.). The urine were collected separately every two hours for 24 h and sampled. They were stored at -20°C prior to determination of the levels of electrolytes, creatinine and urea. Excreted urine volume (EUV) was determined from the ratio of the volume of urine excreted and the volume of fluid overload.

Determination of plasma and urinary electrolytes: Automatic analyzer (Hitachi 902, Roche) was used to determine plasma and urinary electrolytes, creatinine and urea. The determination of sodium and potassium in urine

and plasma was performed by the technique of photometry. Levels of calcium, chlorine and creatinine were performed by the technique of colorimetry. The levels of urea were determined by the principle of kinetics.

Chemicals used: The following reference drugs were used: Furosemide (Lasilix®, Sanofi-Aventis, France), Deoxycorticosterone acetate (DOCA, Sigma). FEFIX and Furosemide were dissolved and diluted in saline solution (NaCl 9‰). DOCA was dissolved in sesame oil on each day of our experiments.

Statistical analysis: The experimental results were expressed as the mean with standard error of mean (m±sem). Data were assessed by the method of analysis of ANOVA followed by Tukey-Kramer test with GraphPad Instat software (Microsoft, San Diego, California, USA). Graphical representations of data were performed by GraphPad Prism 5 software (Microsoft, San Diego, California, USA). The difference between the averages is considered statistically significant when $p<0.05$.

RESULTS

Volume of urine excreted: Urinary excretion induced by furosemide after 2 h was greater than that induced by *F. exasperata* aqueous leaf extract (FEFIX). The values obtained were respectively 32.04±6.87 and 20.04±3.84%. From the fourth hour, FEFIX achieved relatively high urinary volumes and higher than those induced by furosemide administration. After 24 hours, FEFIX caused urinary excretion of 153.32±6.89% while that measured with furosemide was 105.71±9.37% (Fig. 1).

Urinary excretion of electrolytes: After 24 h, FEFIX caused a urinary sodium excretion of 15.77±0.62 mEq (Fig. 2). Furosemide induced urinary excretion of sodium, which amounted to 12.78±0.69 mEq. Urinary potassium levels were 1.54±0.12 and 1.19±0.09 mEq, respectively for FEFIX and furosemide. The rate of chlorine and calcium excreted after 24 h under the action of FEFIX were relatively higher than those induced by furosemide. Concerning chlorine, excretions recorded were 12.35±0.62 and 08.27±0.59 mEq, respectively following FEFIX and furosemide treatments. Calcium excretions were 4.84±0.48 mEq (FEFIX) and 4.32±0.42 mEq (furosemide).

Plasma electrolytes: After 24 h, FEFIX and furosemide caused a decrease in plasma electrolytes (Fig. 3). The serum sodium measured were 115.17±6.06 and 127.50±6.59 mEq L^{-1}, respectively for FEFIX and

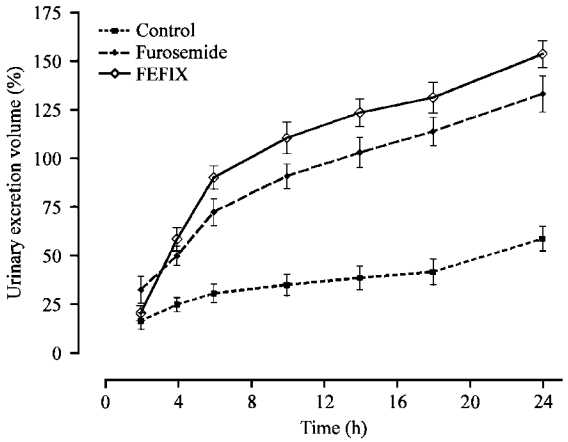

Fig. 1: Evolution of urinary excretion volume measured for three groups of DOCA salt hypertensive rats: Saline solution (NaCl 9 ‰, Control), FEFIX (100 mg kg^{-1} b.wt.) and furosemide (10 mg kg^{-1} b.wt.). Urine output was measured every two hours for 24 h. FEFIX: *F. exasperata* aqueous leaf extract, DOCA: deoxycorticosterone acetate, n = 6, M±SEM

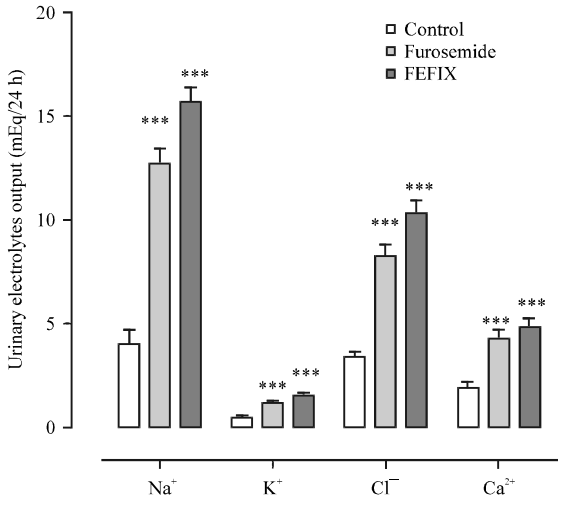

Fig. 2: Rate of electrolyte urinary excretion in rats at 24 hours in three groups of DOCA salt hypertensive rats treated with Saline solution (NaCl 9 ‰, Control), FEFIX (100 mg kg^{-1} b.wt.) and furosemide (10 mg kg^{-1} b.wt.) respectively. Electrolytes were measured in all urine sampled for 24 h after treatment in each group of rats. FEFIX: *F. exasperata* aqueous leaf extract, DOCA: deoxycorticosterone acetate, n = 6, M±SEM, ***: $p<0.001$, **: $p<0.01$, *$p<0.05$

furosemide. The plasma chlorine obtained were 75.00±4.74 mEq L^{-1} (FEFIX) and 76.32±6.51 mEq L^{-1}

Table 1: Effects of FEFIX and furosemide on creatinine and urea in urine output and plasma in DOCA salt hypertensive rat

	Creatinine		Urea	
	$Creatinine_U$ (mmol/24 h)	$Creatinine_P$ (mM)	$Urea_U$ (mmol/24 h)	$Urea_P$ (mM)
Control	0.13±0.02	0.62±0.05	100.07±7.66	3.64±0.17
Furosemide	0.36±0.03**	0.44±0.08	249.42±18.15***	2.54±0.25*
FEFIX	0.40±0.05***	0.41±0.05	290.21±17.48***	2.08±0.33**

Saline solution (NaCl 9 ‰, Control), FEFIX (*F. exasperata* aqueous leaf extract, 100 mg kg^{-1} b.wt.): furosemide (10 mg kg^{-1} b.wt.). DOCA: deoxycorticosterone acetate, u: urine, p: plasma, n = 6, m±sem; ***p<0.001, **p<0.01, *p<0.05

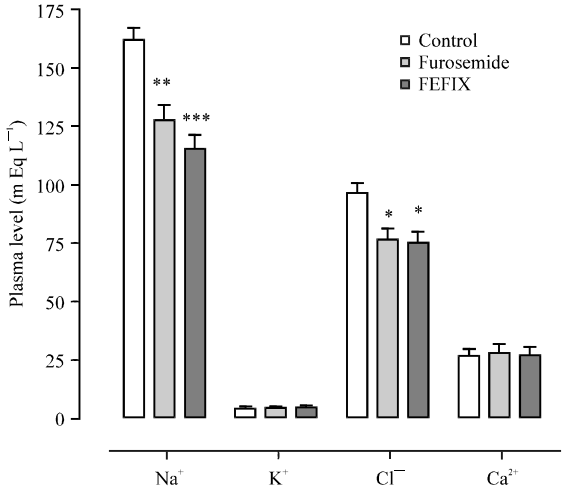

Fig. 3: Plasma levels of electrolytes at 24 h after administration of Saline solution (NaCl 9‰, Control), FEFIX (100 mg kg^{-1} b.wt.) and furosemide (10 mg kg^{-1} b.wt.) in three groups of DOCA salt hypertensive rats respectively. The rate of electrolyte was measured on blood samples. FEFIX: *F. exasperata* aqueous leaf extract, DOCA: deoxycorticosterone acetate, n = 6, M±SEM, ***: p<0.001 , **: p<0.01, *p<0.05.

(furosemide). The serum calcium obtained were 16.50±3.64 mEq L^{-1} (FEFIX) and 17.42±3.14 mEq L^{-1} (furosemide). However, changes in serum potassium induced by furosemide and FEFIX showed no significant difference to that obtained with the control (p>0.05).

Urea and creatinine in urine and plasma: FEFIX and furosemide induced significant urinary excretion of creatinine and urea (Table 1). The creatinine level obtained under FEFIX treatment after 24 h was 0.40±0.05 mmol. That induced by furosemide was 0.36±0.03 mmol. The urea measured in urine were 290.21±17.48 mmol for FEFIX and 249.42±18.15 mmol for furosemide. In the plasma, FEFIX and furosemide also caused significant changes in creatinine and urea. The creatinine levels measured after 24 h were 0.41±0.05 and 0.44±0.08 mM, respectively for FEFIX and furosemide. Uremia obtained in presence of FEFIX (2.08±0.33 mM) was less than that obtained when furosemide (2.54±0.25 mM) was administrated to rats.

DISCUSSION

Ficus exasperata aqueous leaf extract (FEFIX) and furosemide induced urinary excretion volume (EUV) relatively large. The two substances had similar urinary excretion kinetics. However, the urinary excretion obtained with FEFIX was greater than that obtained with furosemide. The increase in urinary excretion induced by FEFIX could result from stimulation of renal excretory function. Similar results were reported in previous works concerning several plants used in Thai Pharmacopoeia (Sripamidkulchai *et al*., 2001). *Urtica dioica* aqueous leaf extract increased urinary excretion according to the dose. For infusions of 4-24 mg/kg/h, this extract increased urine volume by 11 to 84% (Tahri *et al*., 2000). In addition, crude ethanolic extract of leaves *Melothria maderaspatana* protects the kidney in salt hypertensive rats. This protection is manifested by the reduction in histological damage associated with hypertension (Veeramani *et al*., 2012).

Urinary excretion induced by FEFIX and furosemide were associated with significant loss of sodium, chloride and calcium. Electrolytes Urinary excretion caused by FEFIX were relatively higher than those obtained under furosemide treatment. As furosemide, *F. exasperata* aqueous leaf extract inhibited renal reabsorption of electrolytes. Indeed, *Tribulus terrestris* aqueous extract increased the excreted urinary volume. Diuresis induced by this extract was relatively higher than that induced by furosemide (Al-Ali *et al*., 2003). In addition, the infusion of *Salvia scutellarioides* caused high urinary excretion of electrolytes which increased dose-dependent manner (Ramirez *et al*., 2006).

Diuretic effects of FEFIX and furosemide altered the plasma electrolytes. Both substances decreased serum sodium, chloride and serum calcium without affecting significantly the plasma potassium. The decrease in plasma electrolytes could result from their significant urinary excretion. Indeed, previous studies had shown that administration of saponins from *Herniara glabra* decreased arterial blood pressure by reducing the

reabsorption of sodium and water in the renal tubules. Saponins of *H. glabra*, thereby increasing urine flow and excretion of sodium and potassium (Rhiouani *et al*., 1999). Furosemide increased urine volume and urinary excretion of sodium. This increase of diuresis and natriuresis resulted from the inhibition of electrolytes cotransporter along the ascending limb of the nephron (Carmosino *et al*., 2001; Haque *et al*., 2011). In addition, the ethanolic extract of *Tropaeolum majus* and his purified fraction containing isoquercitrin increased diuresis with potassium-sparing. This effect observed in spontaneously hypertensive rats results from inhibition of angiotensin converting enzyme and activity of Na^+/K^+-ATPase (Gasparotto *et al*., 2012). In addition, the Flavangenol extracted from pine bark marine attenuated significantly renal lesions in salt hypertensive rats. This protective effect could be attributed to its antioxidant property which protects against endothelial dysfunction (Kwak *et al*., 2009; Ohkita *et al*., 2011).

CONCLUSION

In salt hypertension, *F. exasperata* aqueous leaf extract induced a diuretic effect relatively large and greater than that induced by furosemide. This diuresis was associated with a significant loss of electrolytes in the urine. These urine outputs of electrolytes, creatinine and urea decreased their levels in plasma.

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