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Diuretic Effects of Aqueous Extract of *Ficus exasperata* Vahl. Leaves in Rat

[1]AK Amonkan, [1]AB Konan, [1]BML Ahui, [2]MN Bleyéré, [2]LK Kouakou and [3]GMK Bouafou
[1]Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire
[2]Laboratory of Physiology, Pharmacology and Phytotherapy, UFR Sciences de la Nature, Nangui Abrogoua University; 02 BP 801 Abidjan 02, Côte d'Ivoire
[3]Division of Life and Earth Sciences, Department of Sciences and Technology, Ecole Normale Supérieure (ENS), 25 BP 663 Abidjan 25, Côte d'Ivoire

Abstract: *Ficus exasperata* is widely used in african pharmacopoeia against hypertension and edema. The hypotensive effects of aqueous extract of *F. exasperata* leaves are reduced in the presence of atropine and methylene blue. The treatment of hypertension often requires the combination of antihypertensive drugs and diuretics substances. The aim of this study was to evaluate the effects of aqueous extract of *F. exasperata* leaves on urinary excretion in rat. Single doses of this extract (50 mg kg^{-1} b.wt.) and furosemide (10 mg kg^{-1} b.wt.) were administered to two groups of rats. A control group receives NaCl 9‰. Urine excretion is collected, measured and sampled for 24 hours. At the end of the experiment, blood is sample. Urinary excretion volume induced by this plant extract was relatively high and greater than that induced by furosemide. Aqueous extract of *F. exasperata* leaves increased urinary excretion of electrolytes, creatinine and urea. However, creatinine and blood urea obtained are similar to those induced by furosemide. At the end of this work, it appears that aqueous extract of *F. exasperata* leaves induced a significant diuretic effect and electrolyte output which does not alter significantly the rate of electrolytes, creatinine and urea plasma.

Key words: Diuretic, urinary excretion, electrolyte, *Ficus exasperata*, furosemide

INTRODUCTION

Ficus exasperata species is commonly used in pharmaceutical preparations for the african pharmacopoeia. It is involved in the treatment of various diseases such as hypertension and edema (Adjanohoun *et al*., 1986). The allegations related to its hypotensive effect were highlighted (Ayinde *et al*., 2007). The aqueous extract of *F. exasperata* leaves reduces blood pressure in a dose-dependent. This effect is reduced in the presence of atropine and methylene blue (Amonkan *et al*., 2010). The treatment of hypertension requires some time the use of diuretics involved in the normalization of blood pressure by reducing blood volume following a major excretion (Knauf and Mutschler, 2005). In addition, phytochemical studies indicate that the leaves of *F. exasperata* contain many chemicals (Dongfack *et al*., 2012). These phytochemical compounds may have many pharmacological effects. Thus, the aim of this study was to evaluate the effects of aqueous extract of *F. exasperata* leaves (FEFIX) on urinary excretion in rats by comparing this effect to a reference diuretic to reveal its diuretic effect which could enhance the hypotensive effect.

MATERIALS AND METHODS

Aqueous extract of *Ficus exasperata* leaves (FEFIX): Fresh leaves of *F. exasperata* were harvested, washed and dried in an oven at a temperature of 40±2°C. They were pulverized to obtain a fine powder which is left to macerate in hexane at a rate of 10 g of powder in 100 mL of 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 Buchi rotary evaporator type (France). A powder of the aqueous extract of leaves of *F. exasperata* (FEFIX) was obtained with a yield of 14.27±3.26 %.

Animals: Male Wistar rats weighing 200 and 250 g were used. They were obtained from animal house, Pasteur Institute, Abidjan, Côte d'Ivoire. The animals were grouped and housed in metabolic cages and maintained

Corresponding Author: AK Amonkan, Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire

under standard laboratory conditions (temperature 25±2°C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. Prior to the start of the experiment all animals were fasted overnight with water which was available *ad libitum*. At the end of experiment, rats bloods were sampled from the inferior vena cava after anesthetized them with ether.

Evaluation of the diuretic: Fluid overload was carried out with distilled water in an amount of 50 mL kg^{-1} b.wt. The Animals divided into three groups of 6 rats received intraperitoneal administration of saline solution (NaCl 9‰, control), FEFIX (50 mg kg^{-1} b.wt.) and furosemide (10 mg kg^{-1} b.wt.), respectively. The urine was collected separately every two hours for 24 h. They were measured, sampled and stored at -20°C for the determination of electrolytes, creatinine and urea. Urinary excretion volume (EUV) was determined from the ratio of urine volume measured and the volume of fluid overload.

Determination of plasma and urinary electrolytes: The content of urinary and plasma electrolytes was determined using an automatic analyzer (Hitachi 902, Roche). The determination of sodium and potassium was performed by the technique of photometry. The determination of the chlorine content of the samples, calcium and creatinine was produced by the technique of colorimetry. The content of urea was determined by the principle of kinetics.

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

Statistical analysis: Data were expressed as means with standard error of mean (M±SEM) obtained from n separate experiments. Statistical analysis of the values and graphical representations of data were performed respectively by GraphPad Instat software (Microsoft, San Diego, California, USA) and GraphPad Prism 5 software (Microsoft, San Diego, California, USA). Differences between the mean statistical validity are assessed through Tukey-Kramer test. The difference between the averages is considered statistically significant at the 5% ($p<0.05$).

RESULTS

Urinary excretion volume (EUV): Urinary excretion volumetric induced by FEFIX and furosemide had the same kinetics during 24 h (Fig. 1). Urinary excretion volume induced by FEFIX after 6 h (25.82±1.82, 47.32±3.27 and 67.42±4.06%) was relatively high but lower than those induced by furosemide (44.17±2.58, 61.88±3.42 and 77.08±3.08%). From 10 h, the EUV obtained due to the effect of FEFIX were higher than those obtained after administration of furosemide. After 24 h, the EUV measured were 142.23±4.85% and 118.13±5.41% for FEFIX and furosemide respectively.

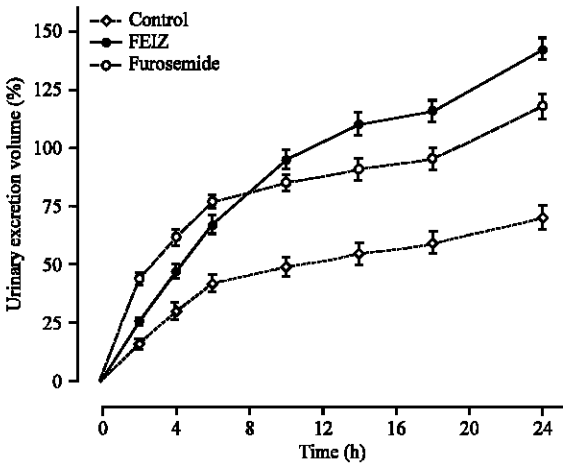

Fig. 1: Evolution of urinary excretion volume measured for three groups of rats: Saline solution (NaCl 9 ‰, control), FEFIX (50 mg kg^{-1}) and furosemide (10 mg kg^{-1}). Urine output was measured every two hours for 24 hours. FEFIX: aqueous extract of *F. exasperata* leaves, n = 6, M±SEM

Urinary excretion of electrolytes: After 24 h, the urinary excretions of electrolytes induced by FEFIX were relatively greater than those induced by furosemide (Fig. 2). FEFIX caused a urinary excretion of sodium (13.92±0.37 mEq), while furosemide induced urinary sodium excretion amounting to 10.88±0.29 mEq. Urinary potassium levels measured were 1.34±0.10 and 1.04±0.14 mEq for FEFIX and furosemide actions respectively. Chlorine and calcium excretion induced by FEFIX during the same period were relatively higher than those induced by furosemide. For chlorine, the amounts excreted were 8.10±0.20 and 7.15±0.39 mEq for FEFIX and furosemide respectively. While those of calcium were 4.70±0.24 mEq (FEFIX) and 3.50±0.54 mEq (furosemide).

Plasma electrolytes: FEFIX and furosemide caused minor variations in plasma electrolytes (Fig. 3). After 24 h, FEFIX decreased serum rate like furosemide. The serum sodium measured was 120.03±5.03 mEq L^{-1}(FEFIX) and 121.50±7.04 mEq L^{-1} (furosemide). Plasma chloride and calcium induced by FEFIX (78.42±3.92 mEq and 4.06±0.36 mEq) were relatively higher than those induced by furosemide (76.55±6.78 and 4.35±0, 29 mEq L^{-1}).

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

	Creatinine		Urea	
	Creatinine $_U$ (mmol/24 h)	Creatinine $_P$ (mM)	Urea $_U$ (mmol/24 h)	Urea $_P$ (mM)
Control	0.19±0.02	0.37±0.04	131.2±9.49	3.02±0.41
FEFIX	0.32±0.02***	0.52±0.06	251.25±12.47***	3.57±0.43
Furosémide	0.28±0.02***	0.47±0.04	209.79±13.96***	3.33±0.23

Saline solution (NaCl 9 ‰, control), FEFIX (50 mg kg^{-1}): aqueous extract of *F. exasperata* leaves, furosemide (10 mg kg^{-1}) U: urine, P: plasma, n = 6, Mean±SEM; ***p<0.001

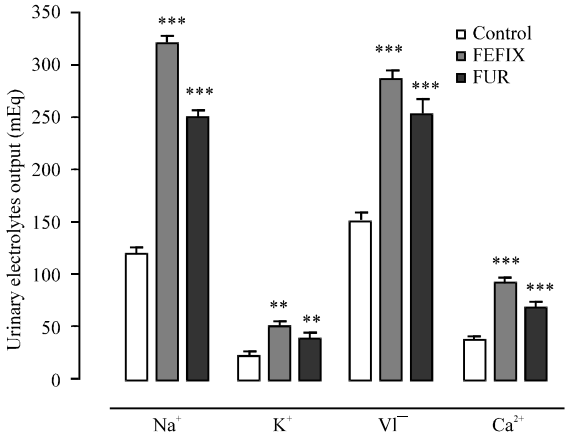

Fig. 2: Rate of urinary electrolyte excretion in rats at 24 h in three groups of rats treated with Saline solution (NaCl 9‰, control), FEFIX (50 mg kg^{-1}) and furosemide (10 mg kg^{-1}) respectively. Electrolytes were measured in all urine sampled for 24 h after treatment in each group of rats. FEFIX: aqueous extract of *F. exasperata* leaves, n = 6, Mean±SEM, ***p<0.001 , **p<0.01

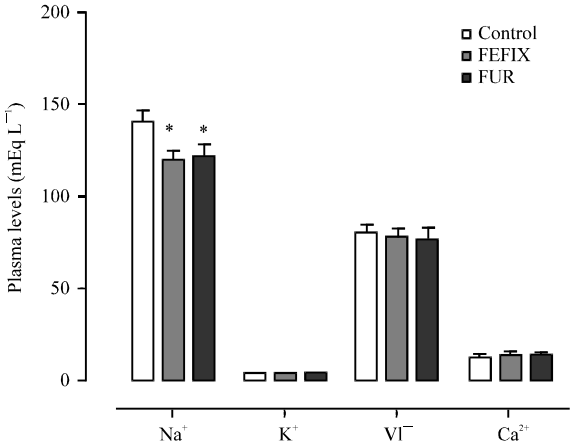

Fig. 3: Plasma levels of electrolytes at 24 h after administration of Saline solution (NaCl 9‰, control), FEFIX (50 mg kg^{-1}) and furosemide (10 mg kg^{-1}) in three groups of rats, respectively. The rate of electrolyte was measured on blood samples. FEFIX: aqueous extract of *F. exasperata* leaves, n = 6, Mean±SEM, *p<0.05

However, these differences were not statistically significant (p>0.05). FEFIX and furosemide alter very little calcium at 24 h (29.20±3.14 and 27.83±3.08 mEq L^{-1}).

Urea and creatinine: The leaves aqueous extract of *F. exasperata* (FEFIX) increased the levels of creatinine and urea in urine output and plasma (Table 1). FEFIX induced a significant increase in the urinary excretion of creatinine and urea (p<0.01). These urinary excretions were greater than those induced by furosemide. After 24 h the creatinine excretion induced by FEFIX was 0.32±0.02 mmol and that induced by furosemide was 0.32±0.02 mmol. Urine urea outputs measured in the same time were 251.25±12.47 mmol (FEFIX) and 209.79±13.96 mmol (furosemide). FEFIX increased plasma creatinine and uremia like furosemide. FEFIX-induced effects were superior to those of furosemide in plasma. However, the differences between these two effects were not significant (p>0.05). After 24 h, creatinine levels were 0.52±0.06 mM and 0.47±0.04 mM for FEFIX and furosemide, respectively. The uremias obtained were 3.57±0.43 mM (FEFIX) and 3.33±0.23 mM (furosemide).

DISCUSSION

Aqueous extract of *F. exasperata* leaves induced relatively high urinary volume (EUV) with similar excretion kinetic observed for furosemide. This EUV was greater than that produced by furosemide. Similar results observed in rats were reported by Lahlou *et al.* (2007). These authors showed that the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* increased urine volume like furosemide. This effect was observed 24 h after administration of 100 mg kg^{-1} of each extract. In addition, the ethanolic extract of *Cocculus hirsitum* leaves increased urine volume in rats. The diuretic effect increased with dose-dependent (Badole *et al.*, 2009). Diuresis induced by FEFIX was associated with significant excretion of electrolytes (Na^+, K^+, $Cl^-$and Ca^{2+}). These urinary excretions were similar to those induced by furosemide. Electrolytes urinary excretions result from

the reabsorption inhibition in the kidney. It has been demonstrated in rats after administration of 10 mL kg^{-1} b.wt. of 5 % decoction of *Stigma maydis* (Maksimovic *et al*., 2004). In addition, orally administered, the aqueous extract of Parsley reduced the activity of Na^+/K^+-ATPase in the cortex and the renal medulla (Kreydiyyeh and Usta, 2002). The inhibition of this ion pump decreases the reabsorption of Na+ by the apical cells and increases intracellular K^+ reducing its excretion. Similar effects have been demonstrated after administration of a loop diuretic such as furosemide (Odlind *et al*., 1983). The rise in urinary electrolytes has also been reported in rats after administration of methanol extract of *Tephrosia purpurea*. In fact, this extract administered at doses of 200 and 400 mg kg^{-1} b.wt. increases the urinary excretion of electrolytes (Ashokkumar *et al*., 2012). In addition, the aqueous extract of *Hemidesmus indicus* roots increased urinary excretion of Na^+ and K^+ like furosemide and hydrochlorothiazide. Natriuretic and kaliuretic effects observed were obtained after 24 h at a dose of 400 mg kg^{-1} b.wt. *per os* (Gadge and Jalalpure, 2011). Urinary excretion volume and urinary excretion of electrolytes induced by FEFIX caused a decrease in the plasma level of these and an increase in serum creatinine and blood urea. FEFIX effects on plasma creatinine and urea were similar to those induced by furosemide. The loop diuretics like furosemide stimulated kidney functions (Assy *et al*., 2006). It results in an increase in creatinine clearance and urea (Shilliday *et al*., 1997; Medcalf *et al*., 2001). This stimulation of renal functions leads to a decrease in plasma creatinine, urea and electrolytes (Badole *et al*., 2009). At the end of this work, it appears that the aqueous extract of *F. exasperata* leaves (FEFIX) induced an important diuresis relatively greater than that induced by furosemide. Urine volume output induced by FEFIX was associated with significant increase of urinary electrolytes and a significant decrease in plasma electrolytes, creatinine and blood urea.

CONCLUSION

The aqueous extract of *F. exasperata* leaves increased urine volume output. This excretion is associated with a loss of sodium, chloride, creatinine and urea. It induced a decrease in plasma sodium and chloride without affecting plasma calcium.

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