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Research Article

Comparative Effects of *Ficus Exasperata* Vahl. Aqueous Leaf Extract and Hydrochlorothiazide on Urinary Excretions in Normotensive Rats

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Abstract: The allegations related to the antihypertensive effect of *Ficus exasperata* in the treatment of hypertension were identified. This increases its use in primary health care for many people and can cause disturbances in the balance of water and electrolytes. The aim of this work was to compare the effects of *Ficus exasperata* aqueous leaf extract (FEFIX) and a thiazide diuretic on urinary excretion in normal rats. Single doses of FEFIX (50 mg/kg b.wt.) and hydrochlorothiazide (5 mg/kg b.wt.) were administered to rats previously received fluid overload (50 ml/kg b.wt.). The urine were collected, measured and sampled for 24 hours. Blood was sampled at the end of the experiment. FEFIX induced a relatively important volume excretion (120.25 ± 5.75%) and higher than that induced by hydrochlorothiazide (91.50 ± 6.38%). FEFIX increases urinary excretion of electrolytes (Na^+, K^+, Cl^- and Ca^{++}), creatinine and urea like hydrochlorothiazide (HCTZ). These urinary excretion of electrolytes decreased serum sodium and serum chloride. At the end of this work, it appears that FEFIX and HCTZ increased urine volume, urinary excretion of electrolytes and decreased plasma electrolytes. However, FEFIX effects are relatively more important.
Keywords: Urinary excretion, Electrolyte, *Ficus exasperata*, Hydrochlorothiazide.

INTRODUCTION

Medicinal plants are commonly used in primary health care in many developing countries. Typically, a given species is used to treat different diseases. These practices are not always safe for their users [1,2]. The use of plants may cause toxic effects or disturbance of electrolyte balance in the body. Indeed, the balance of ions is essential for the proper functioning of the nervous tissue and organs [3, 4]. The balance of electrolytes depended entries, mainly foodborne, and outputs, mainly kidney. According to the needs of the body, electrolytes are either excreted or reabsorbed to maintain their concentration to a physiological level. Medicinal plants contain many bioactive substances. These can change the balance of electrolytes in promoting their excretion [5, 6]. The phytochemical study of the leaves of *Ficus exasperata* revealed the presence of different chemical groups [7]. *F. exasperata* leaves have a hypotensive effect that justifies its use in the treatment of hypertension [8]. However it is necessary to evaluate its effect on the content of electrolytes. This study was carried to compare the effects of *F. exasperata* aquous leaf extract (FEFIX) to those of Hydrochlorothiazide, a thiazide diuretic reference on urinary excretions in normotensive rats.

MATERIALS ET METHODS

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 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.

Ficus exasperata extract preparation

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 [9]. 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 hours. 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.

Evaluation of the diuretic

Fluid overload was carried out with distilled water in an amount of 50 ml/kg b. wt. The Animals divided into three groups of 6 rats received orally saline solution (NaCl 9 ‰, control), FEFIX (50 mg/kg b.wt.) and hydrochlorothiazide (HCTZ, 5 mg/kg b. wt.) respectively. The urine was collected separately every two hours for 24 hours. They were measured, sampled and stored at -20 °C for the determination of electrolytes, creatinine and urea. Urinary excretion volume (UEV) 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 animal use and care [10, 11].

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 volume

After 4 hours, the administration of FEFIX induced urinary volume (53.21 ± 3.07 %) relatively lower than that induced by HCTZ (66.04 ± 3.35 %). After 14 hours, FEFIX caused the elimination of fluid overload (112.08 ± 5.07 %) while that obtained with HCTZ was less than fluid overload (90.58 ± 6.75 %). After 24 hours, the urinary excretion volume (UEV) induced by FEFIX and HCTZ were respectively 120.25 ± 5.75 % and 91.50 ± 6.38 % (Fig. 1).

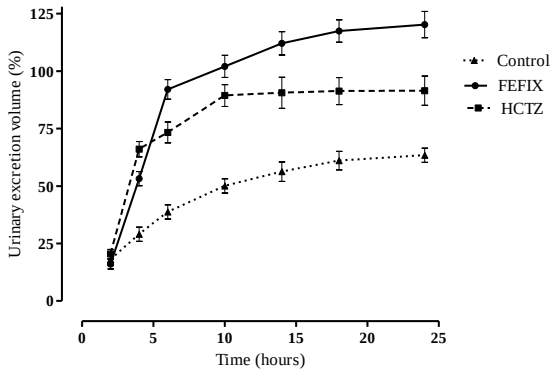

Fig. 1: Evolution of urinary excretion volume measured for three groups of normotensive rats.

Saline solution (NaCl 9 ‰, Control), FEFIX (50 mg/kg b.w.) and hydrochlorothiazide (5 mg/kg b.w.). Urine output was measured every two hours for 24 hours. FEFIX: *F. exasperata* aqueous leaf extract, HCTZ: Hydrochlorothiazide, n = 6, m ± sem.

Urinary excretion of electrolytes

After 24 hours FEFIX and HCTZ increased urinary excretion of sodium and chloride (Table 1). The urinary levels of sodium are highly significant ($p < 0.001$). They were 13.86 ± 0.29 mEq (FEFIX) and 9.79 ± 0.29 mEq (HCTZ). Levels of chlorine measured were 8.19 ± 0.25 mEq and 6.07 ± 0.22 mEq respectively for FEFIX and HCTZ. The urinary excretion of potassium and calcium induced by FEFIX were higher than those obtained with HCTZ. The urinary potassium measured under the action of FEFIX was 1.30 ± 0.11 mEq ($p < 0.001$). While that obtained by the action of HCTZ was 0.68 ± 0.08 mEq ($p < .05$). Urinary calcium levels induced by FEFIX and HCTZ were respectively 4.63 ± 0.16 mEq ($p < 0.001$) and 2.73 ± 0.25 mEq ($p < 0.05$).

Table 1: Rate of electrolyte urinary excretion in rats at 24 hours in three groups of normotensive rats

Urinary electrolytes (mEq/24 h)	Na^+	K^+	Cl^-	Ca^{++}
Control	5.18 ± 0.32	0.67 ± 0.09	4.18 ± 0.22	2.00 ± 0.14
FEFIX	13.86 ± 0.29***	1.30 ± 0.11***	8.19 ± 0.25***	4.63 ± 0.16***
HCTZ	9.79 ± 0.29***	0.68 ± 0.08	6.07 ± 0.22	2.73 ± 0.25*

The rats were treated *per os* with saline solution (NaCl 9 ‰, Control), FEFIX (50 mg/kg b.wt.) and hydrochlorothiazide (5 mg/kg b.wt.) respectively. Electrolytes were measured in all urine sampled for 24 hours after treatment in each group

of rats. FEFIX: *F. exasperata* aqueous leaf extract, HCTZ: Hydrochlorothiazide, n = 6, m ± sem, *** p < 0.001 , ** p < 0.01, * p < 0.05.

Plasma electrolytes

After 24 hours FEFIX and HCTZ changed the plasma sodium and chlorine without affecting significantly the plasma potassium and calcium (Table 2). Serum sodium obtained with HCTZ (103.83 ± 4.13 mEq/L) was lower than that obtained with FEFIX (112.83 ± 3.93 mEq/L). The plasma levels of chlorine decreased significantly under the action of FEFIX and HCTZ ($p < 0.01$). The values obtained were 62.57 ± 3.86 mEq/L and 59.50 ± 3.52 mEq/L respectively for FEFIX and HCTZ.

Table 2: Plasma levels of electrolytes in three groups of normotensive rats

Plasma ions (mEq/L)	Na^+	K^+	Cl^-	Ca^{++}
Control	137.14 ± 4.84	4.24 ± 0.16	83.27 ± 3.82	24.64 ± 0.46
FEFIX	112.83 ± 3.93**	4.18 ± 0.30	62.57 ± 3.86**	28.00 ± 2.74
HCTZ	103.83 ± 4.13***	4.28 ± 0.34	59.50 ± 3.52**	26.84 ± 0.88

Three groups of normotensive rats received *per os* saline solution (NaCl 9 ‰, Control), FEFIX (50 mg/kg b.wt.) and hydrochlorothiazide (5 mg/kg b.wt.) respectively. The rate of electrolyte was measured on blood sampled after 24 hours. FEFIX: *F. exasperata* aqueous leaf extract, HCTZ: Hydrochlorothiazide, n = 6, m ± sem, *** p < 0.001, ** p < 0.01, * p < 0.05.

Creatinine and urea

After 24 hours FEFIX significantly altered the urinary excretion of creatinine and urea ($p < 0.001$). While HCTZ only affected the urinary creatinine (Fig. 2). Creatinine levels induced by FEFIX and HCTZ were respectively 0.31 ± 0.01 mmol ($p < 0.001$) and 0.26 ± 0.02 mmol ($p < 0.01$). Urinary urea levels were 249.75 ± 20.47 mmol ($p < 0.001$) and 171.50 ± 14.15 mmol ($p > 0.05$) respectively for FEFIX and HCTZ. Creatinine and blood urea were not significantly modified by FEFIX and HCTZ (Fig. 3).

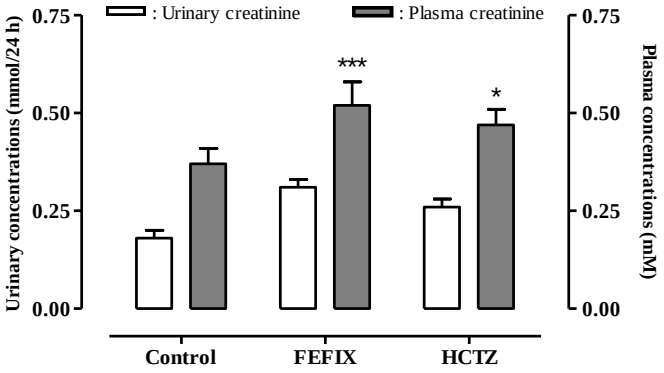

Fig. 2: Levels of creatinine in urine and plasma in normotensive rats treated with saline solution (9‰, control), FEFIX (50 mg/kg b.wt.) and HCTZ (5 mg/kg b.wt.).

Creatinine was measured in all urine sampled for 24 hours after treatment and blood sampled after 24 hours. FEFIX: *F. exasperata* aqueous leaf extract, HCTZ: hydrochlorothiazide, n = 6, m ± sem, *** p < 0.001 , ** p < 0.01, * p < 0.05.

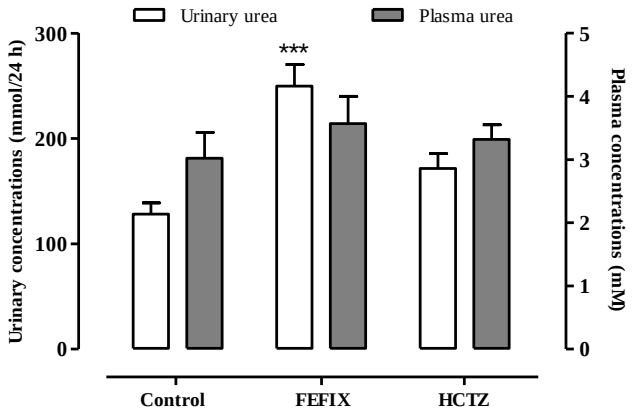

Fig. 3: Levels of urea in urine and plasma in normotensive rats treated with saline solution (9‰, control), FEFIX (50 mg/kg b.wt.) and HCTZ (5 mg/kg b.wt.).

Urea was measured in all urine sampled for 24 hours after treatment and blood sampled after 24 hours. FEFIX: *F. exasperata* aqueous leaf extract, HCTZ: hydrochlorothiazide, n = 6, m ± sem, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

DISCUSSION

These studies shown that *Ficus exasperata* aqueous leaves extract (FEFIX) significantly increased the urinary excretion volume (UEV) like hydrochlorothiazide (HCTZ). However, after 24 hours UEV induced FEFIX was higher than that obtained with HCTZ. Similar results were reported for a mixture of six plants. This mixture increased the diuretic effect of 34 % compared to HCTZ [12]. In addition, it was extracted with soxhlet and maceration of wood roots of *Carissa edulis* at a dose of 50 mg/kg b.wt. increased urine volume excreted. This effect was compared with HCTZ used as diuretic reference [13].

The diuretic effect of FEFIX is associated with significant urinary excretion of electrolytes (Na^+, K^+, Cl-and Ca^{++}). While the diuretic effect of HCTZ is only associated with the urinary excretion of sodium and chlorine. Similar diuretic effects have been reported for the infusion of *Salvia scutellarioides*. This extract induced a rich electrolyte excretion, which increased with dose [14]. In addition, the aqueous extract of *Amaranthus spinosus* increased urinary excretion of electrolytes after 24 hours. Saliurétiques effects were similar to those of a thiazide diuretic [15]. Also, the kaliuretic effects of FEFIX higher than those of HCTZ resulted of the diversity of chemical compounds contained in the extract [9]. These chemicals compounds would act at different levels along the nephron to potentiate its diuretic effect. While, the diuretic effect of HCTZ resulted mainly from his action at the convoluted tubules. It inhibits the mechanism of electroneutral reabsorption of sodium and chlorine [16, 17].

The diuretic effects of FEFIX and HCTZ caused a decrease in serum sodium and serum chloride without affecting potassium and calcium. The decrease in plasma levels of these electrolytes was related to their major urinary excretion. Indeed, *Herniaria glabra* saponins reduced the reabsorption of water and sodium in the renal tubules. Thus saponins increased urinary flow and urinary excretion of electrolytes [18]. In addition, isoquercitrine content in ethanol extract of *Tropaeolum majus* and purified fraction increased diuresis and urinary excretion of electrolytes with potassium-sparing [19].

CONCLUSION

Ficus exasperata aqueous leaf extract (FEFIX) and hydrochlorothiazide increased urine volume excreted. Elevated levels of urinary excretion of electrolytes involved decreases in serum sodium and serum chloride. Diuresis and urinary excretion of electrolytes induced by FEFIX are relatively higher than those induced by hydrochlorothiazide.

COMPETING INTERESTS

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHORS' CONTRIBUTIONS

All co-authors have contributed to the study design, data search and analysis, and write-up of the manuscript.

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