

Effects of *Ficus exasperata* Root Bark Aqueous and Ethanolic 70 % Extracts on Urinary Excretion in Wistar Rat

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Received June 05, 2022; Revised July 13, 2022; Accepted July 19, 2022

Abstract The roots of *Ficus exasperata* Valh (Moraceae) are commonly used in traditional medicine to treat various pathologies including edema and arterial hypertension. Also, this study was conducted to assess the effects of two extracts of the root bark of this medicinal plant on urinary excretion in rats. To do this, 12 groups of 6 water-overloaded rats (50 ml/kg) were treated separately with 0.9% NaCl solution, FURO 5 mg/kg, FEae (15-100 mg/kg) and FEee (15-100 mg/kg). Excreted urine was collected at regular intervals of 2 h for 8 h to determine urinary excretion volume (UEV). Like furosemide, the two plant extracts (FEae and FEee) caused significant urinary excretion in water-overloaded rats. The most marked effect in terms of UEV was observed after the administration of FEae 15 mg/kg. At this dose, an EUV of $76 \pm 0.21\%$ and higher than that of FURO 5 mg/kg ($59 \pm 0.01\%$) was obtained 6 h after the treatment of the animals. These recorded EUV values were significantly higher at $p < 0.001$ than that of 0.9 % NaCl saline solution. *Ficus exasperata* root bark extracts induced significant increases in urinary excretion in Wistar rats. These results could justify the empirical use of this plant as a diuretic in the treatment of edema and hypertension. However, in order to better understand the physiological mechanisms underlying this action, additional studies must be carried out.

Keywords: *Ficus exasperata*, furosemide, diuretic, urinary excretion, arterial hypertension, edema

Cite This Article: Kouao Augustin Amonkan, Brou André Konan, and Kacou Jules Marius Djétouan, "Effects of *Ficus exasperata* Root Bark Aqueous and Ethanolic 70 % Extracts on Urinary Excretion in Wistar Rat." *American Journal of Pharmacological Sciences*, vol. 10, no. 1 (2022): 20-25. doi: 10.12691/ajps-10-1-4.

1. Introduction

To use this template, Arterial hypertension (HTA) is the most widespread cardiovascular disease in black Africa [1,2]. It is the cause of more than 45 % of deaths related to cardiovascular diseases [3], including strokes and heart disease [4]. This pathology is a major public health problem [5,6].

Modern therapy has many drugs to treat hypertension. However, poverty and sociological considerations on the one hand and on the other hand the high cost of pharmaceutical products force people to resort to traditional medicine [7-9]. This medicine is mainly based on plants which are part of the culture of many peoples. Indeed, the use of plants in health care dates back to ancient times [10,11]. Aké-Assi and Guinko [12] showed that medicinal plants are used in the world in general and in Africa in particular for primary health care. Among these medicinal plants is *Ficus exasperata* commonly used to treat edema and arterial hypertension [13,14].

Ficus exasperata (Moraceae) is a very widespread plant species throughout intertropical Africa and mainly in secondary formations [15,16,17]. Several works have

confirmed the allegations related to the use of *Ficus exasperata* leaves in the treatment of edema and arterial hypertension. Amonkan et al. [18,19] also showed the hypotensive, antihypertensive and diuretic effects of *Ficus exasperata* aqueous leaves extract in rats. This would fully militate in favor of the use of the leaves of this plant in the treatment of edema and arterial hypertension.

However, what about the roots that are also used to treat the same pathologies? Indeed, according to the literature, various parts of the plant whose roots are used for this purpose [13,14]. Thus, this study is a contribution to the knowledge of the diuretic properties of the roots of this plant. It was conducted in order to evaluate the effects of two roots bark extracts on urinary excretion in rat.

2. Material and Methods

2.1. Animals and Ethical Considerations

72 males Wistar rats weighing 200-250 g were used. They were obtained from the Vivarium (animal house), Ecole Nationale Supérieure (ENS), Abidjan, Côte d'Ivoire. Animals housed in metabolic cages and maintained under

standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. Animals are fasted 18 hours before the experiment. Rats were treated according to good laboratory practices [20]. The experimental protocols were conducted in accordance with the protocols for the protection of experimental animals of the European Council on Legislation 1201/707 [21].

2.2. Plant Material and Extractions

Fresh leaves and root bark of *Ficus exasperata* were collected in September 2019 in Bingerville forest (Region des Lagunes, Southern region of Côte d'Ivoire). These leaves and root bark of *Ficus exasperata* were certified to be an identical sample at the specimen herbarium (Fourcher n°3309) of Centre National Floristique (CNF), Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). This medicinal plant was authenticated by a Botany expert, Prof. Aké-Assi Emma of the CNF.

The extraction process was implemented according to the method described by Konan et al. [22] with some modifications. Root barks of *Ficus exasperata* were dried under shade and powdered with a machine (Mark RETSCH, type SM 100n Germany). One hundred grams (100 g) of the root barks powder were macerated separately during 24 hours in 1 l water and 2 l ethanol-water (70/30 v/v). The mixtures were filtered (Whatman n°1) for 3 times until complete exhaustion. The filtrates were concentrated under reduce pressure using a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 60°C and dried in a drying oven at $50 \pm 5^\circ\text{C}$. The concentrated extracts obtained (FEae: *Ficus exasperata* aqueous extract and FEee: *Ficus exasperata* ethanolic 70 % extract) were stored at 4°C until experiments. And the concentrations of extracts to be tested were prepared extemporaneously by dilution in 0.9 % NaCl saline solution.

2.3. Chemical Used

Ethanol and Furosemide (Lasilix®) were purchased from Nadal (France) and Sanofi-Aventis (France) respectively.

2.4. Experimental Design

The method described by Konan et al. [22] was employed. Animals were randomly divided into 12 groups, each containing 6 rats and placed individually in metabolic cages. After the induction of the fluid overload (50 ml/kg), animals received drugs by intraperitoneal administration. Group 1 received 0.9 % NaCl saline solution, while Group 2 received furosemide (FURO) at 5 mg/kg. Groups 3, 4, 5, 6 and 7 were treated with FEae (15; 25; 50; 75; 100 mg/kg respectively). Groups 8, 9, 10, 11 and 12 received FEee (15; 25; 50; 75; 100 mg/kg respectively).

2.5. Measurement of Urinary Excretion

The urine was collected every 2 hours for 8 hours. The urinary volumes thus collected made it possible to evaluate the diuretic power of the substances studied by the determination of the urinary excretion volume (UEV).

The UEV was determined from the ratio of the total volume of urine and the volume of water overload [23,24].

2.6. Data Analysis

The values were expressed as mean with standard error of the mean ($m \pm \text{sem}$). The data were evaluated by analysis of variance followed by Tukey-Kramer with GraphPad Instat software (Microsoft, San Diego, California, USA) method. The graphical representations of data were performed by the GraphPad Prism 8 software (Microsoft, San Diego, California, USA). The difference between the averages is considered statistically significant when $p < 0.05$.

3. Results

3.1. Effect of *Ficus exasperata* Aqueous Extract on Urinary Excretion

Figure 1 is the graphic representation of the urinary excretions measured 2 and 6 h after administration of 0.9 % NaCl saline solution, FURO 5 mg/kg and increasing doses of FEae (15-100 mg/kg). After two hours, the measured urinary excretions were different (Figure 1a). The urinary excretions obtained for the doses of FEae (50-100 mg/kg) were lower than that of the 0.9 % NaCl saline solution. Conversely, FURO 5 mg/kg and FEae at doses of 15 and 25 mg/kg induced higher urinary excretions ($p < 0.001$) than that of the 0.9 % NaCl solution ($6.13 \pm 0.8 \text{ ml/kg}$). Urinary excretion induced by FURO 5 mg/kg was $13.67 \pm 0.69 \text{ ml/kg}$. And those induced by FEae at 15 and 25 mg/kg were 14.06 ± 0.56 and $10.67 \pm 0.9 \text{ ml/kg}$, respectively.

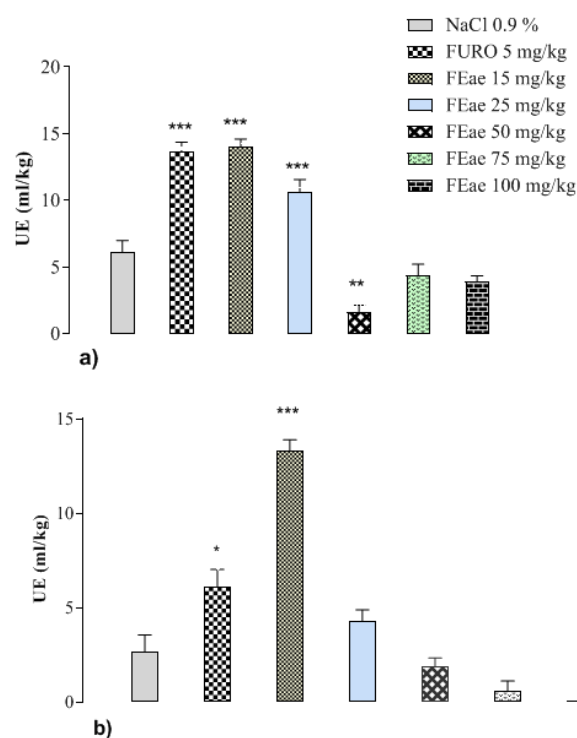


Figure 1. Effects of *Ficus exasperata* aqueous extract and furosemide on urinary excretion in rat (a) 2 hours after treatment, (b) 6 hours after treatment; $m \pm \text{sem}$; $n = 6$; ** $p < 0.01$; *** $p < 0.001$ compared to Control; FEae: *Ficus exasperata* aqueous extract; FURO: Furosemide)

This action of FEae 15 mg/kg persisted longer as shown in Figure 1b, which represents the urinary excretions measured 6 hours after administration of the substances studied. Significant urinary excretion was always observed with FEae 15 mg/kg. It was estimated at 13.33 ± 0.57 ml/kg and was statistically higher at $p < 0.001$ than those of FURO 5 mg/kg and 0.9 % NaCl solution with respective values of 6.13 ± 0.90 ml/kg and 2.67 ± 0.97 ml/kg. The high dose of FEae (100 mg/kg) did not cause urinary excretion in rats 6 hours after its administration.

3.2. Effect of *Ficus exasperata* Ethanolic Extract on Urinary Excretion

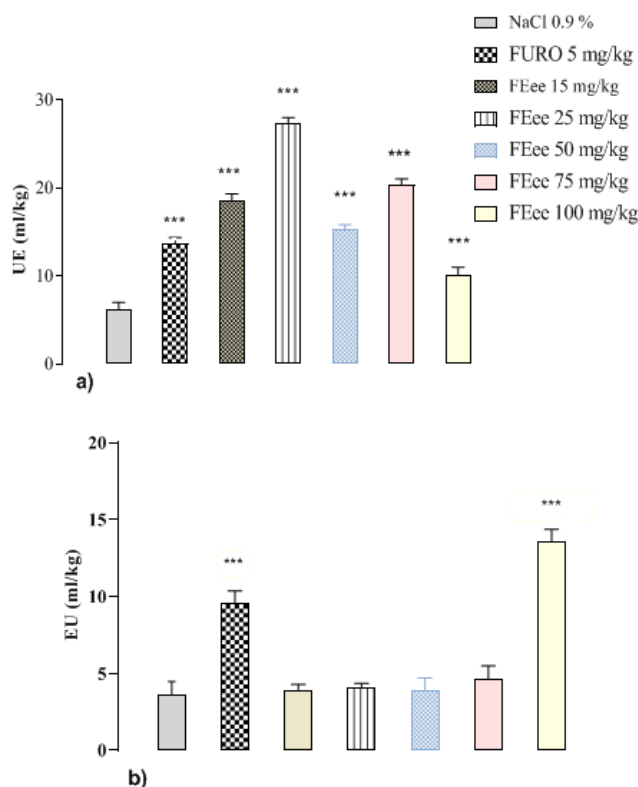


Figure 2. Effects of *Ficus exasperata* ethanolic 70 % extract and furosemide on urinary excretion in rat: a) 2 hours after treatment, b) 4 hours after treatment; $m \pm \text{esm}$; $n = 6$; ** $p < 0.01$, *** $p < 0.001$ compared to Control; FEee : *Ficus exasperata* ethanolic 70 % extract; FURO: Furosemide

The urinary excretions recorded 2 and 4 hours after the administration of increasing doses of FEee (15-100 mg/kg)

in comparison with 0.9% NaCl saline solution and FURO 5 mg/kg are given in Figure 2. The separate administration of the different doses of FEee (15; 25; 50; 75 and 100 mg/kg) and FURO 5 mg/kg induced 2 hours after significant urinary excretions in rats (Figure 2a). The values obtained were all statistically higher ($p < 0.05$) than those of the control rats treated with the 0.9 % NaCl solution. It should be noted, however, that the largest urinary excretions at $p < 0.001$ in comparison with that of 0.9 % NaCl saline solution were caused by the doses of 15, 25 and 75 mg/kg. The values obtained were 18.61 ± 0.7 ml/kg, 27.36 ± 0.61 ml/kg and 20.33 ± 0.69 ml/kg respectively against 6.13 ± 0.8 ml/kg for the 0.9 % NaCl saline solution.

Four hours after the treatment, no difference was observed between the groups of rats treated with doses of 15-75 mg/kg and that of the controls having received the 0.9% NaCl saline solution. However, a significant increase in urinary excretion was observed in rats treated with FEee 100 mg/kg (Figure 2b). It was 13.59 ± 0.77 ml/kg and was significantly higher at $p < 0.001$ than those of NaCl 0.9% saline solution and FURO 5 mg/kg. The urinary excretion induced by FURO 5 mg/kg was 9.53 ± 0.82 ml/kg and 3.60 ± 0.88 ml/kg for the 0.9% NaCl saline solution.

3.3. Effect of *Ficus exasperata* Extracts on Urinary Excretion Volume

The urinary excretion volumes obtained with FEae (15 and 25 mg/kg), FEee (15 and 25 mg/kg), FURO 5 mg/kg and 0.9 % NaCl saline solution are recorded in Table 1. The different doses of plant extracts and furosemide induced urinary excretion volumes that were statistically higher at $p < 0.001$ than that of 0.9% NaCl saline solution. The maximum UEV were measured 8 h after the administration of the doses of the substances studied with the exception of FEae 15 mg/kg. The recorded UEV were 34 ± 0.11 %, 73 ± 0.03 %, 53 ± 0.09 %, 54 ± 0.04 % and 69 ± 0.07 % respectively with 0.9 % NaCl saline solution, FURO 5 mg/kg, FEae 25 mg/kg, FEee 15 mg/kg and FEee 25 mg/kg. With FEae, a peak EUV value of 76 ± 0.21 % was observed 6 hours after treatment. It was even higher than that caused by FURO 5 mg/kg at the 8th hour of treatment (73 ± 0.03 %). FEae 15 mg/kg certainly induced the maximum elimination of the water overload but it did not allow the total elimination of the water overload.

Table 1. Effects of *Ficus exasperata* extracts on urinary excretion volume in Wistar rat

Treated groups	Urinary excretion volume (%)			
	2 hours	4 hours	6 hours	8 hours
NaCl 0.9 %	$12 \pm 0,03$	$20 \pm 0,06$	$25 \pm 0,10$	$34 \pm 0,11$
FURO 5 mg/kg	$27 \pm 0,1^{***}$	$46 \pm 0,03^{***}$	$59 \pm 0,01^{**}$	$73 \pm 0,03^{***}$
FEae 15 mg/kg	$30 \pm 0,08^{***}$	$49 \pm 0,04^{***}$	$76 \pm 0,21^{***}$	$76 \pm 0,21^{***}$
FEae 25 mg/kg	$25 \pm 0,02^{***}$	$36 \pm 0,03^{***}$	$46 \pm 0,04^{***}$	$53 \pm 0,09^{***}$
FEee 15 mg/kg	$37 \pm 0,04^{***}$	$45 \pm 0,04^{***}$	$51 \pm 0,06^{***}$	$54 \pm 0,04^{***}$
FEee 25 mg/kg	$55 \pm 0,07^{***}$	$63 \pm 0,07^{***}$	$68 \pm 0,08^{***}$	$69 \pm 0,07^{***}$

$m = \text{sem}$, $n = 6$, ** $p < 0.01$; *** $p < 0.001$ compared to control, FEae: *Ficus exasperata* aqueous extract, FEee: *Ficus exasperata* ethanolic 70 % extract.

3.4. Comparative Effects of Two Active Doses of *Ficus exasperata* Extracts on Urinary Excretion

The UEV induced by the different substances studied (FURO, FEae and FEee) were greater at $p < 0.001$ compared to that of the 0.9 % NaCl saline solution. The kinetics of action of the three test substances showed some differences compared to that of the 0.9 % NaCl solution. However, the kinetics of *Ficus exasperata* aqueous extract (FEae) was closer to that of furosemide as shown in Figure 3. This figure is the graphic representation of the kinetics of the elimination of water overload in rats by FURO 5 mg/kg and the most active doses of plant extracts (FEae 15 mg/kg and FEee 25 mg/kg). The substances caused a gradual elimination of water overload in rats over time.

Intraperitoneal administration of FURO 5 mg/kg caused progressive elimination of water overload in rats. UEV was estimated at 27 ± 0.01 % and 73 ± 0.03 % respectively 2 and 8 hours after administration. With FEae 15 mg/kg, the EUV which was 30 ± 0.08 % at the 2nd hour gradually increased to reach its maximum value of 76 ± 0.21 % at the 6th hour after its administration. This elimination of water overload remained constant until the 8th hour. FEee 25 mg/kg exhibited water overload elimination kinetic that was different from those of FURO 5 mg/kg and FEae 15 mg/kg. Two hours after administration, FEee 25 mg/kg induced the strongest UEV measured at 55 ± 0.07 % against 27 ± 0.1 %, 30 ± 0.08 % and 12 ± 0.03 % respectively for FURO 5 mg/kg, FEae 15 mg/kg and 0.9 % NaCl saline solution. Eight hours after administration, FEee 25 mg/kg induced EUV estimated at 69 ± 0.07 %.

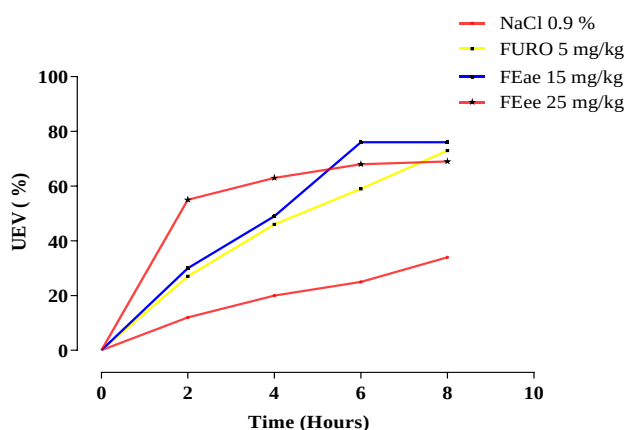


Figure 3. Evolution of urinary excretion volume in the presence of the most active doses of *Ficus exasperata* extracts ($m \pm \text{esm}$; $n = 6$, ** $p < 0.01$; *** $p < 0.001$ compared to saline solution NaCl 0.9 %; UEV: Urinary excretion volume; FEae: *Ficus exasperata* aqueous extract; FEee : *Ficus exasperata* ethanolic 70 % extract; FURO: Furosemide)

4. Discussion

The evaluation of the effects of FEae and FEee on urinary excretion was carried out in rats. Both extracts induced an increase of EUV like furosemide. The greatest urinary excretion was induced by FEae 15 mg/kg. In agreement with many authors, the effects of FEae and

FEee could be explained by the presence of phytochemical compounds contained in the *Ficus exasperata* roots bark. Maghrani *et al.* [25] demonstrated that the metabolites present in plant extracts are responsible of their diuretic activities. Thus, the effects of FEae and FEee would be caused by chemical compounds such as alkaloids, flavonoids, phlobatannins, steroids, tannins and saponins contained in the roots of *Ficus exasperata* [26,27,28].

The results obtained are in agreement with those of previous works which have shown the dose-dependent diuretic effects of various species of *Ficus* used as diuretic substances in traditional medicine. The diuretic effects of *Ficus exasperata* leaves have been reported by several authors [19,29]. Used at 100 mg/kg, *Ficus exasperata* aqueous leaves extract considerably increases the UEV in DOCA-induced hypertensive Wistar rats [19]. Aqueous extract of *Opuntia* flowers (*Ficus indica*) also increases diuresis by 15 % in Wistar rats [30].

Ficus exasperata roots bark extracts (FEae: aqueous extract and FEee: 70% ethanolic extract) showed different action kinetics. This difference would be partly attributable to the solvents used to obtain the extracts. Indeed, the solvents determine the nature of the compounds present in the plant extracts. Solvents with different solubilities and polarities for phytochemicals could fully justify the pronounced effect of one extract compared to another extract from the same part of the plant [31-34].

FEae and FEee induced a significant increase in diuresis like furosemide which is a loop of Henlé diuretic. Loop diuretics inhibit the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter in the ascending limb of the loop of Henlé [35,36]. This results in a leak of Na^+ , Cl^- , K^+ and H^+ during the reabsorption of water at the level of the loop of Henle [37]. Pharmacological substances in general and diuretics in particular modify these different ion transports [38,39]. The phytochemicals contained in FEae and FEee would interact with these ion transporters to produce the effects observed in water-overloaded Wistar rat.

5. Conclusion

Aqueous and ethanolic 70 % extracts of *Ficus exasperata* roots bark caused significant urinary excretion volume in water-overloaded Wistar rats. These results could justify the traditional use of the roots of this plant to treat edema and arterial hypertension. However, additional studies are necessary to elucidate the mechanisms underlying their diuretic action for an efficient and rational use of the roots of *Ficus exasperata* in the treatment of edema and arterial hypertension.

Acknowledgements

The authors are grateful to Prof Emma AKE-ASSI of the Centre National de floristique (Felix Houphouet-Boigny University, Abidjan, Côte d'Ivoire) for botanical identification of *Ficus exasperata* Valh. (Moraceae). Our thanks are also addressed to reviewers for their insightful comments and suggestions for the improvements of this manuscript.

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