

Diuretic Activity of *Hibiscus sabdariffa* L. in Wistar Rats

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Abstract. The diuretic effect of aqueous extract of *Hibiscus sabdariffa* has always been noted by the early botanists who studied the plant. On the other hand, very little scientific work to confirm this state of affairs has been done. In this pharmacological study, we provide scientific evidence to previous observations. Using an electrolyte analyzer to evaluate urinary volume and ions and appropriate experimental protocols on wistar strain rats, we have shown that: The aqueous extract of *Hibiscus sabdariffa* (AEHS) administered at doses of 200, 500 and 700 mg / kg BW induces a dose-dependent and significant increase in urinary excretion in rats. This increase was 12.75 ± 1.971 mL / kg, 23.20 ± 2.082 mL / kg and 29.42 ± 2.097 mL / kg BW, respectively is after 24 hours. That is a respective increase of 55.5, 182 and 333% compared to the control. The aqueous extract of *Hibiscus sabdariffa* (AEHS) uses at the dose of 700 mg / kg BW results in a reduction of the first urination time by 26.30% compared to the control. The determination of electrolytes in rats treated with 700 mg / kg BW after 24 hours showed high concentrations of sodium, potassium and chlorine in the urine. These different concentrations are for sodium of 72.51%, potassium of 76.54% and 58.81% for chlorine relative to the concentration of the controls. Compared to AEHS, hydrochlorothiazide at 25 mg / kg BW results in urinary excretion of 80.79% sodium, 85.30% potassium and 75.97% chlorine. Similarly with furosemide uses at 20 mg / kg BW), the concentrations are 86% sodium, 79% potassium and 71.58% chlorine. Compared with control rats, the Na + / K + ratio did not vary significantly in the treated animals. AEHS is therefore a diuretic substance like hydrochlorothiazide and furosemide.

1. Introduction

Several reports have demonstrated strong antioxidant activity of *Hibiscus sabdariffa* flower petals, which is linked to polyphenolic compounds such as anthocyanins and flavonoids [4] contained in the plant. Its anti-infectious properties make it effective in preventive indications for urinary disorders.

According to Kerharo and Adam [7], calyces possess confirmed antibacterial activity "in vivo" in humans in the case of urinary tract infections. The aqueous extract of the dry petals shows antihypertensive, hypocholesterolemic, hepatoprotective, antioxidant and antimutagenic activity [9], [6], [14].

The plant is very rich in proteins, lipids, minerals, and vitamins C. The aqueous extract of calyces facilitates digestion. During pregnancy, hypertensive women drink without moderately sweet moderation, to reduce their tension and prevent digestive disorders in traditional pharmacopoeia.

The leaves are applied as a poultice to treat incurable wounds and ulcers. The root decoction used as a purge serves as a laxative. The work of Pousset [16] reported urinary and diuretic antiseptic properties of the aqueous extract of *Hibiscus sabdariffa*. It is this diuretic activity of the plant that we will show with the aid of appropriate and modern experiments in the rat.

2. Material and Methods

2.1. Biological material

2.1.1. Plant material

The plant material consists essentially of dried flower petals of *Hibiscus sabdariffa*. The petals are bought at the big market of Yamoussoukro. These dried petals come mostly from Burkina Faso. The flower petals are left to dry in the sun, spread on aluminum foil. The drying time is one to two days in very sunny weather. The crunchy petals are ground with porcelain mortar. To obtain the aqueous extract of *Hibiscus sabdariffa*, the method of decoction was used.

2.1.2. Animal equipment

The rats used are of the species *Ratus norvegicus* (Wistar). They were raised at the animal facility of UFR Biosciences, at a temperature of 25 ° C, under daylight and darkness at night. They are fed with pellets supplied by IVOGRAIN® of Abidjan (Côte d'Ivoire), and have free access to water. They weigh between 150 g and 200 g.

2.1.3. Animal material

Healthy Wistar rats (*Rattus norvegicus*), weighting between 150 and 200 g were used for this study. They were obtained from the Animal house at Felix Houphouet-Boigny University (Abidjan, Côte d'Ivoire) and kept at constant temperature (24 ± 2°C) with 50-55 % of humidity and a photoperiod of 12 hours of daylight and 12 hours of darkness.

They were fed *ad libitum* with pellet (Ivograin, Abidjan, Côte d'Ivoire) and allowed free access to clean water.

All the procedures were conducted in accordance with the guidelines for Care and Use of Laboratory Animals published by the National Institutes of Health (Côte d'Ivoire).

2.2. Methods of study

2.2.1. Preparation of the aqueous extract of *Hibiscus sabdariffa* (Malvaceae)

Fifty grams of dried petals are boiled with one (1) liter of distilled water in a pyrex jar for one hour. The decoction obtained is cooled and then filtered successively on hydrophilic cotton to retain impurities of large dimensions; then on Wattman filter paper No. 2 for small impurities according to the method revised by Mea et al. [10]. The aqueous filtrate is dried with a BUCCHI rotavapor and the pellet is placed in the oven of VENTICELL brand (Medcenter) at 40°C. to accelerate dehydration. After evaporation, a dry and compact base is obtained. This pellet is scraped and then crushed in a porcelain mortar. At the end of this operation, the water-soluble powder which is obtained constitutes the aqueous extract of *Hibiscus sabdariffa* (AEHS). It makes it possible to carry out the experimental doses of the AEHS.

2.2.2. Measurement of diuresis

2.2.2.1. Time of first urination and urinary volume

Four batches of five rats were formed, including three batches and one control bat. To each batch of rats *ad libitum* 1 ml of AEHS, 1 ml of furosemide, 1 ml of hydrochlorothiazide and 1 ml of water for the control batch are given.

The time of first urination is recorded and then, after 24 hours, the urinary volume of each batch of rats.

2.2.2.2. Measurement of electrolytic parameters

After 24 hours of treatment, blood samples were taken by caudal incision in these animals by the method of Kraus [8], in dry tubes and centrifuged at 4500 rpm for 10 minutes. The serum collected in Eppendorff tubes is stored at -20 ° C. in order to analyze the serum parameters. Similarly, the urine collected is stored in Eppendorff tubes for the determination of the electrolytes.

The sodium (Na +), potassium (K +) and chlorine (Cl-) ions are analyzed using the Smartlyte Electrolyte Analyzer (Diamond Diagnostics, USA).

2.2.3. Physiological solutions and pharmacodynamic substances

The pharmacological substances used are synthetic products precisely: Esidrex®), (dihydrochlorothiazide 25 mg) from Novartis Pharma Schweiz AG-Swiss and Lasilix or Laxillix (furosemide 20 mg) from Sanofi-Aventis- France.

2.2.4. Treatment of results

2.2.4.1. Statistical analyzes

The results are analyzed by the ANOVA variance. The Turkey-Kramer multiple comparison test or $p < 0.05$ is considered significant and is exploited. Values are presented as the mean + standard error on the mean (M + ESM).

2.2.4.2. Graphs

Figures and graphs are plotted using GraphPadPrism 5 software (San Diego CA USA).

3. Results

3.1. Effects of AEHS on diuresis in rats

3.1.1. AEHS dose-response effect on urinary excretion in rats

Gavage administration of the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) resulted in urinary excretions that varied considerably with dose (Fig. 1). Indeed, AEHS administered at doses of 200, 500 and 700 mg / kg BW causes a progressive and significant increase in urinary excretion in these treated rats compared to the controls. The data set is summarized in Table 1.

The urinary excretion induced by 200 mg / kg BW is 12.75 ± 1.971 ml / kg BW after 24 hours. However, it was 23.20 ± 2.082 ml / kg and 29.42 ± 2.097 ml / kg BW after 24 hours when the rats were treated with 500 and 700 mg / kg BW. That is a respective increase of 55.5, 182 and 333%.

Urinary excretion induced by AEHS is therefore dose dependent and reaches its maximum after 12 hours and then tends to become stable until the end of the experiment (Fig. 1).

Table 1. Evolution of urinary excretion volume as a function of AEHS dose and time

Time (H)	Controls		AEHS 200 mg/kg BW		AEHS 500 mg/kg BW		AEHS 700 mg/kg BW	
	Average ml/kg BW	ESM	Average ml/kg BW	ESM	Average ml/kg BW	ESM	Average ml/kg BW	ESM
0	0.000	0.000	0.00	0.000	0.00	0.000	0.000	0.000
2	2.450	1.720	4.50	1.819	5.39	2.070	7.150	2.083
4	4.660	1.721	7.69	1.715	10.40	2.100	13.390	2.082
6	5.050	1.610	10.17	1.812	17.28	2.080	19.320	2.093
8	6.100	1.620	11.20	1.818	19.12	2.072	25.000	2.083
10	8.500	1.710	11.60	1.720	20.12	2.072	26.540	2.094
12	8.120	1.615	11.67	1.822	22.01	2.080	27.260	2.092
24	8.200	1.800	12.75	1.917	23.20	2.082	29.420	2.097

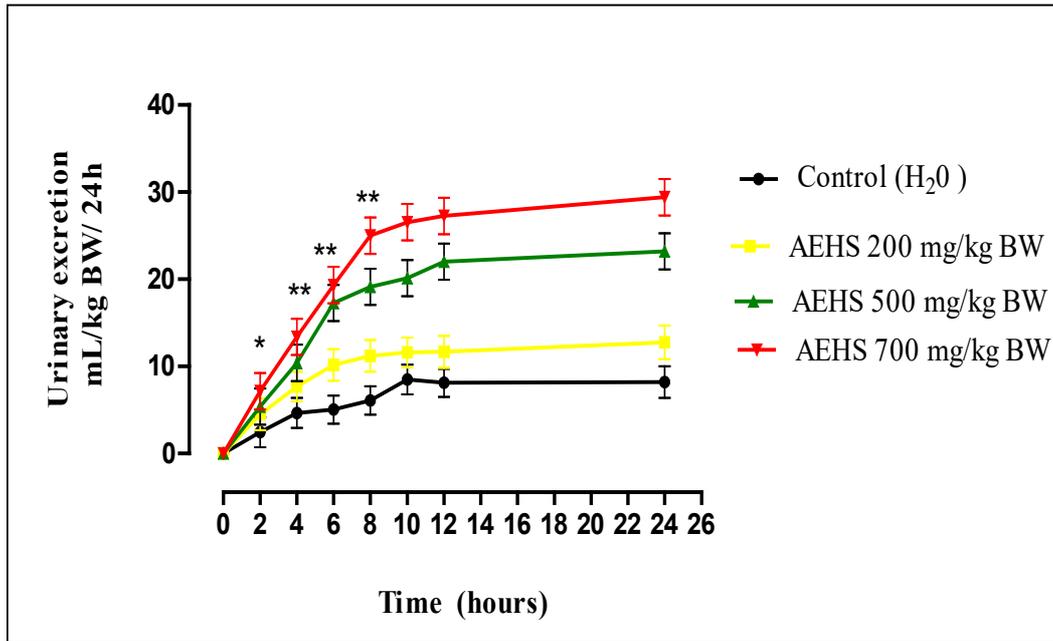


Figure 1. Dose-response effect of aqueous extract of AEHS on excretion in rats in 24 hours
*p< 0.05; **p<0.01

3.1.2. Evaluation of the time of first urination and urinary volume after treatment of rats

The aqueous extract of *Hibiscus sabdariffa* (AEHS) uses at the dose of 700 mg / kg BW results in a reduction of the first urination time by 26.30% compared to the control. Indeed, in the control rats, the time of the first urination is 59.8 ± 2.2 min. On the other hand, in rats treated with 700 mg / kg BW of AEHS, this time was significantly reduced and was 44.07 ± 3.04 min ($p < 0.05$). Similar observations were observed with the administration of hydrochlorothiazide (25 mg / kg BW) and furosemide (20 mg / kg BW), resulting in reductions of 40.80% and 58.99%, respectively, of the first ($P < 0.01$). The results are summarized in Table 1.

The time of first urination decreased in the rats treated with these three substances relative to the control rats. The urinary volume after 24h in control rats was 9.66 ± 1.8 ml / kg BW / 24h.

In rats treated with furosemide, Hydrochlorothiazide and AEHS, the urinary volume was 46.15 ± 2.20 , 48.36 ± 2.99 and 29.40 ± 2.09 ml / kg BW / 24h, respectively. This is an equivalent of an evolution of 377, 400 and 204%. Urinary volume increases in treated patients compared to control rats. Fig. 2 shows the volume of urinary excretion of each batch of rats.

Table 2. Urinary volume excreted in 24 hours and time of first urination

	Time of first urination in minutes	Urine volume in ml / kg BW / 24h
Control rats (H₂O)	59.8 ± 2.20	9.66 ± 1.8
Rats treated with AEHS	$44.07 \pm 3.04^*$	$29.40 \pm 2.09^*$
Rats treated with Hydrochlorothiazide	$35.4 \pm 2.70^{**}$	$48.36 \pm 2.99^{***}$
Rats treated with Furosemide	$24.52 \pm 2.54^{**}$	$46.15 \pm 2.20^{***}$

* P < 0.05, ** P < 0.01, *** P < 0.001 compared to the control

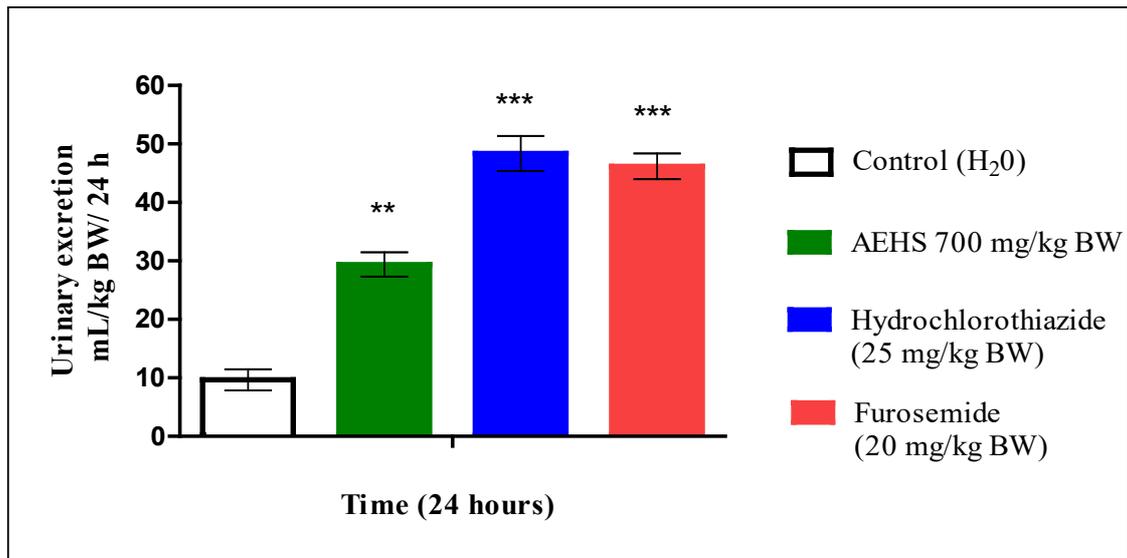


Figure 2. Effect of the aqueous extract of AEHS, hydrochlorothiazide and furosemide on urinary excretion in 24 hours

3.2. Determination of urinary ions

3.2.1. Evolution of electrolytes in the urine of rats treated with AEHS, hydrochlorothiazide and furosemide

Electrolyte dosage showed high concentrations of sodium, potassium and chlorine in the urine of rats treated with aqueous *Hibiscus sabdariffa* at 700 mg / kg BW after 24 hours (Table 3). These different concentrations are for sodium of 72.51%, potassium of 76.54% and 58.81% for chlorine relative to the concentration of the controls. Also, hydrochlorothiazide (25 mg / kg BW) results in a high urinary excretion concentrated to 80.79% sodium, 85.30% potassium and 75.97% chlorine. With furosemide (20 mg / kg BW), the concentrations were 86% sodium, 79% potassium and 71.58% chlorine compared to the electrolyte content of the controls. Results in meq / L / 24h for each batch of treated rats are shown in Table 3.

Table 3. Concentration of electrolytes in urine (meq / L / 24h)

	Na ⁺	K ⁺	Cl ⁻
Control	40.5±3.5	32.4±1.25	50.12±7.7
AEHS 700 mg/kg BW	69.87±4.17**	57.20±3.36**	79.6±4.9**
Hydrochlorothiazide 25 mg/kg BW	73.22±2.5***	60.04±4.75**	88.2±5.2**
Furosemide 20mg/kg BW	75.33±5.3***	58.00±4.42**	86.00±6.43**

*p<0.05 ; **p<0.01 ; ***p<0.001

3.2.2. Evolution of the Na + / K + ratio in the urine of rats treated with AEHS, hydrochlorothiazide and furosemide

Compared to control rats, the Na + / K + ratio did not vary significantly in the treated animals. It is of the order of 1.25 for control rats, 1.22 for rats treated with AEHS, 1.22 for rats treated with hydrochlorothiazide and 1.28 for those treated with furosemide.

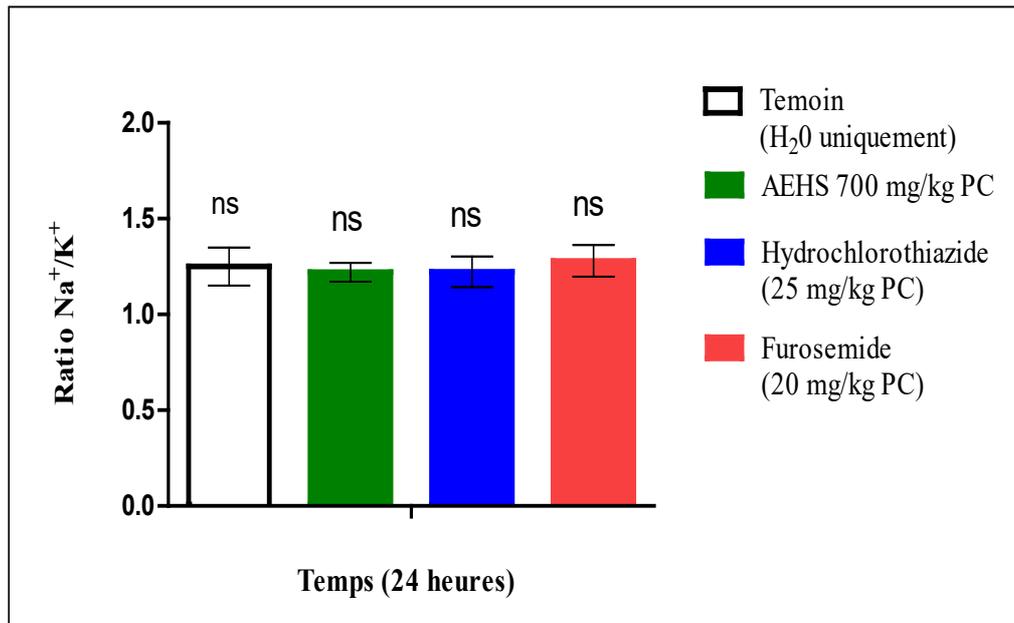


Figure 3. Evolution of the sodium / potassium ratio in urine excreted in rats treated with the aqueous extract of AEHS, hydrochlorothiazide and furosemide after 24 hours. $p \geq 0.05$ relative to control (ns)

4. Discussion

The effects of numerous plant species used in different pharmacopoeias and known as diuretics have already been demonstrated. Amonkan [2] showed that aqueous extract of ficus exasperata (Moraceae) leaves a dose-dependent increase in diuresis.

Similarly, AEHS at the dose of 700 mg / kg BW, significantly increased urinary volume in the rat. These effects are similar to those of furosemide and hydrochlorothiazide.

However, furosemide is a loop diuretic that inhibits water reabsorption at Henle's loop [12]. It influences the dilution-concentration mechanism of the urine to promote a significant diuresis.

Electrolyte dosing showed high concentrations of sodium, potassium and chlorine in the urine of rats treated with AEHS, hydrochlorothiazide and furosemide in substantially the same proportions.

Furosemide inhibits sodium reabsorption at the level of the loop of Henlé by blocking the NKCC (cotransporter $\text{Na}^+ / \text{K}^+ / 2\text{Cl}^-$) at the level of the ascending branch of the loop of Henlé which leads to an increase of urinary excretion Na^+ , K^+ , Cl^- , Mg^{2+} and Ca^{2+} . The natriuretic effect is important and of short duration [3].

Hydrochlorothiazide, directly inhibits the reabsorption of NaCl by competition with the Cl^- site of the co-carrier. They indirectly stimulate the reabsorption of calcium by an increase in tubular reabsorption proximal to that of sodium. Their effect is low; they allow an excretion of 5 to 10% of the filtered sodium [17].

Whereas, compared with control rats, the Na^+ / K^+ ratio did not vary significantly in rats tested by AEHS as well as furosemide and hydrochlorothiazide. This means that aldosterone secretion is not affected in the rats tested. Indeed, it is known that aldosterone stimulation is characterized by a low plasma concentration of Na^+ and a high concentration of K^+ [1] and therefore a considerable decrease in the numerical value of the Na^+ / K^+ ratio.

On the other hand, an inhibition of the secretion of aldosterone follows the opposite conditions, that is to say a high serum sodium and low serum potassium [13], [15], in this case an increase of the numerical value of the Na^+ / K^+ ratio.

The drugs that are intended to counteract the action of aldosterone by blocking it in the renal tubule [18], will result in the elimination of sodium and water and are known as savers of potassium because, unlike other diuretics, they prevent potassium from being eliminated in urine [5].

The high concentrations of sodium, potassium and chlorine in the urine of AEHS-treated rats, hydrochlorothiazide and furosemide compared to control animals shows that AEHS has no effect on serum aldosterone as furosemide and hydrochlorothiazide. Like these pharmacodynamic substances, AEHS can be used as a diuretic drug. Its antihypertensive effects described by Mea et al. [10] find a justification here.

Conclusion

AEHS has compounds with a diuretic effect. It is therefore a diuretic substance. This justifies its use in pharmacopoeia by hypertensive pregnant women. Although not recommended for the treatment of essential hypertension, the use of diuretics is strongly indicated in patients with arterial hypertension associated with chronic renal insufficiency.

Conflict of interest

All authors disclose that the manuscript has any financial or other substantive conflict of interest that might be construed to influence the results or interpretation of the manuscript.

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