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Salidiuretic Effect and Diuretic Mechanism of Action of an Aqueous Extract of *Saccharum Officinarum* Leaves (Poaceae) in Rats

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Abstract *Saccharum officinarum* L (Poaceae) is a plant whose leaves are used as a diuretic and in the traditional treatment of high blood pressure. This study aims to evaluate the salidiuretic effect of the aqueous extract of *Saccharum officinarum* (E*sol*) leaves and detect its mechanism of action in rats. The effect of the dose of 1000 mg / kg BW is compared to those of furosemide (40 mg / kg BW), Esidrex (25 mg / kg BW) and Aldactone (50 mg / kg BW) on the concentration of Na^+, K^+ and Cl^- ions in 24-hour urine produced by rats placed on water overload. The results show that the 24-hour urinary volume increases significantly ($p < 0.001$) with E*sol* (16.22 ± 1.89 mL) as do the diuretics used compared to the control. The determination of Na^+, K^+ and Cl^- ions in the urine of animals treated with E*sol* gives respective concentrations of 2.65 ± 0.34; 0.23 ± 0.05 mEq / L and 2.67 ± 0.253 mEq / L variations significantly close to those of Aldactone. The pH of urine obtained with E*sol* and reference diuretics is basic. The Na^+ / K^+ ratio of E*sol* (11.27) like that of Aldactone (10.63) is greater than 10**.**These parameters confirm that the aqueous extract of *Saccharum officinarum* leaves has salidiuretic properties acting as a sparing agent potassium just like (Aldactone). These results therefore justify the use of *Saccharum officinarum* leaves in traditional medicine.

Keywords: *Saccharum officinarum, salidiuretic, potassium sparing, basic pH, Aldactone*

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1. Introduction

Diuretics are substances that inhibit renal sodium reabsorption and therefore cause urinary elimination of water and sodium chloride [1]. Depending on the site of action of diuretic drugs in the nephron, four classes are distinguished: carbonic anhydrase inhibitors, loop diuretics, thiazides and potassium sparing agents [2].

However, pharmacological studies have shown that synthetic diuretics have numerous side effects (plasma acidosis, ionic imbalance, etc.) and cannot be administered in certain physiological cases such as pregnancy [3]. Also, the accessibility of medicinal plants and their relatively low costs have pushed populations to use them for treatment [4].

Currently, the World Health Organization (WHO) welcomes products from the traditional pharmacopoeia, as an alternative solution to treat diseases [5]. In this context, works has demonstrated the diuretic properties of *Spondias mombin* [6], *Zizyphus mauritiana* [7] and *Oxystelma esculentum* [8]. In our case, we were interested in the leaves of *Saccharum officinarum* which are used in traditional medicine as a diuretic and in the treatment of high blood pressure in Ivory Coast [9,10] and Madagascar [11]. The leaf decoction of *Saccharum officinarum* has an effective diuretic effect and contains chemical compounds that support this [10]. However, the salidiuretic effect as well as its probable mechanism of action remains to be verified.

This present study therefore aims to verify the salidiuretic effect of the aqueous extract of *Saccharum officinarum* leaves in rats placed on water overload and determine the parameters allowing its probable mechanism of action to be known.

2. Material and Methods

2.1. Material

2.1.1. Plant

The plant consists of the leaves *Saccharium officinarum* L. (Poaceae) which were harvested in the town of Bonoua in Kresinboville in June 2022. They were identified at the National Floristic Center (NFC) of the Felix HOUPHOUET-BOIGNY University (Abidjan, Ivory Coast) in comparison with herbarium No. UCJ007975.

2.1.2. Animal

Rats of the *Rattus norvegecus* species (Muridae), of the Wistar strain weighing between 160 grams and 190 grams, were used to carry out the tests on induced water overload. These animals are raised under standard conditions of temperature, nutrition and atmospheric pressure at the Biosciences Formation and Research Unit (FRU) of the Felix HOUPHOUET-BOIGNY University (Abidjan, Ivory Coast).

This study is conducted in accordance with the European directives of November 24, 1986 (86/609/EEC) and decree of April 19, 1988 [12] on animal experiments in research.

2.1.3. Chemical and Drug

The pharmacological substances used in this study are essentially diuretic substances, in this case Furosemide dosed at 40 mg / kg BW (Sanofi Aventis, France), Aldactone dosed at 25 mg / kg BW (Pfizer pfe, France) and Esidrex dosed at 25 mg / kg BW (Norvartis pharma, Switzerland).

2.2. Methods

2.2.1. Preparation of the Aqueous Extract of *Saccharum Officinarum* L. (Poaceae) Leaves

The dry leaves of *Saccharum officinarum* L. (Poaceae) are crushed into small pieces at the Felix HOUPHOUET-BOIGNY University (Abidjan, Ivory Coast). The pieces obtained, 250 g, are brought to the boil in 5000 mL of distilled water for 30 minutes. The homogenate obtained is filtered through hydrophilic cotton and Whatmann No. 1 paper. The filtrate obtained is dried in oven (Venticell Med. Center type, France) at 40°C for 72 hours, in order to obtain the dry extract of the aqueous decoction of *Saccharum officinarum* (E*sol*) leaves. Then, the extract obtained is stored at 4°C.

2.2.2. Comparative Study of the Salidiuretic Effect of E*sol* and Diuretics

The salidiuretic effect of the aqueous extract of *Saccharum officinarum* leaves is compared to that of diuretic substances. This study is based on the method for measuring urinary volume 24 hours after administration of a fluid overload and the substances tested orally [13,3], [14]. The animals were fasted 18 hours before the experiment, with free access to water. Before the experiment, 50 mL / kg BW of distilled water is given to each rat. Then, the animals in the control group each receive two (2) mL of distilled water. The animals of the 4 other batches receive respectively 2 mL: extract (1000 mg / kg BW) for batch 2, Aldactone (50 mg / kg BW) for batch 3, Esidrex (25 mg / kg BW) for the batch 4 and Furosemide (40 mg / kg BW) for batch 5. The animals are then immediately placed in individual metabolism cages. Then, the urine emitted over 24 hours was collected.

The diuretic activity of the extract was determined according to the Diuretic index values (Table 1) [15,16].

The volume of urine emitted in 24 hours having been measured, the diuretic index is calculated according to the formula:

$$\text{Diuretic index} = \frac{\text{Mean urine volume of the treated batch (mL)}}{\text{Mean urine volume of control (mL)}}$$

Table 1. Diuretic power of extracts according to diuretic index values [15] [16]

DIURETIC INDEX	DIURETIC POWER
> 1,50	Potential
1,50 > diuretic index > 1,0	moderate potential
1,0 > diuretic index > 0,72	Low Potential
< 0,72	no potential

2.2.2.1. Evaluation of the Urinary pH

The urinary pH is determined by measuring the pH of urine collected 24 hours after treating the rats with the substances. Thus, two (2) drops of urine are placed on a pH paper. Finally, the coloring of the paper changes and gives a coloring corresponding to the urinary pH value.

2.2.2.3. Determination of Na^+, Cl^- and K^+ in Urine

The effect of E*sol* on the urinary excretion of Na^+, K^+ and Cl^- ions is studied by measuring these ions with a flame spectrophotometer. The 24-hour urine is filtered through hydrophilic cotton, then centrifuged at a speed of 3000 revolutions per minute for 10 minutes to eliminate residues. Then, the supernatant is collected and the urinary level of Na^+, K^+ and Cl^- is directly measured with a flame spectrophotometer (Hycel PHF 104© , India). The Na^+, K^+ and Cl^- results obtained are then multiplied by the volume of 24-hour urine to obtain the daily urinary level of these electrolytes by the animals.

2.2.2.4. Study of the Mechanism of Action of the Aqueous Extract *Saccharum Officinarum* L. (Poaceae)

The extract mechanism of action is determined with the ratios between the rate of the different ions excreted such as: Na^+, Cl^- and K^+ as well as the pH. The sum of Na^+ and Cl^- makes it possible to calculate the salidiuretic activity of a product [17]. The Na^+/K^+ ratio makes it possible to evaluate the natriuretic activity and the Cl- / (Na^+ + K^+) ratio makes it possible to estimate the inhibition of carbonic anhydrase [8] [16-18] (Table 2)

Table 2. Salidiuretic activity according to the values of excreted ions [8] [16-18]

RATIO	INTERPRETATION
$Na^+ + Cl^-$ (Control) < $Na^+ + Cl^-$(treated)	salidiuretic substance
$1 < Na^+ / K^+ < 2$	The product is a satisfactory natriuretic
$2 < Na^+ / K^+ < 10$	The product is a natriuretic promoting the urinary Na+ elimination without excessive loss of K+
$Na^+ / K^+ > 10$	The product is a potassium sparing
$Cl^-/ (Na^+ + K^+) < 0,8$	The product is a carbonic anhydrase inhibitor
$0,8 < Cl^-/ (Na^+ + K^+) < 1$	The product is non-inhibitory of carbonic anhydrase

2.3. Statistical Analysis

The computer program GraphPad *Prism* 5.01 (San Diego CA, USA) is used for statistical analysis of the results. The results are processed by analysis of variance

(ANOVA), followed by Dunnett's multiple comparison test. The difference between two values is considered significant for ($p < 0.05$).

GraphPad *Prism* software version 5.01 (San Diego CA USA) was used to plot the graphs. The diuretic activity graphs are XY and Column type curves from GraphPad *Prism*.

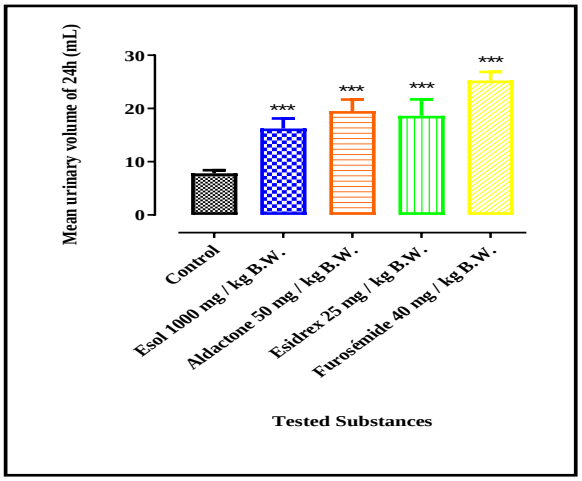
3. Results

3.1. Salidiuretic Effect of the Aqueous Extract of *Saccharum Officinarum* L. (Poaceae) Leaves Compared to That of Diuretics on Urinary Volume in 24 h

After treatment of rats placed on water overload, the 24-hour urinary volume of the group treated with E*sol* increased significantly ($p < 0.001$) compared to that of the control.

In fact, it goes from 7.8 ± 0.59 mL (for the control group) to 16.22 ± 1.89 mL (for E*sol*), an increase of 107.94%.

Likewise, the urinary volume of the batches treated with Aldactone, furosemide and Esidrex are respectively 19.52 ± 2.15; 25.26 ± 1.61 mL and 18.62 ± 3.09 mL Compared to the control, these urine volumes also increased significantly ($p < 0.001$) by 165.07%, 223.84% and 138.71% respectively for Aldactone, furosemide and Esidrex (Figure 1).

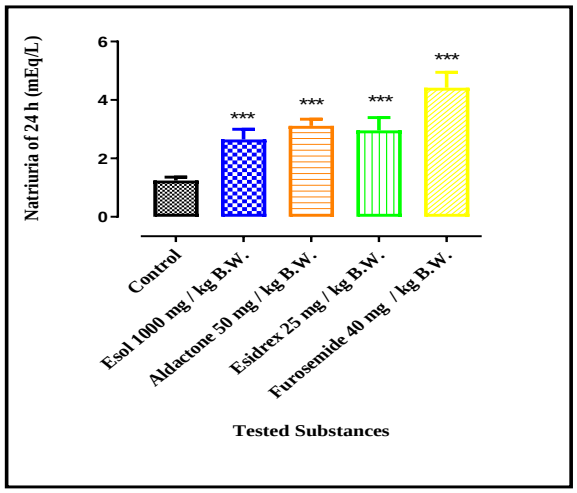


***: $p < 0.001$
n = 5

Figure 1. Variation in diuresis in water overloaded rats treated with the extract or the diuretics compared to controls

3.2. Salidiuretic Effect of the Aqueous Extract of Leaves of *Saccharum Officinarum* L. (Poaceae) Compared to That of Diuretics on Natriuria in 24 Hours

The determination of sodium in the urine of rats placed on water overload and treated with distilled water gives a concentration of 1.24 ± 0.11 mEq / L. In the rats having received E*sol*, Aldactone, Esidrex and Furosemide respectively, the Na^+ concentration increases significantly ($p < 0.001$) compared to that of the control. In fact, the sodium concentration is 2.65 ± 0.34; 3.11 ± 0.22; 2.95 ± 0.11 and 4.42 ± 0.52 mEq/L for E*sol*, Aldactone, Esidrex and Furosemide respectively (Figure 2).



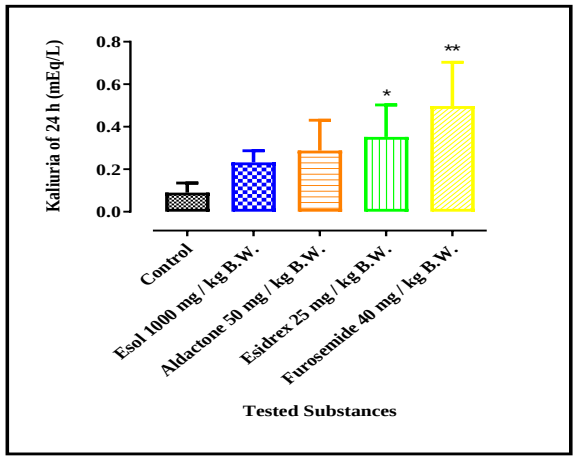
***: $p < 0.001$
n = 5

Figure 2. Variation in natriuria in water overloaded rats treated with the extract or the diuretics compared to controls

3.3. Salidiuretic Effect of the Aqueous Extract of *Saccharum Officinarum* Leaves Compared to That of Diuretics on Kaliuria in 24 Hours

In the control, the K^+ rate is 0.09 ± 0.04 mEq / L. Its concentration does not vary significantly ($p > 0.05$) in the rats treated with E*sol* and Aldactone compared to the control (Figure 3). Indeed, the potassium rate in these batches is respectively 0.23 ± 0.05 mEq / L and 0.28 ± 0.1 mEq / L for E*sol* and Aldactone.

On the other hand, the K^+ rate increases significantly ($p < 0.05$ and $p < 0.01$) in the groups having received Esidrex and furosemide compared to the control with respective rate of 0.35 ± 0.1 mEq / L and 0.49 ± 0.2 mEq/L.



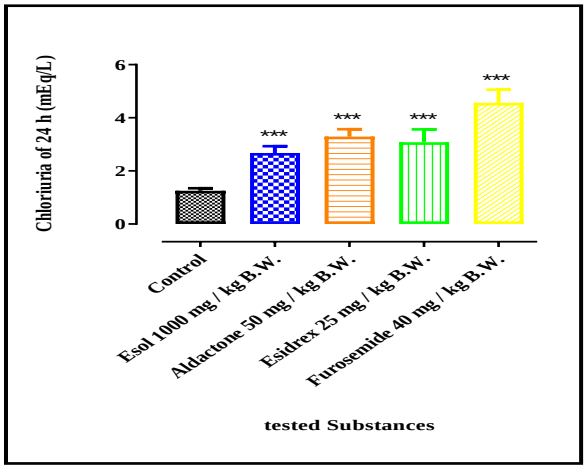
***: $p < 0.001$
n = 5

Figure 3. Variation in Kaliuria in rats placed on water overload treated with the extract or the diuretics compared to controls.

3.4. Salidiuretic Effect of the Aqueous Extract of Leaves of *Saccharum Officinarum* L. (Poaceae) Compared to That of Diuretics on Chloriuria in 24 H

The chlorine level in the 24-hour urine of rats treated with E*sol*, Aldactone, Esidrex and furosemide increased significantly ($p < 0.001$) compared to that of the control.

Indeed, this Cl- concentration is 1.25 ± 0.09 mEq / L for the control. Rats treated with E*sol*, Aldactone, Esidrex and furosemide had respective rate of 2.67 ± 0.253, mEq/L; 3.29 ± 0.273; mEq/L; 3.08 ± 0.48 mEq/L and 4.56 ± 0.49 mEq/L (Figure 4).



***: P < 0,001
n = 5

Figure 4. Variation in chloriuria in water overloaded rats treated with the extract or the diuretics compared to controls

3.5. Mechanism of Action Aqueous Extract of Leaves of *Saccharum Officinarum* L. (Poaceae) and the Diuretics

The diuretic index of E*sol* is identical to that of diuretics, greater than 1.5. The pH of treated rats with E*sol* and diuretics is basic. The sum $Na^+ + Cl^-$ of the treated batch with the aqueous extract of *Saccharum officinarum* like that of the diuretics and is higher than that of the control.

The Na^+ / K^+ ratio of E*sol* like that of Aldactone is greater than 10.

Also, the $Cl^- / (Na^+ + K^+)$ ratio is between 0.8 and 1 for all the substances (E*sol*, Esidrex, Furosemide and Aldactone) (Table 3).

Table 3. Effect of Esol and the diuretics on the diuretic index, pH, sum (Na+ + Cl-), ratio (Na+ / K+) and ratio (Cl- / (Na+ + K+)

Parameters / Batch	Diuretic index	pH	$Na^+ + Cl^-$	Na^+ / K^+	$Cl^- / (Na^+ + K^+)$
Control	1	6.12 ± 0.6	2.504		
E*sol* 1000 mg / kg B.W.	1.62	7.75 ± 0.6	5.327	11.27	0.92
Aldactone 50 mg / kg B.W.	1.82	7.55 ± 0.4	6.408	10.63	0.96
Esidrex 25 mg / kg B.W	1.81	8 ± 0.1	6.040	8.31	0.92
Furosemide 40 mg / kg B.W.	2.42	8.1 ± 0.2	9.018	8.45	0.92

4. Discussion

The salidiuretic effect of the dose of 1000 mg / kg B.W. was compared to those of reference diuretics such as Aldactone, Esidrex and Furosemide. The salidiuretic study is carried out by causing a fluid overload in rats in order to promote reabsorption of electrolytes. Indeed, a water overload dilutes the concentration of electrolytes in the internal environment, thus promoting their reabsorption [3]. It is also known that any salidiuretic substance increases the excretion of electrolytes despite their reabsorption [20]. Our results show that the E*sol* extract has diuretic potential just like the reference diuretics. The extract increases natriuresis, chloriuresis and urinary pH compared to controls but not kaliuresis like Aldactone. The increase in diuresis could be explained by the retention of water in the tubular lumen, following the retention of the Na+ ion, including reabsorption [20]. E*sol* would therefore inhibit water reabsorption. The mechanism of action of the extract of *Saccharum officinarum* L. (Poaceae) was determined according to the ratios between the different ions excreted. The value of the $Cl^- / (Na^+ + K^+)$ ratio of E*sol* (0.92) excludes the inhibition of carbonic anhydrase [16]. Thus, these results show that E*sol* would not act at the level of the proximal tube. Finally, the value of the Na^+ / K^+ ratio shows that the elimination of the Na+ ion is significantly greater than that of K+ with E*sol* (11.27) and Aldactone (10.63). This indicates that E*sol* would spare the potassium ion just like Aldactone. In this case, the aqueous extract of *Saccharum officinarum* L. (Poaceae) would therefore act at the level of the distal convoluted tube [16]. Indeed, Aldactone is an anti-aldosterone diuretic acting at the level of the distal convoluted tubule on the Na^+ / K^+ ATPase-dependent pump [21,22]. Indeed, in the final part of the distal tube and at the level of the collecting tube of the nephron, Na^+ are reabsorbed in exchange with the excretion of potassium or hydrogen ions under the influence of aldosterone [23]. Our results are different from those obtained with the alcoholic extracts of *Oxystelma esculentum* [8] and *Peganum harmala* [17] which promote the elimination of Na^+, K^+ and Cl^-. However, the increase in pH by E*sol* indicates the presence of a high concentration of Na^+ and Cl^- in the urine, making it more alkaline as with the diuretics used. E*sol* has virtually the same effects as Aldactone (spironolactone) on diuresis and natriuria, kaliuria and pH. It is deduced that the aqueous extract of *Saccharum officinarum* would act as an Aldosterone antagonist or as a potassium-sparing diuretic.

5. Conclusion

In order to deepen our research initiated on the diuretic activity of the aqueous extract of *Saccharum officinarum* leaves, we carried out a study of the comparative effect of the extract with reference diuretics.

The results show that this extract promotes an increase in natriuresis, chloriuresis and pH and would act as a potassium sparing agent. It would therefore act on the end of the distal convoluted tube and the collecting tube, thus justifying its use in traditional medicine as a diuretic.

Conflicts of Interest

There are no conflicts of interest.

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