Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Biology and Health

Acute Toxicity and Effect of an Aqueous Extract of *Saccahrum officinarum* (Poaceae) on Diuresis in Rat

Irie Bi Jean Severin[1*], Kouakou Assui Aude Ines[1], Kassi Yomalan[1]

[1]Laboratory of Biology and Health, Felix Houphouet-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire

DOI: 10.36347/sajp.2023.v12i03.001 | **Received:** 16.01.2023 | **Accepted:** 27.02.2023 | **Published:** 06.03.2023

***Corresponding author:** Irie Bi Jean Severin
Laboratory of Biology and Health, Felix Houphouet-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire

Abstract | **Original Research Article**

Saccharum officinarum L (Poaceae) is a plant used as a diuretic and in the traditional treatment of arterial hypertension. This study aims to evaluate the acute toxicity and the diuretic potential of the aqueous extract of *Saccharum officinarum* (E*sol*) leaves on the diuresis of rats subjected to water overload. The toxicity results show that E*sol* is non-toxic at a dose of 2000 mg/Kg B.W. and that the LD50 is greater than this dose. The aqueous extract of *Saccharum officinarum* has many chemical compounds with diuretic and antihypertensive properties such as polyphenols, flavonoids, sterols and polyterpenes and saponosides. E*sol* acts effectively on diuresis with 24-hour urine volumes of 12.4 ± 0.74 mL; 11.3 ± 0.72 mL for the doses of 1000 and 1300 mg/kg B.W. whose volumetric urinary excretion (VUE) is greater than 150 %. The Time to First Micturition (TFM) of 41.7 ± 6.2 min and 41.2 ± 3.8 min respectively for these 2 doses. The pH of the urine of rats treated with the extract is more alkaline between 7 and 7.35. These results therefore indicate that the aqueous extract of *Saccharum officinarum* has a diuretic potential, thus justifying its use in traditional medicine.

Keywords: *Saccharum officinarum*, toxicity, phytochemical screening, diuretic, diuresis.

INTRODUCTION

Plants have always been used by people around the world to heal themselves. Today, they still represent the primary source of therapeutic substances in developing countries (WHO, 2013). Thus, in Africa, 80% of the population use medicinal plants for their health care (WHO, 2002). In Côte d'Ivoire, there are many plants with diuretic properties, prepared in the form of a decoction, from leaves, stem bark.

Diuretics are substances that inhibit the renal reabsorption of sodium and therefore cause urinary elimination of water and sodium chloride (Caruba T and Jacoulet E, 2018). Indeed, their indications are multiple and essentially concern chronic diseases, the main ones being arterial hypertension, heart failure and chronic renal failure (Albert S, 1999; Dumoulin A and Audard V, 2002). Their prescriptions therefore concern pathologies that represent real public health problems. Thus, the prevalence of Chronic Kidney Disease in 2013 was 956,200, which represents an increase of 135% compared to 1990 estimates (Global Burden of Disease Study, 2013). With regard to arterial hypertension (HTA), more than a quarter (26.4%) of the adult world population is hypertensive and this proportion should reach 29.2% by 2025 (Fourcade L and Paule P, 2007).

However, pharmacological studies have shown that synthetic diuretics have many side effects (plasma acidosis, ion imbalance, etc.) and cannot be administered in certain physiological cases such as pregnancy (Beaux D, 1991).

In view of this, the WHO currently welcomes products from traditional pharmacopoeia as an alternative solution to treat diseases (WHO, 2013). Thus, the WHO has put in place a strategic guide to study the effects, but also the toxicities of medicinal plants used in the traditional way (WHO, 1996). The goal is to harmonize study protocols in order to have the most reliable knowledge possible concerning the use of plants.

In this context, our study focuses on the evaluation of the acute toxicity and the diuretic potential of the aqueous extract of *Saccharum officinarum* (Poaceae) in rats, by measuring the 24-hour urinary volume and the urinary pH and by determining

the Volumetric Urinary Excretion (VUE) and Time to First Micturition (TFM).

1-MATERIALS AND METHOD

1.1-Material

1.1.1-Plant

The plant material consists of *Saccharium officinarum* Linée (Poaceae) leaves which were harvested in the town of Bonoua in kresinboville in the month of June 2022. They were identified at the National Floristic Center (NFC) of the Felix Houphouet- Boigny University (Abidjan, Ivory Coast) in comparison with herbarium n°4099.

1.1.2-Animal

Rats of the *Rattus norvegecus* (Muridae) species, of the Wistar strain weighing between 160 grams and 190 grams, were used to carry out the diuresis tests. Mice of the *Mus musculus* (Muridae) species of Swiss strain, whose weight is between 23 and 35 grams, were used to carry out the toxicity tests.

These animals are raised under standard conditions of temperature, nutrition and atmospheric pressure at the UFR Biosciences of the Felix Houphouet-Boigny University of (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 (Anonymous, 1986) on animal experiments in research.

1.2-Methods

1.2.1-Preparation of the aqueous extract of *Saccharum officinarum* L. (Poaceae) leaves

The dry leaves of *Saccharum officinarum* Linée (Poaceae) are ground into small pieces at the Felix Houphouet-Boigny University (Abidjan, Ivory Coast). The pieces obtained, 250 g, are boiled in 5000 mL of distilled water for 30 minutes. The homogenate obtained is filtered through absorbent cotton and Whatmann No. 1 paper. The filtrate obtained is dried in a Med center venticell type oven (France) at 40°C for 72 hours, in order to obtain the dry extract of the aqueous decoction of leaves of *Saccharum officinarum* (E*sol*). Then, the extract obtained is stored at 4°C.

1.2.2-Phytochemical screening of the aqueous extract of *Saccharum officinarum* leaves

The demonstration of secondary metabolites consists of characterization tests of the major groups of chemical compounds contained in E*sol*. The detection of these compounds is based on the principle that they induce chemical reactions in the presence of appropriate reagents (Wagner H and Bladt S, 2001). These tests were carried out using the analytical techniques described in the work of (Lazureski G *et al.*, 2007; Abo K, 2013).

For these tests, a solution of the aqueous extract of *Saccharum officinarum* is prepared by dissolving 5 g of the extract in 50 mL of distilled water. The compounds sought are: Sterols and polyterpenes, polyphenols, flavonoids, saponosides, alkaloids, gallic tannins, catechic tannins and quinone compounds.

1.2.3-Study of the acute oral toxicity of the aqueous extract of *Saccharum officinarum* Linée (Poaceae) leaves

This study is carried out in accordance with the OECD guideline 423 (2001). The method uses pre-determined doses and provides results that allow classification of substances into the Globally Harmonized Classification System for Acute Toxicity.

In this study, three (3) batches of three mice are formed. Doses of 300, 2000 mg/Kg B.W. and 5000 mg/Kg B.W., of the aqueous extract of *Saccharum officinarum* L. (Poaceae), are administered orally at the rate of 1 mL/100 Kg B.W. sequentially. After the administration of a dose of E*sol*, the animals are observed for 48 hours and the number of deaths is noted.

1.2.4-Evaluation of the diuretic potential

1.2.4.1- Study of the effect of the aqueous extract of leaves of *Saccharum officinarum* L. (Poaceae) on the diuresis of rats placed in saline overload (1.8%)

This study was carried out according to the method by Sanogo R *et al.*, (2009). Thus, twenty-five rats (25) are fasted for 18 hours with free access to water. Then they were divided into five (5) batches of five (5) rats each. The measurements of the average weight of each batch are carried out, the treatments are made orally to the animals.

Thus, the rats of each batch are overloaded with saline with 50 mL/Kg B.W. of 1.8% NaCl then immediately with increasing doses of E*sol*.

Thus, the rats of batch 1 (Control) receive distilled water (2 mL/rat). As for those of batches 2, 3, 4 and 5, they receive the aqueous extract of *Saccharum officinarum* at the respective doses of 500 mg/Kg B.W., 800 mg/Kg B.W., 1000 mg/Kg B.W. and 1300 mg/Kg B.W. Then, the rats are each placed in individual metabolism cages in order to measure the urine excreted after the treatment at 2 hours, 4 hours, 8 hours and at 24 hours.

1.2.4.2- Time of First Micturition (TFM)

Once the rat is placed in the individual metabolism cage, it is observed until the appearance of the first drop of urine excreted, the time of appearance of which is noted. Finally, an average is calculated for the animals of the same batch.

1.2.4.3- Evaluation of urinary pH

The effect of E*sol* on urinary pH is determined by measuring the pH of urine collected 24 hours after treatment of rats with the substances.

Thus, on a piece of pH paper is placed two (2) drops of urine. Finally, the coloring of the paper turns and gives a coloring corresponding to a value of the urinary pH.

1.2.4.4- Determination of Volumetric Urinary Excretion (VUE)

Finally, the volumetric urinary excretion (V.U.E) is determined according to the formula of Kau S *et al.*, (1984):

$$V.U.E = \frac{\text{Excreted Volume}}{\text{Administered Volume}} \times 100$$

VA = administered volume in mL.
VE = excreted volume in mL.

The diuretic activity was estimated according to the value of the volumetric urinary excretion according to Kau S *et al.*, (1984).

EUV < 80% = antidiuretic activity;
EUV between 80 – 110% = No activity;
EUV between 110-130% = Low activity;
EUV between 130- 150% = Modest activity
EUV > 150% = Significant activity

1.2.5-Statistical processing of results

The computer program GraphPad *Prism* 5.01 (San Diego CA, USA) is used for the 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 ($p < 0.05$). The values are presented as the mean followed by the error on the mean (M ± SEM). This software was used for the statistical processing of diuretic activity.

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*.

2-RESULTS

2.1-Phytochemical screening of the aqueous extract of *Saccharum officinarum* L. (Poaceae) leaves

Table I summarizes the results of the various tube tests used to characterize the chemical groups present in the aqueous extract of Saccharum officinarum L. (Poaceae) leaves.

According to the results, this extract contains sterols and polyterpenes, polyphenols, flavonoids, saponosides, alkaloids and gallic tannins.

Thus, with the exception of quinone compounds and catechic tannins, the aqueous leaf extract of *Saccharum officinarum* L. (Poaceae) contains all the chemical groups investigated in this study.

2.2-Acute oral toxicity of the aqueous extract of leaves of *Saccharum officinarum* L. (Poaceae)

The study of the acute toxicity of the aqueous extract of leaves of *Saccharum officinarum* L. (Poaceae) according to the OECD guideline 423 revealed that:

- At a dose of 300 mg/Kg B.W. E*sol* administered to mice orally, does not cause death or change in behavior;
- At a dose of 2000 mg/Kg B.W., there is no death. However, the mice huddle together, do not feed for at least two (2) hours, then they return to their normal state;
- At a dose of 5000 mg/Kg B.W., we observed a sedative effect in mice treated with the aqueous extract of *Saccharum officinarum* L. (Poaceae) with swollen eyes, tremors, convulsions and generally died after the second day.

The dose of 2000 mg/Kg B.W. was resumed a second time according to the recommendations of this guideline and there was no death. Thus we can say that the LD50 of E*sol* (Lethal Dose 50%) determined according to the OECD 423 acute toxicity method by the oral route is greater than 2000 mg/Kg B.W. Table II gives the results of the E*sol* toxicity study.

2.3-Effect of the aqueous extract of *Saccharum officinarum* leaves on diuresis

2.3.1-Effect of the aqueous extract of *Saccharum officinarum* L. (Poaceae) leaves on TFM

The Time to First Micturition (TFM) obtained with increasing doses of the aqueous extract *Saccharum officinarum* L. (Poaceae) is shown in Figure 1.

Thus, in rats placed in saline overload then treated with distilled water (batch 1 or Control), the TFM is 56.6 ± 4.8 min.

When these rats are treated under the same conditions with doses of E*sol* of 500 mg / Kg B.W., 800 mg / Kg B.W., 1000 mg / Kg B.W. and 1300 mg / Kg B.W., the TFM is significantly reduced ($P < 0.05$; $P<0.01$; $P<0.001$). The values obtained with these doses are 47.4 ± 5.2 min, 44.3 ± 5.9 min, 41.7 ± 6.2 min and 41.2 ± 3.8 min respectively.

Table I: Results of the phytochemical screening of the aqueous extract of leaves of *Saccharum officinarum* L. (Poaceae)

Researched Compound		***Test or reactif***	**Result**
Sterols et polyterpènes		*Liebermann*	+
Polyphenol		*Chloride ferric*	+
Flavonoid		*Cyanidine*	+
Saponosid		*vigorous shaking*	+
Quinonic compound		*Borntraeger*	-
Alcaloïdes		*Dragendorff*	+
		Bouchardat	_
Tanin	Catechic	*Stiasny*	_
	Gallic	*chlorhydric acid*	+

(+): Presence of the compound
(–): Absence of the compound

Table II: Results of the acute toxicity of the aqueous extract of leaves of *Saccharum officinarum* L. (Poaceae)

Mice number / Doses	**Mice tested number**	**Dead mice number**	**Percentage of dead mice (%)**
300 mg/Kg B.W.	3	0	0
2000 mg/Kg B.W.	3	0	0
5000 mg/Kg B.W.	3	3	100

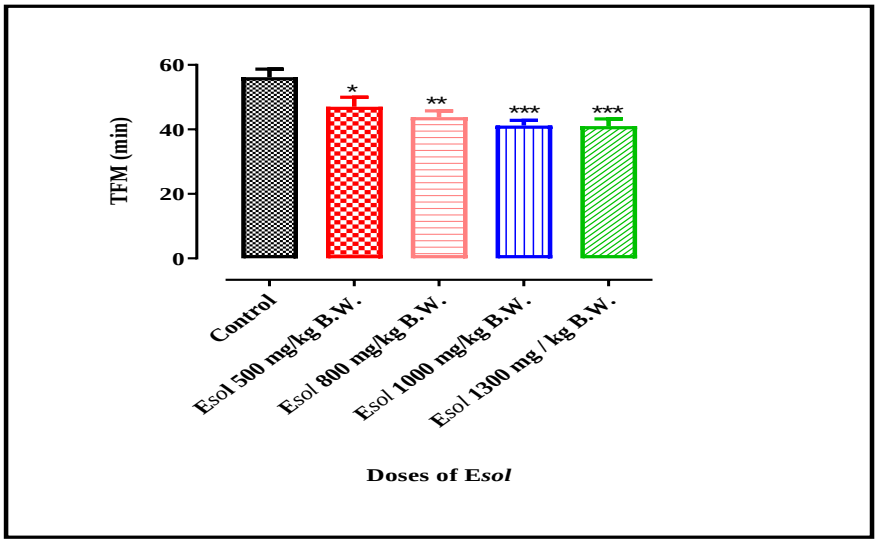

Figure 1: Effect of increasing doses of the aqueous leaf extract of Saccharum officinarum L. (Poaceae) on the time to first micturition (TFM) in rats subjected to water overload
*P<0.05; **: P<0.01; ***: P<0.001
n = 5

2.3.2-Effect of the aqueous extract of *Saccharum officinarum* L. (Poaceae) leaves on 24-hour urine volume

Administration of E*sol* to animals results in a significant dose-dependent increase in diuresis as a function of time compared to that of control rats.

After two (2) hours, the diuresis is 2.9 ± 0.55 mL for the control batch, while it is 3.2 ± 0.75 mL for the batch that received 500 mg/Kg B.W. of E*sol*; either an increase of 10.34% compared to the volume of urine of the control group.

Similarly, in the batches that received 800 mg / Kg B.W., 1000 mg/Kg B.W. and 1300 mg/Kg B.W. of E*sol*, the diuresis was 3.9 ± 0.89 mL, 7 ± 0.91 mL and 7, 2 ± 0.77 mL, corresponding respectively to increases of 34.48%, 141.37% and 148.27% compared to the quantity of urine of the control batch.

Four (4) hours after the diuresis for the control batch is 5.24 ± 0.97 mL. On the other hand, that of the batches treated with 500 mg / Kg B.W., 800 mg / Kg B.W. and 1300 mg / Kg B.W. of E*sol*, is respectively 5.62 ± 2.08 mL, 7.32 ± 0.64 mL and 11 .3 ± 0.72 mL, either increases in volume of 7.10%, 39.14% and 115.22% compared to the control batch. For the dose of

1000 mg/kg B.W., the diuresis is 12.4 ± 0.74 mL, either an increase in urine volume of 136.64% compared to that of the control group.

From eight (8) hours to twenty-four (24) hours, the diuresis varies very little in all the batch. Thus, 24 hours after injection of the various doses, the diuresis is 7.99 ± 1.2 mL in the control group. The urine volume of batches of rats given 500 mg/Kg B.W., 800 mg/Kg B.W. and 1300 mg/Kg B.W. of E*sol* are 10.4±1.01mL, 11.22±0.94 mL, respectively and 14.6 ± 0.89 mL corresponding respectively to increases of 30.16 %, 40.42 % and 82.72 % compared to that of the control batch. The dose of 1000 mg/Kg B.W. leads to a diuresis of 15 ± 1.5 mL, either. an 87.73% increase in the volume of urine compared to that of the control rats at this time.

The evaluation of the effect of the aqueous extract *Saccharum officinarum* L. (Poaceae) on diuresis in rats placed on saline overload is shown in Figure 2.

2.3.3-Effect of the aqueous extract of *Saccharum officinarum* L. (Poaceae) leaves on volumetric urinary excretion (VUE)

After 24 hours, the volumetric urinary excretion (VUE) was determined for each batch of treated rats.

Thus, the volumetric urinary excretion of the control batch obtained is 122.43 ± 3.1. For doses of E*sol*, is 138.39 ± 15.9 %, 148.25 ± 9.2 %, 158.72 ± 10.3 % and 153.59 ± 5.1 % respectively for doses of 500; 800 and 1000 mg/ Kg B.W.

Table III presents the VUE obtained with the doses of 500, 800, 1000 and 1300 mg/kg B.W.

2.3.4-Effect of the aqueous extract of *Saccharum officinarum* L. (Poaceae) leaves on urinary pH after 24 hours

The urinary pH was determined on the 24-hour urine collected from the rats treated with the different substances and the results are mentioned in Table III.

In the control batch having received the salt overload and treated with distilled water, the average pH is 6.63 ± 0.6.

On the other hand, in rats treated with doses of E*sol* of 500, 800, 1000 and 1300 mg/Kg B.W., the pH is increasingly basic with respective values of 7.12 ± 0.13; 7.1 ± 0.42; 7.35 ± 0.17 and 7.35 ± 0.41.

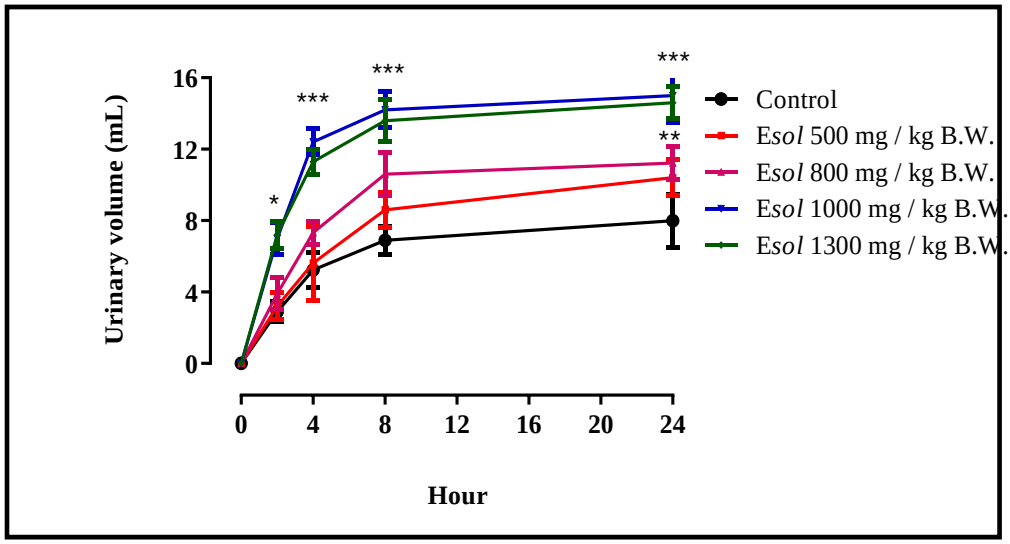

Figure 2: Effect of increasing doses of aqueous extract of Saccharum officinarum L. (Poaceae) leaf on diuresis in rats subjected to water overload

*P<0.05; **: P<0.01; ***: P<0.001

n = 5

Table III: pH and EUV obtained with increasing doses of E*sol* in rats subjected to salt overload

batch / Parameter	Control	E*sol* 500 mg / Kg B.W.	E*sol* 800 mg / Kg B.W.	E*sol* 1000 mg / Kg B.W.	E*sol* 1300 mg / Kg B.W.
P^H	6,63±0,6	7,12±0,13	7,1±0,42	7,35 ±0,17	7,35±0,41
EUV%	122,43±3,1	138,39 ± 15,9	148,25 ± 9,2	158,72 ± 10,3	153,59 ± 5,1

*** : P < 0,001

n = 5

3-DISCUSSION

The comparison of the pharmacodynamic properties of drugs relates to several criteria such as Efficacy (maximum effect, time to obtain effect, duration of effect, resistance, etc.) and Tolerance (acute, chronic toxicity, effects in the long term which makes it possible to establish the benefit/risk balance) (Mager D and Jusko W, 2001). Thus, our study focused on the evaluation of the toxicity and diuretic potential of *Saccharum officinarum*.

The study of the acute oral toxicity of the aqueous extract of leaves of *Saccharum officinarum* L. (Poaceae) was carried out according to the method described in the OECD guideline 423. This study revealed that this extract administered at doses of 300 mg / Kg B.W. and 2000 mg / Kg B.W. does not modify the general behavior and does not cause any death in mice. While at the dose of 5000 mg / Kg B.W. we observed a toxicity effect in mice treated with E*sol*. Thus, the treated animals have swollen eyes, tremors and convulsions; they lie down for long periods and usually die on the second day. In the globally harmonized classification system (GHS) of the OECD, the aqueous extract of the leaves of *Saccharum officinarum* would be placed in category 5 of substances with relatively low toxicity (OECD, 2001). According to this classification, the LD50 of the aqueous extract of *Saccharum officinarum* L. (Poaceae) is much higher than 2000 mg / Kg B.W. There is therefore a good tolerance of the substance at the dose of 2000 mg / kg B.W. and the doses lower than this dose can be used without danger for the pharmacological study.

Phytochemical analysis of the aqueous leaf extract of *Saccharum officinarum* L. (Poaceae) revealed that it contains sterols and polyterpenes, polyphenols, flavonoids, saponins, alkaloids and gallic tannins. On the other hand, an absence of quinone compounds and catechic tannins was noted in this extract. E*sol* therefore contains chemical compounds with enormous pharmacological properties. Indeed, sterols and flavonoids have hypotensive effects (Abo K, 2013).

Polyphenols, saponosides and flavonoids have antihypertensive, vasodilator, diuretic and antiedematous properties respectively (N'guessan K *et al*., 2009). In addition, polyphenolic substances are recognized for their beneficial venotonic and vasculoprotective properties in the prevention of vascular damage likely to occur in hypertensive patients (Sanogo R *et al*., 2009).

The diuretic effect of the aqueous extract of *Saccharum officinarum* L. (Poaceae) was tested in rats subjected to saline overload. It involves a deficit of total body water to total body sodium caused by less water intake than water losses. Indeed, this salt overload has the effect of increasing the concentration of the internal environment of the animals in NaCl and thus allows a reabsorption of water. The urine volume produced by these rats is then reduced. The effect of a diuretic is therefore to increase urine volume despite this reabsorption (Sanogo R *et al*., 2009; Ralahiravo D, 2018). In this study, E*sol* doses of 500, 800, 1000 and 1300 mg/Kg B.W, all resulted in a significant increase in diuresis. Doses of 1000 mg/kg B.W. and 1300 mg/kg B.W. resulted in Vlume urinary excretion (VUE) greater than 150%. At these doses, E*sol* has significant diuretic activity according to the classification of Kau S *et al.*, (1984). Also, the time of appearance of the first drop of urine is reduced significantly with increasing doses of E*sol*. According to Mager D and Jusko W in 2001, an effect can be described by its intensity, its time of appearance and its duration. This would mean that the higher the dose of E*sol*, the faster these effects appear. Therefore, E*sol* would therefore act as a pharmacological substance. It is known that the pharmacological effects of plant extracts are related to their chemical constituents.

However, the increase in pH by E*sol* would indicate the presence of a large concentration of bicarbonate, making it more alkaline as with carbonic anhydrase inhibitor diuretics.

The diuretic action of E*sol* could be due to the presence of alkaloids and flavonoids or polyphenols which would be responsible for the diuretic effects of plants (Al-saikhan F and Ansari M, 2016).

4-GENERAL CONCLUSION

In order to enhance the use of medicinal plants, our study focused on the evaluation of the toxicity and potential diuretic effect of the aqueous extract of *Saccharum officinarum* Linee (Poaceae) leaves on diuresis.

Phytochemical screening, carried out on the aqueous extract of leaves of *Saccharum officinarum* L. (Poaceae) made it possible to highlight the presence of several chemical groups in particular Sterols and polyterpenes, Polyphenols, flavonoids, saponosides, Alkaloids and gallic tannins with the exception of quinone compounds and catechic tannins known for their benefit to human health.

The acute oral toxicity test of E*sol* showed that this extract is non-toxic at a dose of 2000 mg / Kg B.W. but causes deaths at a dose of 5000 mg / Kg B.W.

The study of the effect on diuresis of the aqueous extract of the leaves of *Saccharum officinarum* Linee (Poaceae) has shown that this extract has good diuretic activity.

These results confirm the potential diuretic effect of E*sol* on diuresis in mammals. This would justify the traditional use as a diuretic of the leaves of *Saccharum officinarum* L. (Poaceae).

REFERENCES

- W.H.O. (2013). WHO strategy for traditional medicine 2014-2023. Geneva: World Health Organization.
- W.H.O. (2002). WHO strategy for traditional medicine for 2002-2005. WHO/EDM/TRM/2002, Geneva, 65p.
- Caruba, T., & Jaccoulet, E. (2018). Pharmacology and therapeutics, *Elsevier Masson* SAS, 238p.
- Albert, S. (1999). Uro-Nephrology. Diagnostic guidelines. Ed. *Médi'strophe* (French), chap. III, 31- 46.
- Dumoulin, A., & Audard, V. (2002). Lower limb edema. Référence. *Ed. J.B Baillière* (France), chap. III, 1064-1236.
- Global Burden of Disease Study 2013 Collaborators 2015. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet, 386(9995), 743–800.
- Fourcarde, L., & Paule, P. (2007). Mafart, B. Arterial hypertension in sub-Saharan Africa: current events and perspectives. *Médécine Tropicale*, 67, 559-567.
- Beaux, D. (1991). Pharmacological study of the diuretic properties of extracts of orthosiphon, pilosella, black elderberry, sweet fennel and bearberry in rats. Human medicine and pathology. *Paul Verlaine University - Metz. French*. 285p
- W.H.O. (1996). Guidelines for the evaluation of herbal medicinal products. From WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-fourth report. Geneva, *World Health Organization*, 188-194.
- W.H.O. (2013). World Panorama of Hypertension: a silent killer responsible for a global public health crisis. world health day 2013. *WHO/DCO/2013.2*. 40p.
- Anonymous. (1986). Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities*, 358, 1-28.
- Wagner, H., & Bladt, S. (2001). Plant drug analysis. A thin layer chromatography atlas. *2ème* édition. *Springer*. (Berlin, Allemagne), 384p.
- Lazureski, G. V., Terenteva, I. V., & Chamchurin, A. A. (2007). Praticheskii raboti po khimii soedinienii. *Vischaya Chkola*, (Moscou, Russie), 334p.
- Abo, K. J. C. (2013). From plant to molecule: toxicity, pharmacological effects and mechanism of action of *Justicia Secunda* (Acanthaceae), an antihypertensive plant, on the cardiovascular system of mammals. State doctoral thesis in Natural Sciences, *Félix Houphouët-Boigny University* (Abidjan, Ivory Coast); No. 752/2013, 351p.
- O.C.D.E. (2001). Test guideline 423: OCDE guideline for chemicals. [En ligne], consulté le 20 Août 2022. Disponible sur : https : //www.oecd.org/document/htm.
- Sanogo, R., Karadji, A. H., Dembélé, O., & Diallo D. (2009). Diuretic and salidiuretic activity of a recipe used in traditional medicine for the treatment of arterial hypertension. *Medical Mali*, 4(XXIV), 1-6.
- Kau, S. T., Lokkaddie, J. R., & Andrews, D. (1984). A method for screening diuretic agents in the rat. *Journal of Pharmacologinal Methods*, 11, 67-75.
- N'guessan, K., Kadja, B., Zirihi, N. G., Traoré, D., & Ake-Assi, L. (2009). Phytochemical screening of some Ivorian medicinal plants used in Krobou country (Agboville, Ivory Coast). *Science & Nature*, 6(1), 1-15.
- Ralahiravo, D. Y. (2018). Study of the effect of RG-2 extract on diuresis in rats. Master's thesis, University of Antananarivo, 38p.
- *Mager, D., & Jusko, W. (2001). Pharmacodynamic modeling of time-dependent transduction systems. Clin Pharmacol Ther, 70, 210-216.*
- Al-Saikhan, F. I., & Ansari, M. N. (2016). Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of *Peganum harmala* L. in Wistar albino rats. *Saudi Journal of Biological Sciences*, 23, 749-753.