International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 2065-2068 www.ijprajournal.com ISSN: 2456-4494

Diuretic Effect of Hydroalcoholic Extract of Clematis Mauritiana Lam. (Ranunculaceae) On Rat

T. T. Andriamampianina[1], S. G. Andriamalala[1], T. Rakotondrasoa [1], F. Randimbivololona[1], N. Quansah[2], P. Randrianavony[1]

[1]*Pharmacology Department, Sciences Faculty, University of Antananarivo, Madagascar*
[2]*School for International Training (SIT) Study Abroad Madagascar*

Submitted: 05-06-2022 Revised: 18-06-2022 Accepted: 27-06-2022

ABSTRACT
This study aimed to investigate diuretic activity of Clematis mauritiana extract; 24-hour urine volume and pH were measured; natriuresis and kaliuresis were dosed.
Administered orally, at 100, 200 and 400 mg/kg, it increases diuresis, natriuresis and kaliuresis, while pH remains unchanged. Control group urine is 4.06 ± 0.06 ml, versus 5.57 ± 0.06, 6.56 ± 0.04 and 9.6 ± 0.15 ml ($P<0.05$). Control natriuresis is 0.84 ± 0.41 mEq/L, versus 1.57 ± 0.24, 2.16 ± 0.30 and 3.92 ± 0.30 mEq/L ($P<0.05$). Control kaliuresis is 1.24 ± 0.07 mEq/L, versus 2.38 ± 0.22, 3.48 ± 0.22 and 4.62 ± 0.19 mEq/L ($P<0.05$). Control group urinary pH is 8.45 ± 0.03, versus 8.42±0.03, 8.4±0.18 and 8.41 ± 0.01 (NS).
These results show the diuretic effect of the extract. It might act on Henle loop or the dilution zone.
KEYWORDS: Clematis mauritiana, diuretic, rat

I. INTRODUCTION

Diuretics are predominantly used to treat peripheral edema or edema associated with heart failure, chronic kidney disease, and hepatic cirrhosis or hypertension, hyperkalemia and glaucoma [1].

According to their site and mechanism of action, they are classified as osmotic diuretics (such as mannitol) active on different parts of nephron, especially on the proximal convoluted tubule (PCT). These are prescribed to increase poison elimination. Carbonic anhydrase inhibitors, such as acetazolamides are also active on PCT. They reduce the activity of carbonic anhydrase, responsible for catalyzing the reaction between carbon dioxide and water into carbonic acid, therefore, inhibiting H^+ formation, necessary for Na^+ reabsorption. They also decrease the secretion of aqueous humor, which results in a decrease in intraocular pressure in case of glaucoma [2].

While the "loop diuretics", like furosemide, are the most potent of all diuretics, inhibiting the reabsorption of 25% of filtered sodium, they act predominantly at the apical membrane in the thick ascending limb of the loop of Henle, where they compete with Cl^- for binding to the $Na^+/K^+/2Cl^-$ cotransporter, thereby inhibiting Na^+ , K^+ and Cl^- reabsorption [3]. They also stimulate renal prostaglandin synthesis, particularly the vasodilatory prostaglandin E2 (PGE2), thus increases renal blood flow. Diuretics acting on cortical diluting segment (thiazides or thiazide-like drugs, for example hydrochlorothiazide, metolazone) exert their diuretic effect via blockage of the sodium-chloride (Na/Cl) channel which decreases levels of sodium across the luminal membrane, thereby decreasing the action of the sodium-potassium (Na/K) pump and Na and water passage to the interstitium. Inhibition of the Na/Cl channel in the proximal segment of the distal convoluted tubule results in increased delivery of sodium to the distal segment of the convoluted tubule and the collecting tubule. This Na increase causes the aldosterone-sensitive Na/K pump to increase sodium reabsorption in the principal cells. This exchange increases Na transfer into the interstitium and K transfer into the collecting tubules and lumen [4]. Potassium-sparing agents inhibit Na^+/K^+ exchange in distal convoluted tubule (DCT) (aldosterone antagonist such as spironolactone, or antagonist of angiotensin II "ARAII"). There are also those which inhibit Na^+ reabsorption on DCT independently of aldosterone; they inhibit epithelial Na^+ channel (ENaC) (amilorides) [5].

Although significant numbers of manufactured medicines are available, the Malagasy still use medicinal plants infusion or decoction prepared from different parts of plant as diuretic in Madagascar. For example, leaves of

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 2065-2068 www.ijprajournal.com ISSN: 2456-4494

Amaranthus spinosus (Amaranthaceae) [6] or Lycopodiella cernua (Lycopodiaceae) [7].

Ethnobotanical survey that we have conducted in Antananarivo (Madagascar), enabled us to get data on medicinal plants used in the area to treat oliguria. From this information, we put forward a hypothesis that the leaves of this plant might have diuresis activity. This work aimed to authenticate the traditional use of the plant by evaluating the diuretic effect of the hydro alcoholic extract of Clematis mauritiana (Ranunculaceae) in rat.

II. MATERIALS AND METHODS

- **Preparation of extract**

The leaves were collected in March and identified at the Botany Department of "Parc Botanique et Zoologique de Tsimbazaza" (PBZT), Antananarivo. They were dried in shade, at room temperature, then ground. The powder was macerated in ethanol-water (60:40) for 3 days. The macerate was filtered with Whatman filter paper n°2 and centrifuged at 3 000 rpm. The supernatant was evaporated to dryness with vacuum at 80°C to obtain the extract used in this work.

Experimental protocol

- **Animal experimentation**

Rat of Wistar strain of both sexes, weighing from 250 to 280 g were used. They were kept under standard laboratory conditions, with food and water ad libitum, under a 12 hour light/12 hour dark cycle. They were fastened 12 hours prior to tests.

Protocol has been examined and approved by the Science faculty ethics committee, under the reference n°3/2021"

- **Diuretic activity**

The diuretic activity was evaluated by measuring the 24 h urinary volume. To elucidate its site of action, urine pH was measured using Pierron® pH meter, while natriuria and kaliuria were measured, using flame photometer Systonic®

All the animals were given 50 ml/kg body weight of distilled water, orally. They were then divided into 4 groups, one control group and 3 groups treated with the extract. Animals of the control group received orally 10 ml/kg of de-ionized water, while the 3 treated groups were given orally 100, 200 and 400 mg/kg of extract dissolved in 10 ml/kg of de-ionized water [8].

Immediately after administering the water and extract, the animals were put individually in metabolism cages. Urinary volume of 24 h was collected and measured using graduated vials, pH of the collected urine of 24 hours was also measured, using pH meter, while the natriuria and kaliuria were measured using flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+ [9].

The extracts' diuretic effect was expressed as UVE (urinary volumetric excretion) calculated as a percentage of the initial hydric overload [10].

× 100

With:UVE urinary volumetric excretion
VE volume of urine
VA volume of hydric overload (50 ml)

80% < UVE < 100%: no diuretic activity
100% < UVE < 130%: low diuretic activity
130% < UVE < 150%: mild diuretic activity
150 % >UVE: high diuretic activity

- **Statistical analysis**

The results were expressed as mean ± SEM (standard error of mean). Data was analyzed by using analysis of variance (ANOVA) followed by Student's t-test. The results were considered statistically significant at $P < 0.05$.

III. RESULTS

- **Effect of the extract on diuresis**

Administered orally, the extract increases the volume of urine collected during 24 hours. This increment is dose dependent. The control group urine volume is 4.06±0.06, versus 5.57±0.06, 6.56±0.04 and 9.6±0.15 ml, respectively, for animals treated with the extract at the doses 100, 200 and 400 mg/Kg ($P<0.05$) (figure 1).

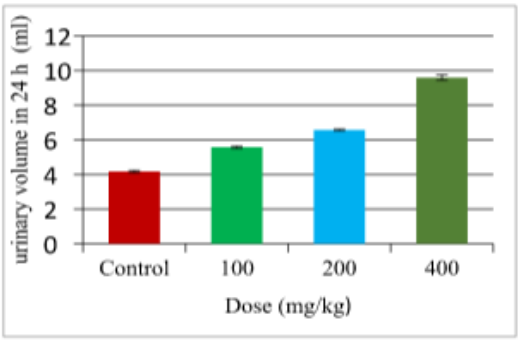

Figure 1. Variation in diuresis in control animals and animals treated with the extract, administered orally, at doses of 100, 200 and 400 mg/Kg after a hydric overload of 50 ml/kg (± sem; n=3 ; $P<0.05$)

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 2065-2068 www.ijprajournal.com ISSN: 2456-4494

After calculation, the urinary volumetric excretion induced by the extract is respectively 96.31, 104.1 and 141.1 % ($P<0.05$). Urinary volume excretion induced with the highest dose (400 mg/kg) is between 130 and 150 %, which indicates a mild diuretic activity of the extract.

- **Effect of the extract on electrolytes**

The extract administered orally increases natriuria and kaliuria. This increment in natriuresis is from 0.84 ± 0.41 mEq/L in control group to 1.57 ± 0.24, 2.16 ± 0.30 and 3.92 ± 0.30 mEq/L in animals treated with the extract at doses 100, 200, and 400 mg/kg respectively ($P<0.05$) (Table I). Kaliuresis increases from 1.24 ± 0.07 mEq/L in control group, to 2.38 ± 0.22, 3.48 ± 0.22 and 4.62 ± 0.19 mEq/L in animals treated respectively with the extract at doses 100, 200 and 400 mg/kg ($P<0.05$) (Table I). These results show the saliuretic activity of the extract.

Table I. 24 h natriuresis and kaliuresis for the animals of control group and treated with the extract administered orally at doses 100, 200 and 400 mg/kg

Doses (mg/kg)	control	100	200	400
Natriuria (mEq/L)	0.84 ± 0.41	1.57 ± 0.24	2.16 ± 0.30	3.92± 0.30
Kaliuria (mEq/L)	1.24 ± 0.07	2.38 ± 0.22	3.48 ± 0.22	4.62± 0.2

- **Effect of the extract on urine pH**

The extract, administered orally, does not affect the urine pH. It is 8.45±0.03 in control group, and 8.42±0.07, 8.4±0.18 and 8.41±0.06 in animals treated with extract at doses 100, 200 and 400 mg/kg (NS).

IV. DISCUSSION

This work aimed to authenticate the traditional use of Clematis mauritiana as diuretic. The study was carried out on rat; its activity was investigated by measuring the urinary volume. While kaliuresis, natriuresis and pH were measured to determine its probable action site.

The results show that the extract possesses mild diuretic activity, therefore supporting its ethno-pharmacological use as a diuretic. Analysis of Na^+, K^+ and H^+ excretion indicates its probable action site. The unchanged pH value indicates that it does not inhibit carbonic anhydrase, because if it did, the pH value would have increased [2]. Which means the extract is not a proximal diuretic. On the other hand, the increase of K^+ excretion indicates that it does not inhibit Na^+/K^+ exchange under aldosterone action at distal convoluted tubule. Because diuretics inhibiting aldosterone spare potassium [5].

Therefore we advance an hypothesis that it might inhibit sodium reabsorption, either in the climbing part of Henle loop or the cortical diluting segment. Diuretics acting at those parts of renal tubules inhibit reabsorption of sodium, potassium and chloride ions. They cause a rapid rise in excretion of sodium and chloride ions from the kidneys and an increase in diuresis [3; 4]. Between the two sites, loop diuretics are more powerful than those acting in diluting zone. Our results show that the extract has a moderate activity, according to the urinary volumetric excretion, which indicates that it might act in the diluting zone [4].

Since the extract contains loads of molecules, further phytochemical researches and dosage of different electrolytes such as Cl^-, Mg^{2+} and Ca^{2+} are required to investigate the active constituents responsible for the activity and to understand the exact mechanism of diuretic effect exhibited by this alcoholic extract of Clematis mauritiana.

V. CONCLUSION

The results obtained show that the alcoholic extract of Clematis mauritiana leaves increases diuresis, natriuresis and kaliuresis. It does not affect the urine pH. The increase of diuresis justify the traditional use of this plant as diuretic. Further investigation will be carried out to elucidate its mechanism of action.

REFERENCES

[1]. Islam, M.S., 2018, "The Art and Science of Using Diuretics in the Treatment of Heart Failure in Diverse Clinical Settings", Adv. Exp. Med. Biol., **1067**: 47-65.

[2]. Supuran; Claudiu, T.; Scozzafava; and Andrea, 2000, "Carbonic anhydrase inhibitors and their therapeutic potential", Expert Opinion on Therapeutic Patents, **10** (5): 575–600.

[3]. Wiss, I.M.; and de Meijer PHEM, 1997, "Loop diuretics—arguments for a choice", NMJ, 50: 75–80.

[4]. Tamargo, J.; Segura, J.; and Ruilope, L.M., 2014, "Diuretics in the treatment of hypertension. Part 1: thiazide and thiazide-like diuretics", Expert Opinion on Pharmacotherapy, **15** (4): 527-547.
[5]. McCormick, J.A.; and Ellison, D.H., 2015, "Distal convoluted tubule", Compr. Physiol., **5**(1): 45-98.
[6]. Pernet, R.; and Meyer, G., 1957, "Pharmacopée de Madagascar", Ed. Pub. Inst. Rech. Sci., Tananarive (Madagascar), **1**: 3 - 6.
[7]. Boiteau, P., 1979, "Précis de matière médicale Malgache", J. Ethnopharmacol., **23**: 165 - 265.
[8]. Diehl, K.H.; Hull, R.; Morton, D.; Pfister, R.; Rabemampianina, Y.; Smith, D.; Vidal, J.M.; and De Vorstenbosch, C.V., 2001, "A good practice guide to the administration of substances and removal of blood, including routes and volumes", J. Appl. Toxicol., **21** (1): 15-23.
[9]. Sanogo, R. ; Halimatou, K.A. ; Dembélé, O. ; and Diallo, D., 2009, "Activité diurétique et salidiurétique d'une recette utilisée en médecine traditionnelle pour le traitement de l'hypertension artérielle", Mali Medical, **24**(4): 1 - 6.
[10]. Kau, S.T.; Keddi, J.R.; and Andrews, D., 1984, "A method for screening diuretic agents in the rats", J. Pharmacol. Meth., **11**: 67–75.