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DIURETIC ACTIVITY OF ETHANOLIC EXTRACT OF *IMPERATA CYLINDRICAL* (L.) P. BEAUV. (POACEAE) LEAVES ON RAT

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ABSTRACT: This work aimed to evaluate the diuretic activity of *Imperata cylindrica* (Poaceae) leaves on rat. The extract was administered orally at doses 100, 200 and 400 mg/kg. The 24h urine volume and its pH were measured, natriuresis and kaliuresis were dosed. The results show that *I. cylindrica* increases diuresis, natriuresis and kaliuresis, but doesn't affect urine pH. Diuresis of 24 h urine increases from 3.23 ± 0.32 ml in control group, to 5.33 ± 0.38, 6.83 ± 1.23 and 75 ± 1.32, respectively, in rats treated with the extract ($P<0.05$). Natriuresis increases from 1.21 ± 0.52 mEq/L to 2.38 ± 0.20, 3.15 ± 0.11 and 5.46 ± 0.72 mEq/L ($P<0.05$). Kaliuresis increases from 0.51 ± 0.71 mEq/L to 0.73 ± 0.65, 1.21 ± 0.51 and 1.75 ± 0.36 mEq/L ($P < 0.05$). The pH does not change, it is 8.52 ± 1.59 in control group and 8.51 ± 1.59, 8.47 ± 1.57 and 8.49 ± 1.58, in groups treated with the extract (N.S). These results show that Imperata cylindrica is a mild diuretic at the doses used, which might act at the ascending limb of Henle Loop or the diluting zone.

INTRODUCTION: Diuretics improve urine output due to sodium excretion by inhibiting its transport along the renal tubule. They are prescribed in case of acute or chronic renal failure [1]. They are also used to treat hypertension, of which complications may cause cardiovascular and renal disease, such as heart attack, heart failure, stroke and kidney diseases [2]. Their action is based on their ability to reduce blood volume and natremia [3]. Edema resulting from cardiac congestive or kidney failure can be treated with diuretics. They are also indicated for detoxification of the organism by renal route [4].

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Diuretics are effective and generally safe, but like any other medications, they may cause side effects. Depending on their site of action, diuretics can influence electrolyte and water balance in the organism. Some diuretics increase excretion of electrolyte-free water, calcium, potassium, chloride, bicarbonate, or magnesium and some can reduce renal excretion of calcium, potassium, or protons. Consequently, electrolyte and acid-base disorders commonly accompany diuretic use. Potential side effects of a diuretic can often be anticipated from its mode of action on the kidney [5].

The loop diuretics are the most effective; they inhibit the sodium and chloride transport in the thick ascending limb of Henle loop and produce large amount of urine. Therefore, they mobilize extracellular fluid in case of edema. In high doses, they are also the drugs of choice in the treatment of both acute and chronic renal failure [6]. Carbonic anhydrase inhibitors, acting in the proximal

convoluted tubule, produce less volume depletion and hypokalemia but commonly induce metabolic acidosis. Loop agents and distal convoluted tubule agents, produce hyponatremia, hypokalemic, hypochloremic and metabolic alkalosis. Dose-related reversible or irreversible ototoxicity may also complicate treatment with loop agents [7]. While potassium-sparing agents, acting in the collecting duct, limit proton excretion, and produce metabolic acidosis. Hyperkalemia is the most complicated of the potassium-sparing agents. Diuretic may also influence the renal handling of calcium, sodium bicarbonate and uric acid. Reported idiosyncratic reactions to diuretics include interstitial nephritis, non-cardiogenic pulmonary edema, pancreatitis, and myalgias [8].

Despite the development of various diuretic medications, communities in different parts of the world still use medicinal plants To take care of edema and difficulty in micturition [9]. For example *Taraxacum officinale* (L.) Weber (Asteraceae) is widely used as a diuretic in Europe, Asia and the America [10], *Orthosiphon aristatus* (Lamiaceae) is used as a tea in Indonesia to treat hypertension and diabetes [11] and in Northeastern Argentina, *Eugenia uniflora* L. (Myrtaceae) is used for its diuretic effect as antihypertensive [12]. According to an ethnopharmacological survey that we have conducted in the northern part of Madagascar, *Imperata cylindrical* (Poaceae) is also largely used for hypertension, oliguria or edema. Decoction prepared with its leaves is taken as beverage to take care of those pathologies. Or, diuretic abuse sometimes leads to tragic consequences. This work aimed to determine this plant's site of action to prevent its negative secondary effects. A hydro alcoholic extract was prepared from the leaves of *I. cylindrica* and administered orally, urine volume and its pH were measured and sodium and potassium were dosed.

MATERIALS AND METHODS:

Plant Material: The leaves used in this work were collected from the northern part of Madagascar and authenticated at the botany department of Parc Botanique et Zoologique de Tsimbazaza (PBZT), Antananarivo. They were dried in the shade at room temperature and ground to powder. This powder was macerated in a hydro-alcoholic mixture in the proportion of 60: 40 water: alcohol for 3 days. The macerate was filtered on cotton cloth, then on Whatman (No.1) filter paper. The filtrate was evaporated to dryness, using a rotary evaporator (Evapotec ®) under reduced pressure at 60°C until dryness.

Experimental Animals: Wistar albino rats of either sex, weighing 180–250g were used for the experiment. They were kept at the animal house of Physiology, Pharmacology and Cosmetology Department, Sciences Faculty, University of Antananarivo, at room temperature and on a 12h light-dark cycle with free access to food and water. The care and handling of animals were in accordance with internationally accepted OECD420 (2008) guidelines for the use of animals. The experiments were conducted following the guidelines of the ethics committee of the Sciences Faculty, University of Antananarivo, Madagascar (Ref: CE/ Fac Sciences/ Pharmacol. /07, 08/18/2021). The selected animals were fasted for 12 hours before the test, with free access to water. They were put into 4 groups of 5 animals per group: one control group and three experimental groups treated with the extract. The control group received distilled water, the second, third and fourth groups received the extract at the dose of 100mg/kg, 200mg/kg and 400mg/kg, respectively. The different products were administered orally in a volume of 10ml/kg [13].

Evaluation of Diuretic Effect of the Extract: All the rats received 50 ml/kg of water overload by oral route to impose a uniform water load and after 30 minutes, the control group received 10 ml/kg of distilled water, while the rest respectively received the extract at the different doses dissolved in 10 ml/kg of distillated water. Immediately after administration, the rats were individually placed in a metabolic cage and urine of 24 hours was collected, its volume was measured, its urinary volumetric excretion (UVE) was calculated, and the product was classified according to its UVE value **Table 1** [14].

U. V. E = (VE / VA) x 100 urinary volumetric excretion

VA: Volume of the liquid administered, VE: Volume of urinary output. The diuretic activity of the extract was evaluated according to UVE calculated in **Table 1.**

TABLE 1: CLASSIFICATION OF A PRODUCT ACCORDING TO ITS U.V.E. VALUE

U. V. E. value	Activity
80 – 110%	no diuretic activity
110-130%	low diuretic activity
130-150 %	mild diuretic activity
U.V.E.> 150 %	high diuretic activity

Evaluation of Extract Effect on Electrolytes Excretion: Sodium and potassium concentrations in urine samples were determined using a flame spectrophotometer (Systonic ®). The machine was calibrated, prior to analysis with different levels of standards of sodium and potassium Merck®. While H^+ excretion was determined by measuring pH directly of fresh urine samples using a pH meter (Pierron ®). Natriuretic and kaliuretic, as well as the ratio of Na+ and K+ were calculated, to express the diuretic activity of the extract **Table 2** [14].

Natriuretic Index: Natriuretic activity in test group/ natriuretic activity in control group.

Kaliuretic Index: Kaliuretic activity in test group/ kaliuretic activity in the control group. Electrolytes ratio Na+/K+ was calculated to evaluate the saliuretic activity of the extract.

TABLE 2: ELECTROLYTIC RATIO AND IT'S INTERPRETATION

Sodium Ratio	Interpretation
$1 < Na^+/K^+ < 2$	Satisfactory natriuretic product
$2 < Na^+/K^+ < 10$	Natriuretic product inducing Na^+ urinary excretion without excessive loss of K^+
$Na^+/K^+ > 10$	Product sparing K^+

Statistical Analysis: Data, expressed as mean ± standard error of mean (SEM), were analyzed using one way ANOVA followed by Student's test. Significant differences were set at p values less than 0.05.

RESULTS:

Effect of the Extract of *Imperata cylindrica*on Urinary Volume: Administration per oral route, of a single dose of the Hydro-alcoholic extract of *I. cylindrical* (100, 200 and 400 mg/kg) significantly increased the volume of urine for 24 hours. The urinary excretion was dose-dependent **Fig. 1**. The urinary volume of the animals in the control group, which received distilled water, is equal to 3.23 ± 0.32 ml/ 24 h, versus 5.33 ± 0.38, 6.83 ± 1.23 and 7.5 ± 1.32 ml/ 24 h, respectively for the animals. That received the extract orally, at doses 100, 200 and 400 mg/kg ($P<0.05$). The dose of 100 mg/kg provokes an increment of 65 %; with the dose 200 mg/kg the urinary volume increases 111 %. The highest dose (400 mg/kg) induced 132 % increase compared to the control group, with a diuretic index of 1.65, 2.11 and 2.32, respectively for 100, 200 and 400 mg/kg doses.

And according to the classification defined by KAU *et al*., with a UEV equal to 150 %, the extract of *I. cylindrical* at dose 400 mg/kg. possesses mild diuretic effect. The results in **Fig. 1** show the improvement of renal excretion of the overload in the presence of *I. cylindrical* Hydro-alcoholic extract.

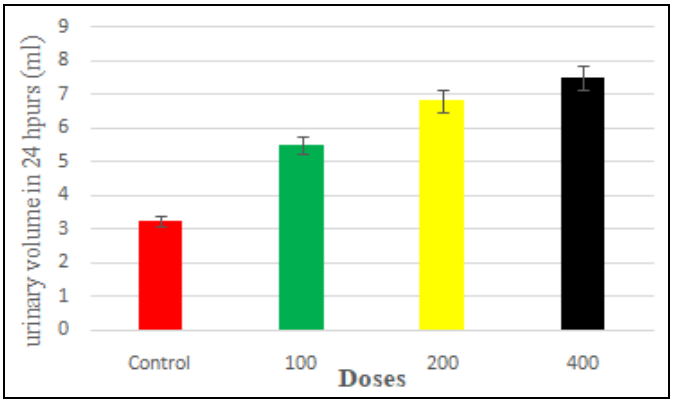

FIG. 1: VARIATION IN 24H URINE VOLUME OF THE CONTROL GROUP (RED) AND RATS TREATED WITH THE EXTRACT, ADMINISTERED ORALLY, AT DOSES OF 100 (GREEN), 200 (YALLOW) AND 400 MG/KG (BLACK) ($\bar{X} \pm \bar{\sigma}$; N= 3; P < 0.05)

Effect of the Extract on Na^+ and K^+ Excretion: Administered orally, Imperata cylindrical leaves' hydro-alcoholic extract increases sodium and potassium urinary excretion. The urinary Na^+ increases with the administered dose **Fig. 2**. It is equal to 1.21 ± 0.52 mEq/L for the control group and 2.38 ± 0.20, 3.15 ± 0.11 and 5.46 ± 0.72 mEq/L, respectively in the groups treated with the extract at doses 100, 200 and 400 mg/kg ($P<0.05$). These results indicate the natriuretic activity of the extract.

It is the same for potassium; the extract increases the urinary excretion of this electrolyte, and its concentration in the urine increases with the administered dose. The excretion of potassium of the control group is 0.51±0.71mEq/L, while the urinary potassium increases to 0,73 ± 0,65 mEq/L

for animals treated at the dose of 100 mg/kg; increases to1,21 ± 0,51 mEq/Lin animals treated at dose 200 mg/kg and increases to 1,75 ± 0,36 mEq/L for the dose 400 mg/kg treated animals **Fig. 2** ($P < 0.05$).

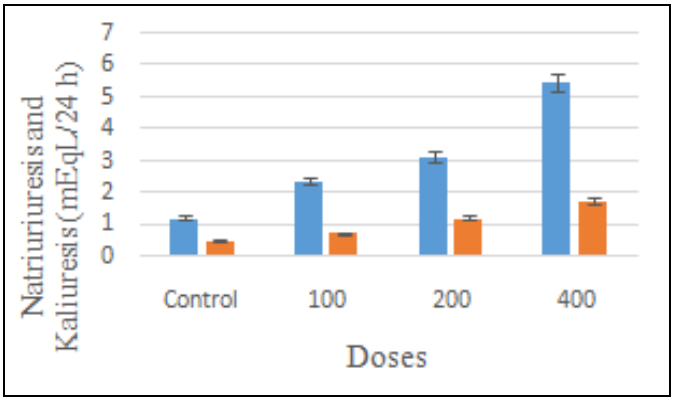

FIG. 2: VARIATION IN NATRIURESIS ( ) AND KALIURESIS ( ) OVER 24 H IN THE CONTROL GROUP AND RATS TREATED WITH THE EXTRACT, ADMINISTERED ORALLY, AT DOSES OF 100,200 AND 400 MG/KG ($\bar{x} \pm \bar{\sigma}$; N= 3 ; P < 0.05).

Analysis of the calculated sodium ratio show a significant natriuretic ratio >3 at the highest dose of 400 mg/kg indicating a significant natriuretic activity.

While the ratio Na^+/K^+ at the highest dose of 400 mg/kg is equal to 3 which is between 2 and 10 **Table 3**. According to the classification of KAU *et al.*, the extract is a natriuretic product which induces sodium excretion without excessive loss of potassium.

TABLE 3: EFFECTS OF THE AQUEOUS EXTRACT OF *ICYLINDRICA* ON URINE OUTPUT INDEX AND ELECTROLYTIC EXCRETION INDEX IN 24 HOURS OF URINE COLLECTION

	100 mg/kg	200 mg/kg	400 mg/kg
Diuretic index	1.65	2.11	2.32
Na^+ index	1.96	2.60	4.51
K^+ index	1.46	2.42	3.5
Na^+/K^+	2.26	2.60	3.12

Effect of the Extract on Urinary pH: The pH value of urine of the animals treated with the extract of *I. cylindrica* and that of the control group don't show any significant difference. It is equal to 8.52 ± 1.59 in the control group, versus 8.51 ± 1.59, 8.47 ± 1.57 and 8.49 ± 1.58 in the animals treated with the extract of *I. cylindrical* (N. S) **Fig. 3**. These results indicate that the extract does not influence H^+ excretion.

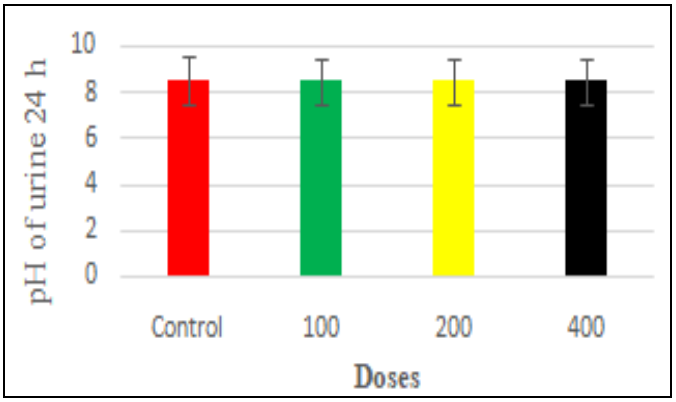

FIG. 3: VARIATION IN PH OF 24 H URINE FOR CONTROL GROUP ( ) AND TREATED ANIMALS WITH THE EXTRACT, ADMINISTERED ORALLY, AT DOSES OF 100 ( ), 200( ) AND 400 MG/KG ( ) ($\bar{x} \pm \bar{\sigma}$; N= 3 ; P < 0.05)

DISCUSSION: The ability to induce negative fluid balance has made diuretics useful in treating various conditions, particularly edematous states and hypertension. This work aimed to evaluate the diuretic activity of hydro alcoholic extract of *I. cylindrica* leaves on rat. First, to justify the traditional use of the plant as diuretic in the northern part of Madagascar and second, to prevent the undesirable effects of the decoction prepared from this plant because local people sometimes abuse the decoction, since it is not bitter and they drink it as beverage. Or depending on the diuretics site of action, their abuse might lead to an imbalance of electrolytes and water in the organism.

To attain the objective, the effect of the extract on diuresis was evaluated by measuring the volume of water excreted. This study showed an increase in the elimination of fluid overload. These results demonstrate that the extract has diuretic activity. And according to the UVE value at the highest dose, it is a mild diuretic [14].

The analysis of excreted urine also shows an increase of Na^+ and K^+. They are significantly higher in animals that received the plant extract than in the control group. These results lead us to advance a hypothesis that this extract might inhibit sodium reabsorption. The increased natriuresis in response to acute treatment by Hydro-alcoholic extract of *I. cylindrica*l leaves may partly explain the diuresis and kaliuresis increase [15]. The increase in excretion of K^+ in animals treated with the

extract compared to the controls indicates that *I. cylindrica* extract does not inhibit its excretion in the distal convoluted tube. At this part of nephron, the increase of sodium concentration stimulates the sodium/potassium pump leading to an increase of potassium excretion [16]. Diuretics acting on this part of nephron reduce urinary K^+, which is not the case here. It means that the extract is not a sparing diuretic acting on distal convoluted tube. On the other hand, there is no significant change in urinary pH of the animals treated with the extract and the control group. These results suggest that *I. cylindrica* extract does not affect H^+ excretion in the proximal convoluted tube, because carbonic anhydrase inhibitors do inhibit bicarbonate uptake by the proximal convoluted tube, resulting in the alkalization of urine [17]. Since, there is no difference between urinary pH of animals treated with the extract and the control, we advance a hypothesis, that this extract does not inhibit the carbonic anhydrase.

At this level, one can say that *I. cylindrica* extract inhibits the reabsorption of sodium. It probably acts by inhibiting its reabsorption in the ascending branch of Henle loop or in the diluting zone. Since diuretics acting in these parts of the tube increase urinary excretion of sodium, potassium, chloride and calcium through renal route, the therapy with the decoction prepared with *I. cylindrica*l eaves may be associated with a variety of fluid and electrolyte complications, including volume depletion, hypokalemia, hyponatremia, hypocalcemia, metabolic alkalosis, hyperuricemia, azotemia and hypomagnesemia. It is necessary to check the concentration of those electrolytes in blood to prevent complications [18] induced by the abuse of the decoction prepared with *I. cylindrical* leaves.

CONCLUSION: In conclusion, the oral administration of a single dose of hydro-alcoholic extract of *I. cylindrica* leaves on rat increased significantly 24 h urine volume. In addition, the treatment increased, in a dose-dependent manner, the excretion of Na^+ and K^+. The stability of urinary pH and the increase of K^+ in the animals receiving the extract show moderate increased diuresis and elevation natriuresis of ascending Henle tube or the diluting zone origin, and probably on the diluting zone.

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