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Diuretic activity of the aqueous roots extract of *Leptadenia hastata* (Asclepiadaceae) in rats

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ABSTRACT

Leptadenia hastata is a plant used in African traditional medicine to treat arterial hypertension. We assessed the acute and subacute diuretic activities of aqueous extract of *L. hastata* roots in rats. Male Adult rats were administered with *L. hastata* roots extract acutely (24 h) and sub-acutely (7 days) at doses 150, 200 and 250 mg/kg (per os). To assess acute diuretic activity, samples of tail vein blood were collected 24h after treatment and urine was collected every 3h. Levels of Na^+, K^+, and Cl^-, urea, and creatinine were also measured. Natriuretic, saluretic, and diuretic indexes were determined. The urine collected for 7 days was processed similarly to assess sub-acute diuretic activity. The extract induced significant increases in urine volume (54.93%, 64.47%, and 77.69% compared to vehicle group for doses 150, 200, and 250 mg/kg, respectively), and urine Na^+ (126.51%, 136.83%, and 133.67%, respectively), Cl^-, and in a lesser extent, K^+ levels 24h after treatment. Creatinine and urea levels increased in urine whereas Blood creatinine and urea levels were decreased. Diuretic, saluretic, natriuretic and ionic indexes were also significative. These effects were maintained along 7 days of treatment, and were comparable with two references drugs effects (furosemide and amiloride hydrochlorothiazide). Altogether, our results suggest that aqueous extract of *L. hastata* roots has strong acute and subacute diuretic activities in rats, which warrant further studies considering the potential for unraveling a novel class of diuretic drugs.

Keywords: *Leptadenia Hastata*, Diuretic, Natriuretic, Saluretic, Chronic Kidney Disease.

INTRODUCTION

Arterial hypertension is among the most frequent pathologies in elderly worldwide, with an incidence ranging from 40% (about 65 years patients) to 90 % (patients older than 85) in developed [1,2]. Diuretics occupy a prominent place among the drugs used against arterial hypertension; they are substances that inhibit the renal reabsorption of sodium, thereby enhancing renal excretion of sodium, water and sodium chloride[3,4]. Diuretics offer the possibility to treat hypertension successfully with limited undesired effects[3,5]. Many medicinal plants with diuretic properties were reported[6,9]. Unraveling medicinal plants with diuretic properties supports the development of evidence-based traditional medicine, particularly in developing countries, and increases the chances for unraveling novel naturally occurring potent diuretic agents with less undesired effects.

Leptadenia hastata (Pers.) Decyne (Asclepiadaceae) is a particularly hard and drought-resistant latex plant commonly consumed as vegetable and used in the traditional medicines of various Sub-Saharan African countries[10,11]. In traditional medicine, latex, leaves and roots are used in decoctions and macerations, alone or in combination with other medicinal plants, to treat high blood pressure, diabetes, stomach ache, jaundice, rheumatism, onchocerciasis, trypanosomiasis, against abdominal pain, otitis, urinary tract infections, infected wounds, ulcers, and snake bite[10,16]. Notably, various reports confirmed anti-inflammatory properties[12,13]. hypoglycemic and antidiabetic properties[15,16]. antiproliferative properties [17]. and anti-trypanosomal activity [14].

In the present study, we assessed the acute and subacute diuretic activities of the aqueous extract of roots of *L. hastata* in rats.

MATERIALS AND METHODS

Animals

Thirty male Wistar rats (3 months old, 200±35.4 g) were used. The animals were obtained from the animal facility of the Faculty of Science, University of Ngaoundere. Experiments started after one week of acclimation to the Laboratory of Medicinal Plants, Health and Galenic Formulation, Department of

Biological Sciences, University of Ngaoundere (25±1°C, 43±10 % relative humidity, 12:12h light/dark cycle). The animals had *ad libitum* access to food and tap water.

The experimental procedures were approved by the institutional review board of the Faculty of Science, University of Ngaoundere. Research activities were conducted following European Community guidelines (86/609/EEC), which encompass the internationally accepted principles for laboratory animal use and care.

Experimental procedures

The animals obtained underwent a preliminary screening aimed at ensuring that they are fit for the study, as previously described[7,8]. Briefly, rats were given distilled water (1ml/100g body weight) and placed individually in metabolic cages for 24h. Urine was collected hourly and cumulated volumes were determined every 3h (3h, 6h, 12h, and 24h). The animals excreting a volume of urine greater than or equal to 40% of the volume of water received were considered fit for the study[7,8]. In this study, all the thirty male Wistar rats tested passed the fitness test.

For the remainder of the study, the animals were maintained in metabolic cages (single housed). They were divided into six experimental groups (N=5 per group), three test groups receiving the aqueous extract of *L. hastata* (administered *per os* with a feeding needle) at doses 150 mg/kg (E150), 200 mg/kg (E200), and 250 mg/kg (E250), two positive control groups administered with furosemide (5 mg/kg, p.o.) or amiloride Hydrochlorothiazide (AHCT, 5 mg/kg, p.o.), and a vehicle group receiving distilled water (p.o.). As mentioned in section 2.1, then, the animals were given seven days for acclimation to metabolic cages and laboratory conditions, after which treatments were administered once daily for seven days. Core body (rectal) temperature of rats was determined 1h before and 5h after treatment on the first day. Urine was collected every 3h and volumes were determined (using graduated precision syringes) to assess extract's acute and subacute diuretic activities. Blood tail vein samples were also collected 24-h after the first treatment to assess plasmatic indicators of diuretic activity.

Plant collection and processing

L. hastata fresh samples (leaves and roots) were collected in March 2017 in Zamay village, Mayo Tsanaga Division, Far North Region, Cameroon. Botanical identification was performed by experts of Cameroon National Herbarium (CNH) and a sample was stored (voucher number: 7805 / SRF / Cam). The roots were processed for aqueous extraction mimicking the traditional medicine use, as follows: fresh roots were shade-dried for a week, and then, ground into powder. Afterwards, 1 kg of powder was macerated in 1 L of distilled water for 12 h. The macerate was filtered using Whatman grade 3 qualitative filter paper, and the filtrate was concentrated with a rotary evaporator for 24 h, at 40°C. At the end of the process, 13.9 g of an oily paste (dry extract) was obtained (yield: 27.8%). The dry extract was stored at -20°C until use. It was reconstituted to prepare the aqueous extract.

To select the doses to use in this study, a preliminary study in few animals where increasing doses of the aqueous extract of *L. hastata* were administered orally (25, 50, 100, 150, 200, and 250 mg/kg) was performed (data not shown). The most active doses (150, 200, and 250 mg/kg) were selected and used in the present study.

Standard analytical tests aimed at characterizing the groups of phytochemicals present were performed on the extract at the National Institute of Medicinal Plants for Medicinal Research (IMPM), Cameroon.

Assessment of acute diuretic activity

Samples of tail vein blood collected in heparinized tubes were centrifuged (3,000 RPM, 15 minutes, 5°C) and the plasma (supernatant) was collected. The plasma obtained and the urine collected 3h, 6h, 12h, and 24h after treatment in dry tubes were processed for standard biochemical assays aimed at determining Na^+, K^+, and Cl^- levels (using flame photometry), but also urea and creatinine levels (using a two-way digital spectrophotometer) (RS 232, Secomam, France). Carbonic anhydrase inhibition, saluretic and natriuretic activities were calculated, as well as natriuretic, saluretic, diuretic, Na^+, K^+, and Cl^- indexes. Urine pH was measured using a pH meter.

Assessment of subacute diuretic activity

Treatments were administered daily for 7 days between 10:00 and 12:00 am. Urine was collected daily, its volume was measured, and Na^+, K^+, and Cl^- levels were determined daily using flame photometry. Standard spectrophotometry was used to determine the urea and creatinine levels. Glomerular filtration rate (GFR) was determined from the creatinine clearance. Plasma creatinine, Na^+, Cl^-, and K^+ levels were determined using the same techniques as for urine.

Statistical analyses

ANOVA followed by LSD test was used to assess the statistical significance of differences between the vehicle group and groups treated with the extract, with furosemide, and with AHCT for all the variables studied. Differences with $P<0.05$ were significant. Data were presented as mean ± SEM.

RESULTS

Phytochemical screening

The phytochemical screening of the aqueous extract of *L. hastata* revealed the presence of flavonoids, alkaloids, tannins, sterols, triterpenes, saponins, phenolic compounds, and carotenoids.

Body temperature

Figure 1A shows the effects of *L. hastata* extract on the difference of body temperature of rats 1 h before and 5 h after treatment. Significant decreases in body temperature were observed in animals treated with the extract compared to values before treatment and to vehicle group values (-0.62% for 150 mg/kg, -0.74% for 200 mg/kg, -0.80% for 250 mg/kg, $P<0.05$ vs. vehicle group). On the other hand, body temperatures of animals treated with amiloride and furosemide were comparable to temperature values before treatment and of the vehicle group ($p>0.05$).

Acute Diuretic Activity

Urine pH and 24-h volume

Figure 1B shows the effects of *L. hastata* extract on urine pH 24 h after treatment. Compared to vehicle group, slight increases were observed in all treatment groups, i.e., animals treated with the extract doses 150 mg/kg (difference of 0.34 representing 3.91%), 200 mg/kg (difference of 0.09 representing 1.02%), and 250 mg/kg (difference of 0.49 representing 5.67%), with furosemide (difference of 0.58 representing 6.75%) or with AHCT (difference of 0.34 representing 3.93%) (Figure 1B).

Figure 1C shows the effects of the aqueous extract of *L. hastata* on the volume of urine excreted in the 24 h following treatment. Increases were observed in all experimental groups 6h after treatment compared to vehicle group. They were significant in the groups treated with extract dose 250 mg/kg and furosemide (23.88% and 21.01% increases compared to vehicle group, respectively, $P<0.05$). The

volume of urine excreted was significantly higher than vehicle group values in all groups 12h (respectively, 24h) after treatment: 28.07% (P<0.05) (respectively, 54.93%, P<0.05) with extract dose 150 mg/kg; 32.01% (P<0.05) (respectively, 64.47%, P<0.01) with dose 200 mg/kg; 50.27% (P<0.05) (respectively, 77.69%, P<0.01) with dose 250 mg/kg; 29.73% (P<0.05) (respectively, 50.83%, P<0.05) with furosemide; and 16.01% (P<0.05) (respectively, 33.75%, P<0.05) with AHCT (Figure 1C).

Urine ion levels in the 24 h following treatment

Figures 2A-C show the effects of *L. hastata* extract on the urinary excretions of K^+ (Figure 2A), Cl^- (Figure 2B), and Na^+ (Figure 2C) in the 24 h following treatment. Increases in levels excreted were observed for all the ions and treatment groups, compared to vehicle group (Figures 2A-C). Post-treatment increases in urine K^+ level compared to vehicle group were: 24.45% (P<0.05) for extract dose 150 mg/kg, 26.31% (P<0.05) for dose 200 mg/kg, 64.09% (P<0.01) for dose 250 mg/kg, 38.13% (P<0.05) for furosemide, and 22.23% (P<0.05) for AHCT (Figure 2A). Instead, post-treatment increases in urine Cl^- level compared to vehicle group were: 115.36% (P<0.001) for extract dose 150 mg/kg, 112.53% (P<0.001) for dose 200 mg/kg, 132.54% (P<0.001) for dose 250 mg/kg, 114.53% (P<0.001) for furosemide, and 65.71% (P<0.01) for AHCT (Figure 2B). And finally, post-treatment increases in urine Na^+ level compared to vehicle group were: 126.51% (P<0.001) for extract dose 150 mg/kg, 136.83% (P<0.001) for dose 200 mg/kg, 133.67% (P<0.001) for dose 250 mg/kg, 129.55% (P<0.001) for furosemide, and 111.96% (P<0.001) for AHCT (Figure 2C).

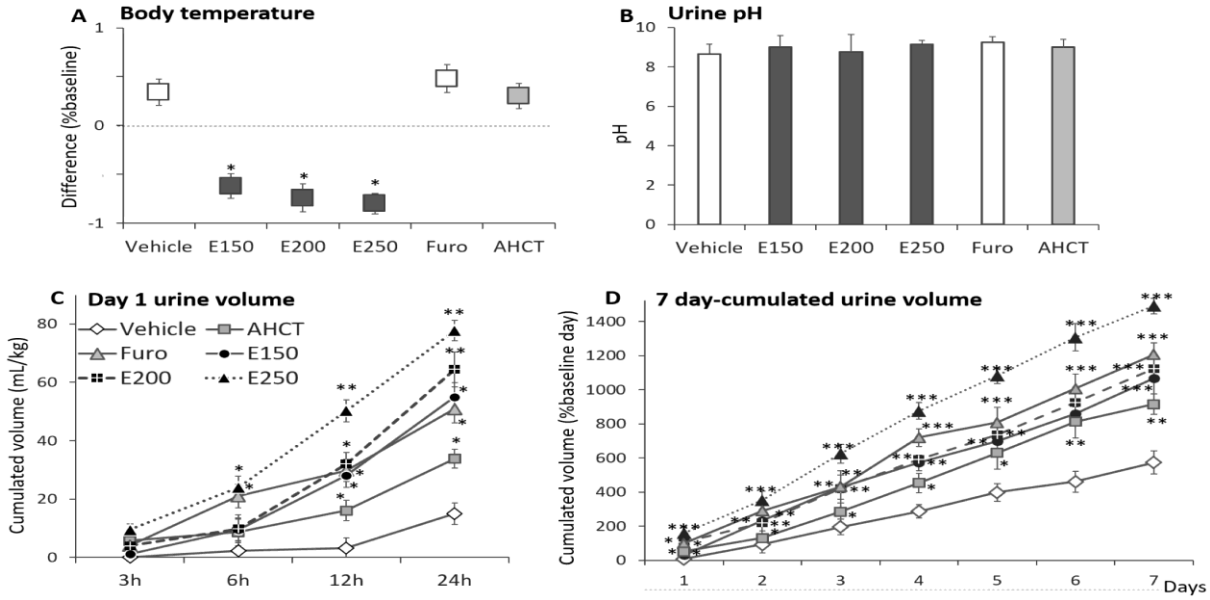

Figure 1: Body temperature and urine volume

Effect of *L. hastata* extract on body temperature (A), urine pH (B), 24-h urine volume (C), and 7-day urine volume (D). Note the decreases in body temperature (A), pH comparable values in all groups (B), and the increases in urine volume (C, D). AHCT: amiloride hydrochlorothiazide. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. Furo: furosemide. Data are mean ± ESM, N=5. ANOVA+LSD test: *P<0.05, **P<0.01, ***P<0.001 vs. vehicle group.

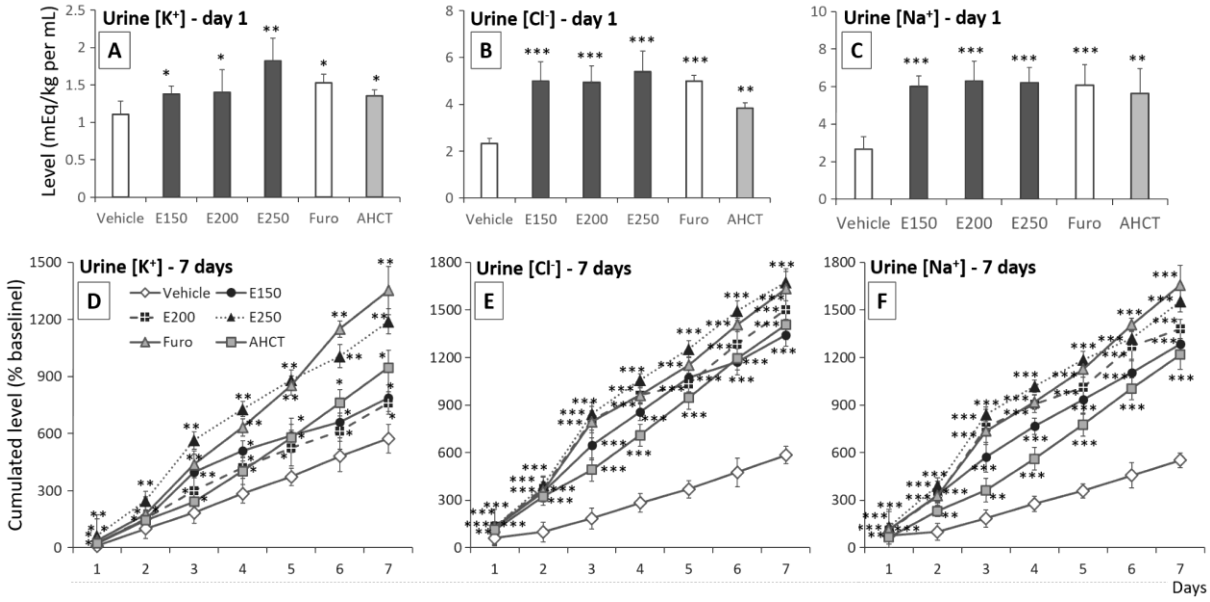

Figure 2: Extract effects on urine ion levels in the 24 h following treatment

A-C. Day 1 excretion of K^+ (A), Cl^- (B), and Na^+ (C). D-F. Day 4 excretion of K^+ (D), Cl^- (E), and Na^+(F). G-I. Day 7 excretion of K^+ (G), Cl^- (H), and Na^+ (I). Note the increases in groups treated with extract doses 150, 200 and 250 mg/kg (E150, E200, E250), with amiloride hydrochlorothiazide (AHCT), and furosemide (Furo). Data are mean ± ESM, N=5. ANOVA+LSD test: *P<0.05, **P<0.01, ***P<0.001 vs. vehicle group.

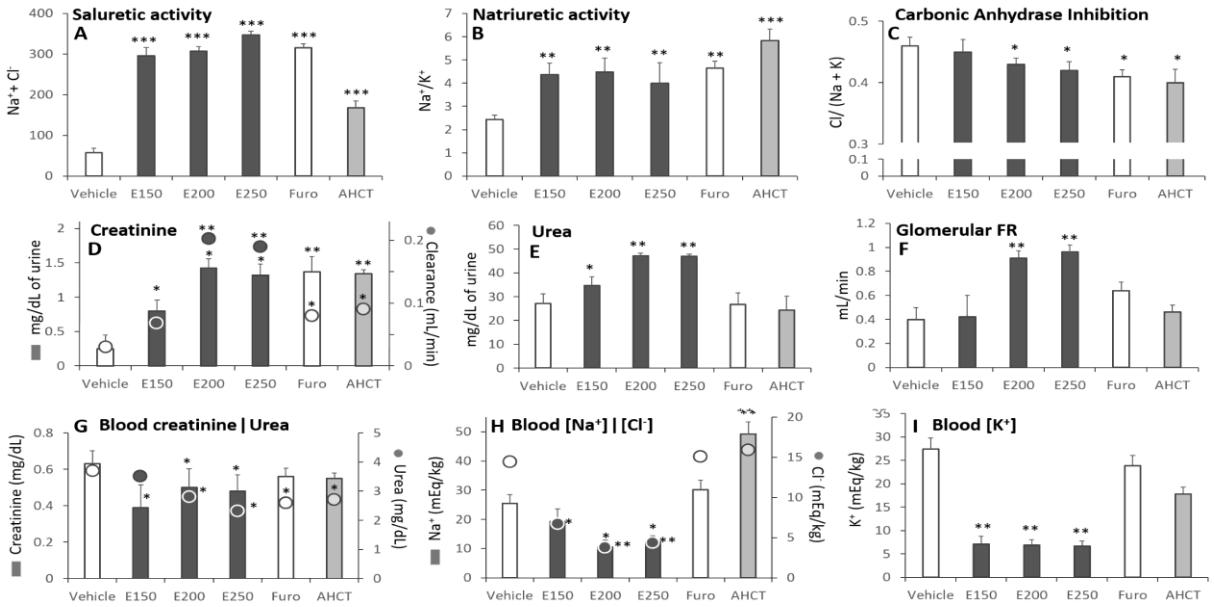

Figure 3: Extract effects on kidney functional indicators

Effects of *L. hastata* extract on saluretic activity (A), natriuretic activity (B), carbonic anhydrase inhibition (CAI) (C), creatinine clearance (D), urea level in urine (E), and glomerular filtration rate (GFR) (F), and blood and urea creatinine (G), Na^+, Cl^- (H), and K^+ (I). Note the increases in saluretic activity (A), natriuretic activity (B), creatinine clearance (D), urea level in urine (E), and the decreases in CAI (C), blood Na^+, Cl^- and K^+ (H,I). AHCT: amiloride hydrochlorothiazide. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. Furo: furosemide. Data are mean ± ESM, N=5. ANOVA+LSD test: *P<0.05, **P<0.01, ***P<0.001 vs. vehicle group.

Table 1: Effect of the extract on urine ion indexes

	Vehicle	Extract doses (mg/kg)			Furo	AHCT
		150	200	250		
Saluretic Index	1.00	5.16***	5.36***	6.06***	5.50***	2.92***
Natriuretic Index	1.00	1.80***	1.85***	1.44***	1.91***	2.40***
CAI Index	1.00	0.97***	0.93***	0.91***	0.89***	0.86***
Diuretic Index	1.00	1.51***	1.54***	1.82***	1.47***	0.66***
Na+ Index	1.00	5.28***	5.63***	6.58***	6.06***	3.25***
K+ Index	1.00	5.02***	5.05***	5.47***	4.85***	2.54***
Cl^- Index	1.00	2.90***	3.00***	4.63***	3.13***	1.34***

AHCT: amiloride hydrochlorothiazide. **CAI**: carbonic anhydrase inhibition. **Furo**: furosemide. Data are mean ± ESM, N = 5. ANOVA+LSD test: *P<0.05, **P<0.01, ***P<0.001 vs. vehicle group

Carbonic anhydrase inhibition, saluretic and natriuretic activities

Figures 3A-C show the effects of *L. hastata* extract on the saluretic activity (Figure 3A), the natriuretic activity (Figure 3B), and the Carbonic anhydrase inhibition (CAI) (Figure 3C). Marked increases were observed in the saluretic (Figure 3A) and natriuretic activities (Figure 3B). Post-treatment increases in saluretic activity compared to vehicle group were about 2-fold (P<0.01) for AHCT, 4-fold (P<0.001) for extract doses 150 mg/kg and 200 mg/kg), and for furosemide, and five-fold for dose 250 mg/kg (Figure 3A). Post-treatment increases in natriuretic activity compared to vehicle group were: 80.16% (P<0.01) for extract dose 150 mg/kg, 85.12% (P<0.01) for dose 200 mg/kg, 64.87% (P<0.01) for dose 250 mg/kg, 91.74% (P<0.01) for furosemide, and 140.50% (P<0.001) for AHCT (Figure 3B). On the other hand, decreases were observed in the CAI compared to vehicle group: -2.17% (not significant) for extract dose 150 mg/kg, -6.52% (P<0.05) for dose 200 mg/kg, -8.70% (P<0.05) for dose 250 mg/kg, -10.87% (P<0.05) for furosemide, and -13.04% (P<0.001) for AHCT (Figure 3C). Overall, the effects on saluretic activity, natriuretic activity, and the CAI (Figures 3A-C) were more marked with increasing doses of extract.

Diuretic and other urine ion indexes

Table 1 presents the effects of *L. hastata* extract on diuretic and other urine ion indexes. Compared to vehicle group, all the extract doses tested increased the natriuretic (5-fold), saluretic (about 2-fold), diuretic (150%), Na^+ (5-fold), K^+ (5-fold), and Cl^- (3-fold) indexes (Table 1). Furosemide, and in a lesser extent AHCT, induced comparable effects (Table 1). Instead, all the extract doses tested, furosemide and AHCT induced decreases in CAI index (about 10%, not statistically significant) (Table 1).

Sub-acute diuretic activity

Cumulated urine volume

Figure 1D shows the effects of *L. hastata* on cumulated urine volume along one week. Vehicle group cumulated urine volume grew linearly, following the equation y=94.13x-88.99 (R^2=0.99) (Figure 1D). Cumulated urine volume grew faster in other groups: 75.83% (P<0.01 vs. vehicle group) faster in animals treated with the extract dose 150 mg/kg (y=165.51x-106.81, R^2=0.99), 81.44% (P<0.01) faster in animals treated with dose 200 mg/kg (y=170.79x-94.24, R^2=0.99), 141.43% (P<0.001) faster in animals treated with dose 250 mg/kg (y= 227.26x-66.86, R^2=0.99), 94.74% (P<0.001) faster in animals treated

with furosemide (y = 183.31x - 81.78, R^2 = 0.99); 54.98% (P<0.05) faster in animals treated with AHCT (y=153.52x-145.88, R^2=0.99) (Figure 1D). Significant differences compared with vehicle group values were observed in all treated groups from day 1 of treatment, with average daily increases of 134.34% (P<0.001) for animals treated with extract dose 150 mg/kg, 279.14% (P<0.001) with dose 200 mg/kg, 469.36% (P < 0.001) with dose 250 mg/kg, 294.27% (P<0.001) with furosemide, and 132.93% (P<0.001) with AHCT (Figure 1D).

Seven days cumulated excretions of K^+, Cl^-, and Na^+

Figures 2D-F show the effects of *L. hastata* extract on cumulated urinary excretions of K^+ (Figure 2D), Cl^- (Figure 2E), and Na^+ (Figure 2F) along one week. The cumulated urinary excretion of K^+ grew linearly: in vehicle group (y=94.24x-90.51, R^2=0.99), in animals treated with the extract dose 150 mg/kg (y=116.61x–53.32, R^2=0.96, 21.31% faster than vehicle group), in animals treated with dose 200 mg/kg (y=128.77x-82.18, R^2=0.98, 33.46% faster), in animals treated with dose 250 mg/kg (y=186.24x-75.67, R^2=0.98, 90.94% faster), in animals treated with furosemide (y=225.04x-237.50, R^2=0.99, 129.74% faster), and in animals treated with AHCT (y=155.11x-77.96, R^2=0.99, 59.81% faster) (Figure 2D). Average daily increases in K^+ cumulated excretions (day 1 to day 7) observed with treatments were: 41.58% (P<0.05 vs. vehicle group) for extract dose 150 mg/kg; 30.19% (P<0.05) for dose 200 mg/kg; 96.76% (P<0.01) for dose 250 mg/kg; 84.05% (P<0.01) for furosemide; and 34.66% (P<0.05) for AHCT (Figure 2D).

The cumulated urinary excretion of Cl^- grew linearly in all treatment groups: vehicle group (y=89.68x-64.51, R^2=0.99), animals treated with the extract dose 150 mg/kg (y=203.98x-20.01, R^2=0.98, 113.18% faster than vehicle group); animals treated with dose 200 mg/kg (y=219.17x-5.83, R^2=0.96, 128.37% faster), animals treated with dose 250 mg/kg (y=258.42x-53.84, R^2=0.98, 167.62% faster), animals treated with furosemide (y=248.5x-74.92, R^2=0.99, 157.70% faster), and animals treated with AHCT (y=217.04x-127.62, R^2=0.99, 126.24% faster) (Figure 2E). Average daily increases in Cl^- cumulated excretions observed with treatments were: 147.07% (P<0.01 vs. vehicle group) for extract dose 150 mg/kg; 138.42% (P<0.01) for dose 200 mg/kg; 174.36% (P<0.001) for dose 250 mg/kg; 161.54% (P<0.01) for furosemide; and 115.75% (P<0.01) for AHCT (Figure 2E).

The cumulated urinary excretions of Na^+ displayed linear increases in all experimental groups, i.e.: in vehicle group (y=82.75x-44.70, R^2=0.99), in animals treated with the extract dose 150 mg/kg (y=193.47x-44.68, R^2=0.99, 109.51% faster than vehicle group), in animals treated with dose 200 mg/kg (y=212.52x-25.72, R^2=0.96, 128.56% faster), in animals treated with dose 250 mg/kg (y=230.95x-2.52, R^2=0.96, 146.99% faster), in animals treated with furosemide (y=256.15x-126.23, R^2=0.99, 172.19% faster), and in animals treated with AHCT (y=193.47x-170.80, R^2=0.99, 109.51% faster), (Figure 2F). Average daily increases in Na^+ cumulated excretions observed with treatments were: 138.83% (P<0.01 vs. vehicle group) for extract dose 150 mg/kg; 119.76% (P<0.01) for dose 200 mg/kg; 170.98% (P< 0.001) for dose 250 mg/kg; 157.28% (P<0.01) for furosemide; and 80.24% (P<0.01) for AHCT (Figure 2F).

Urine biochemical indicators of kidney function

Figures 3D-F show the effects of *L. hastata* extract on creatinine level and clearance (Figure 3D), urea level in urine (Figure 3E), and glomerular filtration rate (GFR) (Figure 3F). Significant increases in creatinine level (2-fold with extract dose 150 mg/kg, (P<0.05) and 4-fold with the other treatments (P<0.01) were observed in all experimental groups compared to vehicle group (Figure 3D). With the exception of the group treated with extract dose 150 mg/kg, statistically significant increases in creatinine clearance were observed in all experimental groups compared to vehicle group (5-fold with extract doses 200 mg/kg and 250 mg/kg, (P<0.01), and 2-fold with furosemide and AHCT, (P<0.05) (Figure 3D).

On the other hand, compared to vehicle group, marked increases in urea where only observed in groups treated with extract doses 150 mg/kg (27.66%, P<0.05 vs. vehicle group), 200 mg/kg (74.17%, P<0.01), and 250 mg/kg (73.80%, P< 0.01) (Figure 3E). Similarly, except for extract dose 150 mg/kg, only groups treated with extract displayed marked increases in GFR (127.5% for dose 150 mg/kg and 140% for 200 mg/kg, P<0.01 vs. vehicle group) (Figure 3F).

Blood creatinine and electrolytes

Figures 3G-I show the effects of *L. hastata* extract on blood levels of urea and creatinine (Figure 3G), Na^+ and Cl^- (Figure 3H), and K^+ (Figure 3I). All the treatments induced decreases in blood creatinine (respectively, blood urea) level compared to vehicle group: -38.10% (P<0.05 vs. vehicle group) (respectively, -4.87%, not significant) with the extract dose 150 mg/kg; -20.63% (P<0.05) (respectively, -24.11%, P<0.05) with dose 200 mg/kg; -23.81% (P<0.05) (respectively,-37.12%, P<0.05) with dose 250 mg/kg; -11.11% (not significant) (respectively, -29.54%, P<0.05) with furosemide; and -12.70% (not significant) (respectively, -26.83%, P<0.05) with AHCT (Figure 3G).

Moreover, compared to vehicle group, treatment with the extract decreased blood Na^+ (respectively, Cl^-) level (Figure 3H): -23.74% (not significant vs. vehicle group) (respectively, -53.43%, P<0.05) with dose 150 mg/kg; -58.73% (P<0.05) (respectively, -73.91%, (P<0.01) with dose 200 mg/kg; -52.38% (P<0.05) (respectively, -69.89%, (P<0.01) with dose 250 mg/kg. Contrarily, increases in blood Na^+ (respectively, Cl^-) level were observed in the other groups compared with vehicle group: 18.87% (not significant) (respectively, 4.58%, not significant) with furosemide; and 93.89% (P<0.01) (respectively, 10.48%, not significant) with AHCT (Figure 3H).

Interestingly, instead decreases in blood K^+ level, compared to vehicle group, were observed with the extract doses 150 mg/kg (-74.24%, P<0.01), 200 mg/kg (-74.82%, P<0.01), and 250 mg/kg (-75.73%, P<0.01), but also with furosemide (12.91%, not significant) and AHCT (-74.82%, P<0.01) (Figure 3I).

DISCUSSION

Our results suggest that *L. hastata* roots have acute and sub-acute diuretic activities in rats. As the diuretic drugs furosemide and amiloride hydrochlorothiazide (AHCT), at all the doses tested the aqueous extract of *L. hastata* increased the volume of urine excreted 3h, 6h, 12h and 24h after treatment, compared to animals receiving the vehicle solution. In addition, the extract increased the 24 h urinary excretions of Na^+, Cl^-, and K^+ also at all the doses tested, in a dose-dependent fashion. Such increase in the elimination of electrolytes in urine and in the volume of urine excreted 3h to 24h after treatment, which typically suggests acute diuretic activity[18,21], was stronger than the effects of furosemide and AHCT. Moreover, the extract increased the saluretic and the natriuretic activities markedly, and induced 5-fold increase in natriuretic index, 2-fold increase in saluretic index, 150% increase in diuretic index, 5-fold increase in Na^+ and K^+ indexes, and 3-fold increase in Cl^- index in the 24 h following the treatment, as furosemide and AHCT. These findings, which also support the acute diuretic activity of the aqueous extract of roots of *L. hastata*, corroborate the established effects of furosemide and AHCT [3,5].

Besides, groups treated with *L. hastata* extract also displayed increases in cumulated urine volume and cumulated urinary excretions of Na^+, Cl^-, and K^+ during one week, comparable and as expected for groups treated with furosemide and AHCT [3-5]. Also supporting diuretic activities, decreases in blood levels of Na^+, Cl^-, and K^+ were observed in extract-treated groups. Unlike the loop diuretic furosemide that mediates its diuretic effects through the inhibition of Na^+ and Cl^- reabsorption in Henle loop ascending branch [4, 5], and the

thiazide diuretic AHCT that acts at the inner medullary collectors to decrease the reabsorption of water, Na^+ and Cl^- [3, 5], the extract increased glomerular filtration rate (GFR), which may indicate an increase in Na^+ concentration in the *macula densa* [22-24].; it increased the urea and K^+ excretions, and decreased their blood levels. Altogether, these findings suggest that aqueous extract of roots of *L. hastata* also have sub-acute diuretic activity, and indicate that the extract may mediate its strong diuretic activities at least partly through other sites than loop diuretic sites of action. Interestingly, the extract also decreased body temperature, carbonic anhydrase inhibition indicator, creatinine blood level, and increased the excretion of creatinine. These findings suggest that the extract could act on the glomeruli (glomerular filtration) [22-25] and warrant mechanistic studies of the diuretic activity of the aqueous extract of roots of *L. hastata*, considering the possibility to unravel a novel class of potent diuretic molecules useful for the treatment of chronic kidney disease. The phytochemical analysis of the aqueous extract of roots of *L. hastata* revealed the presence of various families of molecules with reported diuretic activities, notably flavonoids and triterpenes [18, 19, 21], alkaloids, tannins, and saponins [20, 21, 26], sterols and phenolic compounds [27-29].

CONCLUSION

In this study, levels of Na^+, Cl^-, and in a lesser extent K^+, but also creatinine and urea were decreased in the blood and increased in the urine excreted. These changes, but also the marked increases in the volume of urine excreted that were observed few hours (from 3h) after treatment, were maintained during 7 days of treatment. These effects, which were stronger than furosemide and AHCT effects, suggest that aqueous extracts of *L. hastata* roots have strong acute and subacute diuretic activities. Such activities justify at least partly traditional medicine use to improve high blood pressure, and warrant further studies considering the potential for unraveling novel potent diuretic molecules.

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Abbreviations

%: Percentage; CAI: Carbonic anhydrase inhibition GFR: Glomerular filtration rate; AHCT: Amiloride Hydrochlorothiazide; CNH: Cameroon National Herbarium.

Authors' contributions

NF designed the study and were involved in the drafting and correction of the manuscript, analysis and interpretation of data. It also supervised the study improved the mechanism study and corrected the manuscript. MB carried out the plant selection and collection and performed the experiments. TER provided technical assistance in carrying out biochemical assays, contributed some reagents. VL an SPF helped in recording of diuretic parameters and provided technical assistance. All the authors read the manuscript, critically revised it for important intellectual content and approved the final version of the manuscript.

Conflict of Interest

None declared.

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REFERENCES

1. Al-Lahham S, Jaradat N, Altamimi M, Anabtawi O, Irshid A, AlQub M, et al. Prevalence of underweight, overweight and obesity among Palestinian school-age children and the associated risk factors: a cross sectional study. BMC pediatrics. 2019;19(1):1-3.
2. Mendoza-Herrera K, Pedroza-Tobías A, Hernández-Alcaraz C, Ávila-Burgos L, Aguilar-Salinas CA, Barquera S, et al. Attributable burden and expenditure of cardiovascular diseases and associated risk factors in Mexico and other selected mega-countries. International journal of environmental research and public health. 2019;16(20):4041.
3. Laurent S. Antihypertensive drugs. Pharmacological research. 2017 1;124:116-25.
4. Sica DA, Carter B, Cushman W, Hamm L. Thiazide and loop diuretics. The journal of clinical hypertension. 2011;13(9):639-43.
5. Tamargo J, Segura J, Ruilope LM. Diuretics in the treatment of hypertension. Part 2: loop diuretics and potassium-sparing agents. Expert opinion on pharmacotherapy. 2014 1;15(5):605-21.
6. Menetrier JV, Bonkoski VR, Medeiros KA, Estevan DA, Palozi RA, Livero FA, Velasquez LG, et al. Ethnomedicinal plants used for the treatment of cardiovascular diseases by healers in the southwestern state of paraná, brazil, and their validation based on scientific pharmacological data. Journal of religion and health. 2020;59(6):3004-36.
7. Ntchapda F, Abakar D, Kom B, Nana P, Bonabe C, Kakesse M, et al, Dimo T. Diuretic activity of the aqueous extract leaves of Ficus glumosa Del.(Moraceae) in rats. The Scientific World Journal. 2014 14;2014
8. Ntchapda F, Kakesse M, Fokam MA, Pancha OM, Abakar D, Dimo T, et al. Evaluation of the diuretic effects of crude stem bark extraction of Zanthoxylum heitzii (Rutaceae) in Wistar rats. Journal of integrative medicine. 2015 Sep 1;13(5):326-35.
9. Chukwuma CI, Matsabisa MG, Ibrahim MA, Erukainure OL, Chabalala MH, Islam MS, et al. Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: a review. Journal of ethnopharmacology. 2019 May 10;235:329-60.
10. Mathieu G, Meissa D. Traditional leafy vegetables in Senegal: diversity and medicinal uses. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(4):469-75.
11. Catarino L, Romeiras M M, Bancessi Q, Duarte D, Faria D, Monteiro F, et al. Edible leafy vegetables from West Africa (Guinea-Bissau): Consumption, trade and food potential. Foods. 2019 Oct;8(10):493.
12. Ezike AC, Ufere IK, Akah PA, Ezea SC, Okoli CO. Extracts of Leptadenia hastata Leaf, a Famine Food and Traditional Remedy for Furuncles, Suppress Inflammation in Murine Models. J. of dietary supplements. 2016;13(2):119-35.
13. Nikiema JB, Vanhaelen-Fastre R, Vanhaelen M, Fontaine J, De Graef C, Heenen M. Effects of antiinflammatory triterpenes isolated from Leptadenia hastata latex on keratinocyte proliferation. Phytother res. 2001;15(2):131-4.
14. Malgwi SA, Zango MK, Mbaya AW, Dennis G, Kyari F, Sanda KA, et al. Anti-trypanosomal activity of crude root extract of Leptadenia hastata (Pers) decne in Wistar rats infected with Trypanosoma brucei brucei and associated hematological changes. J Adv Vet Anim Res. 2019; 6(2): 241–246. doi: 10.5455/javar.2019.f339.
15. Saidu Y, Muhammad SA, Abbas AY, Onu A, Tsado IM, Muhammad L, et al. In vitro screening for protein tyrosine phosphatase 1B and dipeptidyl peptidase IV inhibitors from selected Nigerian medicinal plants. J Intercult Ethnopharmacol. 2016 22;6(2):154-157. doi: 10.5455/jice.20161219011346.
16. Sanda KA, Sandabe UK, Auwal MS, Bulama I, Bashir TM, Sanda FA, et al. Hypoglycemic and antidiabetic profile of the aqueous root extracts of Leptadenia hastata in albino rats. Pak J Biol Sci. 2013 15;16(4):190-4.

17. Aquino R, Peluso G, De Tommasi N, De Simone F, Pizza C, et al. New polyoxypregnane ester derivatives from Leptadenia hastata. *J. Nat. Prod.* 1996, 59, 6, 555-564, https://doi.org/10.1021/np960251e.
18. Nascimento YM, Abreu LS, Lima RL, Costa VCO, Melo JIM, Braz-Filho R, et al. Rapid Characterization of Triterpene Saponins from Zornia brasiliensis by HPLC-ESI-MS/MS. Molecules. 2019 ; 24(14): 2519. doi: 10.3390/molecules24142519.
19. Zhang X, Li XY, Lin N, Zhao WL, Huang XQ, Chen Y, Huang MQ, et al. Diuretic activity of compatible triterpene components of Alismatis rhizoma. Molecules. 2017 ;22(9):14-59.
20. Amuthan A, Chogtu B, Bairy KL, Sudhakar, Prakash M, et al. Evaluation of diuretic activity of Amaranthus spinosus Linn. aqueous extract in Wistar rats. J Ethnopharmacol. 2012 27;140(2):424-7. doi: 10.1016/j.jep.2012.01.049. Epub 2012.
21. Ahmed S, Hasan MM, Khan H, Mahmood ZA, Patel S, et al. The mechanistic insight of polyphenols in calcium oxalate urolithiasis mitigation. Biomedicine & Pharmacotherapy. 2018 1;106:1292-9.
22. McClellan JM, Goldstein RE, Erb HN, Dykes NL, Cowgill LD, et al. Effects of administration of fluids and diuretics on glomerular filtration rate, renal blood flow, and urine output in healthy awake cats. Am J Vet Res. 2006;67(4):715-22.
23. Patschan D, Patschan S, Buschmann I, Ritter O. Loop diuretics in acute kidney injury prevention, therapy, and risk stratification. Kidney and Blood Pressure Research. 2019;44(4):457-64.
24. Wang H, Carretero OA, Garvin JL. Inhibition of apical Na+/H+ exchangers on the macula densa cells augments tubuloglomerular feedback. Hypertension. 2003 1;41(3):688-91.
25. Hunter RW, Bailey MA. Hyperkalemia: pathophysiology, risk factors and consequences. Nephrology Dialysis Transplantation. 2019 1;34(Supplement_3):iii2-11.
26. Woldemedhin B, Nedi T, Shibeshi W, Sisay M. Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of the root of Euclea divinorum Hiern (Ebenaceae) in Sprague Dawley rats. J Ethnopharmacol. 2017, 18;202:114-121. doi: 10.1016/j.jep.2017.01.015. Epub 2017.
27. Farzaei MH, Abbasabadi Z, Ardekani MR, Rahimi R, Farzaei F, et al.Parsley: a review of ethnopharmacology, phytochemistry and biological activities. Journal of traditional Chinese medicine. 2013 1;33(6):815-26.
28. Paltinean R, Mocan A, Vlase L, Gheldiu AM, Crișan G, Ielciu I, et al. Evaluation of polyphenolic content, antioxidant and diuretic activities of six Fumaria species. Molecules. 2017;22(4):639.
29. Schlickmann F, de Souza P, Boeing T, Mariano LN, Steimbach VM, et al. Chemical composition and diuretic, natriuretic and kaliuretic effects of extracts of Mimosa bimucronata (DC.) Kuntze leaves and its majority constituent methyl gallate in rats. Journal of Pharmacy and Pharmacology. 2017;69(11):1615-24.

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