The Journal of Phytopharmacology 2022; 11(1):24-31
Online at: www.phytopharmajournal.com

The Journal of Phytopharmacology
(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X
JPHYTO 2022; 11(1): 24-31
January- February
Received: 26-12-2021
Accepted: 22-01-2022
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doi: 10.31254/phyto.2022.11105

Diuretic and antihypertensive activity of the aqueous extract of *Haematostaphis barteri* stem bark in adrenaline-induced hypertensive Wistar Rats

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ABSTRACT

Decoctions of the stem bark of the medicinal plant *Haematostaphis barteri* are used in African traditional medicine to treat hypertension. We assessed the diuretic and antihypertensive activities of *H. barteri* stem bark in Wistar rats. Changes on urine volume and electrolyte elimination induced by acute and subacute treatment of Wistar rats with aqueous extract of the stem bark of *H. barteri* (150, 200 and 250 mg/kg, *per os*). Then, the effects of a week of daily treatments with the extract on indicators of disease progression were assessed in adrenaline-induced hypertensive rats. In a dose-dependent fashion, the extract significantly reduced the urination latency (from 60±2.5 sec in vehicle group to 30±3 sec with dose 250 mg/kg), and increased the urine and electrolyte elimination (more than 2-fold, ($P<0.01$), in both acute and subacute administrations, as furosemide and AHCT. In addition, with stronger activity than propranolol, the extract mitigated the increases in pulse rate (50%), blood urea (90%), LDL, VLDL, and total cholesterol (up to 59%), and decreases in blood triglycerides and HDL (56%). Altogether, our findings suggest that the aqueous extract of *H. barteri* stem bark has diuretic (acute and subacute) and antihypertensive properties, and support the traditional use for the treatment of hypertension.

Keywords: *Haematostaphis barteri*, Diuretic, Electrolytes, Antihypertensive, Adrenaline.

INTRODUCTION

Cardiovascular diseases (CVD) encompass various disorders affecting the heart and blood vessels such as high blood pressure, coronary heart diseases, and cerebrovascular diseases[1,2]. Arterial hypertension is commonly reported as a hallmark of these diseases. Diuretics may treat hypertension successfully with limited undesired effects[3,4]. and therefore, they occupy a prominent place among the drugs used against arterial hypertension. Not surprisingly, medicinal plants with diuretic properties are being reported[5-8]. Various medicinal plants are used to treat arterial hypertension in Cameroonian traditional medicine, mostly plants with diuretic properties[9,10]. However, very few mechanistic studies have evaluated their effect on renal excretion.

Haematostaphis barteri Hook F. (Anacardiaceae) is a dioecious tree with a scaly and brown-gray bark producing edible red-purple berry fruit, which mostly grows on rocky soils [11]. The geographical distribution of this species is limited to West and Central African countries like Ivory Coast, Ghana, Togo, Nigeria, Benin, Gabon, Democratic Republic of Congo, and Cameroon[11]. As supported by several ethnopharmacological works, the stem bark of H. barteri has been used by traditional healers in northern Nigeria, Ghana, and Cameroon for the management of cancer, stomach ache, vomiting, anemia, hemorrhoids, malaria, trypanosomiasis, and hepatitis[12,14]. Phytochemical studies revealed that aqueous extracts of the bark of *H. barteri* contain flavonoids, alkaloids, tannins, and saponins, with 54.5% of fats, 4.11% of crude proteins, 2.4% of crude fibers, and 1% of carbohydrates[15,17]. The aqueous extract of leaves of *H. barteri* was reported anti-inflammatory and antimicrobial properties[16]. Aqueous extracts of leaves and roots were reported in vivo ant plasmodial, anti-inflammatory, and analgesic properties[18,19]. The aqueous extract of H. barteri stem bark was reported antioxidant and in vivo ant plasmodial effects [20, 21] and hepatoprotective activity [22] in laboratory rodents.

The present work aimed at assessing the diuretic and antihypertensive properties of the aqueous extract of the stem bark of *H. barteri* in rat.

MATERIALS AND METHODS

Animals

Thirty male Wistar rats (150.10±3.49 g) were obtained from Yaoundé Pasteur Institute and acclimated for one week before the experiments to the Laboratory of Medicinal Plants, Health and Galenic Formulation, Department of Biological Sciences, University of Ngaoundere. Animals underwent a preliminary screening aimed at selecting only the animals fit for the study as described previously[6,7]. Briefly, rats received 1mL/100 g BW of distilled water and were placed individually in metabolic cages for 24h. The animals excreting a volume of urine 40% of the volume of water received were fit for the study. The animals were single housed in metabolic cages, and they had free access to tap water and standard rat food. The room was under a 12:12h light/dark cycle, with a temperature of 24±2°C and humidity around 45±10%.

All 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.

yield of the extract was calculated and kept under refrigeration in an airtight container after complete evaporation of the solvent until further use.

Plant samples' collection and processing

H. barteri stem bark was harvested in Cameroon Far North region, identified (specimen registration number 36284/HNC, National Herbarium of Yaoundé, Cameroon), and grounded to powder. One kilogram of the stem bark of *H. barteri* was boiled for 20 min in 1 L of distilled water. The solution obtained was filtered with Whatman grade 3 qualitative filter paper. The filtrate was lyophilized at -40°C and 19.5 g of powder was obtained (extraction yield: 1.95%), The extract was stored at -20°C. The powder was used to prepare solutions of aqueous extract.

The bioactive doses 150, 200, and 250 mg/kg of extract were selected for use in the study after preliminary tests of doses 50, 100, 150, 200, and 250 mg/kg in small groups of animals (data not shown). Standard analytical tests for qualitative analysis of families of phytochemicals present were performed on the extract at the National Institute of Medicinal Plants for Medicinal Research (IMPM), Cameroon.

Experimental procedures

All the thirty rats were fit for the study. In the first part of the study, putative acute and subacute diuretic activities of the extract of *H. barteri* were assessed. Animals were randomly distributed in six experimental groups (N=5 per group), namely (*i*) a vehicle group receiving distilled water (p.o.) (*ii*) two positive control groups administered with furosemide (20 mg/kg, p.o.) or amiloride hydrochlorothiazide (AHCT, 20 mg/kg, p.o.) and (*iii*) three test groups receiving the *H. barteri* aqueous extract *per os* (administered with a feeding needle in 0.5 ml/100 g BW) at doses 150, 200, and 250 mg/kg. For subacute diuretic properties assessment, after the first treatment (Day 1), urine volume was measured every 3h, and 24h cumulated urine was processed for the assessment of indicators of acute diuretic activity such as the latency to urination, concentrations of Na^+, K^+, and $Cl^–$, carbonic anhydrase inhibition, saluretic and natriuretic activities. In addition, levels of Na^+, K^+, and Cl^- were assessed in blood samples collected from tail vein 24-h after treatment the first treatment. Core body temperature was determined using a rectal thermometer 1h before and 5h after the first treatment. To assess the subacute diuretic properties of the extract, animals received the same treatments as before, once daily for 6 additional days (a total of 7 days). Urine 24h volume and electrolyte concentrations (Na^+, K^+, $Cl^–$) were determined. Then, animals were allowed two weeks to recover.

In the second phase of the study, the putative antihypertensive activity of the extract of *H. barteri* were assessed using the same animals, now randomly distributed in six new experimental groups (N=5 per group), i.e., 5 groups made hypertensive by intraperitoneal injection under gas anesthesia of adrenaline (0.1 mL), for five consecutive days, and a normotensive control group that was untouched. Increases in pulse rate at rest was used as indicator of successful induction of hypertension. The pulse rate was assessed using a simple clinical method. Briefly, each rat was restrained gently and the examiner placed a finger over rat femoral artery and counted the beats for one minute[23].

Afterwards, the normotensive control and a hypertensive group (hypertensive control group) received normal saline solution (p.o.) a hypertensive group (positive control group) was administered with the hypertensive drug propranolol (5 mg/kg, p.o.), and (*iii*) three hypertensive groups were treated with doses 150, 200, and 250mg/kg of the aqueous extract of *H. barteri* for 1 week. The body weight, serum cholesterol, triglycerides, urea, creatinine, and transaminases were measured. Animals were sacrificed under deep anesthesia and blood was collected intracardially on treatment day 7. Organs were harvested and stored for future studies.

Biochemical tests

Standard biochemical assays were used to determine the Na^+, K^+, and Cl^- levels (flame photometry), transaminases, urea and creatinine levels (two-way digital spectrophotometer) (RS 232, Secomam, France) in urine and sera. Carbonic anhydrase inhibition (Cl/(Na^+K)), saluretic activity (Na^+Cl), and natriuretic activity (Na/K) were calculated. Glomerular filtration rate (GFR) was determined from the creatinine clearance. Urine pH was measured using a pH meter. Serum triglyceride and cholesterol levels were determined using Randox TR 210 kit and Randox CH 200 kit following manufacturer's instructions (Randox Laboratories, Crumlin, UK).

Statistical analyses

ANOVA followed by LSD test was used to assess the statistical significance of differences in the variables studied between: (*i*) the vehicle group and groups treated with furosemide, and with AHCT (positive control groups), or with the extract doses (test groups); and (*ii*) the normotensive group, the hypertensive control group, and the hypertensive groups treated with propranolol (positive control group), or with the extract doses (test groups). Differences with ($P<0.05$) were significant. Data were presented as mean ± SEM.

RESULTS

Assessment of acute diuretic activity

Urination, urine pH, and body temperature

Figure 1 shows the effects of the first daily treatment with *H. barteri* extract on the latency to urination (Figure 1A), 24-h cumulated urine volume (Figure 1B), urine pH (Figure 1C), and core body temperature (Figure 1D). Vehicle group animals urinated 60±2.5 sec after receiving the vehicle solution (Figure 1A). The latency to urination was reduced significantly after administration of the extract (30±3 sec with dose 250 mg/kg, ($P<0.05$) vs. vehicle group; 40±1.5 sec and 32±4.5 sec with doses 150 and 250, respectively, not statistically significant), furosemide and AHCT (23±2 sec and 25±1.5 sec, respectively, ($P<0.05$), vs. vehicle group) (Figure 1A).

Increases in urine volume were observed: (*i*) 3h after administration of furosemide (10-fold), AHCT (7-fold) ($P<0.01$), or extract at doses 150, 200, and 250 mg/kg (2-fold); (*ii*) 6h (cumulated) after administration of furosemide (3-fold), AHCT (3-fold), or extract at

doses 150 mg/kg (68.23%), 200 mg/kg (85.71%), and 250 mg/kg (154.80%) (P<0.05), (*iii*) 12h (cumulated) after administration of furosemide (120.45%), AHCT (190.13%), or extract at doses 150 mg/kg (81.08%), 200 mg/kg (96.85%), and 250 mg/kg (182.10%), (P<0.05), and (*iv*) 24h (cumulated) after administration of furosemide (85.87%), AHCT (115.82%), or extract at doses 150 mg/kg (113.43%), 200 mg/kg (122.90%), and 250 mg/kg (148.93%), (P<0.01), (Figure 1B).

On the other hand, pH values comparable to vehicle group values were observed in all the experimental groups (Figure 1C). Finally, statistically significant decreases in body temperature were observed in animals treated with the extract compared to vehicle group (-288.24% for 150 mg/kg, -341.18% for 200 mg/kg, -247.06% for 250 mg/kg, (P<0.05), vs. vehicle group) (Figure 1D). On the other hand, body temperatures of animals treated with amiloride and furosemide were only slightly different from vehicle group values (-11.77% and 41.18%, respectively, (P>0.05), vs. vehicle group), (Figure 1D).

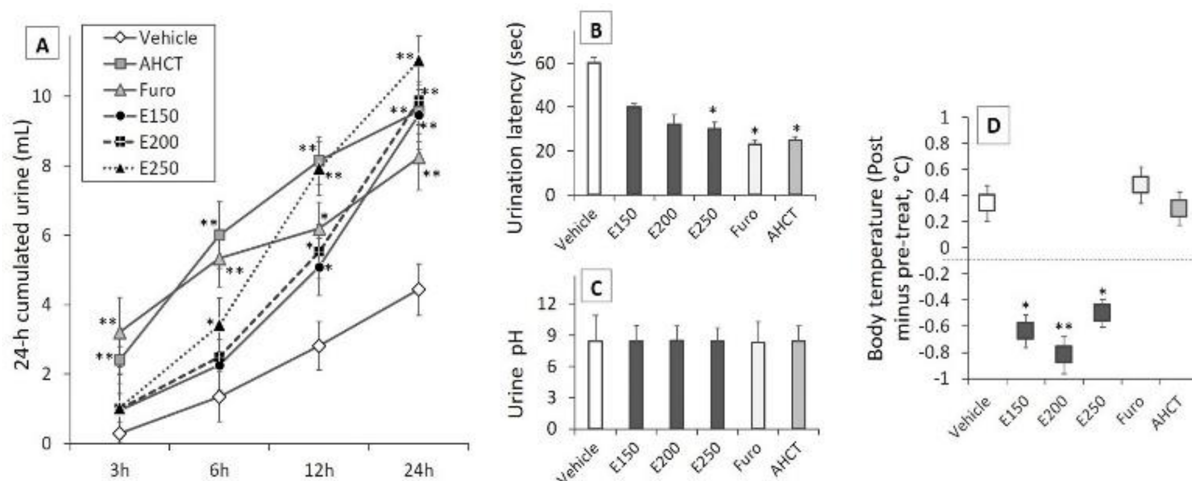

Figure 1: Urination, urine pH, and body temperature Effect of *H. barteri* extract on the latency to urination (A), 24-h cumulated urine volume (B), urine pH (C), and core body temperature (D). Note the decreases in the latency to urination (A), the marked increases in 24-h cumulated urine volume 12-h after treatment (B), comparable pH values in all groups (C), and the marked decreases in body temperature (D). AHCT: amiloride hydrochlorothiazide. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. Furo: furosemide. Data are mean ± SEM, N = 5. ANOVA+LSD test: *P<0.05, ***P<0.001 vs. vehicle group.

Table 1: Urine electrolytes and kidney function indicators 24-h after treatment

	Vehicle	E150	E200	E250	Furosemide	AHCT
Na+, Cl-, and K+ levels						
Na^+ (mEq/kg)	30.4±0.2	161.4±15.1*	164.7±16.7*	170.3±5.7*	185.5±2.8*	99.5±1.2*
K^+ (mEq/kg)	12.7±2.2	23.1±2.6*	24.6±2.8*	27.8±2.9*	39.9±3.5*	17.1±2.8
Cl^- (mEq/kg)	26.7±1.5	103.3±33.8*	120.3±44.4*	143.6±18.0*	129.9±1.2*	68.1±1.4*
CAI, saluretic and natriuretic activities						
Saluretic activity	57.3±1.8	264.7±5.7*	284.9±5.7*	313.7±4.4*	312.5±3.9*	167.6±2.6*
Natriuretic activity	1.4±0.1	7.0±0.7*	6.7±0.5*	6.1±1.2*	4.6±0.8*	5.8±0.4*
CAI	0.6±1.4	0.6±0.8	0.6±1.0	0.7±0.2	0.6±0.2	0.6±0.3
Kidney function indicators						
Creatinine (mg/dL)	0.25±0.5	0.9±0.9	0.9±0.4	1.1±0.8*	1.8±0.05*	1.34±0.05*
Creatinine clearance (mL/min)	0.03±0.01	0.7±0.8*	0.3±0.1*	0.4±0.1*	0.1±0.02	0.1±0.02
GFR (mL/min)	0.4±0.1	0.5±0.9	0.9±0.2*	0.5±0.1	0.6±0.07*	0.5±0.07
Urea (mg/dL)	12.1±2.1	23.5±12.9	23.0±20.0	25.6±12.7*	24.7±1.7	22.4±1.3

AHCT: amiloride hydrochlorothiazide. CAI: carbonic anhydrase inhibition. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. GFR: glomerular filtration rate. Data are mean ± SEM, N = 5. ANOVA+LSD test: *P<0.05, **P<0.01, ***P<0.001 vs. vehicle group.

Electrolyte excretion in urine and kidney function indicators

Table 1 presents urine Na^+, K^+, and Cl^- levels, carbonic anhydrase inhibition (CAI), saluretic and natriuretic activities, and kidney function indicators. From dose 150 mg/kg onward and in a dose-dependent fashion, the extract induced significant increases in urine Na^+, K^+, and Cl^- levels (P<0.05), vs. vehicle group comparable to furosemide, and with the exception of K^+ that did not change markedly, to AHCT (Table 1). Saluretic and natriuretic activities, but not CAI, displayed 4-fold and 7-fold increases, respectively, with all treatments (P<0.05), vs. vehicle group (Table 1).

Moreover, the extracts induced changes in kidney function indicators (Table 1). Creatinine level was increased significantly (P<0.01), vs. vehicle group by furosemide (5-fold), AHCT (3-fold), and the extract, particularly at dose 250 mg/kg (266%) (Table 1). Creatinine clearance was also increased by furosemide (2-fold) and AHCT (166.67%), but significant (P<0.001), were only observed with extract doses 150 mg/kg (22-fold), 200 mg/kg (9-fold), and 250 mg/kg (12-fold) (Table 1). Compared to vehicle group, glomerular filtration rate (GFR) was increased by all treatments, but statistically significant (P<0.05), differences were observed only with extract dose 250 mg/kg (125%) and furosemide (50%), (Table 1). Noticeable increases were observed in urea level compared to vehicle group: 94.21% for extract dose 150 mg/kg, 90.08% for dose 200 mg/kg, 111.57% for dose 250 mg/kg, 104.13% for furosemide and 85.12% for AHCT (Table 1). However, these increases were not statistically significant due to a high interindividual variability.

Blood creatinine, urea, and electrolytes

Table 2 presents blood levels of creatinine, urea, Na^+, K^+, and Cl^- 24-h after treatment. Compared to vehicle group, significant decreases ($P<0.05$) were observed: (*i*) in Na^+ level following treatment with extract doses 150 mg/kg (-73.15%), 200 mg/kg (-74.40%), and 250 mg/kg (-70.78%), with furosemide (-48.59%), and with AHCT (-55.72%); (*ii*) in K^+ level following treatment with extract doses 150 mg/kg (-33.79%), 200 mg/kg (-31.53%), and 250 mg/kg (-25.76%), with furosemide (-45.88%), but not with AHCT (-55.72%), and (*iii*) in Cl^- level following treatment with extract doses 150 mg/kg (-78.24%), 200 mg/kg (-78.17%), and 250 mg/kg (-73.51%), with furosemide (-45.88%), and with AHCT (-53.43%), (Table 2).

Compared to vehicle group, significant decreases ($P<0.05$) were observed in blood creatinine level following treatment with extract doses 150 mg/kg (-61.90%), 200 mg/kg (-61.90%), and 250 mg/kg (-87.30%), but not with furosemide (-11.11%) or AHCT (9.52%) (Table 2). Instead, compared to vehicle group, significant decreases ($P< 0.01$) were observed in blood urea level following treatment with extract doses 150 mg/kg (-62.90%), 200 mg/kg (-61.16%), and 250 mg/kg (-64.32%), with furosemide (-83.23%), and AHCT (-81.94%) (Table The qualitative phytochemical analysis was performed[17].

Assessment of subacute diuretic activity

Body weight

Table 3 presents the effects on the body weight of 7 days of daily treatments with the aqueous extract of *H. barteri* bark. All the treatments administered induced increasingly marked decreases in body weight, with significant decreases following treatments with extract dose 150 mg/kg (-19.87 vs. vehicle group and -17.52% vs. day 1, ($P<0.05$) and with dose 250 mg/kg in treatment day 6 (-17.62 vs. vehicle group and -18.53% vs. day 1, ($P<0.05$) and treatment day 7 (-20.53 vs. vehicle group and -21.40% vs. day 1, ($P<0.05$) (Table 3).

Cumulated urine volume

Table 4 presents the effects on the urine volume of 7 days of daily treatments with the aqueous extract of *H. barteri* bark. The cumulated urine volume displayed marked increases with all the treatments tested compared to vehicle group ($P<0.05$) as follows: 84% (day 1) to 159.57% (day 7) with extract dose 150 mg/kg, 95.04% to 136.53% with dose 200 mg/kg, 117.81% to 216.44% with dose 250 mg/kg, 63.98% to 193.95% with furosemide, and 78.97% to 202.57% with AHCT (Table 4). The cumulated urine volume also displayed marked increases ($P<0.05$) in all the groups compared to treatment day 1: (*i*) 74.69% on day 3 to 180.38% on day 7 in the vehicle group; (*ii*) 66.84% on day 3 to 294.95% on day 7 in the group treated with extract dose 150 mg/kg; (*iii*) 66.35% on day 3 to 240.03% on day 7 in the group treated with extract dose 200 mg/kg; (*iv*) 53.42% on day 2 to 307.35% on day 7 in the group treated with extract dose 250 mg/kg; (*v*) 95.22% on day 2 to 402.61% on day 7 in the group treated with furosemide; and (*vi*) 68.98% on day 2 to 374.02% on day 7 in the group treated with AHCT (Table 4).

Table 2: Blood levels of creatinine, urea, Na^+, K^+, and Cl- 24-h after treatment

	Vehicle	E150	E200	E250	Furosemide	AHCT
Creatinine (mg/dL)	0.63±0.31	0.24±0.10*	0.24±0.19*	0.08±0.31*	0.56±0.16	0.69±0.08
Urea (mg/dL)	15.50±0.14	5.75±1.26*	6.02±1.14*	5.53±0.65*	2.60±0.14*	2.80±0.14*
Na+ (mEq/L)	28.75±0.34	7.72±3.27*	7.36±3.00*	8.4±2.07*	14.78±1.76*	12.73±0.50*
K+ (mEq/L)	27.02±0.51	17.89±1.59*	18.50±1.75*	20.06±2.36	15.72±1.39*	28.18±0.39
Cl- (mEq/L)	13.10±1.61	2.85±0.53*	2.86±0.51*	3.47±0.74*	7.09±0.51*	6.10±0.83*

AHCT: amiloride hydrochlorothiazide. ALT: alanine aminotransferase. AST: aspartate aminotransferase. TC: total cholesterol. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. Data are mean ± SEM, N = 5. ANOVA+LSD test: *$P<0.05$ vs. vehicle group.

Table 3: Body weight changes over 7 days of daily treatments

	Vehicle	E150	E200	E250	Furosemide	AHCT
Day 1	150.4±13	147.2±13.4	145.6±4.7	153.2±5.6	154.8±13.5	149.4±11.3
Day 2	151.8±12.8	145±10.3	145.8±5.6	151.6±12.5	151.6±13.7	146.8±12.6
Day 3	149.8±13.8	143.8±10.1	144±6.28	140.2±7.8	141.8±15.6	143.2±7.6
Day 4	150.2±11.1	141.8±8.6	143.2±5.5	138±5.7	140.2±17.6	140.2±7.1
Day 5	150±12.1	133.2±9.6	131±9.9	130.4±9.2	140.6±16.3	141.4±6.3
Day 6	151.5±10.3	124.2±4.02^{b}	130.6±2.5	124.8±7.4^{ab}	144.8±19.9	135.6±12.3
Day 7	151.5±12.3	121.4±4.4^{ab}	129.4±6.3	120.4±6.2^{ab}	144.6±15.0	135.6±3.5

AHCT: amiloride hydrochlorothiazide. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. Data are mean ± SEM, N = 5. ANOVA+LSD test: $P<0.05$ vs. a day 1 or b vehicle group.

Table 4: Cumulated urine volume (mL/kg BW) over 7 days of daily treatments

	Vehicle	E150	E200	E250	Furosemide	AHCT
Day 1	17.74±2.4	32.69±7.4^{b}	34.60±8.2^{b}	38.64±3.5^{b}	29.09±3.9^{b}	31.75±2.4^{b}
Day 2	22.99±1.3	41.8±2.3^{b}	44.65±2.1^{b}	59.28±5.1^{ab}	56.79±2.5^{ab}	53.65±2.2^{ab}
Day 3	30.99±3.5^{a}	54.54±3.2^{ab}	57.56±2.4^{ab}	75.34±3.2^{ab}	68.89±3.2^{ab}	72.45±3.5^{ab}
Day 4	34.50±6.2^{a}	69.29±5.6^{ab}	69.1±6.1^{ab}	90.31±4.2^{ab}	92.33±6.4^{ab}	91.45±4.3^{ab}
Day 5	40.50±3.5^{a}	87.26±6.4^{ab}	82.67±4.5^{ab}	108.2±6.5^{ab}	105.53±4.3^{ab}	103.45±5.3^{ab}
Day 6	44.75±2.4^{a}	106.11±8.1^{ab}	92.59±2.1^{ab}	125.8±3.2^{ab}	127.85±3.2^{ab}	111.08±1.5^{ab}
Day 7	49.74±1.9^{a}	129.11±2.4^{ab}	117.65±2.8^{ab}	157.4±6.3^{ab}	146.21±4.3^{ab}	150.5±4.6^{ab}

AHCT: amiloride hydrochlorothiazide. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. Data are mean ± SEM, N = 5. ANOVA+LSD test: $P<0.05$ vs. a day 1 or b vehicle group.

Electrolyte excretion

Figure 2 shows the effects of 7 days of daily treatments with the aqueous extract of *H. barteri* bark on the urinary excretion of Na^+ during day 1 (Figure 2A), day 4 (Figure 2B), and day 7 (Figure 2C); of K^+ during day 1 (Figure 2D), day 4 (Figure 2E), and day 7 (Figure 2F); and Cl^- during day 1 (Figure 2G), day 4 (Figure 2H), and day 7 (Figure 2I). Significant increases in excretions of Na^+ and Cl^- were observed in all experimental groups compared to vehicle group on days 1, 4 and 7 (Figures 5A-C and 5G-I). Instead, marked increases (P<0.05 vs. vehicle group) in the excretion of K^+ were observed: (*i*) in all experimental groups on day 1; (*ii*) in groups treated with the extract at dose 250 mg/kg, furosemide, and AHCT on day 4; and (*iii*) in groups treated with the extract at doses 200 and 250 mg/kg, furosemide, and AHCT on day 7 (Figures 5D-F).

Assessment of antihypertensive activity

Body weight and pulse rate

Figure 3 shows the effects of 1 week of treatment with *H. barteri* extract and propranolol on the body weight (Figure 3A). and pulse rate (Figure 3B) of hypertensive animals. Hypertensive control group displayed a decrease of 17.61% in body weight compared to normotensive animals (P<0.05) (Figure 3A). No other group displayed a significant difference with normotensive group. Instead, significant increases (P<0.05) were observed compared to hypertensive control group in animals treated with extract dose 150 mg/kg (20.15%), 200 mg/kg (22.22%), dose 250 mg/kg (18.44%), and propranolol (25.52%) (Figure 3A).

In addition, hypertensive control animals displayed an increase of 50.65% in pulse rate compared to normotensive animals (P<0.01), and no other group displayed a significant difference with normotensive group. Moreover, significant decreases (P<0.05) were observed compared to hypertensive control group in animals treated with extract dose 150 mg/kg (-31.55%), 200 mg/kg (-31.55%), dose 250 mg/kg (-33.45%), and propranolol (-34.83%) (Figure 3B).

Blood creatinine, urea, and transaminases

Figures 4 and 5 show the effects of 1 week of treatment with *H. barteri* extract and propranolol on blood creatinine (Figure 4A), urea (Figure 4B), blood aspartate aminotransferase (ASAT) (Figure 5A), and alanine aminotransferase (ALAT) (Figure 5B) of hypertensive animals. No statistically significant inter-group difference was observed in creatinine levels, although levels were higher in hypertensive control animals compared to the other groups (Figure 4A). Compared to normotensive control animals, increases (P<0.05) in urea were observed in hypertensive control animals (89.57%) and hypertensive animals treated with extract dose 150 mg/kg (69.53%) (Figure 4B). Compared to hypertensive control animals, marked decreases (P<0.05) in urea were observed in hypertensive animals and animal treated with extract doses 200 (-47.39%) and 250 (-58.24%) mg/kg, and propranolol (-50.14%) (Figure 4B).

Slight increases were observed in ASAT and ALAT in hypertensive control animals compared to normotensive animals (Figures 5A,B). Compared to hypertensive control animals, a significant decrease (P<0.05) in ASAT was observed in hypertensive animals treated with extract dose 250 mg/kg (-45.72%) (Figure 5A).

Assessment of effect of extracts on viability of MCF-7 cells and Calculation of IC_{50}.

The MTT assay was done using methanolic extracts of tubers of *A. racemosus* in MCF-7 cells as per [18]. The T25 flask with MCF-7 cells on attaining 70-80 per cent confluency was trypsin zed and seeded in a 96 well plate and exposed to 1280, 640, 320, 160, 80, 40, 20 and 10 µg/mL concentrations of the extract. After 24 hours of incubation with the extract, the media were carefully pipetted out and ten microliters of MTT (5 mg/mL prepared in DPBS) was added to all wells including blanks and covered with aluminium foil and incubated at 37°C for 4 hours, in CO_2 incubator. After incubation, the media containing MTT was removed. Added 200 µL of DMSO to all the wells to dissolve to formazan crystals formed. The plates were gently agitated on orbital shaker for 10 minutes. The absorbance was measured using microplate reader (Varioskan Flash, Thermistor Scientific, Finland) at a wavelength of 570 nm. Per cent cell viability was found out using the formulae and IC_{50} was calculated using online software My curvefit.com.

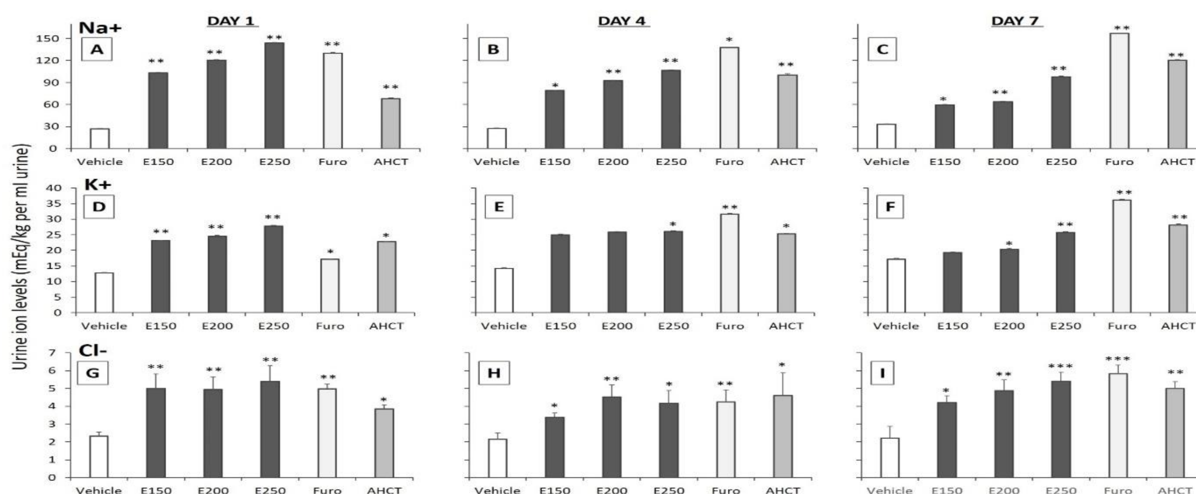

Figure 2: Subacute electrolyte excretion in urine. Cumulated excretion in the urine of Na^+ during day 1 (A), day 4 (B), and day 7 (C); K^+ during day 1 (D), day 4 (E), and day 7 (F); and Cl^- during day 1 (G), day 4 (H), and day 7 (I). Note the increases in groups treated with extract doses 150 mg/kg (E150), 200 mg/kg (E200), and 250 mg/kg (E250), amiloride hydrochlorothiazide (AHCT), and furosemide (Furo). Data are mean ± SEM, N = 5. ANOVA+LSD test: *P<0.05, **P<0.01, ***P<0.001 vs. vehicle group.

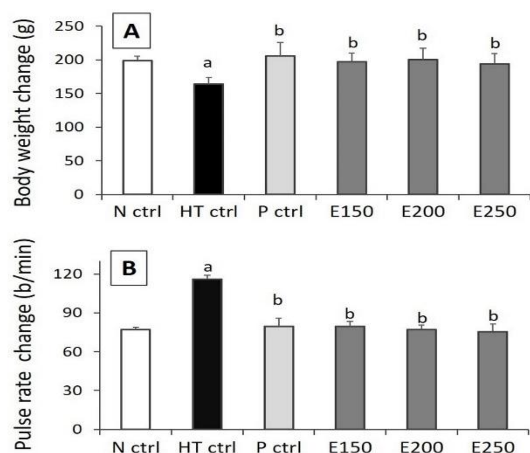

Figure 3: Weight and pulse rate

Effects of *H. barteri* extract on the body weight (**A**) and pulse rate (**B**) of hypertensive animals. Note that the marked decrease in body weight (A) and increase in pulse rate (B) were prevented in hypertensive animals by the extract up to values comparable to normotensive (N ctrl) and positive (P ctrl) control groups. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. HT ctrl: hypertensive control group. Data are mean ± SEM, N = 5. ANOVA+LSD test, P<0.05: [a] vs. normotensive control, [b] vs. hypertensive control.

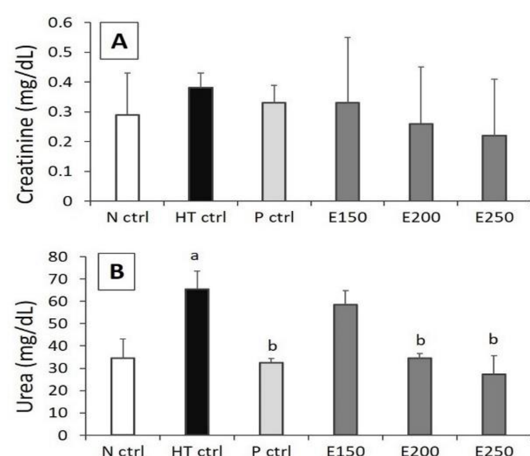

Figure 4: Blood levels of creatinine and urea

Effects of *H. barteri* extract on blood levels of creatinine (**A**) and urea (**B**) of hypertensive animals. Note the comparable levels of creatinine (A) and the prevention of increases in urea level (B) in the hypertensive groups treated with the extract up to values comparable to the normotensive (N ctrl) and positive (P ctrl) control groups. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. HT ctrl: hypertensive control group. Data are mean ± SEM, N = 5. ANOVA+LSD test, P<0.05: [a] vs. normotensive control, [b] vs. hypertensive control.

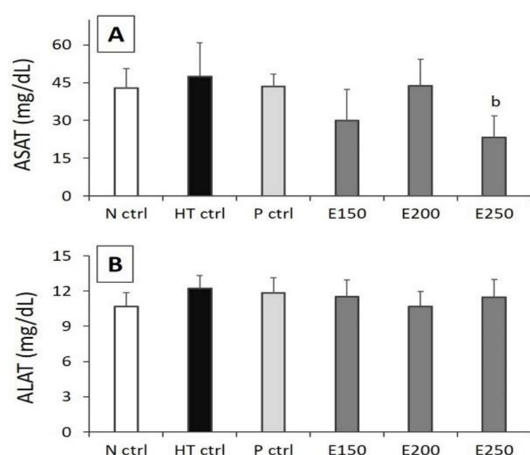

Figure 5: Blood levels of transaminases

Effects of *H. barteri* extract on blood levels of aspartate aminotransferase (ASAT) (**A**) and alanine aminotransferase (ALAT) (**B**) of hypertensive animals. Note the decrease in ASAT (**A**) and the comparable levels of ALAT (**B**) in the hypertensive groups treated with the extract. **E150, E200, E250**: extract doses 150, 200 and 250 mg/kg. **HT ctrl**: hypertensive control. **N ctrl**: normotensive control. **P ctrl**: positive control groups. Data are mean ± SEM, N = 5. ANOVA+LSD test, P<0.05: [a] vs. normotensive control, [b] vs. hypertensive control.

Blood triglycerides and cholesterol

Table 5 shows the effects of 1 week of treatment with *H. barteri* extract and propranolol on blood triglycerides and cholesterol in hypertensive animals. Significant decreases (P<0.05) were observed in triglycerides in all the hypertensive groups compared to the normotensive control group (from -37.80% to -56.25%), however, with a marked decrease in the group treated with extract dose 250 mg/kg compared to the hypertensive control group (-29.66%, (P<0.05) (Table 5). Similarly, compared to normotensive control group, significant decreases (P<0.05) were observed in HDL level in hypertensive control group (-37.80%) and despite treatment with extract dose 150 mg/kg (-26.65%) and propranolol (-25.74%), but not with extract doses 200 mg/kg (-6.98% vs. normotensive control group and 43.09%↑ compared to hypertensive control group) and 250 mg/kg (3.71% vs. normotensive control group and 59.53%↑ compared to hypertensive control group) (Table 5).

A Significant increase (277.93%) (P<0.001), was observed in LDL level of hypertensive control animals compared to the normotensive group (Table 5). LDL level increase was mitigated in hypertensive animals treated with extract doses 200 mg/kg (43.09%) and 250 mg/kg (59.54%) (P<0.05), and in a lesser extent dose 150 mg/kg (12.83%) and propranolol (14.25%) (Table 5). VLDL level was slightly increased in hypertensive control animals compared to normotensive control group (7.46%), while this parameter was markedly decreased (P<0.05) compared to both hypertensive and normotensive control groups following treatment with any of the extract doses (-47.22% to -59.03% and -43.28% to -55.97%, respectively) or propranolol (-31.25% and -26.11%, respectively) (Table 5).

A marked increase in total cholesterol level was observed in hypertensive control animals (28.31), (P<0.05) vs. normotensive group), but not in hypertensive animals treated with extract doses 150 mg/kg, 200 mg/kg, and propranolol (-27.55%, -17.31%, and -30.49%, respectively, vs. hypertensive control group, (P < 0.05), (Table 5).

The ratio of LDL to HDL level showed marked increases (4-fold, P<0.001) vs. normotensive group), in hypertensive control animals (Table 5). Increases in LDL/HDL ratio was mitigated following treatment but not to normotensive values: -57.24% to -67.71% with extract doses, and -71.05% with propranolol vs. hypertensive control group (P<0.05) (and -88.46% to 150%↑, and 69.23% ↑, respectively, vs. normotensive control group, (P<0.01), (Table 5). Finally, the ratio of total cholesterol to HDL level showed a marked increase (P<0.05) vs. normotensive group), which was mitigated to levels comparable with normotensive group values following treatment with extract doses 150, 200, 250 mg/kg, and propranolol (-35.82%, -42.16%, -38.81%, and -39.18%, respectively, vs. hypertensive control group, (P<0.05), (Table 5).

Phytochemical analysis

Qualitative phytochemical analysis revealed the presence of flavonoids, tannins, sterols, triterpenes, saponins, anthraquinones, and phenolic compounds in the aqueous extract of *H. barteri* bark.

DISCUSSION

Our results suggest that the aqueous extract of *H. barteri* stem bark had acute and subacute diuretic activities in rats and induced antihypertensive effects in adrenaline-induced hypertensive rats. Supporting typical acute diuretic activity[24,27], after single treatment with *H. barteri* extract, the latency to urination was reduced significantly in a dose-dependent fashion (from 60±2.5 sec in vehicle group to 30±3 sec following treatment with dose 250 mg/kg). Subacute diuretic activity of the aqueous extract of *H. barteri* bark was confirmed by the maintenance of higher urine volumes and marked urinary excretion of Na^+, Cl^-, and K^+, over 7 days of daily treatments, conversely, with increasingly marked losses in body

Table 5: Blood triglycerides and cholesterol changes in hypertensive animals

	N ctrl	HT ctrl	E150	E200	E250	Propranolol
Triglycerides (mg/dL)	67.2±2.2	41.8±4.9[a]	38.2±3.7[a]	36.8±8.2[a]	29.4±9.9[ab]	40.7±4.8[a]
HDL (mg/dL)	140.3±4.7	91.2±8.1[a]	102.9±4.5[a]	130.5±1.7[b]	145.5±3.7[b]	104.2±3.4[a]
LDL (mg/dL)	36.7±2.4	138.7±6.5[a]	66.5±6.2[ab]	64.1±6.2[ab]	86.2±7.6[ab]	55.7±1.9[ab]
VLDL (mg/dL)	13.4±0.4	14.4±0.9	7.6±0.7[ab]	7.4±1.6[ab]	5.9±1.9[ab]	9.9±0.9[ab]
TC (mg/dL)	190.4±5.1	244.3±18.6[a]	177.0±6.9[b]	202.0±3.8	237.6±5.6[a]	169.8±4.1[b]
Ratio LDL/HDL	0.26±0.5	1.52±0.8[a]	0.65±1.3[ab]	0.49±0.6[ab]	0.59±2.0[ab]	0.44±0.6[ab]
Ratio TC/HDL	1.36±1.2	2.68±1.0[a]	1.72±1.5[ab]	1.55±2.2[b]	1.64±2.8[b]	1.63±1.2[b]

AHCT: amiloride hydrochlorothiazide. HT ctrl: hypertensive control group. N ctrl: normotensive control group.TC: total cholesterol. E150, E200, E250: extract doses 150, 200 and 250 mg/kg. Data are mean ± SEM, N = 5. ANOVA+LSD test: *P<0.05 vs. vehicle group.

weight. As expected, similar findings were observed with the established diuretic drugs furosemide and amiloride hydrochlorothiazide (AHCT)[3,4,28]. including the effective sparing of K^+ by the latter. However, overall, in this study, the effects of the extract at the doses tested were stronger than the effects of furosemide and AHCT. The phytochemical analysis of the aqueous extract of *H. barteri* revealed the presence of various families of molecules with reported diuretic activities, notably tannins, and saponins[26,27,29]. flavonoids and triterpenes[24,25,27]. sterols and phenolic compounds[30,32]. This chemical composition was comparable to previous reports[15,17].

Unlike the thiazide diuretic AHCT that acts at the inner medullary collectors to decrease the reabsorption of water, Na^+ and Cl^- [3,4]. and the loop diuretic furosemide that mediates its diuretic effects through the inhibition of Na^+ and Cl^- reabsorption in Henle loop ascending branch[4,28]. the extract increased glomerular filtration rate (GFR), which may indicate a potential increase in Na^+ concentration in the macula densa[33,35]. These findings suggest that *H. barteri* extract may mediate its strong acute and subacute diuretic activities at least partly through other sites than loop diuretic sites of action. In addition, the extract decreased the body temperature, creatinine blood level (with increases in the urinary clearance of creatinine), and similar to AHCT, it increased the urea and K^+ excretions, with decreased blood levels. These findings suggest that the extract may mediate its effects partly by modulating the glomerular filtration[33,36]. and indicate that the extract contains potent diuretic molecules that may prove useful in chronic kidney diseases.

Following the confirmation of the acute and subacute diuretic activities, in the second phase of the present study, we assessed the effects of the extract of *H. barteri* on the progression of arterial hypertension signs in rats made hypertensive-like by intraperitoneal injections (under gas anesthesia) of adrenaline for five consecutive days. At the end of this induction-phase, animals displayed increases in pulse rate that reached more than 50% in the hypertensive control group during the subsequent week (treatment week for the other groups) compared to normotensive animals. In addition, hypertensive control animals displayed up to 20% decreases in body weight, 89.57% increases in blood urea levels, 56% decreases in blood triglycerides and HDL levels (with concomitant increases in LDL, VLDL, and total cholesterol levels), as well as slight increases in blood creatinine and ASAT and ALAT levels. Although not always to levels comparable to normotensive control group values and as for propranolol, a week of treatment with the extract of *H. barteri* improved all these signs, suggesting that the extract mitigated the progression of arterial hypertension in rats and improved animal condition. Altogether, these findings support that the extract of *H. barteri* has potent diuretic and antihypertensive properties in adrenaline-induced hypertensive rats.

CONCLUSION

In the present study, the volumes of urine excreted were increased and levels of Na^+, Cl^-, K^+, creatinine and urea were decreased in the blood and increased in the urine 24h after single treatment and over 7 days of daily treatments with *H. barteri*. A week of treatment with *H. barteri* improved the condition of adrenaline-induced hypertensive animals and, as compared to hypertensive control group, mitigated the increases in pulse rate, in blood urea, creatinine, LDL, VLDL, and total cholesterol levels, and decreases in body weight, blood triglyceride and HDL levels. These effects, which suggest that aqueous extracts of *H. barteri* have strong acute and subacute diuretic, and antihypertensive activities, were stronger than furosemide and AHCT effects (for diuretic activity) and propranolol (for antihypertensive activity), and justify at least partly the use in African traditional medicine against arterial hypertension and related cardiovascular diseases.

Acknowledgments

This study was partly funded by the Faculty of Science, University of Ngaoundere. Authors acknowledge the support of Cameroon National Institute of Medicinal Plants for Medicinal Research (IMPM) for qualitative phytochemical analysis of the extract, and thank the colleagues of their institutions for proof reading the article.

Conflict of Interest

None declared.

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HOW TO CITE THIS ARTICLE

Ntchapda F, Talla ER, Adjia H, Bonabe C, Kopodjing AB, Miaffo D et al. Diuretic and antihypertensive activity of the aqueous extract of *Haematostaphis barteri* stem bark in adrenaline-induced hypertensive Wistar Rats. J Phytopharmacol 2022; 11(1):24-31. doi: 10.31254/phyto.2021.11105

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