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Diuretic activity of the aqueous leaf extract of *Commelina benghalensis* Linn. in rats

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ABSTRACT

Introduction: *Commelina benghalensis* L., referred to as Benghal dayflower, is used in traditional Chinese medicine for its diuretic, antipyretic, and anti-inflammatory properties. The current study was conducted to evaluate the diuretic activity of *C. benghalensis* extract in rats.

Methods: Thirty-six Wistar rats were randomly assigned to sis equal groups. *C. benghalensis* extract was administrated to groups 1 to 3 at different doses, conventional diuretics, amiloride and furosemide, to groups 4 and 5, respectively, and distilled water to rats in group 6. The urine volume of each rat was measured at 6, 12, and 24 hours after drug administration, and the total urine volume was calculated. After euthanasia of the animals, the plasma and urine samples were used to measure diuretic activity and to perform biochemical analyses.

Results: The phytochemical examination of *C. benghalensis*' aqueous leaf extract revealed phenolics, anthraquinones, flavonoids, and glycosides. The extract promoted diuretic activity in a dose-dependent manner in both female and male rats, comparable to furosemide. Moreover, the extract preserved potassium excretion while increasing sodium excretion, and neither albumin nor glucose was detected in the urine of any of the animals.

Conclusion: The findings indicate that the aqueous leaf extract of *C. Benghalensis* may serve as a promising diuretic agent, corroborating the historic application of this medicinal herb in managing hypertension.

Implication for health policy/practice/research/medical education:
The results of this study provide scientific evidence to understand the diuretic activity of *C. benghalensis* in the management of hypertension. Further research is needed to isolate the bioactive compounds and elucidate the underlying mechanisms.

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Introduction

Hypertension is a major risk factor for cardiovascular diseases and premature death, affecting one in three persons worldwide. This prevalent and lethal condition leads to stroke, myocardial infarction, cardiac failure, renal impairment, and numerous other health complications. From 1990 to 2019, the worldwide population affected with hypertension increased twofold, rising from 650 million to 1.3 billion (1). Moreover, hypertension impacts approximately 30% of male and 50% of female subjects aged 65 to 75 years (2). Approximately fifty percent of people with hypertension worldwide are presently oblivious to their disease. Over 75% of persons with hypertension live in developing countries, where most blood pressure-related fatalities are documented (3). In 2000, it was stated that 80 million individuals in

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Sub-Saharan Africa experienced hypertension, with projections indicating an increase to 150 million by 2025 (4). The World Health Organization (WHO) global report on hypertension indicates that in 2019, the prevalence of high blood pressure among people aged 30–79 years was 37% in Cameroon and 38% in Chad (5).

Therapeutic interventions and lifestyle modifications can effectively manage hypertension, hence reducing the risk of severe health complications. Untreated hypertension can result in disability, diminished quality of life, or potentially fatal cardiovascular events such as heart attacks or strokes. Conventional antihypertensive drugs are used in the management of hypertension, including diuretics, sympatholytic medicines, renin inhibitors, angiotensin-converting enzyme inhibitors, calcium channel blockers, β-adrenergic and α1/β-adrenergic antagonists, and vasodilators (6,7). These drugs exhibit a range of adverse effects, including myalgia, arrhythmia, visual disturbances, dermal rash, emesis, renal failure, profound fatigue, cephalalgia, and edema (8,9). The increasing incidence of hypertension has required a comprehensive strategy for its treatment, encompassing both traditional medical therapies and alternative medicines (10). Due to its historical application and potential antihypertensive properties, traditional and herbal medicine have become appealing choices for hypertension management.

Presently, around 75%–80% of the global population, encompassing individuals with hypertension, utilize herbal remedies owing to the body's positive reaction to these substances and the little occurrence of adverse consequences (11). Ethnobotanical surveys of various medicinal plants demonstrate their extensive application in the management of cardiovascular disorders. The antihypertensive efficacy of plants such as *Cassia occidentalis* (12), *Leersia hexandra* Sw (13) and *Adansonia digitata* (14) has been demonstrated.

Commelina benghalensis, commonly known as the Benghal dayflower, tropical spiderwort, or wandering Jew, is a creeping herb from the Commelinaceae family. Native to tropical Asia and Africa, the plant is used as a traditional medicinal herb, animal feed, and a perennial weed. It is used to address and avert numerous ailments, including jaundice, burns, leprosy, fever, snakebite, sore throat, headache, female infertility, diabetes, rheumatism, and hypertension (15,16). In China, the plant is used as a diuretic, febrifuge, and anti-inflammatory agent (17,18), while in Chad, it is commonly used as pig feed and empirically employed as a diuretic for hypertension treatment. The study of Celestin Baboo, Shijikumar demonstrated its anti-urolithiatic property by inhibiting the formation of the stone in the kidney (19). Phytochemical studies from *C. benghalensis* leaf extracts revealed the presence of alkaloids, total phenolics, anthraquinones, flavonoids, tannins, and cardiac glycosides (20). No previous studies have shown the antihypertensive effects of this plant, while few studies assessed the diuretic activity of *C. benghalensis* (21). Therefore, the present study was conducted to evaluate the diuretic activity of the aqueous leaf extract of *C. benghalensis* in rats. The effects of this extract were also evaluated on the urine electrolytes.

Materials and Methods

Chemicals

All chemicals used in the study were purchased from Sigma–Aldrich Co (St. Louis, MO, USA) and were of analytical grade.

Animals

Wistar rats obtained from the Laboratory of Biological Sciences (University of Ngaoundere, Cameroon) were raised at the Laboratory of Research, Diagnostics, and Scientific Expertise (University of Ndjamena, Chad). The animals were housed in wooded cages under controlled temperature (24 ± 2 °C) and allowed free access to a standard diet (Cameroon National Veterinary Laboratory (LANAVET), Garoua, Cameroon), as well as tap water.

Plant material and preparation of the aqueous leaf extract

The leaves of *C. benghalensis* were harvested in July 2021 in Tchoua, a town in the Tandjile province (Chad). The sample was authenticated at the National Herbarium of Cameroon by comparing it to voucher specimen number 8634 collected by Robert Bruce Faden. After being thoroughly cleaned with water, the leaves were allowed to dry in the shade at room temperature before being ground into a powder with an electric blender. One hundred grams of the resultant powder were macerated in a glass container with 2 liters of hot distilled water (100 °C) for four hours, in accordance with the traditional healer's recommendations. The obtained solution was further filtered through Whatman filter paper No. 3, and the filtrate was dried in a Binder oven at 45 °C for 48 hours. The procedure was conducted thrice, resulting in 16.36 g of extract, which was preserved at -4 °C until required.

Phytochemical analysis of the extract

A qualitative phytochemical screening of the aqueous extract was conducted to ascertain the presence or absence of phytochemical compounds, including phenolics, terpenoids, flavonoids, tannins, alkaloids, sterols, anthraquinones, and cardiac glycosides (20,22,23).

Experimental design

The diuretic efficacy of *C. benghalensis* aqueous extract was assessed using the methodology established by Ntchapda, Barama (12). Sixty rats were selected and randomly allocated into six groups of ten rats each (five males and five females). Each animal was individually kept in a metabolic cage for 24 hours before to the experiment for acclimatization and thereafter fasted overnight while having unlimited access to water. Subsequently, animals in groups 1 to 3 received oral administration of the extract

at dosages of 225, 300, and 375 mg/kg, respectively. Under identical conditions, groups 4 and 5 were supplied conventional diuretics amiloride (14 mg/kg) and furosemide (2 mg/kg), respectively, whereas group 6 was administered distilled water (10 mL/kg). The urine volume of each rat was assessed at 6-, 12-, and 24-hours post-administration of the aforementioned drugs, and the cumulative urine volume was calculated. Subsequently, animals supplied with the aqueous extract (375 mg/kg) and their control group having distilled water (10 mL/kg) were euthanized, and blood samples were extracted from the aorta, followed by centrifugation at 3000 rpm at -4 °C for 10 minutes. The previously obtained urine and plasma were stored at –20 °C for subsequent analysis. The previously collected urine and plasma were stored at –20 °C for further examination.

a. Diuretic activity

The percentage of urine excretion, diuretic effect, and diuretic index were computed for all groups based on the average urine production at the 6th and 24th hours. The assessment of the aforementioned parameters was conducted using the calculations provided below (24):

$$\%\,\text{Urinary excretion} = \frac{\text{Total urinary output}}{\text{Total volume of liquid administered}} \times 100$$

$$\text{Diuretic action} = \frac{\text{Urinary excretion of the test group}}{\text{Urinary excretion of the control group}}$$

$$\text{Diuretic index} = \frac{\text{Diuretic action of the test group}}{\text{Diuretic action of the control group}}$$

The diuretic index was deemed good if the results were greater than 1.5, moderate if the results fell between 1.00 and 1.5, least if the results fell between 0.72 and 0.99, and nil if the results were below 0.72 (25).

b. Biochemical and physiochemical analysis

The analysis of several biochemical parameters related to diuresis was conducted in accordance with the previous study of Ntchapda, Barama (12). The concentrations of sodium and potassium ions in urine and plasma were measured using flame photometry (Jenway PFP 7, Canada). The levels of creatinine, urea, glucose, albumin, and electrolytes in biological fluid specimens analyzed in this study were measured using a two-way digital spectrophotometer (Secomam, Belgique). In animals treated with *C. benghalensis* and reference drugs, urinary osmolarities and natriuresis were measured during the diuretic response, specifically at the peak excretion rate. The osmolarity of plasma and urine samples was quantified by cytometry using an osmometer (Knauer, France). Aldosterone was measured by radioimmunoassay (assay kit Aldo RIACT). Osmolar clearance (Cosm) was calculated using the equation provided by El Menyiy et al (26):

$$\text{Osmolar clearance} = \frac{\text{Urinary osmolarity} * \text{urine flow}}{\text{Plasma osmolarity}}$$

When solutes are eliminated in a greater amount of water than the filtered plasma volume, free water clearance (CH_2O) is positive. This marker was determined using the formula provided by El Menyiy et al (26):

Free water clearance = Urine flow – Osmolar clearance

The natriuretic activity and the saluretic index were respectively calculated using the formulas below (24):

$$\text{Natriuretic activity} = \frac{\text{Concentration of } Na^+ \text{ in the urine of the group}}{\text{Concentration of } Na^+ \text{ in the urine of the same group}}$$

$$\text{Saluretic index} = \frac{\text{Concentration of electrolyte in the urine of the test group}}{\text{Concentration of electrolyte in the urine of the control group}}$$

The glomerular filtration rate (GFR) was calculated from the creatinine clearance by using the following equations (27):

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine concentration} * \text{urine volume at 24th}}{\text{Plasma creatinine concentration}}$$

Glomerular filtration rate (GFR) = Creatinine clearance × 0.81

Data analysis

The results were expressed as the mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) and the Tukey post hoc test for multiple comparisons were used to determine statistical significance. Values of $P < 0.05$ were considered statistically significant.

Results

Phytochemical screening of *C. benghalensis* aqueous leaf extract

The aqueous leaf extract of *C. benghalensis* contained phenolics, anthraquinones, flavonoids, saponins, tannins, and cardiac glycosides (Table 1).

Effects of *C. benghalensis* on urinary excretion

The effects of *C. benghalensis* aqueous leaf extract on urine volume excretion are shown in Figure 1. The reference drug furosemide significantly increased urine volume excretion 6 hours after post-administration compared to both female and male control animals. The aforementioned increases were observed 12 hours after oral administration of Amiloride. Compared to control rats, furosemide increased the cumulative urine volume by 102.56% in females (Figure 1A) and 91.25% in males (Figure 1B) (both $P<0.05$). In the same conditions, the increase was by 106.22% in females and 85.17% in males treated with amiloride compared to their respective controls. Administration of *C. benghalensis* resulted in a

Table 1. Phytochemicals present in *Commelina benghalensis* leaf aqueous extract

Phytochemical compounds	Results
Terpenoids	+
Flavonoids	+++
Total phenolic	+++
Anthraquinones	+++
Tannins	++
Saponins	++
Alkaloids	+
Cardiac glycosides	+++

+++, ++, and +: Present in high, moderate, and low concentrations, respectively.

significant diuresis after 6 hours, comparable to the group given furosemide. The highest cumulative urine volume for the aqueous leaf extract was observed at a dosage of 375 mg/kg, exhibiting an increase of approximately 98.90% in females and 96.57% in males, both ($P < 0.05$) compared to their respective control group (Figure 1C).

As demonstrated in Table 2, the aqueous leaf extract of *C. benghalensis* (300 and 375 mg/kg), together with furosemide and amiloride, significantly increased urine excretion in both females and males compared to control animals. In contrast to rats administered distilled water, the plant extract (375 mg/kg) markedly enhanced urine excretion by 176.28% in females and 187.03% in males. The urine excretion of males treated with furosemide increased by 179.63% in comparison to the control group, whereas that of females treated with amiloride increased by 186.45% ($P < 0.05$). The current findings indicated that when furosemide or amiloride served as controls, the diuretic index produced by the aqueous extract (225 and 300 mg/kg) was below 0.72. At a dosage of 375 mg/kg, the diuretic efficacy elicited by this extract ranged from 0.72 to 0.99 in females, whereas in males it ranged from 1.0 to 1.5, irrespective of the aforementioned reference drug used.

Effects of *C. benghalensis* on urinary electrolyte excretion

The effects of *C. benghalensis* on urinary sodium and potassium excretion are detailed in Table 3. The different doses of *C. benghalensis* extract and the reference drugs delivered in this investigation resulted in a considerable elevation in Na^+ excretion compared to control rats. Amiloride and furosemide treatment improved this Na^+ elimination by approximately 3 and 8 times, respectively, in female and male rats. In comparison to the control group, natriuresis increased by nearly sevenfold in both female and male rats treated with *C. benghalensis* at 375 mg/kg. The current findings indicated that urine K^+ excretion was statistically significant in both female and male rats treated with either furosemide or amiloride compared to their respective controls. Nevertheless, no significant differences in urinary potassium excretion were seen in the treated animals compared to control rats. The natriuretic activity and saluretic index values exhibited a dose-dependent increase in groups administered with *C. benghalensis* compared to the control group. At a

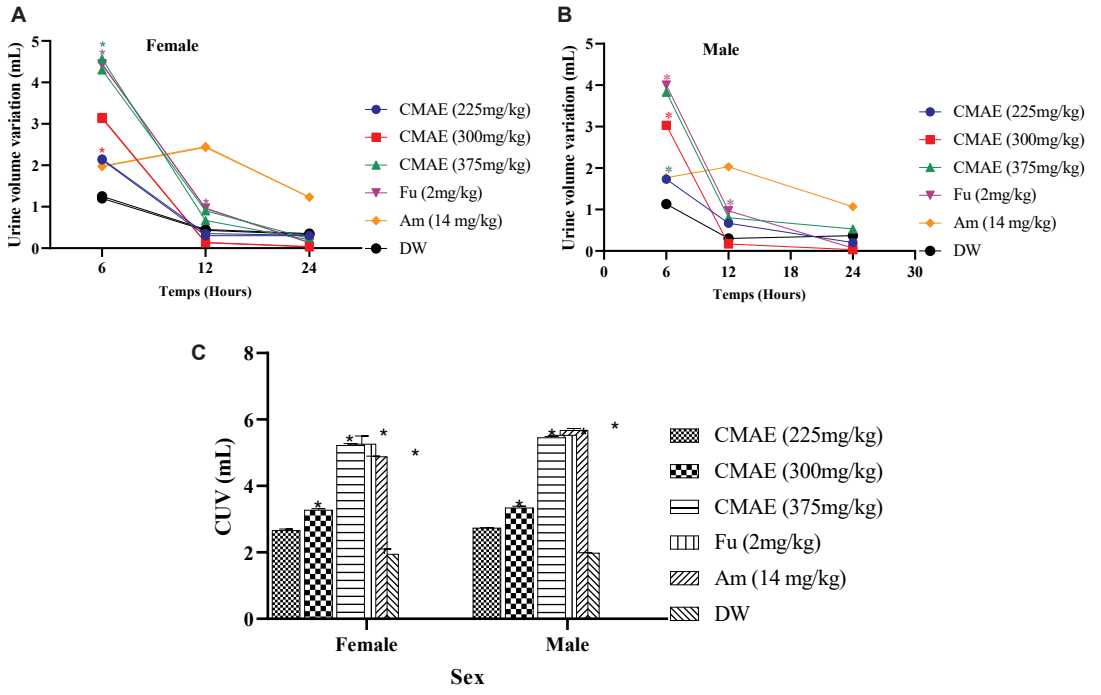


Figure 1. Effects of the aqueous leaf extract of *Commelina benghalensis* (CMAE) on urine volume over time in female (A) and male (B) rats. (C) represents the cumulative urine volume in both sexes. Fu: Furosemide, Dw: Distilled water, CVU: Cumulative urine volume. Each value represents mean ± SEM (n=5/sex); *$P < 0.05$ versus DW (control). Am: Amiloride.

Table 2. Effects of the leaf aqueous extract of *Commelina benghalensis* (CMAE) on urinary excretion, diuretic action, and diuretic activity

		UE (%)	DA	DI (Fu)	DI (Am)
CMAE (225 mg/kg)	Females	136.67 ± 12.22	1.38	0.49	0.48
	Males	131.67 ± 17.78	1.46	0.52	0.54
CMAE (300 mg/kg)	Females	165.00 ± 30.00*	1.67	0.59	0.58
	Males	161.67 ± 31.11*	1.79	0.64	0.66
CMAE (375 mg/kg)	Females	271.67 ± 18.89*	2.76	0.98	0.96
	Males	258.33 ± 28.89*	2.87	1.02	1.06
Fu (2 mg/kg)	Females	276.67 ± 14.44*	2.81	1.00	0.98
	Males	251.67 ± 18.89*	2.79	1.00	1.03
Am (14 mg/kg)	Females	281.67 ± 14.44*	2.86	1.01	1.00
	Males	243.33 ± 8.89*	2.70	0.96	1.00
Dw (10 mL/kg)	Females	98.33 ± 4.44	1.00	-	-
	Males	90.00 ± 3.33	1.00	-	-

Each value represents mean ± SEM (n=5/sex); *P < 0.05 versus distilled water (Dw; Control). Fu: Furosemide, UE: Urinary excretion, DA: Diuretic action, DI (Fu): Diuretic index when furosemide was considered as control, and DI (Am): Diuretic index when amiloride was considered as control.

Table 3. Effects of the aqueous leaf extract of *Commelina benghalensis* (CMAE) on urinary Na^+ and K^+ excretions

		Na^+(mEq/L)	K^+(mEq/L)	Natriuretic activity	Saluretic index
CMAE (225 mg/kg)	Females	08.48 ± 0.94*	4.80 ± 0.01	1.76	1.57
	Males	07.90 ± 0.65*	4.76 ± 0.01	1.65	1.57
CMAE (300 mg/kg)	Females	18.30 ± 1.20**	4.25 ± 0.06	4.30	2.67
	Males	17.60 ± 0.37**	4.13 ± 0.02	4.26	2.70
CMAE (375 mg/kg)	Females	24.72 ± 0.94***	4.56 ± 0.10	5.42	3.47
	Males	24.25 ± 0.50***	4.51 ± 0.12	5.37	3.58
Fu (2 mg/kg)	Females	29.85 ± 0.71***	13.71 ± 0.23*	2.17	5.17
	Males	28.59 ± 0.19***	13.14 ± 0.32*	2.17	5.19
Am (14 mg/kg)	Females	10.95 ± 0.77*	20.66 ± 0.11***	0.53	3.75
	Males	09.96 ± 0.56*	19.77 ± 0.04***	0.50	3.70
Dw (10 mL/kg)	Females	03.68 ± 0.28	4.74 ± 0.24	0.77	-
	Males	03.53 ± 0.26	4.50 ± 0.16	0.78	-

Each value represents mean ± SEM (n=5/sex); *P < 0.05, **P < 0.01, and ***P < 0.001 versus distilled water (Dw; control). Fu: Furosemide and Am: Amiloride.

dosage of 375 mg/kg, the natriuretic response induced by the plant extract increased to 128.57% in females and 111.53% in males. Our findings indicated that the natriuretic activity increased by approximately 180.51% in females and 178.20% in males treated with furosemide, in comparison to their control group. In contrast, treatment with amiloride resulted in a decrease in natriuretic activity by 45.28% in females and 56.00% in males, relative to controls.

Effects of *C. benghalensis* on urinary biochemical markers

Effects of C. benghalensis on albuminuria and glucosuria

The results of the present study revealed that glucosuria and albuminuria tests were negative in all groups.

Effects of C. benghalensis on creatinine and urea

Figure 2 illustrates that the study's findings revealed no significant differences in urinary creatinine levels between animals treated with extracts and the reference drugs, in comparison to those administered distilled water. The amount of urea excreted in urine was markedly increased at 6-, 12-, and 24-hour post-administration of the extract or furosemide in comparison to control rats. The increase was 74.18% and 41.23% in females and 64.36% and 32.23% in males, 24 hours post-administration of the leaf aqueous extract at 375 mg/kg and furosemide, respectively. The administration of amiloride did not result in any significant alterations in the urine urea levels as compared to the control group.

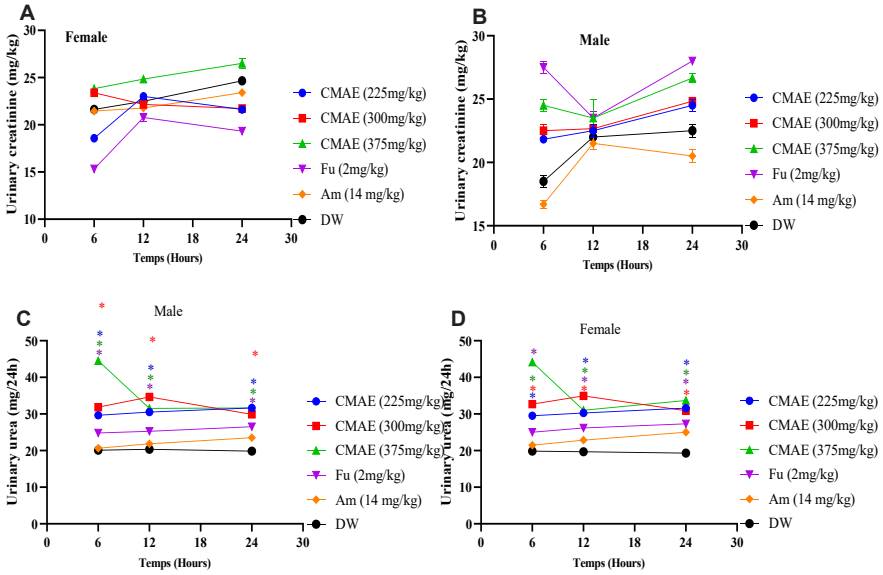


Figure 2. Effects of the aqueous leaf extract of *Commelina benghalensis* (CMAE) over time on urinary urea (A and B) and creatinine (C and D) excretion in male and female rats. Each value represents mean ± SEM (n=5/sex); *P < 0.05 versus distilled water (Dw; control). Fu: Furosemide and Am: Amiloride.

Effects of *C. benghalensis* on plasmatic and urinary osmolarities

Table 4 summarizes the effects of *C. benghalensis* on plasmatic and urine osmolarities. The findings demonstrated no significant variation in plasma osmolarity following the administration of the plant extract (225 and 300 mg/kg) in comparison to the controls. However, a significant increase in the level of this marker was detected in the group treated with the extract at 375 mg/kg, as well as in those receiving furosemide and amiloride, relative to the control rats. Administration of the extract led to a significant and dose-dependent reduction in urine osmolarity compared to the control group. The extract dosage of 375 mg/kg produced a 53.90% reduction in females and a 50.37% reduction in males ($P<0.05$). Under identical conditions, furosemide and amiloride elicited a significant reduction ($P<0.05$) in urine osmolarity levels in both female and male rats compared to controls. The GFR was also affected by the different treatments administered in this study. Our findings indicated that the administration of the plant extract, furosemide, or amiloride led to a reduction in GFR levels compared to the control group.

Effects of *C. benghalensis* on osmotic and free water clearances

As illustrated in Figure 3, *C. benghalensis* did not induce any alterations in osmotic clearance values compared

Table 4. Effects of the aqueous leaf extract of *Commelina benghalensis* (CMAE) on plasmatic and urinary osmolarities as well as glomerular filtration

Treatment	Sex	Osmolarity		
		Plasma	Urine	GFR
CMAE (225 mg/kg)	Females	255.00 ± 0.67	110.00 ± 0.67**	1.50 ± 0.01
	Males	257.00 ± 0.67	114.00 ± 0.67**	1.58 ± 0.01
CMAE (300 mg/kg)	Females	264.67 ± 2.22	112.00 ± 1.33**	1.46 ± 0.01
	Males	267.00 ± 2.00	116.00 ± 0.67**	1.48 ± 0.02
CMAE (375 mg/kg)	Females	280.67 ± 2.22*	128.00 ± 1.33**	1.35 ± 0.01*
	Males	285.00 ± 2.67*	133.00 ± 0.67**	1.38 ± 0.01*
Fu (2 mg/kg)	Females	276.67 ± 1.56*	160.67 ± 2.89**	1.31 ± 0.01*
	Males	278.67 ± 1.11*	163.33 ± 2.44**	1.33 ± 0.01*
Am (14 mg/kg)	Females	290.00 ± 0.67*	165.67 ± 3.56**	1.27 ± 0.01*
	Males	291.33 ± 1.11*	170.67 ± 0.89**	1.31 ± 0.02*
Dw (10 mL/kg)	Females	260.33 ± 1.78	197.00 ± 1.33	1.54 ± 0.04
	Males	270.00 ± 3.33	200.33 ± 1.11	1.61 ± 0.01

Each value represents mean ± SEM (n=5); *P < 0.05 versus DW (control). Fu: Furosemide, Dw: Distilled water, GFR: Glomerular filtration rate ,and Am: Amiloride.

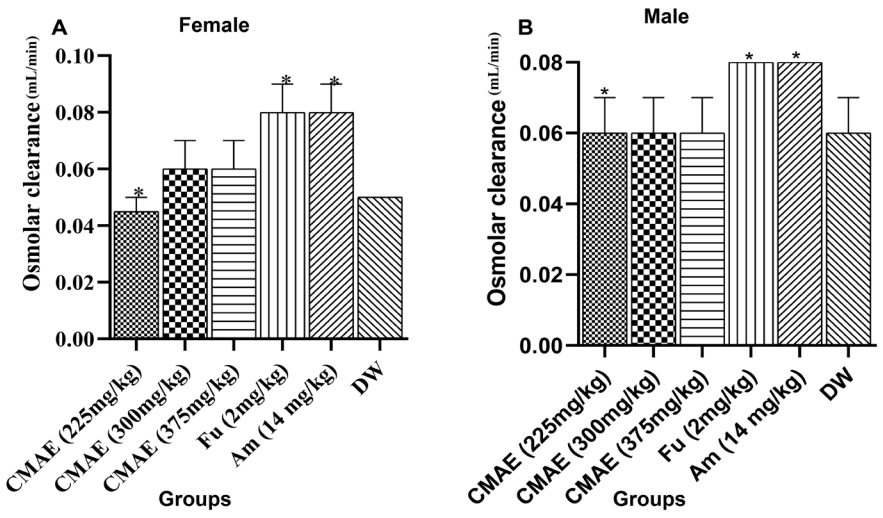


Figure 3. Effects of the aqueous leaf extract of *Commelina benghalensis* (CMAE) on osmolar clearance in (A) female and (B) male rats. Each value represents mean ± SEM (n = 5); **P* < 0.05 versus distilled water (Dw; control). Fu: Furosemide and Am: Amiloride.

to the control group. An exception was noted in males administered the extract (375 mg/kg), as osmotic clearance increased by approximately 16.66% ($P<0.05$) relative to the control group. Administration of furosemide or amiloride resulted in an increase in osmotic clearance of 28.56% ($P<0.05$) in females and 16.66% ($P<0.05$) in males compared to their respective controls.

Effects of *C. benghalensis* on biochemical blood markers

The effects of *C. benghalensis* extract (375 mg/kg) on blood parameters are presented in Table 5. The results indicated that the levels of plasma urea, albumin, aldosterone, and osmolarity were markedly elevated in the extract-treated groups compared to the control animals. The blood levels of Na^+ and creatinine were considerably reduced by 34.63% and 38.09% in males and by 35.63% and 44.26% in females treated with the extract, respectively, compared to controls. No significant change was seen in the levels of glucose and K^+ between the extract-administered rats and their controls.

Discussion

Diuretics, often referred to as water pills, are crucial antihypertensive medications employed in the treatment

Table 5. Effects of the aqueous leaf extract of *Commelina benghalensis* (CMAE) on some blood biochemical markers

Biochemical markers	Sex	Dw (10 mL/kg)	CMAE (375 mg/kg)
Glucose (mg/dL)	Males	94.98 ± 0.59	101.96 ± 0.58
	Females	93.15 ± 0.57	91.97 ± 0.58
Creatinine (mg/dL)	Males	0.63 ± 0.02	0.39 ± 0.01**
	Females	0.61 ± 0.01	0.34 ± 0.02**
Urea (mg/dL)	Males	24.49 ± 0.72	34.83 ± 0.28*
	Females	22.06 ± 0.29	32.00 ± 1.23*
Albumin (g/L)	Males	42.50 ± 0.72	48.99 ± 1.12
	Females	40.78 ± 0.34	46.50 ± 2.38
Plasmatic osmolarity (mosmol/kg)	Males	262.50 ± 2.50	279.00 ± 1.00*
	Females	258.50 ± 0.50	275.50 ± 0.50*
Aldosteronemia (pg/mL)	Males	292.00 ± 1.00	309.00 ± 1.00*
	Females	188.50 ± 0.50	201.50 ± 0.50*
Na^+ (mEq/L)	Males	18.71 ± 0.11	12.23 ± 1.00*
	Females	15.80 ± 0.07	10.17 ± 0.06*
K^+ (mEq/L)	Males	1.94 ± 0.05	2.00 ± 0.01
	Females	1.73 ± 0.04	1.99 ± 0.01

Each value represents mean ± SEM (n=5); **P* < 0.05 and ***P* < 0.01 versus distilled water (Dw; Control).

of hypertension by promoting the excretion of surplus salt and water from the body. Medicinal plants have been used by several populations around the world for the management of hypertension (13,28). Thus, the present study investigated the diuretic activity of *C. benghalensis* in rats. The phytochemical analysis of this extract revealed bioactive components including alkaloids, total phenolics, anthraquinones, flavonoids, tannins, and cardiac glycosides (20). Several studies also indicated that *C. benghalensis* contains polyphenols, flavonoids, alkaloids, and tannins (29). These phytochemicals may elucidate the traditional use of *C. benghalensis* for the management of hypertension.

In this study, treatment with *C. benghalensis* led to a significant increase in diuresis, urine excretion, diuretic effect, and diuretic index, comparable to furosemide or amiloride, when compared with the control groups. The current findings indicated that the diuretic index of the extract was below 0.72 at the lower doses tested. At the higher dose examined, the diuretic index ranged from 0.72 to 0.99 in females, whereas it ranged from 1.0 to 1.5 in males. Consequently, the diuretic index values indicate *C. benghalensis* exhibits diuretic activity. The aqueous extract of *C. benghalensis* leaves may exhibit minor effects at lower dosages; however, at higher levels, the activity is classified as mild in females and moderate in males (25). This finding indicates that males exhibited greater sensitivity to the diuretic effects of the extract compared to females. Under identical conditions, the diuretic impact of furosemide is categorized as moderate in both male and female rats, but in the amiloride-treated group, the effect is mild in females and moderate in males. These results are in agreement with previous studies (30,31).

Accordingly, with an increase in urine production, *C. Benghalensis* also elicited a significant increase in Na^+ excretion in urine, including elevated natriuretic activity and saluretic index, as stimulated by furosemide in comparison to controls. Nonetheless, this extract did not produce significant variations in urine potassium excretion relative to control rats. Thus, present findings suggested that *C. benghalensis* aqueous leaf extract may exert its diuretic activity through the conjunction of its potassium-sparing, natriuretic, and/or saluretic effects (32). Moreover, the Na^+/K^+ ratio was commonly considered an indicator of natriuretic activity, suggesting adequate and advantageous natriuresis, as well as beneficial K^+ sparing activity when the values exceed 1, 2, and 10, respectively (33). The index values for natriuretic activities in the present investigation demonstrated that the extract displayed adequate natriuresis at low doses and beneficial natriuresis at elevated doses. Moreover, the extract (375 mg/kg) markedly enhanced both urine output and osmolarity, suggesting that at elevated dosages, it may function as an osmotic diuretic akin to mannitol (34). This class of diuretics raises the osmolarity of the blood and renal filtrate, which prevents the reabsorption of water and sodium. Moreover, this study revealed that the oral administration of *C. benghalensis* resulted in a dose-dependent decrease in GFR, and this was comparable to the effects produced by furosemide and amiloride. The observed reduction in GFR in extract-treated animals may be attributed to blood volume depletion, activation of the renin-angiotensin system (RAS), and/or elevations in intratubular or interstitial pressures (35). In comparison to controls, the oral administration of the extract markedly reduced blood creatinine levels and elevated blood aldosterone concentrations.

Creatinine is essential for evaluating renal function due to its notable characteristics. In blood, it serves as an indicator of GFR. The reduction in blood creatinine levels caused by the extract indicates that it may also confer protective benefits on renal function. The elevation in aldosterone levels indicates that *C. benghalensis* may possess phytochemicals that influence renal excretory function, functioning as facultative diuretics in conditions of elevated plasma aldosterone (36).

This study was limited in that we did not specifically examine which compounds from *C. benghalensis* contributed to ameliorating diuretic activity, despite the presence of various active compounds. Despite this limitation, the results of this study indicated that *C. benghalensis* had a potent diuretic property.

Conclusion

The findings of this investigation indicate that *C. benghalensis* leaves exhibit significant diuretic activity in both male and female Wistar rats, thereby demonstrating their use in traditional medicine as a diuretic agent. This activity may be due to the presence of phytochemical compounds, including alkaloids and flavonoids, known for their diuretic properties. Thus, we are currently conducting further experiments to isolate phytochemical constituents to elucidate the specific mechanism underlying the diuretic activity of *C. benghalensis*.

Authors' contribution

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Conflict of interests
The authors declare no conflict of interests in this work.

Ethical considerations
Experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee of the University of Ngaoundere (Reference N°. FWIRB 00001954).

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