

Original Research Article

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Diuretic Activity of the Aqueous Extract Leaves of *Ceiba pentandra* (Bombacaceae) in Rats

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ABSTRACT

Ceiba pentandra is a plant belonging to the family; Bombacaceae. In Cameroon it is used in traditional medicine for the treatment of arterial hypertension and a good number of other cardiovascular complications. This work is aimed at evaluating the sharp diuretic activity of the aqueous extract of *Ceiba pentandra* leaves in the rat. Male rats weighing between 150 and 180g were chosen to carry out this study. Five doses of (100, 150, 200, 250 and 300mg/kg) of the aqueous extract of *Ceiba pentandra* leaves were used. The selected animals were then distributed in to eight batches or groups of five rats each. The volume of urinary excretion, food and water intake were determined. The rats were the killed (sacrificed) 24hrs after treatment and the blood collected in heparinated tubes. The concentration of creatinine, urea, glucose, ALAT, ASAT and electrolytes (Na⁺, K⁺, Cl⁻) in blood and/or in the urine was evaluated with use of a spectrophotometer. Aqueous leave extract just like Furosemide and Amiloride brought in rats a significant increase (p< 0.05) and dependent dose of volume of urinary excretion compared to the negative control group. Meanwhile the dose of 300 mg/kg of the aqueous extract in rats brought a significant increase (p< 0.05) of urinary excretion in 24hrs compared to groups or batches treated with Furosemide (20.86%) and 69.50% Amiloride. This increase in urinary excretion is accompanied with a significant elimination (p< 0.05) of Na⁺ and Cl⁻ ions while sparing K⁺ and Ca²⁺ ions. The aqueous extract equally brought a significant increase (p< 0.05) of the natriuretic, saluretic activities alongside the inhibition of carbonic anhydrase. Furthermore, we equally noted a significant increase (p< 0.05) of metabolic wastes (urea and creatinine) in the urine. The evaluation of glycemia revealed a significant decrease (P<0.05) in animals (rats) treated with the aqueous leaves extract from 33.75, 29.65 and 40.92% respectively for the doses of 200, 250 and 300mg/kg compared to the control group. The concentration in transaminase show a great decrease (P<0.05) of the concentration of ASAT from 22.46, 46.79 and 21.73%, respectively for the doses of 250, 300 mg/kg and Amiloride compared to control group. In contrast no significant change (P>0.05) in the rate of ASAT was observed in animals treated with the aqueous extract at the dose of 200 mg/kg as well as in the animals treated with Furosemide compared to the control group. The significant increase (p< 0.05) of urinary excretion as well as that of Na⁺ and Cl⁻ ions justify the use of this plant for it diuretic property in the treatment of arterial hypertension.

Keywords

Ceiba pentandra,
Diuresis,
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Saluretic

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Introduction

Cardiovascular diseases (CVDs) constitute a set of problems affecting the heart and the blood vessels. Among these pathologies we can cite coronary cardiopathy, cerebrovascular diseases, peripheral arteriopathy, rheumatismal cardiopathy, cardiac congenital malformation, deep (abstruse) veinous thrombosis and pulmonary embolism. Cardiovascular diseases are the major cause of death (mortality) in the world, each year it kills more people, more than the other diseases. It is estimated that 17.5 million is the number of death attributed to cardiovascular diseases, say, 31% of the global mortality rate^[3]. In most countries undergoing development, the victims of cardiovascular diseases are two times higher than those caused by HIV/AIDS, malaria and tuberculosis put together^[4]. In 2005, approximately eighteen million people died as a result of cardiovascular diseases in the world, about 80% of these deaths came from countries undergoing industrialization (development)^[5]. It is estimated that in 2020, cardiopathy is going to reach 120% in women and 137% in men in countries undergoing development if something is not done^[6]. Some of the principal causes of cardiovascular diseases are: arterial hypertension, dyslipidemia, metabolic syndrome and cigarette consumption.^[7] Of these principal factors, arterial hypertension is known as one of the major risk factors responsible for cardiovascular disease^[8].

Arterial hypertension is a very important height (elevation) which persists although the subject is at rest from the force exerted by blood on the wall of arteries. Usually arterial pressure is evaluated by two digits: the first, the most elevated shows systolic arterial pressure or maximal blood pressure (that is when the heart contracts and pumps blood in the arteries and the second digit, the lowest corresponds to the minimal blood pressure or

diastolic arterial pressure (that is when the heart relaxes)^[9]. Arterial hypertension increases the risk of cerebral vascular disorder, coronary diseases, cardiac insufficiency, renal insufficiency and cognitive problems^[10]. In Africa the prevalence rate of arterial hypertension is about 46 % and about 37% in Cameroon^[11]. The antihypertensive treatment still appeals to hygiene diet measures; the correction of metabolic associated abnormalities like hypercholesterolemia, diabetes etc at different levels of medication^[11].

In 1950, diuretics were the first medications administered for the treatment of arterial hypertension^[12]. Nowadays, because of their efficiency, diuretics still serve in the treatment of light arterial hypertension, during first line care. In most countries undergoing development, access to conventional medicine remain limited to great agglomerations, difficulties to movement, little or no qualified staff, undesirable secondary effects, the high cost of accessing conventional medicine services and the socio-economic factors that does not give any other choice to the population than to go in for traditional medicine^[13, 14].

Due to an increase in these diseases and generally the inability to afford conventional medicine, the World Health Organization, WHO AFR/RC50/R3 resolution of 31st august 2000, encourages African countries to elaborate regional strategies on traditional medicine in order to undertake research on medicinal plants and to promote their optimal usage in care and health service systems^[15]. To reach the goal, a good number of researchers turned to ethno pharmacological approach especially (notably) in the cardiovascular domain. It is in this light that we undertook the evaluation of the diuretic properties of the aqueous extract of *Ceiba pentandra* leaves on rats.

Ceiba pentandra is a big tree of the family Bombacaceae, reaching up to 50m in height and 250 cm in diameter. The leaves, flowers and fruits when dried are used vegetables or ingredients cuisine (kitchen, cooking) ^[16]. The leaves, flowers and fruits have medicinal properties and allow for the alleviating painful affliction like fever, abscess, paronychia, mental ailments, conjunctivitis, dizziness, headache (cephalagia) and arterial hypertension. The bark is aphrodisiac, febrifuge and galactologue. It also allows for the treatment of sterility in women, cough, malaria, diarrhoea, gastralgia, gingivae (gingivitis), haemorrhoids etc.

This present study therefore has the principal aim of evaluating the diuretic properties of aqueous extract of *Ceiba pentandra* leaves in rats for 24hrs per administration.

Materials and Methods

Plant Material

In the course of this work, we used dried leaves of *Ceiba pentandra* (Bombacaceae). This plant was harvested (collected) in the locality of Dschang, Menoua division, West region of Cameroon then identified at the Herbarium National of Cameroon by comparing with a registered sample under the number 3012/SRSK.

Animal Material

Forty (40) male albino rats of the Wistar strain, aged 2, 5 to 3 months, weighing between 150 and 180 g, domesticated in the laboratory of the Department of Biological Sciences of the Faculty of Science, were used for our study (work) University of Ngaoundere. These animals were acclimatized for about a week before being manipulated upon in the laboratory of Medicinal Plants, Health and Galenic Formulation of the

University of Ngaoundere. In the course of the process (manipulation), the animals were under the control and care of veterinary technician (doctor) of the Science and Veterinary Medicine, University of Ngaoundere. The animals were placed and controlled under the temperature of about 25 ± 4 °C) and relative humidity of about $45\% \pm 10\%$. They had access to *ad libitum*, to food and water. The procedure as well as the experimental protocol was endorsed (approved) by the Institution in charge of Animals and the research was approved by the Ethnic Committee of Animals of the University of Ngaoundere.

Reference pharmacological substance

Medicine such as Amiloride Hydro-Chloro-Thiazide (AHCT) and Furosemide were used as reference diuretics.

Preparation of aqueous extract

Freshly harvested leaves of *C. pentandra* were dried under a shade then ground in order to obtain it powder and thereafter we introduced (added) 50g of the powder in 1L of cold water. We macerate for 12hrs on an agitator and the maceration process, the mixture was filtered with the help of the Wattman paper n°3. The filtrate was then dried freeze at -40°C , with the help of a lyophilisation machine, this permitted us to obtain 13,9g of the powder giving a yield of about 27, 8%.

Phytochemical study

A phytochemical study was carried out to determine the different families of chemical compounds found in the bark of the trunk of *C. pentandra* responsible for its diuretic activity. For the identification of the different groups of metabolites in the raw (crude) aqueous extract, an analytical examination (test) at the Institute of Medicinal Plants for Medicinal research of

Cameroon. As a result, the method describes by Chen *and al.*, in 2011^[17] was used. A preliminary test of the phytochemical study was conducted for the identification of the different structures of the chemical compounds responsible for the diuretic activity of *C. pentandra*. Briefly and for instance, an essential oil of the aqueous extract of *C. pentandra* was extracted with hexane

Evaluation of diuretic activity

At the beginning of our manipulation, we realized a preliminary screening in order to proceed to the selection of rats that « urinated well ». As such, it consisted the oral administration of distilled water of 1 mL/100g body weight to rats. Rats treated as such were individually placed in their metabolic cages, the urine was collected and the urinary volume measured at the end of 6hrs. The animals haven excreted a volume above 40% of the volume of water were selected while those that excreted a volume less than 40% were excluded^[18]. The selected animals were allocated (distributed) in 6 groups of 5 rats each individually placed in the metabolic cages for 7 days for acclimatization and after the period of acclimatization, the first three groups respectively received the aqueous extract of *C. pentandra* leaves at doses of 200, 250 and 300mg/kg. The last two groups made up of positive control group received the reference diuretics and the negative control group received distilled water. The volume of urinary excretion was determined at 3hrs, 6hrs, 12hrs and 24hrs after the administration of a single dose of the different extracts. 24hrs after the administration of the different substances, the animals were killed (sacrificed) and blood was subsequently collected in EDTA tubes. All experimental procedure was approved by the Institution of Ethnic Committee of the Department of Biological Sciences of the University of Ngaoundere (ECDBSUN 15/01/2015/ UN/FS/DSB).

Determination of the different parameters

The treated animals were killed by decapitation (beheading) and the blood collected in EDTA tubes. The serum was separated by centrifugation at 3500 tr / min for 15 minutes then stored at -20 ° C for the dosage of biochemical parameters such as creatinine, urea, sodium ion, potassium ion, chloride ion, ALAT and ASAT.

The urinary concentrations of sodium and potassium ions were determined with the aid of photometric flame (Jenway, PFP 7). The creatinine concentrations (Creat), urea and electrolytes in the samples of serum and urine were evaluated with the help of a bidirectional mass spectrophotometer. (Secomam RS 232C, France). In animals treated with the extract reference substance, urinary osmolarity and natriuresis were measured during the diuretic respond, particularly at maximal excretion levels. Osmolarity samples of plasma and urine was measured by cytometry with the help of an osmometer (Knauer). Osmolar clearance (Cosm) was determined from plasmatic osmolality (POSM), from urinary osmolarity (Uosm) and the urinary flow (debit, rate) (V) according to the relation (formula): $\text{Cosm} = \text{Uosm} \cdot \text{V} / \text{POSM}$. The glomerular filtration rate (flow) (DFG) was evaluated by creatinine clearance (Creat). The quantity of Na + ion and K + ion was calculated according to the parameter for the saluretic activity. The relation $\text{Na} + / \text{K} +$ was calculated for the natriuretic activity. To estimate the inhibition activity of carbonic anhydrase the following relation was used $\text{Cl} / (\text{Na} + + \text{K} +)$ ^[19].

Determination of rectal temperature

A digital thermometer was used to measure the rectal temperature. As a result, the transducer (sensor) end of the thermometer was introduced in the anus of the animal for a

minute. All animals within the temperatures range of 35-38°C at the beginning of the experiment was selected meanwhile those that were having temperatures below or above the margin were eliminated. These temperatures were measured before and after 24hrs of administration of the different substances.

Statistics analysis

Each of these results are expressed or articulated as average \pm standard deviation (n=5). The different statistics between the negative control group and the test groups were obtained thanks to ANNOVA test followed by Dennett's t test. The software analysis for variances had an origin of 8.0. The difference is significant if $P < 0.05$ and insignificant if $P > 0.05$.

Results and Discussion

Phytochemical analysis

The phytochemical study carried out on crude extracts with methanol and ethyl acetate the presence of several primary and secondary metabolites such as flavonoids, tannins, alkaloids, steroids and triterpens, saponins, les anthraquinons, phenol compounds and coumarins. Phenol compounds, notably triterpens, volatile oils and sterols were equally in the extract.

The presence of flavonoids and alcaloids is remarkable. The phytochemical results have been presented in table 1 below. Four fractions were collected and analysed to determine the presence of alcaloids, tanins, flavonoids and saponins.

These fractions were finally grouped into four and numbered A, B, C and D base on their predominant phytochemical reactions as indicated in table 1 below. These first observations and findings suggest that the

aqueous extract of *C. pentandra* leaves contain several chemical compounds with potential biological activity, meriting as such more and advanced research.

The effect of aqueous extract of *Ceiba pentandra* leaves on body temperature

During the experiment, no significant difference ($P > 0.05$) was observed on the body temperature animal (Table 2).

Effect of the aqueous extract of *Ceiba pentandra* leaves on the lag phase of urinary excretion

Figure 1 below presents the effect of the aqueous extract of *C. pentandra* leaves on the lag phase of urinary excretion of rats. We remarked on this figure that there is a significant reduction ($P < 0.05$) and the dependent dose of the lag phase of urinary excretion of (60 minutes for control at 32, 30, 20, 19 and 18 minutes respectively for the doses of 200, 250, 300 mg/kg, Furosemide and Amiloride).

Effect of aqueous extract of *Ceiba pentandra* leaves on screening urinary volume

Table 3 below shows the effect of aqueous extract of the bark of *C. pentandra* on screening urinary volumes. From this table we observed that there is a significant increase ($P < 0.05$) of urinary excretion at time period of 6hrs, 12hrs and 24hrs in animals treated with the aqueous extract of different doses compared to the control group (i.e. treated with distilled water).

Meantime the volume of urinary excretion at doses 200 mg/kg (18, 06 mL/kg), 250 mg/kg (21, 9 mL/kg) and 300 mg/kg (31, 94 mL/kg) showed an exponential increase of 117.06, 163.22 and 283.9% respectively at a time

period of 24hrs compared to the control group (8, 32 mL/kg).

Effect of aqueous extract of *Ceiba pentandra* on cumulative urinary excretion

Cumulative urinary excretion increases significantly and at all time periods in animals treated with Furosemide and Amiloride compared to the control group. This significant increase varies at different time periods of 3hrs, 6hrs, 12hrs and 24hrs) from 291,9%, 188,9%, 39,37% and 41,10% for furosemide and 106,06%, 46,12%, 83% and 173,07% for Amiloride respectively compared to the control group (Table 4). In animals treated with the aqueous extract of different doses 200, 250 and 300 mg/kg), no significant difference ($P > 0.05$) was observed on urinary excretion volume at a time period of 3hrs compared to the control group.

Contrary to the 3hrs time period, the administration of the plant extract of different doses brought a significant increase and in the dose dependent manner of the urinary excretion at different time intervals of 6hrs, 12hrs and 24hrs. However it was equally noted that the plant extract at a dose of 300 mg/kg brought a significant increase ($P < 0.05$) in the urinary volume at time interval of 12hrs (26,48mL/kg/time interval) and 24h (31,94mL/kg/time interval) compared to Furosemide (9,88 mL/kg/time interval and 11,74 mL/kg/time interval) and Amiloride (8,08 mL/kg/time interval and 22,72 mL/kg/time interval). During the manipulation process, no significant change of urinary pH was observed in animals treated at different doses.

Effect of the aqueous extract of *Ceiba pentandra* leaves on the excretion of electrolytes

Table 5 below shows the effect of the aqueous extract of the bark of *C. pentandra* trunk on

the excretion of electrolytes. We remarked in this table that the urinary excretion of Na^+ ions increased in a significant manner in animals treated with the aqueous extract at doses of 250 mg/kg (45,64 mmol/L) and 300 mg/kg (58,6 mmol/L) say 5,8% and 35,89% respectively, as well as in animals haven received pharmacological reference substances (8,9% and 27,41% respectively for Amiloride and Furosemide) compared to the control group (43,12 mmol/L). Rats treated with the aqueous extract at different doses just like the rats treated with Furosemide and Amiloride showed an insignificant increase in the urinary excretion of K^+ compared to the rats treated with distilled water.

Animals treated with the aqueous extract showed a significant increase in the excretion of chloride ions from 33%, 44, 96% and 86, 18% for the doses of 200, 250 and 300 mg/kg respectively compared to animals in the negative control. Likewise, Amiloride and Furosemide brought in rats a significant increase in the excretion of Cl^- ions of 24, 38% and 73, 36% compared to distilled water.

The urinary concentration Ca^{2+} ions significantly increased as in animals treated with the aqueous extract of different doses than those that received reference diuretics.

Furthermore, it was equally noted that the aqueous extract of the bark of *C. pentandra* trunk at a dose of 300 mg/kg brought a significant excretion ($\Delta P < 0.05$) Na^+ and Cl^- ions respectively of 23, 53% and 49, 69% compared to Amiloride and 6, 66% and 7, 40% compared to Furosemide.

Effect of the aqueous extract of *Ceiba pentandra* leaves on diuretic index and electrolytes indices in 24hrs

Table 6 below shows the effect of the aqueous extract of *C. pentandra* leaves on the diuretic and electrolytes indices in 24hrs. We

remarked from this table that the diuretic index increases in a dose dependent manner in animals treated with the aqueous extract as well as in those treated with reference medicine compared to the control.

Likewise, an increase in the index of Na^+ , K^+ , Cl^- as well as Ca^{2+} ions was observed in rats treated with the plant extract than in those treated with furosemide and amiloride compared to the control rats meanwhile, the dose of 300 mg/kg showed a more elevated electrolytes and diuretic indices than the other doses.

Effect of the aqueous extract of *Ceiba pentandra* leaves on the natriuretic, saluretic and CAI activity

Natriuretic, saluretic activity as well as CAI was evaluated 24hrs after the administration of substances as seen in table 7. Saluretic activity significantly increased and of the dose dependent manner in animals treated with plant extract (15.29%, 23.73% and 58.89% doses for 200, 250 and 300 mg/kg) respectively as well as in those that were treated with reference pharmacological substances (16.03% and 48.42% respectively for furosemide and amiloride) compared to the animals treated with distilled water.

Likewise, CAI significantly increases in animals with the aqueous extract from 27.39%, 31.50% to 35.61% for the doses of 200, 250 and 300 mg/kg respectively as well as in the animals treated with reference pharmacological substances (12.32% and 37.26% for furosemide and amiloride) respectively in comparison to the control.

The dose of 300 mg/kg of saluretic showed a significant increase of 36.94% compared to furosemide and 7.05% compared to amiloride. The doses 200 and 250 mg/kg showed no significant change in the natriuretic activity compared to control. On the other hand, the

dose 300 mg/kg as well as reference medicines brought a significant increase of 19.53%, 17.57% and 35.15% for the dose 300 mg/kg, furosemide and amiloride) respectively of the natriuretic activity compared to the control group.

Effect of the aqueous extract of *Ceiba pentandra* leaves on the functioning indices of the kidneys

Table 8 shows the values of biochemical parameters in urine. We remarked on this table that no significant change ($P>0.05$) in the rate of creatinine was observed in animals treated with aqueous extract of doses 200 and 250 mg/kg as well as in those haven received furosemide compared to the control. In contrast, a significant increase ($p<0.05$) in the rate of creatinine from 47,56 mg/dL to 44,65mg/dL respectively for the groups treated with the extract at the dose 300 mg/kg and in those treated with amiloride compared to the control (36,43 mg/dL) was observed (be it a respective increase rate of 30.55% and 22.56%).

Creatinine clearance varies in animals treated with neither aqueous extract nor reference medicine. The aqueous extract administered at the doses 200 and 250 mg/kg increased significantly ($p<0.05$) creatinine clearance compared to the control (a respective percentage of 102, 5% and 90% for instance). Likewise, furosemide and amiloride brought an increase of this clearance of $0,08\pm 0,02$ and $0,09\pm 0,01$ compared to $1,002\pm 0,12$ respectively for the control.

The glomerular filtration rate increases significantly ($P<0.05$) and in a dose dependent manner in animals treated with the aqueous extract of $1,15\pm 0,12$, $1,23\pm 0,07$, $1,58\pm 0,09$ for the of doses 200, 250 and 300 mg/kg respectively compared to the control ($0,93\pm 0,12$).

In the same light, reference pharmacological substances brought a significant increase ($p < 0.05$) of glomerular filtration rate (1, 16 ± 0 , 12 for furosemide and 1, 48 ± 0 , 15 for amiloride) compared to the control (0, 93 ± 0 , 12).

The administration of the aqueous extract just like furosemide and amiloride brought a increase in the concentration of urea in urine.

This concentration significantly increased ($p < 0.05$) and in a dose-dependent manner in animals treated with the aqueous extract from 7,75%, 25,28% to 66, 37% for doses 200, 250 and 300 mg/kg respectively compared to the control group. In the same light, a significant increase in the concentration of urea in urine was observed in animals treated with 86, 78% furosemide and 60, 34% amiloride compared to the control.

Uosm significantly reduced ($p < 0.05$) in animals treated with the aqueous extract as well as in animals treated with reference pharmacological substances (42,30%, 40,10%, 35,51%, 8,79% and 15,93% for the doses 200 mg/kg, 250 mg/kg, 300 mg/kg, furosemide and amiloride) compared to the animals control group.

The extract dose of 300 mg/kg, for furosemide and amiloride increased significantly ($p < 0.05$) the Cosm from 4, 25%, 59, 57% to 42,55% compared to distilled water. The Cosm of rats treated with the aqueous extract of doses 250 mg/kg and 300 mg/kg reduced significantly ($p < 0.05$).

The C_{H_2O} significantly increased from 15, 78%, 22, 88% to 19, and 29% respectively for the significant dose of extract, furosemide and amiloride (Table 8).

Effect of the aqueous extract of *Ceiba pentandra* leaves on blood parameters

Table 9 below shows the effects of the aqueous extract of *Ceiba pentandra* leaves on the biochemical parameters in the blood. On this table we notice a significant reduction in the concentration of urea from 74.07, 74.07, 51.85, 51.85 to 37.03% respectively for the doses of 200, 250, 300 mg/kg respectively, Furosemide and Amiloride compared to the control.

During the manipulation process, a significant reduction ($P < 0.05$) of glycemia was observed treated with the aqueous extract from 33.75%, 29.65% and 40.92% for the doses 200, 250 and 300mg/kg respectively compared to the control.

Likewise, we also observed a significant reduction ($P < 0.05$) in glycemia of 27.76% in animals treated with Amiloride compared to the control rats. No significant change in glycemia was observed in animals treated with Furosemide. Similarly, no significant change ($P > 0.05$) in the concentration of creatinine was observed in the animals during the manipulation process.

The concentration of ions (Na^+ , Ca^{2+} et Cl^-) in the blood reduced in a significant manner ($P < 0.05$) as in animals treated with the aqueous extract of the plant than in those that received Amiloride compared to the group treated with distilled water.

Similarly, we also noticed a significant increase in the Posm in animals treated with the aqueous extract with doses of 250 and 300 mg/kg, with furosemide and amiloride compared to those treated with distilled water. Contrarily, no significant change ($P > 0.05$) in the concentration of potassium ions in the blood was observed.

Table.1 Phytochemical analysis of the aqueous extract of *C. pentandra* leaves

Chemical compound	Indication of leaves extract	Content (%)	Fractions			
			A	B	C	D
Anthocyanin	+	0.693±0.012	-	-	-	-
Glycoside	+	0.312±0.025	-	-	-	-
Flavonoid	+++	16.210±0.162	+++	-	-	-
Alkaloid	++	11.530±0.187	-	++	-	-
Phenol	++	0.457±0.035	-	-	-	-
Steroids	++	0.374±0.033	-	-	-	-
Tannins	+	0.268±0.048	-	-	-	++
Saponins	++	5.345±0.057	-	-	++	-

+++, the presence of a very strong concentration of bioactive compounds;
 ++, the presence of a strong concentration of bioactive compounds;
 +, the presence of bioactive compounds.

Table.2 Effect of extract aqueous of the bark of *C. pentandra* on the body temperature

Doses (mg/kg)	control	200	250	300	Furosemide (5mg/kg)	Amiloride (14mg/kg)
T initial (°C)	36,76 ±1,26	36,88 ±0,64	37,34 ±0,79	36,78 ±1,01	37,3 ±0,43	37,54 ±0,85
T final (°C)	36,7 ±0,99	36,66 ±0,73	36,66 ±1,39	36,48 ±0,47	36,96 ±0,53	37,24 ±1,15

Each value represents the average ± standard deviation, n=5. T= temperature

Table.3 Effect of aqueous extract of *C. pentandra* on screening urinary volume

		Urinary volume (mL/kg)			
		3hrs	6hrs	12hrs	24hrs
Extract (mg/kg)	Control	0,99±0,18	2,45±0,9	7,25±0,7	8,32±1,32
	100	5,72±1,90*	4,56±1,11	8,27±2,01	11,33±2,00*
	150	1,52±0,70	5,47±0,47*	12,14±1,5*	13,93±2,3*
	200	1,01±0,33	6,42±1,42*	14,28±2,00*	18,06±1,58*
	250	1,2±0,21	6,8±1,8*	16,09±2,09*	21,9±2,91*
	300	0,67±0,20	13,87±1,51*	26,48±3,00*	31,94±2,94*

Each value represents an average ± standard deviation. *P < 0.05, a significant difference compared to control group (that is treated with distilled water).

Table.4 Effect of aqueous extract of *Ceiba pentandra* leaves on the cumulative urinary excretion volume

		Urinary volume (mL/kg/time interval)				pH value
		3hrs	6hrs	12hrs	24hrs	
control		0,99±0,18	2,45±0,9	7,25±0,7	8,32±1,32	7,38±0,15
Extract (mg/kg)	200	1,01±0,33	6,42±1,42*	14,28±2,00*	18,06±1,58*	7,58±0,16
	250	1,2±0,21	6,8±0,21*	16,09±2,09*	21,9±2,91*	8,55±0,14
	300	0,67±0,20	13,87±1,51*	26,48±3,00*▲	31,94±2,94*▲	7,75±0,2
Amiloride (14 mg/kg)		2,04±0,94*	3,58±1,40*	8,08±1,94*	22,72±2,72*	7,50±0,18
Furosemide (5mg/kg)		3,88±0,86*	7,08±1,07*	9,88±1,40*	11,74±1,80*	7,76±0,18

Each value represents an average ± standard deviation. *P < 0.05, a significant difference compared to the control group (distilled water); ▲P < 0.05, a significant difference compared to Amiloride and Furosemide.

Table.5 Effect of aqueous extract of *Ceiba pentandra* leaves on urinary excretion of electrolytes

		Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	Ca ²⁺ (mg/dL)
Control		43,12±2,46	6,05± 0,77	36,34±2,5	41,96±2,26
Extract (mg/kg)	200	43,28±1,1	7,96±0,17	48,33±2,6*	50,38±2,23*
	250	45,64±2,01*	9,06±0,77	52,68±5,63*	53,54±63,92*
	300	58,6±2,1*▲	9,56±1,51	67,66±2,2*▲	63,92±2,2*
Amiloride (14 mg/kg)		47±1,31*	7,68±0,15	45,2±6,08*	66,82±1,52*
Furosemide (5 mg/kg)		54,94±4,11*	7,93±0,1	63±2,25*	59,56±1,21*

Each value represents an average ± standard deviation. *P < 0.05 a significant difference compared to the control (distilled water); ▲P < 0.05 a significant difference compared to Amiloride and Furosemide.

Table.6 Effect of the aqueous extract of *Ceiba pentandra* on electrolytes and diuretic indices in 24hrs

		Diuretic index	Index of Na ⁺	index of K ⁺	Index of Cl ⁻	Index of Ca ²⁺
Control		1,00	1,00	1,00	1,00	1,00
Extract (mg/kg)	200	2,17	1,003	1,31	1,32	1,20
	250	2,63	1,05	1,49	1,44	1,27
	300	3,83	1,35	1,58	1,86	1,52
Furosemide (5 mg/kg)		1,41	1,08	1,26	1,24	1,59
Amiloride (14 mg/kg)		2,73	1,27	1,31	1,73	1,41

Each value shows an average per dose.

Table.7 Effect of the aqueous extract of *Ceiba pentandra* leaves on natriuretic, saluretic and CAI activities

		Saluretic activity (Na ⁺ +Cl ⁻)	Natriuretic activity (Na ⁺ /K ⁺)	CAI Cl ⁻ / (Na ⁺ +K ⁺) activity	Index saluretic	Natriuretic index	CAI index
Control		79,46±4,96	5,12± 3,19	0,73±0,77	1,00	1,00	1,00
	200	91,61±3,7*	5,43±2,47	0,93±2,04*	1,15	1.06	1,27
Extrcit (mg/kg)	250	98,32±7,64*	5,03±2,61	0,96±2,02*	1,23	0.98	1,31
	300	126,26±4,3*▲	6,12±1,39*	0,99±0,60*	1,58	1.16	1,35
Furosemide (5 mg/kg)		92,2±7,39*	6,11±1,73*	0,82±1,16*	1,16	1.19	1,12
Amiloride (14 mg/kg)		117.94±6.36*	6,92±1,1*	1,002±0,54*	1,48	1.23	1,37

Each value shows an average ± standard deviation. *P < 0.05 a significant difference compared to the control (distilled water); ▲P < 0.05 a significant difference compared to Amiloride and Furosemide.

Table.8 Effect of the aqueous extract of *Ceiba pentandra* leaves on the functioning index of the kidneys.

Dose (mg / kg)	control	200	250	300	Furosemide	AHCT
Creatinine (mg/dl)	36,43±0,44	38,78±0,53	41,2±0,74*	47,56±2,68*	40,46±1,23*	44,65±0,79*
Creat Cl (ml/min)	0,44±0,08	0,83±2,04*	0,96±2,02*	0,99±0,60*	0,82±1,16*	1,002±0,54*
GFR (ml/min)	0,93±0,12	1,15±0,12*	1,23±0,7*	1,58±0,09*	1,16±0,12*	1,48±0,15*
Urea (mg/dl)	348±0,44	375±0,2	436±0,6	579±0,5*	650±0,5*	558±0,4*
Uosm (mOsmol/kg)	182±9	105±11*	109±15*	121±12*	166±12*	153±16*
Cosm (mL/min)	0.047±0.005	0.044±0.012	0.046±0.012	0.049±0.017*	0.075±0.021*	0.067±0.011*
CH₂O (mL/min)	0.057±0.011	0.056±0.012	0.058±0.018	0.066±0.016*	0.070±0.011*	0.068±0.013*

Each value represents the mean ± ESM, n = 5. * P < 0.05 significant difference compared to control (ED = Distilled Water). Cl Crea = Creatinine Clearance, GFR = Glomerular Filtration Rate, AHCT = Amiloride Hydrochlorothiazide.

Table.9 Effect of aqueous extract of *Ceiba pentandra* leaves on blood parameters

	Urea	Creatinine	Glucose	Na ⁺	K ⁺	Cl ⁻	Ca ²⁺	Posm (mOsmol/kg)	
Control	27 ± 0,5	0,46 ± 0,02	131,12±1,6	107,79±1,13	2,52 ± 0,02	97,94±1,44	98,20±0,77	250.12±21.21	
	200	7 ± 0,3*	0,55 ± 0,04	86,86±3,92*	99,97±0,89*	2,32 ± 0,43	94,80±3,27*	65,28±1,54*	255.23±2.23*
Extract	250	7±0,2*	0,63 ± 0,03	92,23±3,7*	97,60±1,11*	2,82 ± 0,16	76,40±4,82*	54,46±1,42*	260.43±31.43*
	300	13±0,4*	0,76 ± 0,04	77,46±1,27*	86,85±1,67*	3,33 ± 0,44	53,32±5,66*	62,18±1,33*	265.62±42.67*
Furosemide (5mg/kg)		13±0,2*	1,21 ± 0,18	129±1,78	103,64±3,78	3,29 ± 0,20	101,20±4.05	124,66±3,14	266.82±32.84*
Amiloride (14mg/kg)		17±0,2*	1,26 ± 0,15	94,72±3,99*	83,98±1,24*	3,47 ± 0,30	63,40±4,86*	61,20±0,27*	265.35±44.57*

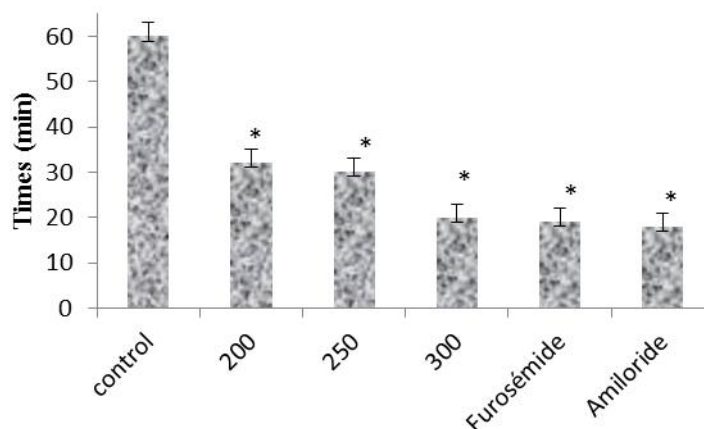
Each value represents the mean ± ESM. *P < 0.05 a significant difference compared to the control (distilled water).

Table.10 Effect of the aqueous extract of *Ceiba pentandra* leaves on the rate of transaminases

		Extract (mg/kg)					
		Control	200	250	300	Furosemide	Amiloride
blood	ASAT (UI)	19,32 ± 0,63	18,74 ± 0,70	14,98 ± 1,08	10,28 ± 1,22	17,12 ± 0,57	15,12 ± 1,35
	ALAT (UI)	8,76 ± 0,85	12,64 ± 1,90	12,96 ± 0,71	14 ± 1,58	8,68 ± 1,01	7,20 ± 1,30

Each value represents the mean ± ESM.

Fig.1 Effect of aqueous extract of *C. pentandra* leaves on the lag phase of urinary excretion. Each bar represents the average ± standard deviation. *P < 0.05, significant compared to the control.



Effect of the aqueous extract of *Ceiba pentandra* leaves on the rate of transaminases

The rate of transaminases in the blood evaluated in the course of our manipulation and recorded in the table 10 shows a significant reduction (P<0.05) in the concentration of ASAT from 22.46, 46.79 and 21.73% of doses of 250, 300 mg/kg respectively and Amiloride compared control group. In contrast, no significant change (P>0.05) in the rate of ASAT in animals treated with the aqueous extract with a dose of 200 mg/kg as well as in those animals that were treated with Furosemide compared to the control. In the same light, the concentration of ALAT showed no significant change in the different test groups compared to the control.

The phytochemical analysis results done on the aqueous extract of *Ceiba pentandra* leaves

showed the presence of some bioactive compounds. Amongst these families of compounds, some had a capital role as far as the diuretic power of the plant is concerned. Flavonoids were the compounds that induce diuresis by reacting either individually or in association with other substances^[20].

Rectal temperature evaluated in the course of the experiment showed no significant change in the animals. This relates the fact that the aqueous extract of *Ceiba pentandra* leaves have no anti pyretic activity. These same results were obtained by Ntchapda *et al.*, in 2015^[18] with aqueous extract of the bark of *Zanthoxylum heitzii* trunk had no antipyretic activity on rats.

The increase in the urinary excretion observed during screening in animals treated with the aqueous extract with doses of 200, 250 and 300 mg/kg relate to the fact that these doses

are effective doses of medicine. The dose of 300 mg/kg of the extract brought a strong increase in urinary excretion at time interval 6hrs, 12hrs and 24hrs compared to both positive and negative test control groups. This increase in diuresis could be due to the presence of families of chemical compounds such as flavonoids, saponosids and triterpens^[21]. It is probable that this increase in urinary excretion brought by the aqueous extract of *C. pentandra* leaves may have come from the synergic effects of several active substances in the extract and these effects could have been due to active secondary metabolites^[18].

According or based on our results, we noticed that the elimination of urine was accompanied by a high elimination of electrolytes. The high elimination of Na⁺ and Cl⁻ ions observed with the dose of 300 mg/kg of the extract and the group treated with furosemide show their natriuretic and saluretic effects. The elimination of Na⁺ ions by the different doses of the extract of *C. pentandra* just like furosemide is explained by the effect of the aqueous extract of furosemide on different segment of the nephron^[22]. So this may be explained by the fact that the aqueous extract of *C. pentandra* is acting by inhibiting the reabsorption of Na⁺ and water at the level of the descending loop of Henle^[23]. The presence of the sodium pump between the tubular cell and the interstitial liquid prevent sodium from migrate towards the tubular cell^[24]. The energy necessary for the functioning of the sodium pump is supplied by membranous ATPase. The urinary excretion of sodium and to a lesser extend potassium seems to indicate that the extract has an inhibitory effect on this sodium pump^[21].

The elimination of sodium ions may be equally due to the fact that the effect of the aqueous extract on the distal convulated tubules and collecting duct of the nephron by inhibiting the reabsorption of water and

sodium^[25]. Furthermore, the increase in the excretion Na⁺ ions may equally be due to the inhibiting effect of the aqueous extract, which is acting by inhibiting the conversion of the enzyme angiotensine, bringing a reduction in the secretion of aldosterone, favouring in this way the increase in urinary excretion of sodium^[26]. Moreover, an increase in the excretion of sodium reduces speed of glomerular filtration by increasing the charge in Na⁺ necessary for the exchange of Na⁺ ↔ K⁺ and also by stimulating these exchanges by intermediary of hyperaldosteronism it causes and in this way, reducing the mass of blood^[18]. In fact, a good diuretic is one that induces a great elimination of sodium while sparing potassium^[27, 28]. Our results showed a high elimination of sodium ions and a low elimination of potassium ions. It could be that all the potassium filtered by the glomerulus either actively reabsorbed at the proximal tubules. The low quantity of potassium seen in the urine result from the secretion by the distal tubules and the collecting ducts in exchange with the reabsorption of sodium and in competition with the secretion of sodium ions. The reduction in the excretion of K⁺ ions explains hypokaliuretic effect of the aqueous extract of *C. pentandra* and thereby justifying the increase in natriuretic activity.

Our study showed a significant increase (p<0.05) in the natriuretic activity with a dose of 300 mg/kg of the extract just like the reference medicines compared to the negative test groups. The work led by Ntchapda *and al.*, in 2015^[18], showed that when the relation (Na⁺/K⁺>02), of the aqueous extract has an important natriuretic activity, bringing then a more elevated urinary excretion of sodium than that of potassium.

Our results corroborate the study of Sanogo *and al.*, 2009^[29], on diuretic and salidiuretic activity of a process used in traditional medicine for the treatment of arterial

hypertensions. Similarly, when the activity of $CAI < 0, 8$, this shows a reduction in the diuretic activity^[18]. Our plant extract just like amiloride and furosemide showed an activity of $CAI > 0, 8$, hence a strong diuretic activity.

Creatinine and urea are markers for the kidney functioning. The results obtained during our work on these two markers showed a significant change of the rate of creatinine in the groups that have received furosemide and amiloride compared to the negative control group. Moreover, analysis also showed that the concentration of urea and creatinine in the urine presents a significant difference between groups treated with the aqueous extract and negative control group. In fact, creatinine just like urea is essentially eliminated from blood by glomerular filtration. An increase in the rates of urea and creatinine in urine observed in the batches treated with the aqueous extract could be due to plant effect on the glomerulus bringing then a good glomerular filtration, thereby eliminating metabolic wastes^[28].

The analysis of results showed a reduction in dependent dose in glomerular filtration flow obtained from creatinine clearance. This reduction could be due to an increase in the excretion of Na^+ ions which serves to reduce DFG by increasing the charge of Na^+ ions available for the exchange of Na^+/K^+ , stimulating such an exchange beyond by hyperaldosteronism which causes a reduction in the volume of blood^[30].

The biochemical parameters in the blood showed a decrease in dependent dose of glycemia and uremia. Urea, considered as a marker for renal dysfunctioning, generally has a normal serum concentration^[31]. The decrease in the rate of urea noticed in the blood in animals treated with the aqueous extract could be due to its significant elimination from the kidneys. The decrease of glycemia, uremia and electrolytes in animals

treated with the aqueous extract can be attributed to the fact that, besides its diuretic effect *C. pentandra* also possesses antihyperglycemic ability, has an effect in the elimination of metabolic wastes and contribute to the excretion of electrolytes (Na^+ and Cl^-)

The aqueous extract of *Ceiba pentandra* leaves has brought no significant reduction of serum transaminases. This decrease can be explained by the presence of some chemical substances such as flavonoids, saponosids and alkaloids in the plant extract which are known for their anti oxidizing activity hence hepato protective^[32]. Similarly, a study led by Barrakat *and al.*, in 2011^[33] showed that diuretics have anti oxidizing properties, so this can also be that *C. pentandra* have anti oxidizing and as such behaves as an antihypertensive.

The outcome of our work shows that the aqueous extract of *Ceiba pentandra* leaves effectively possesses diuretic properties and more over reduces arterial pressure during excretion of urine, it equally contributes to the excretion of electrolytes as well as elimination of metabolic wastes.

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