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EVALUATION OF THE ANTIHYPERTENSIVE EFFECTS OF AN AQUEOUS EXTRACT OF LEAVES OF *CEIBA PENTANDRA* (BOMBACACEAE) IN WISTAR RATS

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ABSTRACT: *Ceiba pentandra* is a plant in the African pharmacopoeia known for its antihypertensive properties. The aim of this study was to evaluate the antihypertensive effects of an aqueous extract of leaves of *Ceiba pentandra* (AECp) in Wistar rats. To do this, rats made hypertensive, by subcutaneous injection of Deoxycorticosterone acetate (DOCA) dosed at 25 mg/kg of body weight (BW) associated with a 1% sodium chloride (NaCl) drink for three weeks, were used to determine systolic blood pressure (SBP), diastolic blood pressure (DBP) and respiratory rate (RR) using a Contec 08A-VET type sphygmomanometer. Treatment of hypertensive rats for three weeks with AECp at 1000 mg/kg B.P. and Nifedipine, Captopril and Propranolol, all at 20 mg/kg B.P., promoted a highly significant ($P \leq 0.001$) reduction in PAS, DBP and RF of hypertensive rats. Analysis of biochemical markers revealed that AECp improved highly significantly ($P \leq 0.001$) the levels of creatine kinase (CK-MB), aspartate amino transferase (AST), alamine amino transferase (ALT), triglycerides (TG) and total cholesterol in hypertensive rats. AECp also reduced left ventricular mass index (LVMI). These effects would be due to the alkaloids, flavonoids and polyphenols contained in this extract. AECp induces a restoration of hemodynamic and biochemical parameter values in hypertensive rats close to those of normotensive rats. These effects could justify its use in the management of hypertension in traditional medicine.

INTRODUCTION: Medicinal plants play a vital role in the discovery and development of new molecules of therapeutic interest^{1, 2}. With this in mind, the World Health Organization (WHO) has been encouraging research initiatives on medicinal plants for decades, with a view to making improved, safe and low-cost phytopharmaceutical products available to the public³.

This strategy gives priority attention to chronic diseases, including hypertension, which is a major public health problem worldwide, with a prevalence varying between 30 and 50% in 2023^{4, 5}. Medication management of this condition requires the use of antihypertensive pharmaceutical specialties⁶.

However, due to their high cost, sometimes associated with serious side effects, and also for cultural reasons, Ivorian populations frequently turn to medicinal plants as therapeutic alternatives. Indeed, numerous scientific studies have already highlighted the pharmacological potential of certain medicinal species in the Ivorian pharmacopoeia, such as *Phyllanthus amarus* (Euphorbiaceae), *Lippia*



multiflora (Verbenaceae), *Ficus sycomorus* (Moraceae) and *Ceiba pentandra* (Bombacaceae), in the management of hypertension⁷⁻¹⁰. *Ceiba pentandra* is found in many parts of the world, including South and Central America, the West Indies and West Africa¹¹. It is used in traditional medicine to treat a variety of conditions such as mental disorders, fever¹², and hepatitis¹³. It also has diuretic, hemostatic and antidiarrheal properties¹⁴. At the cardiovascular level, previous studies have shown that the hydroethanolic extract of leaves of *Ceiba pentandra* was capable of inducing vasorelaxation *ex-vivo*, on arteries of pigs and rats¹⁵. Furthermore, the methanolic extract of bark of *Ceiba pentandra* (75 and 150 mg/kg BW), showed efficacy in the treatment of cardiometabolic syndrome in rats, by improving markers of oxidative stress¹⁶. Despite these data, the aqueous extract of leaves of *Ceiba pentandra*, used in the Ivorian pharmacopoeia in the form of decoction or maceration, for the treatment of high blood

pressure (HBP), has been the subject of little scientific investigation. Thus, the general objective of this study was to evaluate the antihypertensive effects of an aqueous extract of leaves of *Ceiba pentandra* in rat models made hypertensive by subcutaneous administration of Deoxycorticosterone acetate, associated with a salt diet (DOCA-salt).

MATERIALS AND METHODS:

Materials:

Plant Material: The plant material consisted of fresh leaves of *Ceiba pentandra* (Bombacaceae) **Fig. 1**, collected in the GBANGUIE forest, in the South-east of Côte d'Ivoire, in April 2019. This plant was identified and authenticated at the National Floristic Center (NFC), at the Félix Houphouët Boigny University, where plant samples are available, respectively under herbarium numbers 56 and 14179¹⁷.



FIG. 1: PHOTOGRAPHS OF CEIBA PENTANDRA (BOMBACACEAE)¹⁷. 1- Leafy trunk of *Ceiba pentandra*; 2- Spiny trunk of *Ceiba pentandra*

Animal Materials: A total of thirty-six (36) rats of the species *Rattus norvegicus* (Muridae) of the Wistar strain, with an average weight ranging from 128 to 138 g and an aged eight (8) weeks, were used in the experiments. They were raised and acclimated in the animal house of the Animal Physiology Teaching and Research Unit (TRU) at the Félix Houphouët-Boigny University (Abidjan, Ivory Coast). They were exposed to natural lighting (12 h light/dark cycle), with an average temperature of 25°C, and had free access to water and food. The animals were treated in accordance with the principles of the Scientific Ethics Committee for Biology concerning the use of laboratory animals in experimental tests at the Félix Houphouët-Boigny University.

Methods:

Preparation of the aqueous extract of leaves of *Ceiba pentandra*: One hundred (100) grams of crushed dried leaves of *Ceiba pentandra* were macerated using a RH type magnetic stirrer, IKA (Labortechnik, Berlin, Germany), in two thousand (2000) milliliters (ml) of distilled water for 24 hours. The macerate was filtered through Whatman N°3 paper. The filtrate obtained was evaporated under vacuum at 70°C using a rotary evaporator (Buchi Rotavapor, Shanghai, China), then freeze-dried using a freeze-dryer (Alpha 3-4 LSC basic, Berlin, Germany). The lyophilisate thus obtained constituted the powder of the aqueous extract of leaves of *Ceiba pentandra* (AECp).

Experimental Induction of Hypertension in Wistar Rats by Deoxycorticosterone Acetate Salt: Experimental induction of hypertension in rats was done according to the protocol described by Yao *et al.*⁷, with minor modifications. Thirty-six (36) normotensive rats were subcutaneously administered Deoxycorticosterone acetate (DOCA), solubilized in olive oil (1ml/100g), at a dose of 25 mg/kg body weight, twice (2) weekly for three (3) weeks. DOCA administration was combined with a 1% NaCl beverage, and treated rats were given food and water *ad libitum* for three (3) weeks. Hemodynamic blood pressure parameters were measured regularly once a week, before and during the induction of hypertension in rats, by the non-invasive method, using a CONTEC08A-VET plethysmograph (Contec Medical Systems, Beijing, China) **Fig. 2**.

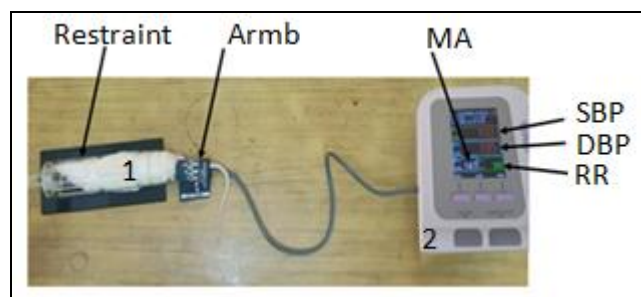


FIG. 2: PHOTOGRAPH OF THE DEVICE FOR NON-INVASIVE BLOOD PRESSURE MEASUREMENT IN RATS⁹. 1- Rat, *Rattus norvegicus* (Muridae), in the restraint cage, 2- Electronic sphygmomanometer

DBP: Diastolic blood pressure; MAP: Mean arterial pressure; SBP: Systolic blood pressure; RR: Respiratory rate.

Rats blood is collected at each parameter recording for analysis of biochemical and serum parameters before and during hypertension induction. At the end of the induction period, rats with PAS $\geq$ 140 mm Hg and PAD $\geq$ 90 mm Hg are considered hypertensive and selected for pharmacological testing.

Curative Effects of Aqueous Extract of Leaves of *Ceiba pentandra* in DOCA Salt Induced Hypertensive Rats: The rats were divided into two large groups: a control group of two (2) lots and a test group of four (4) lots, each batch consisting of six (6) rats. Rats in Lot 1, consisting of normotensive rats (NR), were treated subcutaneously with 1ml/100g olive oil P.C., food

and water. Lot 2 used as a positive control, consisted of untreated hypertensive rats (UTHR), which received DOCA by subcutaneous injection, associated with a high-salt diet. Lots 3, 4, 5 and 6 consisted of rats made hypertensive under similar conditions as those of Lot 2, then treated respectively with AECp at 1000 mg/kg BW, Nifedipine, Captopril and Propranolol, all dosed at 20 mg/kg BW, for three (3) weeks. During treatment, systolic and diastolic blood pressures and respiratory rate were recorded weekly in treated rats, using the plethysmographic method as previously described.

Determination of Biochemical Parameters of Treated Hypertensive Rats: After 3 weeks of treatment, all animals were fasted and anesthetized with diethyl ether. Blood was collected from rats at the orbital sinus of the eye using a Pasteur pipette, then centrifuged at 3,000 rpm for ten (10) minutes using a YSCF-TD4 centrifuge (YSENMED, Beijing, China). The sera obtained were stored in dry tubes for biochemical analysis.

Quantitative determination of triglycerides (TG), total cholesterol (Chol-T), high-density lipoproteins (HDL), low-density lipoproteins (LDL), creatine kinase (CK-MB), aspartate amino transferase (AST), alanine amino transferase (ALT) and electrolytes such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), chlorine (Cl^-) was carried out using a HITACHI Cobas C311/C501 (16FZ-16, Berlin, Germany).

Determination of Left Ventricular Mass Index and Relative Left Kidney Mass of Hypertensive Rats: Once the rats' hearts have been removed, they are dissected. Fat and other tissues are removed, and the heart is weighed. Then the left ventricle is weighed after cutting the atria and the right ventricle with scissors. Left ventricular mass index (LVMI) is a surrogate for left ventricular hypertrophy and a predictor of cardiac morbidity and mortality in hypertensive adults⁸. LVMI is determined by calculating the ratio of left ventricular mass to heart mass:

$$\text{LVMI} = \text{LVM (g)} / \text{MH (g)}$$

LVMI: Left ventricular mass index; LVM: Left ventricular mass; MH: Whole heart mass.

Statistical Analysis: Statistical processing of the data and graphical representation of the values were carried out using Graph Pad Prism 8 (San Diego, California, USA) and Graph Pad Prism 4 (San Diego, California, USA) software respectively. Statistical differences between the means were determined by analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance level of $p < 0.05$. All values are presented as Mean $\pm$ SEM (Standard Error on the Mean). In the test, when $p > 0.05$, the difference observed is insignificant; $p \leq 0.05$, the difference observed is significant; $p \leq 0.01$, the difference observed is highly significant; $p \leq 0.001$, the difference observed is highly significant; (*) symbol for significance compared to the normal control; (#) symbol for significance compared to the untreated disease control.

RESULTS:

DOCA Salt Induces Changes in Hemodynamic and Biochemical Parameters in Normotensive Wistar Rats: Table 1 shows the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP) and respiratory rate (RR) of normotensive rats during induction of arterial hypertension in wistar rats by DOCA-salt. The mean normal values of these parameters at D0 of hypertension induction were 123.12 ± 1.56 mm Hg for PAS, 90.02 ± 2.48 mm Hg for DBP and 87.63 ± 1.49 cycles/min for RR. These values increased highly significantly ($p < 0.001$) during induction. The values obtained at the end of induction were 152.56 ± 1.77 mm Hg for SBP, 120.12 ± 1.16 mm Hg for DBP and 119.05 ± 1.33 Cycles/min for RR on the 21st day. This represents increases of 30.84%, 28.79% and 32.49% respectively.

TABLE 1: VARIATIONS OF HEMODYNAMIC PARAMETERS IN NORMOTENSIVE RATS DURING INDUCTION OF ARTERIAL HYPERTENSION

		Hemodynamic Parameters		
Induction duration	Lots	SBP (mm Hg)	DBP (mm Hg)	RR (Cycles/min)
D0	Normotensive rats	121.5 ± 1.9	87.75 ± 1.61	87.75 ± 1.61
	Treated rats	121.1 ± 1.59	91.33 ± 1.30	87.66 ± 2.64
D7	Normotensive rats	123.12 ± 1.56	90.02 ± 2.48	87.63 ± 1.49
	Treated rats	139.76 ± 1.82	$111.32 \pm 2.17^*$	$100.11 \pm 2.35^*$
D14	Normotensive rats	119.41 ± 1.63	88.75 ± 2.25	88.73 ± 1.92
	Treated rats	$152.56 \pm 1.77^{**}$	$117.43 \pm 1.89^{**}$	$114.21 \pm 1.27^{**}$
D21	Normotensive rats	121.56 ± 1.67	87.85 ± 2.03	89.88 ± 1.91
	Treated rats	$158.45 \pm 2.01^{***}$	$120.12 \pm 1.16^{***}$	$119.05 \pm 1.33^{***}$
PV	Normotensive rats	+0.49%	+0.11%	+4.76%
	Treated rats	+ 30.84 %***	+ 28.79 %***	+ 32.49 %***

Rats receiving DOCA-salt for twenty-one (21) days of treatment were all hypertensive. DOCA induced a highly significant increase in the values of SBP, DBP and RR of the treated rats compared to those of normotensive rats. The values expressed represent the mean followed by the standard error of the mean ($m \pm SEM$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, $n = 6$).

Table 2 shows the variations of hepatic, cardiac, lipid and electrolyte parameters of RN during induction of arterial hypertension. The mean normal values of the hepatic parameters ALT, AST and CK-MB recorded on D0 of the induction of

hypertension were respectively 35.67 ± 0.18 , 39.21 ± 0.27 and 37.80 ± 0.22 U/L. DOCA-salt induced highly significant increases ($p < 0.001$) in these values compared to those of NR.

TABLE 2: VARIATIONS OF THE LEVELS OF HEPATIC, CARDIAC, LIPID AND ELECTROLYTE BIOCHEMICAL PARAMETERS IN NORMOTENSIVE RATS DURING INDUCTION OF ARTERIAL HYPERTENSION BY DOCA-SALT

		Biochemical Parameters										
Induction duration	Lots	ALT (UI/L)	AST (UI/L)	CK-MB (UI/L)	TG (g/L)	HDL (g/L)	LDL (g/L)	Chol-T (g/L)	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Ca ²⁺ (mg/L)	Cl ⁻ (mEq/L)
D0	NR	35.78 ± 0.47	38.45 ± 0.15	37.52 ± 0.05	0.75 ± 0.01	0.72 ± 0.01	1.34 ± 0.01	2.01 ± 0.01	145.5 ± 1.55	3.74 ± 0.01	94 ± 1.29	95.75 ± 0.85
	TR	35.67 ± 0.18	39.21 ± 0.27	37.80 ± 0.22	0.73 ± 0.00	0.74 ± 0.01	1.36 ± 0.02	2.05 ± 0.02	145.0 ± 1.62	3.73 ± 0.01	94.96 ± 0.82	95.54 ± 0.28
	NR	37.24 ± 0.18	38.13 ± 0.15	38.31 ± 0.05	0.74 ± 0.01	0.70 ± 0.01	1.41 ± 0.01	2.10 ± 0.01	144.0 ± 1.55	3.77 ± 0.01	93 ± 1.29	94.25 ± 0.85
	TR	35.67 ± 0.18	39.21 ± 0.27	37.80 ± 0.22	0.73 ± 0.00	0.74 ± 0.01	1.36 ± 0.02	2.05 ± 0.02	145.0 ± 1.62	3.73 ± 0.01	94.96 ± 0.82	95.54 ± 0.28

		0.38	0.64	0.51	0.01	0.01	0.02	0.01	1.32	0.01	0.71	0.62
D21	TR	43.59 ± 0.48**	45.06 ± 0.76**	45.35 ± 0.78**	1.18 ± 0.02***	0.43 ± 0.01**	2.35 ± 0.01***	3.04 ± 0.03**	157.3 ± 2.53*	2.45 ± 0.07**	100.8 ± 0.23*	101.1 ± 0.30*
	NR	+ 4.08 %	- 0.8 %	+ 2.10 %	-	- 2.77 %	+ 5.22 %	+ 4.40 %	- 0.34 %	+ 0.80 %	- 1.06 %	- 1.56 %
PV	TR	+ 22.20 %**	+ 14.91 %**	+ 19.99 %**	+ 61.64 %***	- 41.41 %**	+ 72.79 %***	+ 48.29 %***	+ 8.48 %*	- 34.31 %**	+ 6.14 %*	+ 5.81 %*

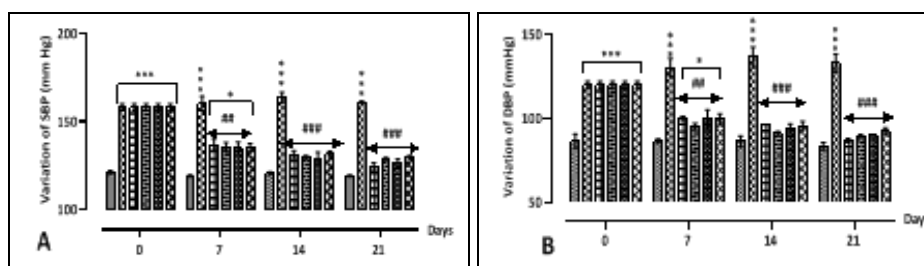
DOCA caused a highly significant increase ($p < 0.01$) in ALT, AST and CK-MB levels, a highly significant increase in TG, LDL, Chol-T levels, a highly significant decrease in HDL level, a significant increase ($p < 0.05$) in serum Na^+ , Ca^{2+} , Cl^- levels and a significant decrease ($p < 0.01$) in potassium level after twenty-one (21) days of induction in hypertensive rats compared to NR. The values expressed represent the mean followed by the standard error of the mean ($m \pm \text{SEM}$; ** $p < 0.01$, $n = 6$). ALT: Alamine Amino Transferase; AST: Aspartate Amino Transferase; Ca^{2+} : Calcium; Chol-T: Total Cholesterol; CK-MB: Creatine Kinase-MB; Cl^- : Chloride; DOCA: Deoxycorticosterone Acetate; D0 and D21: Duration of treatment with deoxycorticosterone acetate + salt; HDL: High Density Lipoprotein; K^+ : Potassium; LDL: Low Density Lipoprotein; Na^+ : Sodium; PV: Percentage of variation; NR: Normotensive Rat; SEM: Standard error of the mean; TG: Triglyceride; TR: Treated Rat with deoxycorticosterone acetate + salt.

They increase respectively to 43.59 ± 0.48 , 45.06 ± 0.76 and 45.35 ± 0.78 U/L on the 21st day, respectively, representing percentage increases of 22.20%, 14.91% and 19.99% compared with NR. The mean normal values of TG, HDL, LDL and Chol-T recorded from NR at D0 of induction were respectively 0.73 ± 0.00 , 0.74 ± 0.01 , 1.36 ± 0.02 and 2.05 ± 0.02 g/L. These values increase to 1.18 ± 0.02 , 0.43 ± 0.01 , 2.35 ± 0.01 and 3.04 ± 0.03 g/L, i.e. highly significant increases ($p < 0.001$) of 61.64%, 72.79% and 48.29% respectively for TG, LDL and Chol-T, and hypertensive rats show a highly significant decrease ($p < 0.001$) of 41.41% for HDL compared to NR. Finally, DOCA-salt induced a significant increase ($p < 0.05$) in the serum level of Na^+ , Ca^{2+} and Cl^- of 8.48%, 6.14% and 5.81% compared to NR. On the other hand, the serum level of K^+ indicates a highly significant decrease ($p < 0.001$) of 34.31%.

Ceiba pentandra Leaf Aqueous Extract Reduces Hemodynamic Parameters in Hypertensive Rats

DOCA-Salt: The hypertensive animals present mean values of SBP, DBP and RR of 152.56 ± 1.77 , 120.12 ± 1.16 mm Hg and 119.05 ± 1.33 Cycles/min respectively. The administration of AECp at 1000 mg/kg BW to hypertensive rats induced a highly significant decrease ($p < 0.001$) in SBP compared to that of RHNT, this decrease was

124.8 ± 2.06 mm Hg, i.e. a reduction of 18.19%. Treatment of rats with 20 mg/kg BW of Nifedipine, Captopril and Propranolol also induced highly significant decreases ($p < 0.001$) in the SBP of these animals compared to untreated hypertensive rats (UTHR). These decreases were respectively 15.44%, 16.88% and 14.91%. The SBP values of the treated animals showed no significant difference compared to those of the NR at the end of the 21 days of treatment **Fig. 3A**. AECp at 1000 mg/kg BW induced a significant decrease in DBP of treated rats compared to that of UTHR. The value is 87.00 ± 1.15 mm Hg, a reduction of 27.57%. The administration of a dose of 20 mg/kg BW of Nifedipine, Captopril and Propranolol, induces a respective reduction of 25.28%; 24.86% and 22.78% of DBP. These DBP values are not statistically different from those of NR at the end of 21 days of treatment **Fig. 3B**. The RR of rats treated with 1000 mg/kg P.C. of AECp showed a significant reduction compared with that of UTHR, with a value of 102.50 ± 1.93 Cycles/min, i.e. a 14.66% reduction. Nifedipine, Captopril and Propranolol, at a dose of 20 mg/kg BW, induced reductions in RR values of 13.74%, 13.96% and 12.44% respectively, which were not statistically different from those of NR **Fig. 3C**.



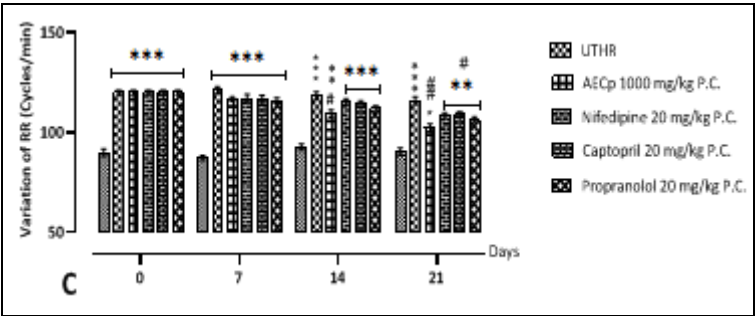


FIG. 3: HISTOGRAMS OF VARIATIONS IN HEMODYNAMIC PARAMETERS OF HYPERTENSIVE RATS DURING TREATMENTS WITH AQUEOUS EXTRACT OF LEAVES OF *CEIBA PENTANDRA*, NIFEDIPINE, CAPTOPRIL AND PROPRANOLOL. A: Histograms of systolic blood pressure variation, B: Histograms of diastolic blood pressure variation, C: Histograms of respiratory rate variation. AECp, Nif., Capt. and Prop. induced highly significant decreases ($p < 0.001$) in SBP, DBP and RR of hypertensive rats compared to those of UTHR. The values of SBP and DBP were not statistically different from those of NR, however the RR showed statistical differences from those of NR after 21 days of treatment. The values are expressed as means followed by the standard error of the mean ($m \pm SEM$, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$ vs. NR; $\#p < 0.05$; $##p < 0.01$; $###p < 0.001$ vs. UTHR; $n = 6$).

Aqueous Extract of Leaves of *Ceiba pentandra* Improves Serum Concentrations of Liver and Cardiac Parameters in Hypertensive Rats DOCA-Salt: Hypertensive animals had mean AST, ALT and CK-MB levels of 45.06 ± 0.76 , 43.59 ± 0.48 and 45.38 ± 0.74 U/L. Administrations of AECp at 1000 mg/kg BW and 20 mg/kg BW of Nifedipine, Captopril, Propranolol to hypertensive rats induce highly significant decreases ($p < 0.001$)

in AST and CK-MB levels and very significant decreases ($p < 0.001$) in ALT levels compared to those of UTHR, these levels increase respectively to 37.57 ± 0.32 , 39.72 ± 0.23 and 38.67 ± 0.46 U/L, i.e. reductions of 16.63%, 11.19% and 14.73% **Table 3.** The levels of AST, ALT and CK-MB of treated rats showed no significant difference ($p > 0.05$) compared with those of NR.

TABLE 3: VARIATIONS OF HEPATIC AND CARDIAC PARAMETERS IN HYPERTENSIVE RATS AFTER TREATMENT WITH DOSES OF AQUEOUS EXTRACT OF LEAVES OF *CEIBA PENTANDRA* AND REFERENCE ANTIHYPERTENSIVES AFTER 21 DAYS

Liver and Cardiac Parameters				
Duration	Treatments	ALT (U/L)	AST (U/L)	CK-MB (U/L)
D0	NR	37.24 ± 0.38	38.13 ± 0.64	38.31 ± 0.51
	UTHR	$43.50 \pm 0.30^{***}$	$45.06 \pm 0.93^{***}$	$45.38 \pm 0.74^{***}$
	AECp 1000 mg/kg P.C.	$43.62 \pm 0.31^{***}$	$45.21 \pm 0.91^{***}$	$44.90 \pm 0.85^{***}$
	Nif. 20 mg/kg P.C.	$43.51 \pm 0.37^{***}$	$44.78 \pm 0.94^{***}$	$45.12 \pm 0.89^{***}$
	Capt. 20 mg/kg P.C.	$43.56 \pm 0.40^{***}$	$44.97 \pm 0.88^{***}$	$44.99 \pm 0.97^{***}$
	Prop. 20 mg/kg P.C.	$43.57 \pm 0.33^{***}$	$45.14 \pm 0.79^{***}$	$45.33 \pm 0.95^{***}$
D21	NR	37.48 ± 0.21	37.54 ± 0.18	37.8 ± 0.29
	UTHR	$45.38 \pm 1.66^{***}$	$46.02 \pm 0.78^{***}$	$46.82 \pm 0.88^{***}$
	AECp 1000 mg/kg P.C.	$35.48 \pm 0.87^{###}$	$37.57 \pm 0.32^{###}$	$38.67 \pm 0.46^{###}$
	Nif. 20 mg/kg P.C.	$37.2 \pm 0.61^{###}$	$38.23 \pm 0.61^{###}$	$39.49 \pm 0.54^{###}$
	Capt. 20 mg/kg P.C.	$37.05 \pm 0.57^{###}$	$36.77 \pm 0.57^{###}$	$37.84 \pm 0.36^{###}$
	Prop. 20 mg/kg P.C.	$36.8 \pm 0.58^{###}$	$38.26 \pm 0.64^{###}$	$38.02 \pm 0.81^{###}$

Treatments of hypertensive rats with the different substances induced very significant decreases ($p < 0.001$) in the ALT, AST and CK-MB levels of these rats compared to those of the UTHR at D21 and were not statistically different from those of the NR. Values are expressed as means followed by the standard error of the mean ($m \pm SEM$, $***p < 0.001$ vs. NR; $###p < 0.001$ vs. UTHR; $n = 6$).

Aqueous Extract of leaves of *Ceiba pentandra* Improves Lipid Marker Concentrations in DOCA-salt Hypertensive Rats: Table 4 summarizes the lipid profile results obtained after treatment of hypertensive rats with AECp at 1000 mg/kg BW, Nifedipine, Captopril and Propranolol

at 20 mg/kg BW. The mean values of serum levels of TG, HDL-Chol, LDL-Chol and Chol-T in hypertensive rats were 1.1 ± 0.07 , 0.38 ± 0.01 , 2.38 ± 0.01 and 2.97 ± 0.01 g/L, respectively. Treatments of hypertensive rats with EACp, Nifedipine, Captopril and Propranolol resulted in

highly significant decreases ($p < 0.001$) in TG, LDL-Chol and chol-T levels and a highly significant increase ($p < 0.001$) in HDL-Chol levels

compared to those of UTHR at D21. However, these rates do not present any statistical difference compared to those of NR.

TABLE 4: VARIATIONS OF LIPID PARAMETERS IN HYPERTENSIVE RATS AFTER TREATMENT WITH DOSES OF AQUEOUS EXTRACT OF LEAVES OF *CEIBA PENTANDRA*, NIFEDIPINE, CAPTOPRIL AND PROPRANOLOL FOR 21 DAYS

		Lipid Parameters			
Duration	Treatments	TG(g/L)	HDL-Chol(g/L)	LDL-Chol(g/L)	Chol-T(g/L)
D0	Normotensive rat	0.75 ± 0.01	0.54 ± 0.01	1.41 ± 0.02	2.1 ± 0.02
	UTHR	1.1 ± 0.07***	0.38 ± 0.01***	2.38 ± 0.01***	2.97 ± 0.01***
	AECp 1000 mg/kg P.C.	1.2 ± 0.01***	0.38 ± 0.01***	2.39 ± 0.05***	2.99 ± 0.05***
	Nifedipine. 20 mg/kg P.C.	1.1 ± 0.07***	0.37 ± 0.04***	2.36 ± 0.01***	2.96 ± 0.01***
	Captopril. 20 mg/kg P.C.	1.2 ± 0.03***	0.39 ± 0.03***	2.40 ± 0.04***	2.95 ± 0.06***
	Propranolol. 20 mg/kg P.C.	1.2 ± 0.03***	0.38 ± 0.01***	2.38 ± 0.01***	2.98 ± 0.03***
D21	Normotensive rat	0.71 ± 0.01	0.51 ± 0.02	1.26 ± 0.05	1.94 ± 0.04
	UTHR	1.12 ± 0.04***	0.32 ± 0.03***	2.36 ± 0.01***	2.90 ± 0.13***
	AECp 1000 mg/kg P.C.	0.75 ± 0.01###	0.55 ± 0.02###	1.29 ± 0.06###	1.99 ± 0.03###
	Nifedipine. 20 mg/kg P.C.	0.72 ± 0.01###	0.53 ± 0.01###	1.34 ± 0.01###	2.02 ± 0.01###
	Captopril. 20 mg/kg P.C.	0.72 ± 0.07###	0.51 ± 0.01###	1.35 ± 0.02###	2.08 ± 0.03###
	Propranolol. 20 mg/kg P.C.	0.81 ± 0.04###	0.56 ± 0.02###	1.38 ± 0.03###	2.10 ± 0.03###

Treatments of hypertensive rats with the different substances induced highly significant decreases ($p < 0.001$) in TG, LDL-Chol, and Chol-T levels and a highly significant increase ($p < 0.001$) in HDL-chol compared to those of UTHR at D21 and were not statistically different from those of NR. Values are expressed as means followed by the standard error of the mean ($m \pm SEM$, *** $p < 0.001$ vs. NR; ### $p < 0.001$ vs. UTHR, $n = 6$).

Aqueous Extract of Leaves of *Ceiba pentandra* Normalizes Serum Electrolyte Concentration in Hypertensive Rats DOCA-Salt: The mean serum levels of Na^+ , K^+ , Ca^{2+} and Cl^- of hypertensive rats were 157.3 ± 2.53 , 2.450 ± 0.04 , 101.3 ± 1.31 and 101.1 ± 0.01 mEq/L, respectively. At D21, treatments of hypertensive rats with AECp at 1000 mg/kg BW and 20 mg/kg BW of Nifedipine,

Captopril and Propranolol, resulted in a significant decrease ($p < 0.05$) in the Na^+ level, highly significant ($p < 0.001$) in the Ca^{2+} and Cl^- levels and a highly significant increase ($p < 0.001$) in the K^+ level of these animals compared to those of UTHR. These rates are statistically close to those of NR **Table 5**.

TABLE 5: ELECTROLYTE LEVELS OF HYPERTENSIVE RATS AFTER TREATMENT WITH DOSES OF AQUEOUS EXTRACT OF LEAVES OF *CEIBA PENTANDRA*, NIFEDIPINE, CAPTOPRIL AND PROPRANOLOL FOR 21 DAYS

Duration	Treatments	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{2+} (mg/L)	Cl^- (mEq/L)
D0	NR	144 ± 1.32	4.408 ± 0.01	93 ± 0.71	94.2 ± 0.62
	UTHR	157.3 ± 2.53*	2.450 ± 0.04***	101.3 ± 1.31*	101.1 ± 0.01*
	AECp	156.2 ± 2.60*	2.459 ± 0.03***	100.6 ± 2.05*	99.9 ± 1.88*
	Nif.	158.1 ± 1.97*	2.467 ± 0.07***	102.0 ± 2.01*	101.4 ± 1.53*
	Capt.	157.2 ± 1.94*	2.439 ± 0.02***	100.9 ± 1.04*	102.5 ± 2.16*
	Prop.	158.5 ± 1.99*	2.438 ± 0.04***	101.5 ± 1.11*	100.6 ± 1.13*
D21	NR	143.5 ± 1.70	4.155 ± 0.02	94.5 ± 0.50	95 ± 1.47
	UTHR	152.8 ± 0.64*	2.875 ± 0.05***	101.8 ± 1.54*	101.8 ± 0.75*
	AECp	142.3 ± 0.25#	3.455 ± 0.14###	94 ± 1.22#	94.5 ± 1.44#
	Nif.	143.5 ± 1.04#	3.368 ± 0.05###	92.75 ± 0.47#	91.75 ± 0.75#
	Capt.	145.5 ± 1.84#	3.215 ± 0.03###	93.5 ± 1.19#	92.75 ± 1.18#
	Prop.	144.5 ± 0.84###	3.326 ± 0.05###	93.0 ± 0.81#	91.75 ± 0.81#

Treatments in hypertensive rats induce a significant decrease ($p < 0.05$) in the Na^+ level, a highly significant decrease ($p < 0.001$) in the Ca^{2+} and Cl^- levels and a highly significant increase ($p < 0.001$) in the K^+ level compared to those of UTHR at D21 and not statistically different from those of NR. Values are expressed as means followed by the standard error of the mean ($m \pm SEM$, * $p < 0.05$, *** $p < 0.001$ vs. NR; # $p < 0.05$, ### $p < 0.001$ vs. UTHR; $n = 6$).

Aqueous Extract of Leaves of *Ceiba pentandra* Decreases Left Ventricular Mass Index of

DOCA-Salt Hypertensive Rats: At the end of the treatments, the left ventricular mass indices

(LVMI) of hypertensive rats treated with AECp 1000 mg/kg BW, Nifedipine, Captopril and Propranolol at 20 mg/kg BW showed respectively very significant ($p < 0.01$) and significant ($p < 0.05$) decreases compared to those of UTHR **Table 6**. The LVMI of UTHR indicated a very significant

increase ($p < 0.01$) in their left ventricle compared to that of NR. The LVMI of hypertensive rats treated with AECp 1000 mg/kg BW was not statistically different from that of NRs, whereas the LVMI of those treated with reference antihypertensives were statically different.

TABLE 6: LEFT VENTRICULAR MASS INDICES OF HYPERTENSIVE RATS AFTER TREATMENT WITH AQUEOUS EXTRACT OF LEAVES OF CEIBA PENTANDRA, NIFEDIPINE, CAPTOPRIL AND PROPRANOLOL

Masses of the Heart and Left Ventricle			
Treatments	MVG (g)	MC (g)	IMVG
Normotensives Rats (NR)	1.43 ± 0.06	2.32 ± 0.06	0.61 ± 0.06
Untreated hypertensive rats (UTHR)	$3.17 \pm 0.07^{**}$	$3.61 \pm 0.07^{**}$	$0.87 \pm 0.07^{**}$
AECp 1000 mg/kg P.C.	$1.67 \pm 0.02^{##}$	$2.39 \pm 0.02^{##}$	$0.69 \pm 0.02^{##}$
Nifedipine 20 mg/kg P.C	$2.29 \pm 0.07^{*#}$	$2.51 \pm 0.07^{*#}$	$0.91 \pm 0.07^{*#}$
Captopril 20 mg/kg P.C	$2.12 \pm 0.28^{*#}$	$2.62 \pm 0.28^{*#}$	$0.80 \pm 0.28^{*#}$
Propranolol 20 mg/kg P.C.	$2.03 \pm 0.06^{*#}$	$2.58 \pm 0.06^{*#}$	$0.78 \pm 0.06^{*#}$

AECp induced very significant ($p < 0.001$) decreases in hypertensive rats and significant ($p < 0.01$) decreases in those treated with reference antihypertensives in LVMI compared to that of UTHR after 21 days. On the other hand, the LVMI of rats treated with AECp 1000 mg/kg BW were not statistically different from that of NR. Values are expressed as means followed by the standard error of the mean ($m \pm SEM$, * $p < 0.05$, ** $p < 0.01$ vs. NR; # $p < 0.05$, ## $p < 0.01$ vs. UTHR; $n = 6$).

DISCUSSION: Induction of hypertension in normotensive rats by DOCA-salt induced very significant changes of hemodynamic parameters of blood pressure (SBP, DBP, and RR) and serum biochemical parameters (renal, cardiac, lipid, and electrolyte) compared to those in normotensive rats (NR). All these changes confirm the validity of the experimental model obtained in this study⁸. DOCA-salt resulted in a highly significant increase ($p < 0.001$) in SBP, DBP, and RR of RN, which increased from 121.50 ± 1.00 to 158.45 ± 2.01 mm Hg, from 87.75 ± 1.61 to 120.12 ± 1.16 mm Hg, and from 87.75 ± 1.61 to 119.05 ± 1.33 cycles/min, respectively. DOCA-salt would promote the release of vasopressin, a hormone which in turn stimulates the release of endothelin I, a substance known for its vasoconstrictor properties¹⁸. The increase in blood pressure could also be due to an alteration of endothelial activity (cardiac, vascular), an increase in the level of angiotensin II, an increase in intestinal absorption of sodium and a decrease in renal excretion of NaCl¹⁹.

Daily administrations of AECp at 1000 mg/kg BW and 20 mg/kg BW of Nifedipine, Captopril and Propranolol, as treatments to hypertensive rats, induced highly significant ($p < 0.001$) decreases of SBP, DBP and significant decreases of RR of these hypertensive rats compared to those of untreated hypertensive rats (UTHR). The values of SBP and DBP are not statistically different from those of

NR, on the other hand the RR present statistical differences from those of NR after 21 days of treatment. Our results are similar to those of Kouadio⁹ with aqueous and hydroethanolic extracts of leaves of *Ficus sycomorus* L. (Moraceae) in mammals, which resulted in normalization of blood pressure values in hypertensive rats at 250 mg/kg B.P., and to those of Kouakou et al.²⁰ with extract of *Bidens pilosa* (Asteraceae), which generated small variations in RR within the range of normal values in animals. These results suggest that the aqueous extract of leaves of *Ceiba pentandra* contains bioactive compounds capable of maintaining or restoring the hemodynamic parameters of treated hypertensive rats to values similar to those of NR. Indeed, polyphenols would be able to reduce the serum level of angiotensin converting enzyme (ACE)²¹ and to stimulate the synthesis of nitric oxide (NO), a vasodilator compound²², by endothelial cells. Also tannins and flavonoids would also exert a strong inhibitory activity of ACE²³.

The aqueous extract of bark of *Ceiba pentandra* would contain sterols and polyterpenes, polyphenols, flavonoids, saponins, alkaloids and catechin tannins²⁴. Like this extract, AECp could contain polyphenols, tannins and flavonoids which would be responsible for the beneficial effects of AECp on the hemodynamic parameters of treated hypertensive rats. Several previous studies have

highlighted the determining role of CK-MB, as a cardiovascular marker in the diagnosis of high blood pressure (HBP), whose elevated activity would indicate induction of oxidative stress in the heart, leading to cardiac lesions such as cardiac infarction, for which it is a more sensitive marker²⁵, and inflammation of the heart muscle²⁶. Overexpression of CK-MB levels in DOCA-salt hypertensive rats was normalized in rats treated with doses of 1000 and 20 mg/kg BW, respectively, of AECp and reference antihypertensives. A similar effect was obtained with aqueous and hydroethanolic extracts of the leaves and ripe fruits of *Ficus sycomorus* (Moraceae), which, at a dose of 500 mg/kg BW, caused a decrease in the CK-MB level of approximately 15.56%, in rats made hypertensive by administration of 80% fructose⁹.

Serum concentrations of hepatic markers (AST and ALT) were significantly disturbed during the onset of hypertension, but treatment of these rats with 1000 mg/kg BW of AECp and 20 mg/kg BW of Nifedipine, Captopril and Propranolol significantly improved the level of these hepatic markers after 3 weeks of treatment. These results are in agreement with the work of N'Guessan *et al*²⁷, who showed that the aqueous extract of *Blighia sapida* (Sapindaceae) normalized the hepatic parameters of hypertensive Wistar rats by administration of 70% fructose, after 30 days of treatment. DOCA-salt administration to rats caused a significant increase in serum levels of TG, LDL-Cholesterol and Chol-T, with a significant decrease in HDL-Chol levels, compared to NR. AECp promoted a significant improvement in the level of these lipid markers in DOCA-salt hypertensive rats after 3 weeks of treatment, just like reference antihypertensive drugs. These results are comparable to those obtained with ethanolic extracts of the leaves and fruits of *Ficus carica* (Moraceae), which improved TG, LDL-Chol, Chol-T and HDL-Chol concentrations, in Sprague Dawley hypertensive rats²⁸.

The improvement of lipid parameters induced by the aqueous extract of leaves of *Ceiba pentandra* is similar to that obtained with its methanolic extract administered at doses of 75 and 150 mg/kg of BW, in the hypertensive rat model by administration of sodium monoglutamate, for four (4) weeks¹⁶. DOCA-salt induced a significant increase of Na⁺,

Ca²⁺ and Cl⁻ levels, with a significant decrease of K⁺ levels, compared to NR. In the presence of AECp at 1000 mg/kg BW, serum levels of Na⁺ decreased, with an improvement of K⁺ levels, in treated hypertensive rats compared to UTHR and ionogram values were not statistically different from those of NR. The absence of significant difference in serum concentrations of these electrolytes in treated hypertensive rats would suggest that the secretory capacity of the kidney and the normal functioning of the organs, in relation to these parameters affected by the onset of HBP, have been restored. AECp, like Nifedipine, Captopril and Propranolol, would restore the dynamic balance of these electrolytes. Our results are similar to those of Ibrahim *et al.*²⁹, who showed that the administration of the ethanolic extract of leaves of *Markhamia tomentosa* (Benth) K. Schum (Bignoniaceae) allowed to maintain the dynamic balance existing between the different serum concentrations of Ca²⁺, Cl⁻, K⁺ and Na⁺ in rats.

All these results corroborate the efficacy of the aqueous extract of leaves of *Ceiba pentandra* in the management of cardiometabolic diseases.

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Author's Contributions: This work was carried out in collaboration among all authors.

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