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Acute and subacute toxicities of an aqueous leaves extract of *Ceiba pentadra* (bombacaceae), a antihypertensive plant, and its ethanolic fraction in wistar rats

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Abstract
Ceiba pentandra is an African pharmacopoeia plant known for its antihypertensive properties. The aim of this study was to evaluate the oral toxicity of the aqueous leaves extract of *Ceiba pentandra* (EAqCp) and its ethanolic fraction (F1). These toxicity tests were carried out in accordance with guidelines 423 and 407 of the Organisation for Economic Co-operation and Development (OECD). Extracts of EAqCp and F1 were administered as a single dose (2000 and 5000 mg/kg body weight (BW)) and continuously (200, 400, 800 and 1000 mg/kg B.W) for 28 days, enabling a serial of haematological and biochemical analyses to be carried out. The results of the tests and analyses show that EAqCp and F1 are non-toxic and have a lethal dose 50% (LD50) greater than 5000 mg/kg B.W. This absence of oral toxicity would argue in favour of its use in various therapies in traditional medicine.

Keywords: *Ceiba pentandra*, acute toxicity, subacute toxicity

Introduction
According to the World Health Organisation, traditional medicine is defined as « the sum total of all the knowledge, skills and practices based on the theories, beliefs and experiences of different cultures used in the preservation of health » [1]. Around 80% of the world's population and over 90% of the population of developing countries rely on medicinal plants for their primary health care [2].
In fact, more than 13,000 species of medicinal plants are used as traditional remedies by various cultures around the world. There are a number of reasons why people turn to herbal medicine, including the high cost of pharmaceutical products, their socio-cultural habits and the existence of diseases for which there are no effective modern treatments [3, 4]. Thus, notwithstanding their undoubted therapeutic effects, toxicological studies are needed to improve the use of these plants from the African pharmacopoeia in people's health care.
In Côte d'Ivoire, ethnobotanical surveys have shown that *Ceiba pentandra* (Bombacaceae) is one of the plants used to treat high blood pressure. In general, the aqueous bark extract of *Ceiba pentandra* has anti-diabetic, antioxidant, anti-hyperglycaemic and anti-haemolytic properties [5], but to date, few scientific studies have focused on the biotolerance of this plant.
The general aim of this study will therefore be to evaluate the acute and subacute toxicity of an aqueous leaves extract of *Ceiba pentandra* (Bombacaceae) and its ethanolic fraction in rats in order to contribute to the valorization of the use of plants used in traditional medicine to improve the health of populations.

Materials and Methods
Plant Material
Végétal Matérial
The plant material consists of fresh leaves of *Ceiba pentandra* (Bombacaceae) collected in the GBANGUIE forest in the South-East of Côte d'Ivoire in April 2019. These leaves were authenticated at the National Floristic Center (CNF) of Abidjan of the Félix Houphouët Boigny University (UFHB), by Aké-Assi and Adjanohoun whose herbarium number is 56 identified in Tiélehoula on 26/03/1972 and in the Adiopodoumé forest on 01/07/1978 by Aké-Assi at number 14179.

Animal material

The animals used in our experiments were Wistar rats of the species *Rattus norvegicus* (Muridae). They weighed between 128 and 138 g and were eight (8) weeks old. They were raised in the animal house of the Teaching and Research Unit in Animal Physiology Laboratory of Biology and Health in Biosciences Training and Research Unit of the Félix Houphouët Boigny University of Abidjan. The room had natural lighting and an average temperature of 25 °C. The animals were placed in cages with free access to water and food.

Methods

Preparation of the aqueous leaves extract of *Ceiba pentadra*

One hundred (100) grams of dry, crushed leaves of *Ceiba pentandra* are macerated in two thousand (2.000) millilitres (ml) of distilled water for 24 hours using a RH-type magnetic stirrer (IKA, Labortechnik, Germany). The macerate was filtered through Whatman paper N° 3. The filtrate obtained was evaporated under vacuum at 70°C using a "Bucchi" type Rotavapor evaporator. The paste obtained was frozen and then freeze-dried. A powder was obtained. This powder was dissolved in distilled water to prepare the different doses used of extract.

Fractionation of aqueous leaves extract of *Ceiba pentandra*

The method used is that of Kechar and Hellal [6], which separates the chemical substances contained in the extract according to their properties. Twenty-eight (28) grams (g) of aqueous extract of *Ceiba pentandra* are dissolved in one litre of 70% ethanol (70/30; V/V). The water-ethanol mixture was stirred with a magnetic stirrer for six (6) hours and left to stand in a separating flask for twenty four (24) hours. Two phases are obtained: an ethanolic phase (F1), which is the supernatant, and a residual aqueous phase at the bottom of the settling flask. These two phases are collected separately and evaporated using a Bucchi rotary evaporator at 60 °C. The ethanolic phase obtained is dried in an oven at 50 °C.

Study of the acute and subacute toxicities of aqueous leaves extract of *Ceiba pentadra* and its ethanolic fraction by oral administration in wistar rats

Study of the acute toxicity of aqueous leaf extract of *Ceiba pentadra* and its ethanolic fraction by the oral administration in wistar rats

The acute toxicity study of aqueous leaves extract of *Ceiba pentandra* (EAqCp) and its ethanolic fraction (F1) in wistar rats was carried out in accordance with guideline 423 of the Organisation for Economic Co-operation and Development (OECD) [7].

The acute toxicity test was performed on non-pregnant Wistar females of eight (8) weeks hold and homogenous weight. Nine (9) animals were randomly divided into three (03) batches of three (03) rats each. The rats were fasted for eighteen (18) hours. The rats in batch 1 (Control) were given a distilled water solution at 10 ml/kg. Rats in batches 2 and 3 received 2000 mg/kg bw of EAqCp and F1 respectively.

The extract was administered orally at a rate of two (2) mL of solution per animal. The observation time between the different treatments administered to the rats in each batch was fortyeight (48) hours. The rats were observed at thirty (30) minute intervals until the fourth (4^{th}) hour. After four (4) hours, the rats were fed water and food. Finally, the rats were observed once every twenty-four (24) hours until the fourteenth (14^{th}) day. After 24 hours with no apparent signs of toxicity, doses of 5000 mg/kg bw EAqCp and F1 were administered to the different test batches (2 and 3).

Clinical signs of toxicity were diarrhoea, lethargy, aggressiveness, excitability, drowsiness and death. The mean weights of the fasting rats in each group were determined before administration of the substances by gavage, in order to calculate weight loss or gain during the experimental period.

Study of the subacute toxicity of aqueous leaves extract of *Ceiba pentadra* and its ethanolic fraction by the oral administration in wistar rats

The subacute toxicity study was carried out according to the experimental protocol described by Ramirez-Farias *et al*. [8]. Fifty-four (54) Wistar rats were divided into nine (9) batches of six (6) rats. Each batch contained an equal number of adult males and females with an average weight of between 128 and 138 g. The nine (9) batches of rats were divided into 02 test groups of four (4) batches of six (6) rats, 3 per sex (male and female) each, and one (1) control group (batch I) of six (6) rats. The rats in batch I, serving as controls, were given distilled water at a rate of 10 ml/kg. Rats in batches II, III, IV and V received doses of 200, 400, 800 and 1000 mg/kg B.W. of EAqCp or F1 at a rate of two (2) ml of solution per animal. These different doses of extracts were administered daily and at the same time. The animals were observed daily to detect any physiological and/or behavioural changes.

After twenty-eight (28) days, the rats were fasted and anaesthetised with diethyl ether. Their blood was then collected from the orbital sinus, without damaging the eye, using a Pasteur pipette and collected in EDTA (Ethylene Diamine Tetra Acetic) tubes for haematological tests. These tests were carried out using a Beckman Coulter (FRANCE) automated system to determine the haematocrit percentage, haemoglobin concentration, erythrocyte and reticulocyte count, leucocyte formula and platelet count in the samples taken. The blood collected in dry tubes was used to measure biochemical parameters. Organs such as the liver, kidneys and pancreas are preleved and weighed.

The relative weight of each organ was determined using the following formula

$$PR = PO / PC \times 100$$

With
PR: Relative weight of the organ (%)
PO: Organ weight (g)
PC: Body weight of rat (g).

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±SEM (Standard Error on the Mean).

The following notation is used for any significant difference

1. Not significant (ns): $p > 0.05$.
2. Significant (*): $p < 0.05$.

3. Highly significant (**): $p < 0.01$.
4. Highly significant (***): $p < 0.001$.

Results and Discussion

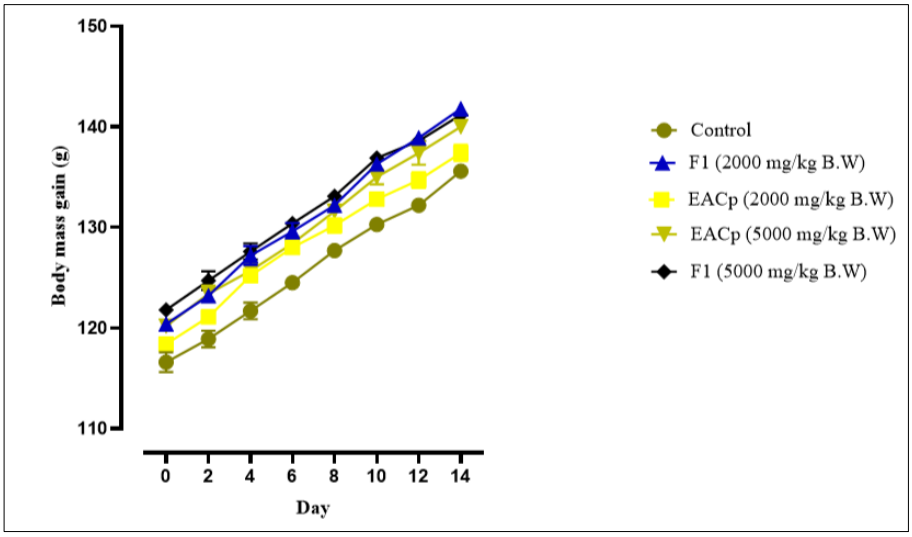
Acute toxicity of an aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction in wistar rats

Effects of an aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on the behaviour of wistar rats

Administration by gavage of single doses of 2000 and 5000 mg/kg bw of EAqCp and F1 to the animals did not result in any significant change in their behaviour after 30 minutes, 1, 2, 4, 8 and 24 hours of observation. Nevertheless, a few clinical signs such as tremors and polypnoea were observed for 2 to 3 days, without leading to the death of the animals. Also, no deaths were observed in rats treated with these doses of 2000 and 5000 mg/kg bw of the different extracts after two (2) weeks of observation.

Effects of an aqueous extract of *Ceiba pentandra* leaves and its ethanolic fraction on the body weight of rats after 14 days of administration

Figure 1. Shows the changes in body mass of rats after administration of EAqCp and F1 after fourteen (14) days of observation. The results show a weight gain in control and treated animals after fourteen (14) days of observation. However, this weight gain was not statistically significant ($p > 0.05$).



EAqCp and F1 at 2,000 to 5,000 mg/kg body weight caused non-significant body weight gain in rats after 14 days of observation (Mean ± SEM; n = 3)
EAqCp: Aqueous leaves extract of *Ceiba pentandra*;
F1: Ethanolic fraction of the aqueous leaves extract of *Ceiba pentandra*

Fig 1: Body weight variation curves for female rats fourteen (14) days after administration of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction

Effects of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on haematological parameters and serum parameters in wistar rats after fourteen (14) days of administration

Effects of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on haematological parameters in wistar rats

Table I shows the variations of haematological parameters of rats after 14 days of single administration of doses (2000 and 5000 mg.kg bw) of EAqCp and its ethanolic fraction. Our results show that these extracts cause no change in haematological parameters compared with those of control rats.

Effects of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on liver and kidney biomarkers in wistar rats

Variations in liver and kidney biomarker levels of rats after single administration of 2000 and 5000 mg/kg B.W of EAqCp and its ethanolic fraction are shown in table II. Gavage of rats with this different doses of EAqCp and F1 did not result in any variation in creatinine, urea, ALT and AST levels compared with controls.

Subacute toxicity of an aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction in wistar rats

Effects of an aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on the behaviour of wistar rats after 28 days of administration

The subacute oral toxicity study of EAqCp and F1 caused no mortality and no signs of distress after twenty-eight (28) days of gavage. EAqCp and F1 administered orally caused no deaths at doses of 200, 400, 800 and 1000 mg/kg B.W.

Effects of an aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on the body weight of rats after 28 days of administration

Table III shows the variations in body weights of the animals during twenty-eight (28) days of daily administration of doses of EAqCp and its ethanolic fraction. After twenty-eight (28) days, all the doses used resulted in a non-significant weight gain in the animals compared with their starting weights. Indeed, the female control rats showed a weight gain of 42.42±0.34 g, and those in the test batches showed respective gains of 49.79±0.15g, 46.78±0.77g and 45.87±0.39g for doses ranging from 200 to 1000 mg/kg bw EAqCp. For female rats receiving F1 at the same doses we observed weight gains of 45.67±0.71g, 42.93±1.27g, 43.40±0.55g and 46.67±0.16g.

Table 1: Values of haematological parameters of rats after fourteen (14) days of administration of an acute oral dose of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction

	D_0					D_{14}				
Haematological parameters Extracts	**WBC (10^3/µU)**	**RBC (10^6/µL)**	**Hg (g/dl)**	**Ht (%)**	**BP (10^6/µL)**	**WBC (10^3/µU)**	**RBC (10^6/µL)**	**Hg (g/dl)**	**Ht (%)**	**BP (10^6/µL)**
Distilled water (Control)	13.42±0.53	7.60±0.1	14±0.15	38±0.60	784.9± 3.33	13.65±0.58	7.81±0.48	13±0.50	37±0.34	787±0.04
EAqCp 2000 mg/kg P.C.	13.56±0.08	6.82±0.58	13±0.48	39±0.70	793±1,12	14±0.38	7.23±0.18	13.85±1.88	38±2.80	795±1.37
F1 2000 mg/kg P.C.	12.76±0.56	6.27±0.07	13.15±0.77	40.55±0,53	794.5±4.15	13.80±1.28	6.78±0.28	13.05±0.35	41±0.28	798±2.28
EAqCp 5000 mg/kg P.C	14±0.88	8±0.18	14±0.58	38±0.90	789±0.52	13±0.28	7.89±0.58	13.95±0.57	39±3.20	788±2.56
F1 5000 mg/kg P.C.	13±0.56	7.01±0.18	13.25±0.58	39±1.05	790±0.75	12.89±0.87	7.35±0.08	13.78±0.48	40±1.28	785±0.28

EAqCp and its ethanolic fraction did not alter the haematological parameters of the rats after fourteen (14) days of observation (Mean±SEM, ns; n= 3)

D0 and D14: 1st and 14th days of extract administration; SEM: Standard Error of the Mean; WBC: White Blood Cells; RBC: Red Blood Cells; Hg: Haemoglobin; Ht: Haematocrit; BP: Blood Platelets; EAqCp: Aqueous leaves extract of *Ceiba pentandra;* F1: Ethanolic fraction of the aqueous leaves extract of *Ceiba pentandra;* Distilled water (control): rats given 2ml of distilled water; EAqCp (2000 and 5000 mg/kg B.W): Rats treated with aqueous leaves extract of *Ceiba pentandra* at doses of 2000 and 5000 mg/kg B.W; F1 (2000 and 50000 mg/kg B.W): Rats treated with the ethanolic fraction of EAqCp at doses of 2000 and 5000 mg/Kg B.W

Table 2: Rate of variation of biochemical parameters of rats after administration of a single dose of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction after 14 days of observation

	DO				D14			
Biochemical parameter Treatments	**CREA (mg/l)**	**UREA (g/l)**	**AST (UI/L)**	**ALT (UI/L)**	**CREA (mg/l)**	**UREA (g/l)**	**AST (UI/L)**	**ALT (UI/L)**
Distilled water (control)	238±2.10	6±0.58	186±5.84	43±4.16	240±0.10	5.90±1.58	188±3.84	40±2.56
EAqCp 2000 mg/kg B.W	233±5.12	6.33±0.5	185±4.93	37.67±3.84	232±3.75	6.15±0.45	184±3.73	38.35±2.74
F1 2000 mg/kg B.W	230±6.28	5.85± 0.82	182±5.32	40.30±4.5	235±7.01	5.85± 0.82	182±5.32	39.23±1.5
EAqScB 5000 mg/kg B.W	234± 3.12	6.33±0.5	180±4.93	38.67±3.84	230± 2.05	6.33±0.5	180±4.93	38±0.864
F1 5000 mg/kg B.W	231±5.28	6.43± 0.82	184±5.32	42.30±4.5	230±0.28	6.05± 0.82	183±5.02	40.30±6.3

EAqCp and F1 cause no change in the levels of biochemical parameters (Mean±MSE, n= 3)

D0 and D14: 1st and 14th days of extract administration; SEM: Standard error of the mean; CREA: Creatinine (mg/l); AST: Aspartate Amino Transferase (U/L); ALT: Alanine Amino Transferase; EAqCp: Aqueous leaves extract of *Ceiba pentandra;* F1: Ethanolic fraction of the aqueous leaves extract of *Ceiba pentandra;* Distilled water (control): Rats given 2ml of distilled water; EAqCp (2000 and 50000 mg/kg B.W): Rats administered aqueous leaves extract of *Ceiba pentandra* at doses of 2000 and 5000 mg/kg B.W; F1 (2000 and 5000 mg/kg B.W); Rats administered the ethanolic fraction of EAqCp at doses of 2000 and 5000 mg/kg B.W

Tableau 3: Variation of body weight of rats during 28 days of daily administration of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction

	D0SE (mg/kg BW)	**D0**		**D14**		**D28**	
		Weight Females (g)	**Weight Males (g)**	**Weight Females (g)**	**Weight Males (g)**	**Weight Females (g)**	**Weight Males (g)**
EAqCp	Controls (distilled water)	138.98±1.32	138.83±0.47	160.48±0.85	160.87±0.24	181.40±0.50	181.52±0.01
	200	132.28±0.22	132.43±0.16	155.88±0.74	153.75±0.28	182.07±2.37	180.54±0.65
	400	135.19±0.05	135.30±0.79	156.33±1.25	154.83±1.58	181.95±1.36	182.61±3.18
	800	133.39±0.21	133.58±0.28	153.23±2.58	154.66±0.96	179.26±1.97	181.53±1.59
	1000	136.37±3.21	134.43±2.16	160.48±3.85	151.75±2.28	182.29±2.92	181.54±1.65
F1	Controls (distilled water)	138.98±1.32	138.83±0.47	160.48±0.85	160.87±0.24	181.40±0.50	181.52±0.01
	200	137.50±0.04	137.63±0.07	155.78±0.07	157.74±0.33	183.17±1.75	182.76±0.15
	400	136.34±0.14	136.43±0.21	156.69±2.58	156.86±1.62	179.27±0.13	179.07±0.12
	800	137.20±0.10	137.31±0.08	156.04±0.74	156.44±0.25	180.60±2.75	180.60±0.02
	1000	135.50±2.04	136.73±2.21	155.78±4.07	157.86±3.62	182.17±1.75	182.67±3.12

EAqCp and its ethanolic fraction induced non-significant body weight gains after twenty-eight (28) days of observation (Mean±SEM; n= 6)

D0, D14 and D28: 1st, 14th and 28th days of extracts administration

EAqCp: Aqueous leaves extract of *Ceiba pentandra*;

F1: Ethanolic fraction of the aqueous leaves extract of *Ceiba pentandra*

Controls (distilled water): Rats given 2ml of distilled water

200,400, 800, 1000: Doses of EAqCp and F1 administered to rats

Body weight gain in control EAqCp and F1 rats was approximately 42.69±0.54 g. Rats given the different doses (200, 400, 800 and 1000 mg/kg bw) of EAqCp and F1 had body weight gains ranging from 42.69±0.54 g to 48.11±0.95 g.

Effects of the aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on relative weights of organ in wistar rats: Table 4 summarizes the relative weights of rat liver, heart and kidney after increasing daily doses of EAqCp and F1 for twenty-eight (28) days. Daily administration of

these extracts did not result in any significant variation in the relative weights of the test batches compared with those of the control rats.

Effects of the aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on the hematological and serum parameters of wistar rats after twenty-eight (28) days administration

Effects of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on haematological parameters in wistar rats after twenty-eight (28) days administration

Table V summarizes the changes in haematological parameters in wistar rats after twenty-eight (28) days administration of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction at doses of 200, 400, 800 and 1000 mg/kg B.W. These variations show that EAqCp and F1 did not cause any significant changes in the haematological parameters of the test batches compared with the control batches during 28 days of administration.

Effects of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction on liver and kidney biomarkers in wistar rats after twenty-eight (28) days of administration

Table VI shows the variations in the levels of liver and kidney parameters rats during 28 days of administration of EAqCp and F1 at increasing doses of 200, 400, 800 and 1000 mg/kg bw. The values for serum creatinine, urea, ALT and AST indicate that these doses of extracts did not cause any significant variation ($p > 0.05$) in liver and kidney parameters compared with those of the control batches.

Tableau 4: Relative weights of rats liver and kidney after twenty-eight (28) days of increasing doses of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction

Extracts	Genders	Doses (mg/kg BW)	Body mass (g)	Absolute masses of organs (g)		Relative masses of organs (%)	
				Foie	Reins	Foie	Reins
EAqCp	Females	Controls	181,40±0,50	2,36±0,09	0,36±0,04	1,30±0,01	0,20±0,01
		200	182,07±2,37	2,38±0,03	0,38±0,06	1,31±0,01	0,21±0,01
		400	181,95±1,36	2,38±0,08	0,40±0,09	1,31±0,03	0,22±0,02
		800	179,26±1,97	2,31±0,07	0,38±0,02	1,29±0,02	0,21±0,01
		1000	182,29±2,92	2,37±0,04	0,36±0,04	1,30±0,01	0,20±0,02
	Males	Controls	181,52±0,01	2,44±0,01	0,40±0,03	1,33±0,01	0,22±0,02
		200	180,54±0,65	2,42±0,02	0,43±0,01	1,34±0,31	0,24±0,03
		400	182,61±3,18	2,46±0,05	0,44±0,01	1,35±0,09	0,24±0,05
		800	181,53±1,59	2,54±0,06	0,40±0,02	1,40±0,06	0,22±0,05
		1000	181,54±1,65	2,46±0,05	0,43±0,07	1,36±0,07	0,24±0,06
F1	Females	Control	181,40±0,50	2,44±0,01	0,36±0,06	1,30±0,01	0,20±0,01
		200	183,17±1,75	2,40±0,09	0,38±0,07	1,31±0,02	0,21±0,02
		400	179,27±0,13	2,35±0,11	0,39±0,02	1,31±0,01	0,22±0,01
		800	180,60±2,75	2,35±0,13	0,38±0,05	1,30±0,03	0,21±0,01
		1000	182,17±1,75	2,37±0,13	0,40±0,01	1,30±0,02	0,22±0,01
	Males	Controls	181,52±0,01	2,44±0,01	0,40±0,03	1,33±0,01	0,22±0,02
		200	182,76±0,15	2,44±0,06	0,38±0,01	1,34±0,04	0,21±0,01
		400	179,07±0,12	2,40±0,01	0,39±0,02	1,34±0,03	0,23±0,01
		800	180,60±0,02	2,43±0,02	0,38±0,01	1,35±0,02	0,24±0,03
		1000	182,67±3,12	2,43±0,02	0,38±0,02	1,33±0,04	0,21±0,04

The different doses of EAqCp and F1 did not cause any significant change in relative weights of organ after 28 days of administration (mean±SEM; $p>0.05$, n=6).
SEM: Standard Error of the Mean,
EAqCp: Aqueous leaves extract of *Ceiba pentandra*
F1: Ethanolic fraction of the aqueous leaves extract of *Ceiba pentandra*.
Controls: Rats given 2 ml distilled water;
200,400, 800, 1000: Doses of EAqCp and F1 administered to rats

Tableau 5: Variation in haematological parameters in rats after administration of increasing doses of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction after 28 days of treatment

Extracts	Genders	Dose (mg/kg P.C.)	D0					D28				
			WBC (10^3/µL)	RBC (10^6/µL)	Hg (g/dl)	Ht (%)	BP (10^6/µL)	WBC (10^3/µL)	RBC (10^6/µL)	Hg (g/dl)	Ht (%)	BP (10^6/µL)
EAqCp	Fem.	Controls	12.42±0.28	7.60±0.21	13.91±0.01	41.05±0.35	794.5±4.30	12.65±0.35	7.81±0.07	14.55±0.21	41.15±0.35	796.8±4.42
		200	12.28±0.14	6.27±0.07	13.85±0.79	40.55±0.07	793.5±7.77	14.22±1.27	7.18±1.04	15.53±1.13	41.43±0.28	793.5±4.13
		400	12.97±0.70	6.72±0.23	13.44±0.14	41.71±0.84	790±54.4	15.55±0.98	7.35±0.65	15.35±1.62	41.15±0.21	790.6±1.62
		800	12.76±0.56	6.82±0.07	13.75±0.77	42.52±1.41	797.5±8.38	14.55±1.20	7.85±0.26	15.15±0.21	43.65±1.20	797.9±7.53
		1000	12.67±1.03	7.43±0.38	13.45±1.04	41.87±2.34	797.6±9.45	13.15±0.68	7.46±0.72	15.01±0.03	43.11±3.44	799.7±6.77
	Mâles	Controls	11.91±0.14	8.43±0.14	14.22±0.14	48.21±0.42	715.5±4.71	11.33±0.14	8.45±0.13	14.24±0.28	48.35±0.49	716.5±4.79
		200	11.55±0.07	8.55±0.07	14.86±0.22	48.64±1.13	710.5±8.55	12.88±0.84	7.15±0.34	14.72±0,69	49.15±4.59	717.2±3.10
		400	12.51±1.27	7.65±0.47	13.85±0.49	48.65±3.88	718.5±8.79	12.84±1.27	7.91±0.67	14.35±0.07	49.61±1.41	712.1±8.61
		800	12.65±0.77	8.25±0.21	14.35±1.06	48.55±1.34	712.5±7.48	12.05±0.49	7.17±0.55	14.25±2.61	48±0.70	715.5±4.54
		1000	11.74±0.43	8.67±0.46	13.37±2.11	48.21±1.83	711.7±4.76	12.98±0.92	8.72±0.47	14.78±1.97	48.77±2.87	713.4±7.86
F1	Fem.	Controls	2.42±0.28	7.60±0.21	13.91±0.01	41.05±0.35	794.5±4.30	12.65±0.35	7.81±0.07	14.55±0.21	41.15±0.35	796.8±4.42
		200	11.81±0.70	6.95±0.08	13.05±0.77	42.25±0.35	793.5±3.89	13.11±0.98	7.95±0.54	13.96±0.98	42.35±0.21	795.3±6.98
		400	12.87±0.77	6.62±0.21	12.82±1.27	41.89±3.67	799.1±8.61	13.55±0.28	7.98±0.21	14.77±0.14	42.31±2.12	794.5±5.87
		800	13.75±0.12	6.72±0.05	12.13±1.13	42.57±2.26	797.8±4.14	12.32±0.60	7.65±0.77	14.93±0.28	43.45±6.92	790.2±7.19

		1000	13.54±1.17	7.27± 1.11	13.44±1.38	41.74±2.75	790.7±2.76	12.98±1.44	7.93±0.82	14.17±1.73	42.82±1.84	789.7±3.21
	Mâles	Controls	11.91±0.14	8.43±0.14	14.22±0.14	48.21±0.42	715.5±4.71	11.33±0.14	8.45±0.13	14.24±0.28	48.35±0.49	716.5±4.79
		200	10.41±0.22	8.15±016	14.15±0.35	47.33±1.13	713.5±3.84	12.75±0.64	7.68±0.68	14.63±1.55	49.15±4.59	725.7±4/42
		400	10.43±0.32	7.31±0.31	13.66±0.70	47.25±1.62	711.3±7.83	10.15±1.76	8.14±0.41	14.35±0.07	49.61±1.41	712.5±3/47
		800	10.45±2.33	7.25±0.19	13.11±0.56	48.35±3.04	711.6±4.66	12.75±0.77	7.67±0.14	14.25±0.77	49.72±0.70	716.5±7.98
		1000	11.02±2.74	8.22±0.94	14.55±1.35	47.03±2.18	715.3±6.17	12.95±1.77	8.43±0.47	14.72±1.92	48.77±2.87	727.4±3.35

EAqCp and F1 cause no change in the level of haematological parameters after twenty-eight (28) days of observation (Mean±SEM, ns; n= 6)
D0 and D28: 1st and 28th days of extract administration; SEM: Standard Error of the Mean; Fem: Female; WBC: White Blood Cells; RBC: Red Blood Cells; Hg: Haemoglobin; Ht: Haematocrit; BP: Blood Platelets; EAqCp: Aqueous leaves extract of *Ceiba pentandra;* F1: Ethanolic fraction of the aqueous leaves extract of *Ceiba pentandra;* Control: Rats given 2ml of distilled water; 200, 400, 800 and 1000 mg/kg B.W: Controls: Rats given 2ml distilled water; 200,400, 800 and 1000: Doses of EAqCp and F1 administered to rats

Tableau 6: Variation in liver and kidney biomarker levels in rats after administration of increasing doses of aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction during twenty-eight (28) days of administration

Extracts	Genders	Dose (mg/kg BW)	DO				D28			
			CREA (mg/L)	UREA (g/L)	ALT (UI/L)	AST (UI/L)	CREA (mg/L)	UREA (g/L)	ALT (UI/L)	AST (UI/L)
EAqCp	Females	**Controls**	5,80±0,98	0,58±0,05	45,60±1,02	33,30±1,41	5,35±1,55	0,59±0,07	45,60±1,02	34,50±2,68
		200	5,65±0,22	0,58±0,02	45,15±0,34	33,25±0,21	5,50±2,33	0,59± 0,09	45,95±2,89	37,70±1,09
		400	5,30±0,07	0,57±0,25	45,95±1,09	33,80±1,97	5,25±1,62	0,57±0,01	45,00±1,67	34,65±1,86
		800	5,45±0,07	0,57±0,08	45,15±0,23	33,80±0,84	5,50±5,30	0,56±0,02	45,05±0,99	33,35±1,11
		1000	5,76± 0,34	0,56±0,02	44,21±1,65	33,44±0,21	5,04±0,32	0,55±0,72	46,03±0,56	36,87±1,28
	Mâles	Controls	6,05±0,49	0,46±0,09	47,40±0,21	37,70±1,43	6,20±0,14	0,47±0,01	47,40±0,21	38,45±5,30
		200	6,60±0,07	0,47±0,08	47,80±0,45	37,35±0,63	6,30±0,70	0,47±0,03	47,40±0,21	38,50±1,41
		400	6,45±0,14	0,47±0,07	47,85±0,73	37,10±1,69	6,65±2,19	0,45±0,06	47,20±0,36	33,86±2,89
		800	6,40±0,49	0,46±0,04	47,35±0,33	37,30±1,41	5,65±2,47	0,46±0,01	47,05±5,56	37,80±1,60
		1000	6,13±0,23	0,45±0,04	48,21±0,27	38,45±0,99	5,56±0,27	0,42±0,06	46,87±0,74	38,09±1,08
F1	Females	Controls	5,80±0,98	0,58±0,08	46,70±0,69	46,70±0,69	5,35±1,55	0,59±0,07	46,70±0,69	34,50±2,68
		200	5,70±0,12	0,57±0,08	45,65±0,23	45,65±0,23	5,90±0,43	0,59±0,04	44,50±2,05	34,60±0,19
		400	5,75±0,07	0,57±0,02	46,95±0,83	46,95±0,83	5,10±0,67	0,58±0,01	51,30±3,66	34,50±2,93
		800	5,70±0,13	0,58±0,07	45,60±0,37	45,60±0,37	5,20±0,84	0,58±0,02	46,90±5,78	33,25±2,94
		1000	5,95±0,48	0,59±0,01	47,98±3,12	47,98±3,12	6,02±0,97	0,57±0,09	47,56±1,74	34,56±1,0
	Mâles	Controls	6,05±0,49	0,46±0,09	47,80±0,42	47,80±0,42	6,20±0,14	0,47±0,01	47,80±0,42	38,45±5,30
		200	6,30±0,14	0,46±0,07	49,00±0,32	49,00±0,32	6,00±0,42	0,45±0,01	47,80±0,42	38,05±1,14
		400	6,25±0,14	0,47±0,20	48,65±0,45	48,65±0,45	6,15±0,49	0,46±0,02	51,20±1,44	38,60±1,98
		800	6,30±0,28	0,45±0,00	49,95±0,71	49,95±0,71	6,25±0,07	0,44±0,04	49,30±2,90	38,45±0,28
		1000	5,91±0,34	0,46±0,02	48,04±1,22	48,04±1,22	6,02±0,53	0,48±0,04	48,01±0,43	37,59±0,44

EAqCp and F1 cause no change in the levels of biochemical parameters (Mean±MSE, n= 3)
D0 and D14: 1st and 14th days of extract administration; SEM: Standard Error of the Mean; CREA: Creatinine (mg/l); AST: Aspartate Amino Transferase (U/L); ALT: Alanine Amino Transferase; EAqCp: Aqueous leaves extract of *Ceiba pentandra;* F1: Ethanolic fraction of the aqueous leaves extract of *Ceiba pentandra;* Controls: Rats given 2ml of distilled water; 200,400, 800 and 1000: Doses mg/kg BW of EAqCp and F1 administered to rats

Discussion

The toxicological studies of the aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction involved determining the lethal doses of these extracts in normal wistar rats.

Oral administration of doses of 2000 and 5000 mg/kg B.W of EAqCp and F1 produced no signs of toxicity during the four (4) hours of observation and no death at the end of all experiments. The lethal dose 50 (LD50) of the extract and its fraction would be greater than 5000 mg/kg B.W. According to the OECD globally harmonised classification system, the aqueous leaves extract of *Ceiba pentandra* and its fraction can be classified in category 5 and considered to be non-toxic by the oral route. This absence of toxicity by gavage is also observed with other plant extracts from the traditional African pharmacopoeia, such as the aqueous of fresh leaves extract of *Cymbopogon citratus* (Poaceae) [9], the acetic extract of *Morinda morindoides* (Rubiaceae) [10] and the aqueous extract of the whole plant of *Crotalaria retusa* (Fabaceae) [11]. These authors have shown that these different extracts have LD50 values greater than 5000 mg/kg B.W.

Single oral administration of 2000 and 5000 mg/kg B.W. of these extracts resulted in non significant weight gain after fourteen (14) days observation in animals given EAqCp and F1 compared with controls. The same was true of daily doses of 200, 400, 800 and 1000 mg/kg B.W of these extracts. In this study, both male and female rats showed weight gain, which could be explained by the physiological adaptation of these animals. These results are similar to those of the oral administration to rats of the aqueous leaves extract of *Calceata Psychotria* (Rubiaceae) at doses of 300, 1200, 2000 and 5000 mg/kg B.W., which did not result in death but in a non-significant increase in the weight of the rats compared with the control [12].

A variation in the relative weight of organs is an indication of toxicity, which generally translates into a disturbance in the normal functioning or a specific lesion of these organs [13]. In this study, the relative weights of vital organs such as the liver and kidneys of animals treated with EACp and its F1 fraction revealed that they did not differ from those of control animals. This suggests that our extract does not interfere with the normal function of these organs. These results are similar to those obtained by Ukwubile *et al.* [14] on the evaluation of the toxicity of leaves of *Camellia sinensis* (Liliaceae) in mice and those of Yamssi *et al.* [15] on the acute toxicity of aqueous extracts of *Pentaclethra macrophylla* (Fabaceae) and *Psidium guajava* (Myrtaceae). These authors showed that these extracts did not significantly alter the weight of the animals and the relative weight of the organs.

Blood cells are the main targets of toxicity, so analysis of blood parameters in toxicology studies is relevant for assessing the safety of a substance [16]. The results of the haematological parameter assays show that EAqCp and F1 did not cause any significant changes ($p > 0.05$) in haematological parameters (WBC, RBC, Hg, Ht and BP) throughout the experiments. These results are similar to those obtained by Ouolouho *et al*. [17] on aqueous and ethanolic seeds extracts of *Ceiba pentandra* and contrary to those of Gbagbo *et al*. [18] who showed that the administration of 500 and 1000 mg/kg B.W. of the phytomedicine obtained from the total aqueous of stem bark extract of *Spondias mombin* L. (Anacardiaceae) resulted in a significant decrease in the number of RBC, Hg levels and an increase in BP in rats.

Analysis of liver and kidney function is very important in assessing the toxicity of extracts, because they are necessary for the survival of an organism [19]. Creatinine and urea are excellent markers of renal function. Their increase indicates renal dysfunction [15]. Our results showed that serum creatinine, urea, ALT and AST levels at doses of 200, 400, 800 and 1000 mg/kg B.W of EAqCp and F1 did not vary significantly ($p > 0.05$) from those of control batches. Similar results were obtained by Mukinda *et al*. [20] with the aqueous extract of *Aztemisia afra* (Asteraceae) in rats. However, these results are contrary to those of Gbagbo *et al*. [18], who showed a gradual increase in liver markers in rats with prolonged administration of the total aqueous of the stem bark extract of *Spondias mombin* L (Anacardiaceae). AST and ALT levels increase in cases of myopathy and myocardial infarction, and particularly AST levels increase in cases of hemolysis [21]. The maintenance of creatinine, urea, ALT and AST levels in rats after daily administration of increasing doses of EACp and F1 could indicate that these extracts have no effect on the kidneys or liver. This reinforces the non-toxicity of this plant by oral administration at the doses studied.

Conclusion

A study of the acute and subacute oral toxicity of the aqueous leaves extract of *Ceiba pentandra* and its ethanolic fraction showed that they are non-toxic, with LD50 values greater than 5000 mg/kg B.W. Analyses of haematological and biochemical parameters confirmed the non-toxicity of these extracts at levels ranging from 200 to 5000 mg/kg B.W.

These results are therefore favourable to the use of this plant in traditional medicine for the treatment of diseases.

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Conflict of Interest

There are No potential conflicts of Interest.

Author's contributions

This work was carried out in collaboration among all authors.

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