



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2019; 8(4): 211-217

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www.thepharmajournal.com

Received: 26-02-2019

Accepted: 30-03-2019

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Assessment of acute and subacute oral toxicity of Ethanollic fraction of root barks of *Anogeissus leiocarpa* (DC) Guill & Perr in albinos wistar rats

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Abstract

This study was designed to examine the influence of ethanollic fraction of the root barks of *Anogeissus leiocarpa* on the toxicity profile. Acute and sub-acute toxicity studies were carried out in rats by using OECD guideline 423 and OECD guideline 407 respectively with slight modifications. In acute oral toxicity study, ETHA was administered by oral route at 2000 and 5000 mg/kg body weight and animals were observed for toxic signs at 0, 0.5, 24 h and for next 14 days. In sub-acute oral toxicity study, ETHA was administered orally at 100, 300 and 500 mg/kg body weight/day. Animals were observed for mortality, morbidity, body weight changes and organ's relative weight, feed and water intake. Hematology, clinical biochemistry, and electrolytes were performed.

Data analysed and graphical representation of data was performed using Graph Pad Prism 7.0. The results are expressed as mean \pm standard error of means. Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Differences were considered significant at p-value less than 0.05. At acute oral toxicity, no death or adverse effects were recorded. ETHA is orally non-toxic at a LD₅₀ up to 5000 mg/kg body weight and should be classified into category 5. The subacute oral toxicity test observations during 28 days did not significantly affect the weight gain, electrolytes, hematological and biochemical parameters of rats compared to the control. Food consumption, body weight, organ weight, hematological parameters and biochemical parameters revealed no abnormalities. Therefore, it would not have a negative influence on cells blood and renal, hepatic and cardiac function and structure. This study shows that ETHA is well tolerated at a LD₅₀ up to 5000 mg/kg body weight by the organism. Definitely, ETHA has no adverse effects on the normal functioning of the body, it's orally safe.

Keywords: *Anogeissus leiocarpa*, acute subacute toxicity, nontoxic

1. Introduction

In low and middle-income countries, modern medicine is not easily accessible compare to traditional medicine. Practitioners and medicinal plants are considered as an important resource for population health. That is why, plants still continue to be used by nearly 80 % of the population as a source of primary health care [1]. Although offering alternative therapeutic solutions, plants are not always free of toxicological effects. Yet rightly or wrongly, the plants consumed as medicines or dietary supplements benefit from the image of a natural as well as natural medicine [2]. As a result, populations tend to believe that plant remedies are free of undesirable side effects [3]. Indeed, various plants used in traditional medicine have exhibited toxic effects [4]. Adverse and toxic effects are diverse and include digestive, neurological, gyneco-obstetric and cutaneous adverse effects [5]. These effects can sometimes be serious, ranging from simple intoxication to death [6]. Thus, in view of the undeniable importance of the safety and the safety of chemicals, including plant extracts, following the recommendations of several meetings of experts, international organizations such as Organization for Economic Co-operation and Economic Development of European Union has established guidelines for testing in animal models like mice, rat, guinea pig, dog, rabbit, monkey etc. It is therefore essential, before any use in medicinal form of plants extracts, to evaluate its toxicological profile to ensure its safety.

Anogeissus leiocarpa known in Côte d'Ivoire, under the name of "kerèkètè or kalama" in Malinké, "souroubouet" in Baoulé and "Niga'm" in Sénoufo, was selected for this study. This plant is variously used according to the geocultural spheres. In Côte d'Ivoire, it is used for the treatment of diarrhea [7], hemorrhoids, fever, malaria, urinary schistosomiasis

and amoebic dysentery [8]. It is also used in the treatment of constipation [6], helminthosis, shistosomosis and leprosy [9]. Pharmacological studies attribute antimicrobial [10], antihelminthic [11], trypanocidal [12] antiplasmodial [13], leishmanicidal [14], antioxidant and hepatoprotective [15] activities for this plant.

The aim of this study was to examine the toxicological profile of the ethanolic fraction of *Anogeissus leiocarpa* root bark with relevant pharmacological activities through acute and subacute oral toxicity in albino Wistar rats.

2. Materials and methods

2.1 Material

2.1.1 Plant material

The root barks of *A. leiocarpa* were used. These organs were harvested in January 2013 in Kouto (Bagoué region), a town located at 725 km north of Abidjan. The plant has been authenticated by Professor Aké-Assi of the National Floristic Center of Félix Houphouët-Boigny University and compared to the voucher specimen N° CNF 14798.

2.1.2 Animals

Albinos white rats, male and female, of Wistar strain aged 2 to 3 months and weighing between 165 to 225 g were used.

2.2 Methods

2.2.1 Preparation of ethanol fraction

The liquid-liquid partition chromatography or chromatography based on solute partition in two immiscible liquid phases was used. It required the use of three solvents of different polarity namely dichloromethane, ethyl acetate and ethanol. To carry out this chromatography, 20 g of aqueous extract of *A. leiocarpa* prepared according to the method described by Guédé-Guina *et al.* (1993) [16] are added in 100 mL of a mixture of distilled water-solvent (v/v). The whole was homogenized for 24 hours at $27 \pm 2^\circ\text{C}$, using a magnetic stirrer type IKAMAG-RCT After decantation in a separator funnel, a residual aqueous phase and an organic phase was obtained. This operation is repeated 4 times in a row. The organic fractions were collected and concentrated under vacuum at 60°C using a rotary evaporator (Büchi R110 Brand, Type MKE 6540/2) and then dried in an oven at 45°C . The lower aqueous phase was then recovered and extracted with the given solvent polarity. The toxicity of the ethanolic fraction that demonstrated good pharmacological activity in previous studies is tested in this investigation.

2.2.2 Experimental design

2.2.2.1 Acute oral toxicity test

The acute oral toxicity test was performed following the guidelines 423 of Organization for Economic Co-operation and Development (OECD) for testing of chemicals [17], with minor modifications. The acute toxicity testing involves the determination of lethal dose, the dose that kills 50 % of the tested group of rats called the median lethal dose or LD_{50} . Nine female rats nulliparous and non pregnant, weighing between 165-180 g were randomised into three groups (3 per group), control and two test groups. All the animals were fasted overnight before the commencement of the experiment. Control group received 1 mL of distilled water as vehicle whilst the first test group received ETHA single oral dose of 2000 mg/kg body bw. All the experimental animals were observed for mortality and clinical signs of toxicity (general behaviour, respiratory pattern, cardiovascular signs, motor

activities, reflexes and changes in skin and fur texture) at 30 min and 24 hours and thereafter once a day for the next 14 days following vehicle or ETHA administration. After 24 hours, no death and signs of toxicity were recorded, then a limit test at a single oral dose of 5000 mg/kg bw was conducted with three other animals under the same conditions as previously. All observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern were systematically recorded and maintained with an individual record. In addition, consideration was given for observations of convulsions, tremors, diarrhea, salivation, cough, loss of appetite, drivetrain, drowsiness, lethargy, sleep, coma and mortality. The LD_{50} was determined as previously described by OECD guidelines 423.

2.2.2.2 Subacute oral test

OECD test Guideline 407 [18], with some modifications, was used for this analysis. The sub-acute oral toxicity testing implies the determination of long-term effects of the test compound upon repeated administration. The test substance is administered daily orally (gavage) at different dose levels to several batches of animals, at a dose level per batch, for a period of 28 days. Twenty-four (24) rats of both sexes aged two to three months and weighing between 180 and 225 g on average were used. The rats were divided into four lots of six rats each according to their weight. The rats in the control group received individually and daily 1 mL of distilled water by gavage during the 28 days of treatment. The three test lots (ETHA 100, ETHA 300 and ETHA 500) received respectively by gavage 100; 300 and 500 mg/kg bw of ETHA daily for 28 days. The volume of ETHA administered daily in a single dose was 1 mL. During the 28 days of treatment, the animals were observed daily for clinical signs, symptoms of toxicity (before, immediately and three hours after administration of ETHA). The rats were weighed every week to determine the impact of the fraction on their weight gain. At the 29th day of the experiment, ie the day after the last day of treatment, all rats were anesthetized using ether and blood samples were collected in dry tubes and EDTA tubes by caudal amputation for hematological and biochemical analyzes. All animals were sacrificed by cervical dislocation. The vital organs mainly the liver, kidneys, heart and spleen were removed, cleaned with saline (NaCl 0.9 %) and weighed.

2.2.2.3 Hematological parameters

The hematological analysis was carried out using an automatic hematology analyzer ((URIT®-2900 PLUS). The parameters including Red Blood Cells (RBC) count, White blood cells (WBC) count, Hemoglobin (Hb), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean cell volume (MCV), Mean hemoglobin corpuscular rate (MHCR), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and platelet count were determined.

2.2.2.4 Biochemical parameters

The blood contained in the dry tubes was centrifuged using a centrifuge at 3000 rpm/min for 5 minutes. The serum was separated from non-heparinized blood and the serum biochemical parameters including Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Blood glucose, Blood urea, Creatinine, Creatine kinase (CK), Total protein (TP), Total cholesterol

(TC), HDL cholesterol (HDL), LDL-cholesterol (LDL) and electrolytes: K⁺, Ca²⁺, Mg²⁺ and Na⁺ were analysed by using semi-automatic ROBONIK® PRIETES TOUCH.

2.3 Statistical analysis

Results expressed as a mean ± standard error of the mean. The differences between groups of acute and subacute toxicity to control group were determined by one-way analysis of variance (ANOVA) followed by Dunnett multiple comparison test. Differences were considered significant at p less than 0.05.

3. Resultats

3.1 Acute oral toxicity of ETHA in healthy albinos Wistar rats

Two limits doses (2000 and 5000 mg/kg bw) of the ethanolic

fraction of *A. leiocarpa* were used and administered as a single oral dose to rats. The effects of oral administration of single doses of ETHA in rats are summarized in Table 1 and Table 2.

Table 1 and Table 2 showed the rat mortality rate, and the overall appearance and clinical manifestations of acute toxicity during 14 days of observation, respectively. On one hand the ethanolic extract of *A. leiocarpa* at both doses investigated in the present study did not result in mortality and any macroscopic differences in the rat’s skin, coat, eyes and mucous membranes compared to the normal control animals. On other hand, the median lethal dose (LD50) of ETHA was found be up to 5000 mg/kg bw.

Table 1: Animal mortality rate caused by ethanolic fraction of root bark of *Anogeissus leiocarpa* in acute oral test

Lots	Number of animals / Lot	Number of deaths					Total deaths	Percentage of deaths (%)
		30 min	24 h	48 h	72 h	14 j		
Control	3	0	0	0	0	0	0	0
2000	3	0	0	0	0	0	0	0
5000	3	0	0	0	0	0	0	0

Table 2: General appearance and clinical manifestations of toxicity observed in rats during acute oral toxicity

Observations	Control témoin		Tets lots expérimentaux	
	6 h	Jour 14	6 h	Jour 14
Skin and coat	Normal	Normal	Normal	Normal
Eye	Normal	Normal	Normal	Normal
Mucosa and membrane	Normal	Normal	Normal	Normal
Behavior	Normal	Normal	Normal	Normal
Appearance of feces	Normal	Normal	Normal	Normal
Drivetrain	Normal	Normal	Normal	Normal
Loss of appetite	NO	NO	NO	NO
Salivation	NO	NO	NO	NO
Léthargy	NO	NO	NO	NO
Drowsiness	NO	NO	NO	NO
Coma	NO	NO	NO	NO
Tremors	NO	NO	NO	NO
Cough	NO	NO	NO	NO

NO: not observed

3.2 Subacute oral toxicity in albinos Wistar rats

3.2.1 Effect of ETHA on the weight of rats and organs

The acute toxicity analyses of ETHA administration up to 500 mg/kg doses did not result into any change of behavior or mortality of the rats. One can see that the drugs treated animals did not show any decrease in body weight after 28 days of treatment, but they displayed only a general slight increase in body weight (Fig 1).

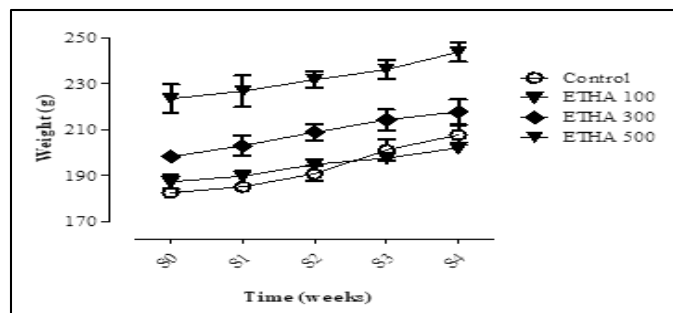


Fig 1: Effect of the ethanolic fraction of *Anogeissus leiocarpa* on the weight in subacute oral toxicity

The values are expressed as the mean affected by the standard

error on the mean (m ± esm). Each lot includes 6 animals (n = 6/lot). ETHA 100:lot treated with ETHA at dose of 100 mg/kg bw, ETHA 300:lot treated with ETHA at dose of 300 mg/kg bw, ETHA 500:lot treated with ETHA at dose of 500 mg/kg bw.

Table 3 showed the weekly weight gain rate from the first week (W1) to the fourth week (W4). One can see no significant difference in body gain rate of treated animals compared to control from W1 to W4.

Table 4 reports the measures of the weights of the heart, liver, kidney and spleen of the drugs treated animals for 28 days and the control, respectively.

The study showed no significant difference (p> 0.05) in the weight of the organs of animals group treated with ETHA compared to the control. The physical appearance of all internal organs examined at all doses of ETHA treated animals did not show any macroscopic differences in size, color and texture compared to the normal control animals. The study showed that by comparison with their respective initial body weights of the rats, there is an increase of the final body weights during the examination period. No significant difference (p> 0.05) in relative weights of treated animals compared control group these organs were also observed.

Table 3: Effect of ETHA on the weight gain rate of animals in subacute oral toxicity.

Lots	Weekly weight gain rate (%)			
	W1	W2	W3	W4
Control	1.81±0.17	4.49±0.12	6.18±.55	8.81±0.69
ETHA 100	1.68±0.65 ^{ns}	4.03±0.71 ^{ns}	5.45±0.61 ^{ns}	7.84±0.36 ^{ns}
ETHA 300	2.44±0.54 ^{ns}	5.38±0.80 ^{ns}	6.11±0.19 ^{ns}	8.28±0.42 ^{ns}
ETHA 500	1.74±0.48 ^{ns}	3.68±0.95 ^{ns}	5.90±0.56 ^{ns}	8.05±0.64 ^{ns}

W: week, ETHA: éthanolic fraction of *Anogeissus leiocarpa*. The values are expressed as the mean affected by the standard error on the mean (m ± esm). Each lot includes 6 animals (n = 6/lot). The statistical analyzes are made comparing to the

control lot. ns:no difference at p> 0.05 compared to control. ETHA 100:lot treated with ETHA at dose of 100 mg/kg bw, ETHA 300:lot treated with ETHA at dose of 300 mg/kg bw, ETHA 500:lot treated with ETHA at dose of 500 mg/kg bw.

Table 4: Effect of ETHA on the weight of organs harvested from rats in subacute oral toxicity.

Organes	Weight of organs (g)				Relative weight of organs (%)			
	Control	ETHA 100	ETHA 300	ETHA 500	Control	ETHA 100	ETHA 300	ETHA 500
Spleen	0.56±0.07	0.56±0.04 ^{ns}	0.63±0.01 ^{ns}	0.56±0.04 ^{ns}	0.27±0.05	0.28±0.00 ^{ns}	0.29±0.01 ^{ns}	0.25±0.01 ^{ns}
Heart	0.75±0.05	0.76±0.00 ^{ns}	0.77±0.02 ^{ns}	0.90±0.00*	0.36±0.01	0.37±0.06 ^{ns}	0.35±0.02 ^{ns}	0.37±0.22 ^{ns}
Kidney	0.57±0.02	0.62±0.03 ^{ns}	0.56±0.03 ^{ns}	0.62±0.03 ^{ns}	0.27±0.03	0.31±0.10 ^{ns}	0.26±0.03 ^{ns}	0.25±0.02 ^{ns}
Liver	5.91±0.81	6.03±1.01 ^{ns}	6.18±0.5 ^{ns}	6.89±0.22 ^{ns}	2.84±0.09	2.98±0.35 ^{ns}	2.84±0.10 ^{ns}	2.83±0.26 ^{ns}

The values are expressed as the mean affected by the standard error on the mean (m ± esm). Each lot includes 6 animals (n = 6/lot). ns:no difference at p> 0.05 compared to control. * p < 0.05:significant difference compared to control at p < 0.05. ETHA 100:lot treated with ETHA at dose of 100 mg/kg bw, ETHA 300:lot treated with ETHA at dose of 300 mg/kg bw, ETHA 500:lot treated with ETHA at dose of 500 mg/kg bw.

3.2.2 Effect of ETHA on the hematological profile of healthy albinos Wistar rats

The hematological profile of treated and control group are summarized in Table 5. The ETHA at all doses examined in the study did not significantly (p> 0.05) affect the red blood cells count, white blood cells (RBC), hemoglobin (Hb),

hematocrit (HCT), mean corpuscular volume (MCV), mean cell volume, mean corpuscular hemoglobin rate (MCHR), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration (MCHC). In contrast, however, the platelets (PLT) at dose of 500 mg/kg bw daily during 28 days increased significantly (p < 0.05 and p < 0.001).

Table 5: Effect of ETHA on hematological profile in subacute oral toxicity

Parameters	Value of hematological parameters			
	Control	ETHA 100	ETHA 300	ETHA 500
WBC (×10 ³ /μL)	11.34±0.04	11.29±0.13 ^{ns}	11.98±1.05 ^{ns}	12.08±0.54 ^{ns}
RBC (×10 ⁶ /μL)	7.08±0.11	7.68±0.08 ^{ns}	7.77±0.79*	7.33±0.20 ^{ns}
Lymphocytes (%)	73.88±1.56	70.78±3.79 ^{ns}	74.33±4.24 ^{ns}	69.85±2.38 ^{ns}
Monocytes (%)	10.88±0.41	11.34±0.30 ^{ns}	11.84±1.63 ^{ns}	11.03±0.32 ^{ns}
Neutrophils (%)	22.75±0.35	23.21±1.06 ^{ns}	22.33±2.11 ^{ns}	24.50±0.29 ^{ns}
Eosinophils (%)	10.01±0.01	10.33±0.23 ^{ns}	10.33±0.33 ^{ns}	10.43±0.30*
PLT (×10 ³ /μL)	261.8±30.49	270.7±16.26 ^{ns}	290.3±27.12 ^{ns}	422.8±15.43***
Hb (g/dL)	15.6±0.12	16.38±0.05 ^{ns}	14.67±1.24 ^{ns}	15.9±0.46 ^{ns}
HCT (%)	41.56±0.44	43.65±0.33 ^{ns}	39.8±4.70 ^{ns}	40.75±0.85 ^{ns}
MCV (fL)	57.48±1.37	58.85±2.32 ^{ns}	56.67±0.98 ^{ns}	56.15±1.12 ^{ns}
MCHR (pg)	21.27±0.19	21.00±0.35 ^{ns}	20.81±0.54 ^{ns}	21.83±0.68 ^{ns}
MCHC (g/dL)	37.7±3.33	35.58±2.35 ^{ns}	35.37±2.35 ^{ns}	39.45±2.62 ^{ns}
PCT (ng/mL)	0.33±0.01	0.3±0.00 ^{ns}	0.20±0.06 ^{ns}	0.23±0.04 ^{ns}

The values are expressed as the mean affected by the standard error on the mean (m ± esm). Each lot includes 6 animals (n = 6 / lot). ns:no difference at p> 0.05 compared to control. *p < 0.05; ***p< 0.001: significant difference compared to control respectively at p < 0.05 and p < 0.001. WBC: White Blood Cells (×103/μL); RBC: Red Blood Cells (×106/μL); PLT: Platelets (×103/μL); MCHC: Mean corpuscular hemoglobin concentration (g/dL); Hb: Hémoglobine (g/dL); HCT: Hématocrite (%); MCV: Mean corpuscular volume (FL/cell); MCHR: Mean corpuscular hemoglobin rate (pg); PCT: procalcitonine (ng/mL). ETHA 100:lot treated with ETHA at dose of 100 mg/kg bw, ETHA 300:lot treated with ETHA at dose of 300 mg/kg bw, ETHA 500:lot treated with ETHA at dose of 500 mg/kg bw.

3.2.3 Effect of ETHA on biochemical markers in healthy albinos Wistar rats

Table 6 summarized the effect of the ethanolic fraction of *A. leiocarpa* after 28 days of treatment on the rat's biochemical parameters. One can see no significant difference (p> 0.05) on all biochemical parameters such as renal bio makers (urea and creatinine), hepatic bio makers (ASAT activity, ALAT activity, alkaline phosphatase activity, and total protein), lipid bio makers (HDL, LDL, cholesterol total and triglycerides) and cardiac bio makers (Creatine kinase activity) of ETHA treated animals compared to the normal control group. Effect

of ETHA on the blood electrolytes of healthy albinos Wistar rats

Effect of ETHA on serum levels of electrolytes (Na⁺, K⁺, Cl⁻, Ca²⁺ and Mg²⁺) were summarized in Table 7.

All the parameters showed normal values and was no significant (p> 0.05) different in the drug treated groups when compared to the control group. However, at the end of the treatment, the high dose of 500 mg/kg bw of ETHA caused a significant decrease (p < 0.001) in the level of Mg²⁺ compared to control. This same dose of ETHA also increased significantly (p < 0.05) serum level of Cl⁻ compared to control.

Table 6: Effect of ETHA on biochemical parameters of rats in subacute oral toxicity

Biochemical parameters		Value of biochemical parameters			
		Control	ETHA 100	ETHA 300	ETHA 500
Blood Sugar	Glucose (g/L)	0.77±0.01	0.77±0.08 ^{ns}	0.75±0.06 ^{ns}	0.80±0.00 ^{ns}
	PT (g/L)	92.09±1.01	92.76±1.62 ^{ns}	92.89±3.27 ^{ns}	94.31±0.36 ^{ns}
Liver profile	ASAT (UI/L)	140.56±1.28	144.20±21.4 ^{ns}	127.3±8.57 ^{ns}	123.87±4.87 ^{ns}
	ALAT (UI/L)	57.81±3.14	55.83±6.42 ^{ns}	58.53±10.17 ^{ns}	63.47±5.33 ^{ns}
	ALP (UI/L)	87.50±2.33	90.50±4.79 ^{ns}	82.75±4.01 ^{ns}	85.5±3.18 ^{ns}
Renal profile	C (mg/L)	4.37±0.63	4.63±0.23 ^{ns}	4.51±0.33 ^{ns}	4.58±0.51 ^{ns}
	U (g/L)	0.30±0.03	0.30±0.02 ^{ns}	0.29±0.01 ^{ns}	0.28±0.00 ^{ns}
Lipid profile	TC (g/L)	0.80±0.00	0.83±0.04 ^{ns}	0.82±0.00 ^{ns}	0.80±0.00 ^{ns}
	TG (g/L)	0.92±0.20	0.90±0.16 ^{ns}	0.92±0.18 ^{ns}	0.97±0.52 ^{ns}
	HDL (g/L)	0.61±0.01	0.60±0.04 ^{ns}	0.58±0.03 ^{ns}	0.61±0.00 ^{ns}
	LDL (g/L)	0.10±0.02	0.09±0.00 ^{ns}	0.08±0.01 ^{ns}	0.12±0.03 ^{ns}
Heart Profile	CK (UI/L)	1118.9±197.30	1104.90±221.01 ^{ns}	1346.57±202.6 ^{ns}	1398.75±421.08 ^{ns}

The values are expressed as the mean affected by the standard error on the mean ($m \pm esm$). Each lot includes 6 animals ($n = 6 / \text{lot}$). ns:no difference at $p > 0.05$ compared to control. * $p < 0.05$.

ASAT: Aspartate aminotransferase (UI/L); ALAT: Alanine aminotransferase (UI/L); U:Urea (mg/L); C: creatinine (mg/L); LDH: Lactate dehydrogenase (g/L); CK: creatine kinase (IU / L); ALP: Alkaline phosphatase (UI/L); TP: Total Protein (g/L); TC: Total cholesterol (g/L); TG: Triglycerides

(g/L) HDL: High density lipoproteins; LDL: Low density lipoproteins (g/L). ETHA 100:lot treated with ETHA at dose of 100 mg/kg bw, ETHA 300:lot treated with ETHA at dose of 300 mg/kg bw, ETHA 500:lot treated with ETHA at dose of 500 mg/kg bw.

Table 7: Effect of ETHA on blood electrolytes of rats in subacute oral toxicity.

Electrolytes	Serum electrolytes level			
	Control	ETHA 100	ETHA 300	ETHA 500
Na ⁺ (mmol/L)	141.11±1.07	139.20±1.83 ^{ns}	140.3±3.43 ^{ns}	142.5±0.71 ^{ns}
K ⁺ (mmol/L)	15.85±0.88	15.29±0.84 ^{ns}	16.02±0.20 ^{ns}	16.43±0.75 ^{ns}
Cl ⁻ (mmol/L)	101.6±3.03	100.3±1.32 ^{ns}	104.6±2.47 ^{ns}	104.53±0.47*
Ca ²⁺ (g/L)	128.71±3.00	128.3±3.54 ^{ns}	131.3±5.89 ^{ns}	132.80±3.21 ^{ns}
Mg ²⁺ (g/L)	22.83±0.88	22.76±1.37 ^{ns}	22.93±0.49 ^{ns}	21.67±0.08***

The values are expressed as the mean affected by the standard error on the mean ($m \pm esm$). Each lot includes 6 animals ($n = 6 / \text{lot}$). ns:no difference at $p > 0.05$ compared to control.

* $p < 0.05$; *** $p < 0.001$: significant difference compared to control respectively at $p < 0.05$ and $p < 0.001$. Potassium (K⁺), Magnesium (Mg²⁺), Calcium (Ca²⁺), Sodium (Na⁺), Chlorine (Cl⁻).

4. Discussion

The results of the acute toxicity study revealed that administration of oral ETHA at single doses of 2000 and 5000 mg/kg bw did not cause any sign of toxicity or mortality in rat during the 14 days of observation. According to OECD Test Guideline 423, LD₅₀ was greater than 5000 mg/kg bw. According to the OECD Globally Harmonized Classification System [16], this fraction is classified as category 5 of non-toxic substances orally. These results are in agreement with those of Agaie *et al.* (2007) [19]. These authors found a LD₅₀ of the aqueous leaf extract of *A. leiocarpa* greater than 3200 mg/kg bw in the rats.

Subacute oral toxicity study was performed followed OECD test guideline 407 in both male and female Wistar albinos rats. In this present study, ETHA was administered at doses of 100-500 mg/kg bw. No significant changes ($p > 0.05$) in body weight were observed in the treated animals compared to control group. There were no significant changes in animal behavior, food and water consumptions as well as in body weight gain. This result therefore suggests that the secondary metabolites contained in ETHA did not have significant impacts on lipid metabolism, more specifically on lipid accumulation. Changes in organs weights were also signs of toxicity in animals.

The hematopoietic system serves as an important target for toxins and chemicals. It is a sensitive index of disease states in both humans and animals [20]. The results showed that

ETHA did not cause any change in the parameters of the hematological profile. This indicated that ETHA did not affect blood cells or their production. Indeed, according to Guyton and Hall (2000) [21], normal levels of mean cell volume (MCV) and mean hemoglobin corpuscular concentration (MCHCC) indicated that the morphology and osmotic fragility of red blood cells are unaffected.

Relatively normal and consistent during the 28 days of experimentation indicated a possible absence in this fraction of hemolytic effect. Gome *et al.* (2011) [22] also made the same observations with the aqueous extract of *Passiflora foetida*. The significant increase in platelet count observed at a dose of 500 mg/kg bw, could be due to the stimulatory effect of the fraction on thrombopoietins.

Biochemical parameters were performed to evaluate the effect of ETHA on blood glucose levels and to assess its effect on vital organs through serum markers of kidney, liver and heart. In fact, the liver and the kidney, by virtue of their roles in purifying the body [23] and their roles in the metabolism of xenobiotics, constitute privileged targets for certain toxic compounds of medicinal plants.

Transaminases (ALT and AST) are good indicators of liver structure and function, and biomarkers for predicting drug toxicity [24]. ALT is a liver-specific cytosolic enzyme that is a very sensitive indicator of hepatotoxicity [25]. ASAT is also an indicator of hepatocyte destruction, although in addition to the liver, it is found in the heart, skeletal muscles, lungs and

kidneys [26]. In fact, the activities of transaminases increase in case of hepatic toxicity [27] and under the conditions that favor an abnormal permeability of the hepatocyte membrane. The results of this study showed no changes in activities of ALAT and ASAT. This assumed that ETHA did not affect liver function, structure or metabolism.

Alkaline phosphatase (ALP) is an excellent biomarker of the endoplasmic reticulum membrane and is present in cells lining the bile ducts of the liver [28]. The results obtained with ETHA showed that ALP activity of the treated rats did not significantly ($p > 0.05$) vary compared to control. This might testify that ETHA did not cause cholestatic hepatobiliary pathologies by obstructing the bile ducts.

Total protein levels are commonly used to highlight hepatocellular damages [29]. This parameter reflects the nutritional status and is taken into account in the screening and diagnosis of kidney, liver and other diseases. In this study, no significant change in serum total protein levels was observed compared to the control group. This could suggest a good functioning of the liver and kidneys.

The state of the structure and renal function are also appreciated by the urea and creatinine levels. Serum concentrations of urea and creatinine, markers of renal function [30] did not significantly change during the 28 days of administration of the ethanolic fraction of *A. leiocarpa*. This observation suggests that structural integrity and renal function were not damaged during the 28 days of exposure to ETHA.

Changes in the serum level of electrolytes indicated renal dysfunction [31]. Also, no disturbance of serum electrolyte levels (Ca^{2+} , K^+ , Mg^{2+} , Na^+ and Cl^-) was observed between the control and the treated groups. This observation confirmed no adverse effect on electrolytes homeostasis and no damage to the structure of the kidney, as well as to glomerular function. In addition, the blood glucose level was not influenced during the experimental period, suggesting that ETHA had no hyper or hypoglycemic effect. As a result, the blood glucose control system has not been disrupted.

The evaluation of the effect of the ethanolic fraction of *A. leiocarpa* on the lipid profile was also performed through the determination of serum plasma levels. Indeed, the serum triglyceride concentration is useful for assessing atherothrombotic risk, but also, in case of a sharp increase, the risk of acute pancreatitis. Administration by gavage of doses ranging from 100 to 500 mg/kg bw, of the ethanolic fraction of *A. leiocarpa* to the rats during 28 days, did not result in significant variations ($p > 0.05$) of the lipid plasma parameters levels ($p > 0.05$) compared to control. This allows to deduce that the lipid metabolism was not affected by ETHA. Indeed, according to Akpanabiatu *et al.* (2005) [32], if hepatic synthesis or plasma lipid degradation is not stimulated, the rates of these parameters do not undergo any observable changes.

Like the activities of aspartate aminotransferase and lactate dehydrogenase, creatine kinase activity could be used in evaluating the integrity of the cardiac tract in biotransformation, and drug metabolism [33]. The present subacute oral toxicity study did not demonstrate a significant change in serum creatine kinase activity, indicating the maintenance of cardiac and skeletal muscle integrity.

In view of all the results obtained, it appears that ETHA was nontoxic for the majority of organs studied. Therefore, it would have no negative influence on blood cells as well as on renal, hepatic and cardiac structure and functions.

5. Conclusion

The current study provided valuable data on the acute and subacute oral toxicity of the ethanolic fraction of *Anogeissus leiocarpa*. The fraction did not produce any clinical signs of toxicity or mortality. It appears to be no toxic with LD_{50} greater than 5000 mg/kg bw. The no changes in biochemical and hematological parameters confirms that ETHA can not cause any damage or adverse effect on in the internal organs, like kidney, liver, spleen, and heart of the rats. The present investigation demonstrates at least in part, the safety of ETHA.

6. Acknowledgments

The author is grateful to Mr. Ahoussi Corneille of the department of Bacteriology-Virology of the Central laboratory of University Hospital of Treichville, Abidjan Côte d'Ivoire and Dr Gnahoue Goue of the Ecole Normale Supérieure (ENS) in Abidjan (Côte d'Ivoire) for their assistance.

7. Conflicts of interest

The authors declare no conflict of interest.

8. Ethical approval

The experiments were conducted according to the ethical guidelines of Ethics Committee of Pasteur Institute of Côte d'Ivoire (Charter of Ethics of the Pasteur Institute, Text of September, 2012).

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