Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2021; 9(4): 129-135

Received: 22-05-2021
Accepted: 24-06-2021

N'dia Kouadio Frédéric
Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nature Sciences Training and Research Unit, Nangui Abrogoua University, PO Box 801 Abidjan 02, Côte d'Ivoire

Gnangoran Boua Narcisse
Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nature Sciences Training and Research Unit, Nangui Abrogoua University, PO Box 801 Abidjan 02, Côte d'Ivoire

Goze Nomane Bernard
Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nature Sciences Training and Research Unit, Nangui Abrogoua University, PO Box 801 Abidjan 02, Côte d'Ivoire

Yapo Angoué Paul
Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nature Sciences Training and Research Unit, Nangui Abrogoua University, PO Box 801 Abidjan 02, Côte d'Ivoire

Corresponding Author:
N'dia Kouadio Frédéric
Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nature Sciences Training and Research Unit, Nangui Abrogoua University, PO Box 801 Abidjan 02, Côte d'Ivoire

Effects of repeated administration of butanolic fraction of *Blighia unijugata* Bak. (Sapindaceae) leaves on some haematological parameters in wistar rats

N'dia Kouadio Frédéric, Gnangoran Boua Narcisse, Goze Nomane Bernard and Yapo Angoué Paul

DOI: https://doi.org/10.22271/plants.2021.v9.i4b.1322

Abstract
Blighia unijugata is a very widespread forest species in tropical Africa. It is usually used to treat various pathologies including hypertension. This study aims at evaluating the safety use of butanolic fraction of *Blighia unijugata* leaves (BuF) on some haematological parameters, body weight and relative organ weights in *Wistar* rats. Twenty-four adult male rats were randomly divided into four groups of six rats each. The control group was repeatedly administered by oral route with distilled water at 10 mL/kg bw for 28 days while test groups 2, 3 and 4 were repeatedly gavaged with BuF extract at 50, 500 and 1000 mg/kg bw respectively. Blood withdrawls were performed at days 7, 14, 21 and 28 using EDTA tubes by ocular puncture from fasted rats previously anesthetized with ether in order to assess BuF extract influence on some haematological parameters. At the end of the twenty-eight days of treatment, rats were weighed and sacrificed. Kidneys, liver, spleen and heart were removed and their relative organ weights were determined. The results showed that BuF extract induced a non-significant decrease (P>0.05) in some haematological parameters such as erythrocytes, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leucocytes, lymphocytes, monocytes, granulocytes and significant (P<0.05) in hematocrit and thrombocytes in the treated rats compared to the control group. However, no significant changes (P>0.05) were observed in body weight and the relative organ weights such as heart, liver, kidneys and spleen during this study. This study indicates that butanolic fraction of *Blighia unijugata* leaves (BuF) administered to rats decreases hematocrit and thrombocyte levels at 50, 500 and 1000 mg/kg bw and is safe for other hematological parameters and body weight in rats.

Keywords: *Blighia unijugata*, butanolic fraction, haematological parameters, body weight, relative weights, rats

1. Introduction
The use of medicinal plants in Africa in the treatment of many ailments is an ancient times practice. African flora is rich in plant species used to treat various pathologies. This practice, considered to be without apparent danger by the populations, is part of their behaviors and customs and is, moreover, less expensive [1]. Today, in several African countries, traditional medicine occupies a prominent place in their national health development plan. In Côte d'Ivoire, since 1972, a scientific research program on "natural substances for pharmaceutical and cosmetic uses" has been initiated to study this rich flora heritage for the purposes of its development and rational use by the populations. *Blighia unijugata* (Sapindaceae), a species widespread in tropical and equatorial regions of Africa [2], has been widely used for its multiple therapeutic properties. In Côte d'Ivoire, Nigeria and Congo, this plant is widely consumed as a vegetable and also used in the treatment of fever, nausea and vomiting, leprosy, eye pain, cough, migraines, rheumatism, kidney pain and joint stiffness, dizziness and hypertension [3, 4]. However, despite the increased use of this medicinal plant, data on its efficacy and safety are not yet available, exposing people to all kinds of dangers. Scientific studies and rational toxicological evaluation of plants commonly used in traditional medicine could ensure their best use and reduce the risk of accidents. Therefore, a systematic evaluation

of medicinal plants for their potential toxicity is a necessary step for the validation of their regular therapeutic use [5]. Previous studies reported that the butanolic fraction of *B. unijugata* leaves (BuF) had hypotensive and antihypertensive activities in rabbits (*Oryctolagus cuniculus*) [6]. In addition, the acute toxicity study revealed that BuF extract is non-toxic by oral and intraperitoneal routes with an LD_{50} greater than 5000 mg / kg bw [7]. These same authors have revealed the presence of sterols, saponins, tannins, flavonoids, polyphenols and polyterpenes in BuF extract. Considering its use as a vegetable and drink, excessive consumption of the products of this plant may present risks of intoxication for consumers. To our knowledge, very few scientific studies have been undertaken to establish the toxicity risks of this plant on haematological parameters. The objective of this work is to carry out the safety use of butanolic fraction of *Blighia unijugata* leaves (BuF) on some haematological parameters, body weight and relative organ weights in *Wistar* rats.

2. Materials and Methods

2.1. Material

2.1.1. Plant material

Fresh leaves of *B. unijugata* were collected in Abidjan (Cocody) in June 2009. Taxonomic identification of these leaves was established by us using available herbaria and a book written by [8]. The species was then authenticated by the National Floristic Center of Felix Houphouët Boigny University of Cocody-Abidjan, Côte d'Ivoire, with voucher no165 in Côte d'Ivoire National Herbarium.

2.1.2. Animals

Experiments were carried on twenty-four albino male Wistar rats (*Rattus norvegicus*), healthy, three months old and weighing 148.00 ± 7.07 g. These animals came from the Animal House of Pasteur Institute of Côte d'Ivoire located in Adiopodoumé (Abidjan) and have been put in reproduction in the Animal House of the Ecology Research Center of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). They were exposed to 12 h dark/light cycle at room temperature (22–25 °C) and given standard food for rats (Ivograin® pellets) and water *ad libitum*. Subacute toxicity research was conducted in accordance with the good laboratory practices [9].

3- Methods

3.1. Extraction methods

3.1.1. Preparation of the total ethanolic extract of *Blighia unijugata* leaves

This extract was prepared according to the method of [10]. The fresh leaves of *Blighia unijugata* were washed thoroughly with tap water and then dried at room temperature for two weeks. They were powdered with a grinding machine (RETSCH, type SM 100, Germany). Cold maceration was carried out with 100 g of leaf powder in 2 liters of 96% ethanol, for 48 h, with magnetic stirring. The resulting solution was first filtered through Buchner and then through Wattman No. 1 filter paper. This operation was repeated twice on the same powder residue. The filtrates obtained were added and concentrated under reduced pressure at 45 °C. using a rotary evaporator (Büchi R110, type MKE 6540/2, Germany). The concentrated product, obtained after evaporation, was collected in a container and then stored in desiccators (Mark Culatti, France) at 45 ° C for 48 h. 13.3 g of powder were obtained and corresponded to the total ethanolic extract of *Blighia unijugata* (TEE). It was stored at -5 °C in a tightly closed jar.

3.1.2. Preparation of the fractions of the total ethanolic extract of *Blighia unijugata*

The different fractions of the total ethanolic extract (TEE) are obtained by successive liquid-liquid extractions, with five solvents of increasing polarities. In this order, hexane, chloroform, ethyl acetate and n-butanol were used according to the modified method of [11].

Ten grams of the total ethanolic extract (TEE) are dissolved in 200 mL of hot distilled water. The whole is homogenized by magnetic stirring for 15 minutes at 27 ± 2 °C. The mixture is then filtered. The aqueous filtrate obtained is then exhausted for 10 minutes at 27 ± 2 °C with 200 mL of hexane, thus giving two phases after decantation (a hexane phase and a residual aqueous phase). The residual aqueous phase is again treated for 10 minutes at 27 ± 2 °C with 200 mL of chloroform to in turn give two phases (a chloroform phase and a residual aqueous phase). The same operation is continued by treating the residual aqueous phase successively with ethyl acetate to also give an ethyl acetate phase and a residual aqueous phase, then with n-butanol to finally obtain a butanol phase and a residual aqueous phase after decantation. Each of the organic phases and the residual aqueous phase obtained was recovered and concentrated under reduced pressure using a rotary evaporator (Büchi R110, type MKE 6540/2, Germany). The various concentrated products obtained were collected in containers and stored in desiccators (Culatti, France) at 45 °C for 24 h according to the method described by [12]. 0.6 g of hexane fraction (HexF), 1.1 g of chloroform fraction (ChlF), 1.6 g of ethyl acetate fraction (AEF), 3.18 g of butanol fraction (BuF) and 3.12 g of aqueous fraction were obtained. The extractions were repeated several times in order to obtain a sufficient amount of extract to perform the tests. All of these extracts were previously tested on rabbit arterial blood pressure in the Physiology, Pharmacology and Pharmacopoeia laboratory. The butanol fraction (BuF) was the most active and was selected for subacute toxicity study.

3.2. Subacute toxicity study

3.2.1. Preliminary tests

Acute oral toxicity results were used as a basis for the selection of BuF doses which were administered and tolerated during animals' gavage. The initial dose level, selected on the basis of this guiding study, is well below the maximum tolerated dose (MTD). At the end of these tests, 50, 500 and 1000 mg/kg bw were selected to be the doses to be used during this experiment.

3.2.2. Grouping and treatment

The animals were divided into four homogeneous groups of six rats per group. This homogeneity of the different groups depends on the body weight of the animals. The tests were carried out on a control group and three group of treated animals. According to the method described by [13], each rat in the control group received by gavage distilled water at a rate of 10 mL/kg bw. The butanolic fraction (BuF), diluted in distilled water was administered by gavage to each rat from the three test groups, respectively at doses of 50, 500 and 1000 mg/kg bw. The volume of BuF administered to the animals in each group was 1 mL/100 kg bw. These animals received distilled water or BuF daily by force-feeding every morning between 7 and 8 am for 28 days. During treatment, clinical signs of toxicity and behavioral changes were

recorded in the animals.

3.2.3. Collection of blood samples
On days 7, 14, 21 and 28 of treatment animal were slightly anesthetized with cotton wool soaked in Ether, 0.5 to 2 mL of blood samples from all four fasted rat groups were collected by ocular puncture in tubes containing an anticoagulant (EDTA), using Pastor pipettes according to the technique described by [14]. The blood samples were immediately used for haematological analysis.

3.2.4. Determination of haematological parameters
Complete blood count (CBC) was performed from blood samples collected in tubes containing EDTA using an automatic analyzer (Sysmex-KX-21N, Japan). The CBC determined the levels of erythrocytes, Haemoglobin (Hb), hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), leukocytes, lymphocytes, monocytes, granulocytes and thrombocytes.

3.2.5. Determination of body weight and organ weight of rats
Body weight measurement of rats was performed using a scale (Mettler Toledo, PB 153-L, Switzerland). The mean body weights of the rats were determined on days 7, 14, 21 and 28. At the end of the experiment, all rats were subjected to a general autopsy. Organs from each animal such as the liver, kidneys, heart, and spleen were carefully isolated and weighed. The relative organs weights of the liver, kidneys, heart and spleen were assessed by the following formular used by [15].

$$\text{Relative organ weight (\%)} = \frac{\text{organ weight (g)}}{\text{body weight (g)}} \text{ X } 100$$

3.3. Statistical analysis
Data were performed using Graph Pad Prism 5.01 software (San Diego, USA) and presented as mean ± standard error on mean (M ± SEM). Comparisons between treated groups and controls were made using Student's t test and one-way analysis of variance (ANOVA). Tukey comparison test was used as post-hoc test. Values were considered statistically significant when $P<0.05$.

4. Results
4.1. Effect of the butanolic fraction of *Blighia unijugata* leaves on some haematological parameters in rats
4.1.1. Effect of the butanolic fraction of *Blighia unijugata* leaves on erythrocyte parameters
Repeated oral administration of the butanolic fraction of *Blighia unijugata* leaves (BuF) at 50; 500 and 1000 mg/kg bw, did not significantly ($P>0.05$) affect erythrocytes, haemoglobin, MCV, MCH and MCHC rates when compared to the control group during the experimental periods. On the other hand, a significant decrease ($P>0.05$) in the hematocrit rate was observed only on day 28 in rats treated with BuF at all the studied doses compared to the control group (Table 1).

Table 1: Evolution of erythrocytes and erythrocytes indices after 28 days of treatment in rats

Erythrocyte Parameters	Doses of BuF (mg/kg bw)	Duration			
		Day 7	Day 14	Day 21	Day 28
Erythrocytes (10^6/µL)	0	07.22 ± 0.20	07.45 ± 0.24	06.94 ± 0.85	07.48 ± 0.23
	50	07.23 ± 0.13	06.40 ± 0.91	07.25 ± 0.17	06.61 ± 0.35
	500	05.31 ± 1.02	06.66 ± 0.15	06.62 ± 0.71	06.72 ± 0.26
	1000	05.18 ± 1.30	06.87 ± 0.30	05.93 ± 1.44	05.86 ± 1.38
Hb (g/dL)	0	12.86 ± 0.21	13.26 ± 0.16	12.09 ± 0.63	12.06 ± 0.43
	50	12.54 ± 0.43	11.74 ± 0.30	10.56 ± 0.60	11.56 ± 0.87
	500	11.84 ± 0.63	11.28 ± 1.64	11.22 ± 0.38	11.92 ± 1.16
	1000	11.74 ± 0.51	12.54 ± 0.65	11.09 ± 1.20	11.50 ± 1.25
HCT (%)	0	40.84 ± 1.10	38.58 ± 1.98	40.12 ± 2.72	42.62 ± 0.65
	50	35.18 ± 3.65	39.10 ± 1.30	41.56 ± 1.96	37.8 ± 0.66*
	500	31.36 ± 5.22	43.08 ± 0.73	37.27 ± 0.80	37.48± 0.68*
	1000	30.32 ± 5.61	34.38 ± 8.10	36.42 ± 1.78	38.38± 2.02*
MCV (fL)	0	57.74 ± 4.43	59.18 ± 1.17	57.08 ± 2.38	58.46 ± 1.04
	50	56.18 ± 1.14	58.82 ± 1.47	55.83 ± 1.76	58.82 ± 1.05
	500	62.88 ± 5.27	55.94 ± 2.03	59.04 ± 4.03	57.74 ± 0.84
	1000	62.32 ± 6.95	56.54 ± 1.00	60.44 ± 3.72	58.86 ± 0.41
MCH (pg)	0	21.32 ± 4.89	18.44 ± 0.36	19.03 ± 2.34	18.38 ± 0.78
	50	17.24 ± 0.50	18.28 ± 0.60	18.07 ± 1.33	17.12 ± 1.00
	500	31.3 ± 12.27	17.04 ± 0.55	17.62 ± 2.05	16.06 ± 1.73
	1000	32.34 ± 9.88	15.22 ± 2.31	16.35 ± 1.47	15.94 ± 1.23
MCHC (g/dL)	0	35.64 ± 4.69	31.10 ± 0.23	33.16 ± 3.14	31.42 ± 0.94
	50	30.72 ± 0.48	31.04 ± 0.33	32.70 ± 2.20	29.40 ± 1.54
	500	45.54± 12.57	27.16 ± 3.87	32.24 ± 1.55	27.80 ± 2.89
	1000	45.60 ± 9.44	30.04 ± 0.30	31.18 ± 1.23	27.40 ± 1.93

Hb: Haemoglobin; HCT: hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; BuF: butanolic fraction of *Blighia unijugata* leaves. * $P<0.05$: significant difference between the values of the same column of the group treated with BuF and those of the control group (distilled water), n = 6.

4.1.2. Effect of the butanolic fraction of *Blighia unijugata* leaves on leukocyte parameters in rats
Table 2 showed changes in leukocytes and leukocyte indices rates when BuF was administered at 50, 500 and 1000 mg/kg bw. From day 7 to day 28, BuF, at 50 and 500 mg/kg bw did not induced any significant variation ($P>0.05$) in leukocytes, lymphocytes, monocytes and granulocytes rates in rats when compared to the rats of control groups. Oppositely, BuF at 1000 mg/kg bw induced a significant reduction ($P<0.05$) in leukocyte indices (lymphocytes, monocytes and granulocytes)

rates on day 7 before normalizing from day 14 to day 28.

Table 2: Change in leukocytes and leucocytes indices after 28 days of treatment in rats

Leucocyte parameters	Doses of BuF (mg/kg bw)	Duration			
		Day 7	Day 14	Day 21	Day 28
Leucocytes (10^3/µL)	0	14.72 ± 2.50	14.04 ± 1.48	14.68 ± 2.05	12.38 ± 1.72
	50	15.60 ± 2.10	15.48 ± 2.00	16.02 ± 1.74	13.46 ± 1.10
	500	17.30 ± 2.05	16.48 ± 2.21	16.38 ± 1.48	14.50 ± 1.21
	1000	14.84 ± 0.79	14.06 ± 1.95	14.76 ± 2.56	12.54 ± 1.65
Lymphocytes (%)	0	12.30 ± 1.28	11.16 ± 1.17	11.55 ± 2.06	09.70 ± 1.42
	50	10.08 ± 1.20	10.46 ± 1.28	10.34 ± 1.42	08.06 ± 1.08
	500	08.44 ± 1.76	07.44 ± 1.68	08.23 ± 1.84	07.96 ± 1.08
	1000	7.18 ± 0.73*	06.84 ± 1.20	08.09 ± 1.18	07.08 ± 1.43
Monocytes (%)	0	0.22 ± 0.09	0.08 ± 0.07	0.13 ± 0.06	0.06 ± 0.03
	50	0.15 ± 0.01	0.09 ± 0.04	0.10 ± 0.01	0.07 ± 0.03
	500	0.10 ± 0.01	0.07 ± 0.03	0.08 ± 0.02	0.10 ± 0.02
	1000	0.04 ± 0.03 *	0.07 ± 0.03	0.08 ± 0.01	0.05 ± 0.03
Granulocytes (%)	0	03.20 ± 1.17	02.93 ± 0.84	02.64 ± 1.03	02.57 ± 0.31
	50	03.39 ± 0.91	02.72 ± 1.32	02.77 ± 0.62	02.37 ± 0.26
	500	02.76 ± 0.46	02.81 ± 0.69	02.53 ± 0.57	02.44 ± 0.48
	1000	01.82 ± 0.6 *	02.41 ± 0.87	01.97 ± 0.76	02.33 ± 0.52

BuF: butanolic fraction of *Blighia unijugata* leaves. * $P<0.05$: significant difference between the values of the same column of the group treated with BuF and those of the control group (distilled water), n = 6.

4.1.3. Effect of the butanolic fraction of *Blighia unijugata* leaves on thrombocytes in rats

Oral administration of BuF to the rats at 500 and 1000 mg/kg bw, induced a significant decrease ($P<0.05$-0.01) in thrombocytes rates when compared to the control rats from day 7 to day 28. However, no significant change ($P>0.05$) was observed at all study periods in rats treated with BuF at 50 mg/kg bw compared to control rats (Table 3).

Table 3: Variation of thrombocyte rates after 28 days of treatment in rats

Haematological parameter	Doses of BuF (mg/kg bw)	Duration			
		Day 7	Day 14	Day 21	Day 28
Thrombocytes (10^3/µL)	0	1003 ± 76	971 ± 27	750 ± 45	763 ± 67
	50	784 ± 48	498 ± 95	645 ± 22	691 ± 20
	500	692 ± 71**	329 ± 28**	618 ± 40*	487 ± 88*
	1000	639 ± 41**	313 ± 37**	454 ± 18**	420 ± 50**

BuF: butanolic fraction of *Blighia unijugata* leaves. * $P<0.05$; ** $P<0.01$: significant difference between the values of the same line of the group treated with BuF and those of the control group (distilled water), n = 6.

4.2. Clinical signs after oral administration of the butanolic fraction of *Blighia unijugata* leaves in rats

Daily oral administration of the butanolic fraction of *Blighia unijugata* leaves (BuF) at 50; 500 and 1000 mg/kg bw for 28 days did not cause any obvious symptoms of toxicity in rats compared to control group. No alteration of locomotion or piloerection activity was recorded during treatment. In addition, no mortality was recorded in the different treated groups during the 28 days compared to control group. Faeces observation did not reveal any diarrhea during the experiment. The general behavior as well as the coat of the rats of the different group were also identical.

4.3. Effect of the butanolic fraction of *Blighia unijugata* leaves on body weight in rats

In general, an increase in body weight was observed in all treated groups during all periods of the experiment (Table 4). However, the increase in body weight seen in rats treated with BuF at all doses was not significant ($P>0.05$) compared to control group from day 7 to day 28. Therefore, BuF at all doses does not induced any change in body weight.

Table 4: Evolution of body weight after 28 days of treatment in rats

Time (days)	Body weight (g)			
	BuF 0	BuF 50	BuF 500	BuF 1000
Day 0	143 ± 15.10	147 ± 14.50	150 ± 15.10	152 ± 15.50
Day 7	149 ± 16.40	149 ± 11.80	154 ± 13.30	155 ± 15.50
Day 14	157 ± 16.50	157 ± 11.50	160 ± 13.00	159 ± 15.20
Day 21	168 ± 16.50	165 ± 10.70	166 ± 12.60	163 ± 14.90
Day 28	178 ± 16.30	171 ± 10.70	170 ± 12.40	167 ± 14.90

BuF: butanolic fraction of *Blighia unijugata* leaves; No statistical significant difference ($P>0.05$) between values of the same line of the group treated with BuF and control (day 0).

4.4. Effect of the butanolic fraction of *Blighia unijugata* leaves on the relative organ weights in rats

The variations in the weight of the liver, heart, kidneys and spleen compared to the variations in the absolute weight of the rats of the different group showed that the repeated oral administration of BuF did not affect significantly ($P>0.05$) the relative weights of these organs of the treated rats compared to those of the control group. A non-significant increase ($P>0.05$) in the relative weights of certain organs such as kidneys, liver, heart and spleen were recorded during this

study (Table 5).

Table 5: Variation of the relative organ weights in rats

Organs	Relative organ weights (g/100g pc)			
	Control	BuF 50	BuF 500	BuF 1000
Kidneys	0.62 ± 0.06	0.64 ± 0.03	0.64 ± 0.04	0.78 ± 0.09
Liver	3.29 ± 0.31	3.57 ± 0.24	3.48 ± 0.32	4.41 ± 0.53
Heart	0.35 ± 0.04	0.33 ± 0.01	0.37 ± 0.03	0.43 ± 0.03
Spleen	0.24 ± 0.03	0.27 ± 0.02	0.25 ± 0.02	0.28 ± 0.02

No statistical significant difference (P>0.05) between values of the same line of the group treated with BuF and control.

5. Discussion

The butanolic fraction of *Blighia unijugata* leaves (BuF) possesses hypotensive and antihypertensive activities in rabbits [6]. However, prolonged administration of this extract could have deleterious effects on the body, in particular on the haematopoietic system. Indeed, this system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological state. The blood profile usually gives vital information about the body's response to injury or stress [16, 17]. The various haematological parameters examined in this study are some useful indicators to assess plant extracts toxicity in animals and help to decide the extent of the detrimental effects of the extracts on animal blood [18]. It is for this reason that the evaluation of haematological parameters is vital among various toxicity biomarkers to know whether the tested substances affect the haematopoietic system [19]. To ensure the safety of this extract on the haematopoietic system, the subacute toxicity study was undertaken in rats. The results of this study showed that repeated oral administration of BuF at 50, 500 and 1000 mg/kg in rats did not induced any change in erythrocytes, haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) rates. These results could indicate that the balance between production rates (erythropoiesis) and destruction of red blood cells was not affected by the administration of BuF extract. Therefore, the general absence of significant changes in BuF extract on erythrocytes and erythrocyte indices could mean that neither the incorporation of haemoglobin into red blood cells, nor the morphology and osmotic fragility of the red blood cells were altered, which was shown by [20]. However, BuF extract caused an insignificant decrease in erythrocytes correlated with a significant reduction in hematocrit at the end of this study. This observation suggests that BuF extract would have a selective effect on erythrocyte parameters. More elaborate experiments are necessary to confirm or refute this hypothesis.

Secondary metabolites such as saponins contained in BuF extract would have caused haemolytic anemia. However, it is known that saponins have a hemolytic property which could weaken the membrane of red blood cells in experimental animals and cause anemia as reported by [21]. This hypothesis is more plausible as [7] revealed the presence of saponins in this extract. However, more elaborate studies could confirm or deny the anemic hemolytic activity of this extract. In addition, the interaction of saponosides and sterols induces an increase in membrane permeability and ions movement. The entry of Na^+ ions and water, followed by the exit of K^+, causes the membrane to burst. This results in the release of hemoglobin [22]. These results are almost similar to those of [23] who showed that the ethanolic extract of the stem bark of *Blighia sapida* at 250, 500 and 750 mg/kg bw did not cause any significant variation in erythrocyte parameters. Likewise, the results of this study partly agree with [24]. These authors did not reveal any significant change in these parameters after oral administration of an ethyl acetate extract (Active fraction) of *Lophira lanceolata*, an antihypertensive plant, for 28 days in male rats. However, these results differ from those of [25] who showed a significant increase in haemoglobin in rats treated with the aqueous extract of fresh leaves of *Blighia unijugata* at 200, 300, 400 mg/kg bw for 7 study days.

Leukocytes are involved in the body's defense against all foreign substances. They are evaluated to assess the influence of BuF extract on the body's defense capacity of rats. Repeated oral administration of BuF extract significantly altered lymphocyte, monocyte and granulocyte rates on day 7 only at 1000 mg/kg before normalizing on day 14 and day 28. This decrease observed in this study suggests that BuF at high dose would have caused a failure of the defense system for a certain time. The insignificant effect of the level of lymphocytes, monocytes and granulocytes observed from day 14 to day 28 would mean that the activity of BuF extract would be transient and would cancel out over time. BuF extract would therefore not have altered the body's defense system in rats at all the studied doses. These results are close to the subacute toxicity study conducted by [26] who showed that oral administration of ethyl acetate extract from the leaves of *Holarrhena floribuna* did not modify leukocyte parameters in rats at the high dose of 1000 mg/kg bw.

BuF extract, at doses of 500 and 1000 mg/kg bw, induced a significant decrease in thrombocytes. This decrease in thrombocytes is greater at 1000 mg/kg. BuF therefore caused thrombocytopenia. Previous studies showed that certain substances such as flavonoids are antiplatelet agents [27, 28]. It is probable that the decrease in the level of thrombocytes would be due to the flavonoids present in BuF extract as revealed by [7]. BuF could therefore be an antiplatelet agent. In addition, during arterial hypertension, an increase in the level of thrombocytes is observed. These thrombocytes will agglomerate to form thromboses leading to the formation of clots. These clots block the blood vessels, preventing normal blood flow [29]. Therefore, any antihypertensive substance would reduce the level of thrombocytes. However, [6] showed the antihypertensive activity of BuF, hence the significant decrease in the level of thrombocytes observed in this study.

Flavonoids are thought to act on the level of thrombocytes by preventing thrombosis linked to the movement of thrombocytes. Some studies have suggested that flavonoids exert their antiplatelet properties by lowering intracellular Ca^{2+} levels, altering the metabolism of cAMP and thromboxane A2 [30]. The antiplatelet property of BuF could be mediated by lowering intracellular Ca^{2+} levels or by inhibiting key aspects of cAMP and thromboxane metabolism. These results are similar to those of [31] who indicated that the flavonoids in grape juice significantly decrease the activity and the level of thrombocytes. [32] also showed that flavonoids also facilitate blood circulation by inhibiting the activity of thrombocytes. In addition, [16] and [33] have shown, respectively, that aqueous extracts of *Ocimum suave* at doses ranging from 250 to 1000 mg / kg bw and *Arctotis arctotoides* at doses between 500 and 2000 mg/kg bw

cause a significant decrease in thrombocytes in rats and mice. Treatment of the rats with BuF extract did not cause any significant change in the body weight and relative organ weights of the rats compared to the control. These results suggest that BuF has absolutely no effect on body weight and nutrient utilization by rats. Generally, reductions in body weight gain and internal organ weights are simple and sensitive indicators of toxicity after exposure to toxic substances [34]. Moreover, according to [35], an increase in the relative organ weights would indicate inflammation while a decrease could be attributed to cell constriction. BuF therefore does not cause inflammation or cell constriction. The lack of a significant effect on the relative organ weight suggests that this fraction had no toxic effect on the organs studied in rats during 28 days of experimentation. These results are comparable to those of [24] and [23] who showed respectively that the ethyl acetate extract of Lophira lanceolata leaves at 250, 500 and 1000 mg/kg bw and the ethanolic extract of the stem bark of *Blighia sapida* at 250, 500 and 750 mg/kg bw did not cause any significant change in the body weight of the rats. In addition, macroscopic observation of the internal organs of the rats did not reveal any abnormalities.

This study showed that the butanolic fraction of *Blighia unijugata* leaves (BuF) is likely to cause a disturbance of the blood count in rats, in particular by the non-significant drop in certain haematological parameters in repeated administration such as red blood cells, haemoglobin, MCH, MCHC and white blood cells. BuF also caused a significant decrease in hematocrit and thrombocytes in this study. This fraction showed antiplatelet effects. However, further studies should be carried out on suitable models to confirm these activities. The misuse of this plant therefore causes disruptions which should be taken into account during the purification and isolation of the active compounds. This study could be continued through pathological studies to better assess the safety of this fraction of *Blighia unijugata* and guarantee the safety use of this extract by populations.

6. References

1. Ambady R, Chamukuttan S. Early diagnosis and prevention of diabetes in developing countries. Reviews in Endocrine & Metabolic Disorders 2008;9(3):193-201.
2. Hyde MA, Wursten B. Flora of Zimbabwe: Species information: *Blighia unijugata.* CITIS ed., Harare 2002, 317-318.
3. Adjanohoun E, Aké Assi L. Contribution au recensement des plantes médicinales de Côte d'Ivoire. Centre National de Floristique, Abidjan, Côte d'Ivoire 1979, 272-274.
4. Burkill HM. The useful plants of West tropical Africa. 2nd ed., Volume 5, Families SZ, Addenda Royal Botanic Gardens, Kew, United Kingdom 2000, 11-13.
5. El Kabbaoui MA, El-Akhal J, Azdad O, Mejrhit N, Aarab L, Bencheikh R *et al*. Acute and sub-chronic toxicity studies of the aqueous extract from leaves of *Cistus ladaniferus* L. in mice and rats. Journal of Ethnopharmacology 2017;209:147-156.
6. N'dia KF, Bléyéré NM, Kouakou KL, Abo KJC, Yapo AP, Ehilé EE. Acute toxicity in mice and effects of a butanol extract from the leaves of *Blighia unijugata* Bak. (Sapindaceae) on electrocardiogram of rabbits. Scholars Academic Journal of Pharmacy 2013a;2(6):429-435.
7. N'dia KF, Kouakou KL, Bléyéré NM, Yapo AP, Ehilé EE. Hypotensive effects of a butanol active fraction from leaves of *Blighia unijugata* bak. (Sapindaceae) on arterial blood pressure of rabbit. World Journal of Pharmacy and Pharmaceutical Sciences 2013b;2(6):6693-6705.
8. Aké Assi L. Boissiera 58. Flore de la Côte d'Ivoire : catalogue systématique, biogéographie et écologie II. Collection Mémoires de botanique systématique, Edition Conservatoire et Jardin Botanique Ville de Genève 2002, 117-124.
9. OCDE. Série sur les principes de bonnes pratiques de laboratoire et vérification du respect de ces principes. ENV/MC/CHEM (98) 1998;17:22-23.
10. N'dia KF, Traoré F, Kouakou KL, Ehilé EE. Effets pharmacologiques d'un extrait aqueux de *Mirabilis jalapa* L. (Nyctaginaceae) sur le système cardiovasculaire, la respiration et l'activité mécanique intestinale de mammifères. Afrique Science 2009;05(2):330-348.
11. Sakandé J, Nacoulma OG, Nikiéma JB, Lompo M, Bassené E, Guissou IP. Etude de l'effet antipyrétique d'extraits des inflorescences mâles du rônier *Borassus aethiopum* Mart (Arecaceae). Médecine d'Afrique Noire 2004;51(5):280-282.
12. N'Guessan JD, Bidie AP, Lenta BN, Weniger B, André P, Guédé- Guina F. *In vitro* assays for bioactivity-guided isolation of *antisalmonella* and antioxidant compounds in *Thonningia sanginea* flowers. African Journal of Biotechnology 2007;6(14):1685-1689.
13. Watcho P, Nchegang B, Nguelefack TB, Kamanyi A. Evaluation of the prosexual effects of extracts of *Bridelia ferruginea* in naive male rat. Andrology 2010;20(3):209-215.
14. Lima LB, Vasconcelos CFB, Maranhão HML, Leite VR, Ferreira PA, Andrade BA *et al*. Acute and subacute toxicity of *Schinus terebinthifolius* bark extract. Journal of Ethnopharmacology 2009;126(3): 468-473.
15. Nkono BLNY, Sokeng SD, Dzeuflet DPD, Frida L, Kamtchouing P. Subchronic toxicity of aqueous extract of *Alstonia boonei* from Wild. (Apocynaceae) stem bark in normal rats. International Journal of Pharmacology and Toxicology 2015;3(1):5-10.
16. Tan PV, Mezui C, Enow-Orock G, Njikam N, Dimo T, Bitolog P. Teratogenic effects, acute and sub chronic toxicity of the leaf aqueous extract of *Ocimum suave* Wild (Lamiaceae) in rats. Journal of Ethnopharmacology 2008;115(2):232-237.
17. Mukinda JT, Eagles FK. Acute and sub-chronic oral toxicity profile of the aqueous extract of *Polygala fruticosa* in female mice and rats. Journal of Ethnopharmacology 2010;128(1):236-240.
18. Yakubu MT, Akanji MA, Oladiji AT. Haematological evaluation in male albino rats. Following chronic administration of aqueous extract of *Fadogia argrestis* stem. Pharmacognosy Magazine 2007;3(9):34-38.
19. Bashir L, Oluwatosin KS, Agboola AR, Ibrahim AO, Adeniyi AK, Prince CO. Effect of methanol extract of *Telfairia occcidentalis* on Haematological parameters in Wister rats. Journal of Medical Sciences 2015;15(5):246-250.
20. Adebayo AH, Abolaji AO, Opata TK, Adegbenro IK. Effects of ethanolic leaf of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino Wistar rats. African. Journal of Biotechnology 2010;9(14):2145-2150.
21. Bissinger R, Modicano P, Alzoubi K, Honisch S, Faggio C, Abed M *et al*. Effect of saponin on erythrocytes. International Journal of Hematology 2014;100(1):51-59.
22. Majester-Savornin B, Elias R, Diaz-Lanza AM,

Balansard G, Gasquet M, Delmas F. Saponins of the ivy plant, *Hedera helix* and their leishmanicidic activity. Planta Medica 1991;57(3):260-262.
23. Adekola MB, Areola JO, Omisore NO, Asaolu FT, Ogunleye SG, Apalowo OE *et al*. Sub-chronic toxicity study of ethanol stem-bark extract of *Blighia sapida* (Sapindaceae) in wistar rats. Heliyon 2020;6(2):e02801. https://doi.org/10.1016/j.heliyon.2019.e02801
24. Oussou NJB, Asiedu-Gyekye IJ, Bla KB, Kouakou KL, Yapi HF, Yapo AF *et al*. Bio-tolerance of an ethyl acetate fraction of *Lophira lanceolata* (Ochnaceae) leaves in albino rats. American Journal of Bio-pharmacology Biochemistry and Life Sciences 2015;4(2):80-92.
25. Agbafor KN, Offor CE, Engwa GA. Haemoglobin level, total white blood cell and packed cell volume in the albino rats treated with aqueous extract of fresh l eave of *Blighia unijugata*. IOSR Journal of Environmental Science, Toxicology and Food Technology 2015;9(9):134-137.
26. Kouassi KB, Gnangoran BN, Adjambri AE, Koné M, Yapo AP. Influence of an ethyl acetate extract of *Holarrhena floribunda* (G. Don) Durand and Schinz (Apocynaceae) on blood cells in normal rats, plant used in traditional medicine for treatment diabetes in Côte d'Ivoire. World Journal of Pharmacy and Pharmaceutical Sciences 2017;6(3):97-108.
27. Harnafi H, Amrani S. Flavonoids as Potent Phytochemicals in Cardiovascular Diseases Prevention. Pharmacognosy Reviews 2007;1(2):193-202.
28. Chen J, Du CYQ, Lam KYC, Zhang WL, Lam CTW, Yan AL *et al*. The standardized extract of *Ziziphus Jujuba* fruit (jujube) regulates pro-inflammatory cytokine expression in cultured murine macrophages: suppression of lipopolysaccharide-stimulated NF-kB activity. Phytotherapy Research 2014;28(10):1527-1532.
29. Di Matteo J, Vacheron A. Cardiologie. Ed. Expansion Scientifique française, Paris, France 1987, 587-603.
30. Kang WS, Chung KH, Lee JY, Park JB, Zhang YH, Yoo HS *et al*. Antiplatelet activity of green tea catechins is mediated by inhibition of cytoplasmic calcium increase. Journal of Cardiovascular Pharmacology 2002;38(6):875-884.
31. Freedman JE, Parker III C, Li L, Perlman JA, Frei B, Ivanov V *et al*. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation 2001;103(23):2792-2798.
32. Ghedira K. Flavonoids: Structure, biological properties, prophylactic role and uses in therapy. Herbal medicine 2005;3(4):162-169.
33. Jimoh FO, Adepapo AA, Sofidiya MO, Masika PJ, Afolayan AJ. Safety evaluation of the extract from the shoots of *Arctotis arctotoides* in rats and mice. African Journal of Biotechnology 2008;7(18):3173-3177.
34. Sellers RS, Mortan D, Michael B, Roome N, Johnson JK, Yano BL *et al*. Society of Toxicology Pathology position paper: organ weight recommendations for Toxicology studies. Toxicologic Pathology 2007;35(5):751-755.
35. Moore KL, Dalley AF. Clinical oriented anatomy (4th Edition). Lippincot Williams & Wilkins; a Woller Klumner Corporation, Philadelphia, USA 1999, 263-271.