Journal of Pharmaceutical Research International

24(4): 1-10, 2018; Article no.JPRI.36463
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Study of Acute and Subacute Toxicities of *Wakouba* on Rats

Tiekpa Wawa Justine[1*], Koutou Anatole[2], Bahi Calixte[3] and Coulibaly Adama[1]

[1]Training and Research Unit of Biological Sciences, Peleforo Gon Coulibaly University, PO Box 1328, Korhogo, Côte d'Ivoire.
[2]La Mé Research station, National Agricultural Research Centre, 01 PO Box 1740, Abidjan, Côte d'Ivoire.
[3]Laboratory of Biochemical Pharmacodynamy, Biosciences Training and Research Unit, Felix Houphouët-Boigny University, 22 PO Box 582, Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out with all authors' collaboration. Author TWJ designed the study, wrote the protocol, performed the laboratory investigations, and wrote the first draft of the manuscript with assistance of author KA. Author BC achieved the statistical treatment, managed the literature searches, and assisted the results interpretation in collaboration with authors CA and TWJ. Author CA supervised the full study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2018/36463
Editor(s):
(1) Dr. Mostafa Abbas Mostafa Shalaby, Professor, Pharmacology, Faculty of Vet. Med., Cairo, University and Previous Head of Pharmacology, Veterinary Medicine, Cairo University, Egypt.
Reviewers:
(1) Esraa Ashraf Ahmed ElHawary, Ain Shams University, Egypt.
(2) Leonardo Basco, Aix-Marseille University, France.
(3) Israel O. Obaroh, Kebbi State University of Science and Technology, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/27309

Original Research Article

Received 29 August 2017
Accepted 21 January 2018
Published 19 November 2018

ABSTRACT

Aims: *Wakouba* is a salty substance used till medieval ages in traditional medical practices for the preventive treatment of hypertension. The current research focuses on the acute and sub-acute toxicity of this salt using laboratory Wistar rats.
Study Design: Wakouba prepared using rank 17 fronds from Elaeis guineensis plant's crown: fronds harvested, dried, incinerated; then the ash dissolved into water and duly filtered. Then various *Wakouba* doses orally given to adult Wistar rats and acute and sub-acute toxicity parameters measured for 28-days.
Place and Duration of Study: Investigations performed at La Mé Research Station for Oil-Palm (National Agronomical Research Centre, CNRA) and Laboratory of Biochemical Pharmacodynamy

**Corresponding author: E-mail: tiekpajustine@yahoo.fr;*

(Felix Houphouët-Boigny University), between Mai 2014 and February 2015.
Methodology: For acute toxicity assessment, *Wakouba* given separately to rats at doses varying from 5,000 to 8,000 mg/kg body weight (BW) by oral route, and animals observed for behavioral changes or mortality. For sub-acute toxicity study, animals also orally administered with various *Wakouba* doses between 950 to 2,500 mg/kg BW, and then weekly examined, during 28 days, for toxicity symptoms dealing with hematological parameters (numbers of red blood cells, white blood cells, and hemoglobin; percentage of hematocrit, mean corpuscular volume, mean corpuscular rate, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, lymphocytes, and platelet count), biochemical characteristics (urea, creatinine, aspartate aminotransferase, alanine aminotransferase, total cholesterol triglycerides, alkaline phosphatase, lactate dehydrogenase, and creatine phosphokinase), and histopathological traits (liver, heart, and kidney).
Results: *Wakouba* appeared a non-toxic substance because it revealed LD50 and MTD respective values of 6,308.57 and 5,000 mg/kg BW. Beyond the 28-days assessment, the rats were sacrificed for hematological, biochemical and histopathology concerns. Any significant variations in the overall hematological traits and some biochemical parameters (AST, ALP, CPK, LDL, and TP) were observed. However, at the rate of 2,500 mg/kg BW, *Wakouba* induced significant ($P<0.001$) increases in urea, creatinine, and HDL-cholesterol and decrease drop in the LDL-cholesterol from the treated animals. Histological examination of vital organs showed normal architecture suggesting no morphological trouble in the heart, kidney, and liver.
Conclusion: The oral administration of *Wakouba* did not produce any significant toxic effect from rats. Such observations are significant safety margin in the uses of *Wakouba* that could therefore be valorized in therapeutic approach through pharmaceutical formulations.

Keywords: Wakouba; acute toxicity; subacute toxicity; tissue histology.

1. INTRODUCTION

Elaeis guineensis (Jacq) known as oil palm plant is a monocotyledon crop belonging to plant family of Arecaceae. It's a tree reaching 15 to 30 m of height [1], consisting of a single trunk without any branch, and displaying a consistent crown of 40-50 leaves of 5 to 8 m long [2]. Palm oil culture is a very important livelihood for many populations from its growing lands where it records several food, cosmetic, and therapeutic properties. Several studies from the oil palm plant were reported regarding the methanolic extract of leaves. According to Salleh et al. and Runnie et al. [3,4], this methanolic extract fights against atherosclerosis and hypercholesterolemia by inhibiting the oxidization of low density lipoprotein (LDH). It also exhibits vasodilatative, antihypertensive, and wound healing effects [5;6]. In vitro trials attempted on the same extract by Chong et al. [7] revealed the inhibition of the Gram positive bacteria growth at Minimum inhibitory concentration (MIC) between 6.25 mg/mL and 12.5 mg/mL; while Kweifio-Okai [8] reported the anti-inflammatory effect of the total aqueous extract of oil palm leaves.

Some pharmacological interests are also involved with other derivatives from the oil palm. Indeed, the palm sap is used to highlight the rabies virus by immunofluorescence [9]. In Senegal, it is used against aches and rheumatism, whereas it's used against hepatotoxicity in Nigeria [10,11]. In Côte d'Ivoire, the salt obtained from fronds of oil palm, named ‘*'Wakouba''*, is used in traditional treatment of hypertension. In fact, the arterial hypertension is a disease whose treatment by modern conventional medicine is very expensive and inaccessible. An antihypertensive used in the traditional medicine, and accessible for all, deserves deeper and sound scientific investigations to avoid treatment risks since such concerns often seem to be tragical for populations. The purpose of this study was to investigate the bio-toxicological parameters of this salt.

2. MATERIALS AND METHODS

2.1 Plant Material

Wakouba was prepared according to the method described by Zirihi et al. [12], using the palms of rank 17 from the oil palm crown. These palms were harvested, washed, carved into approximate 1 cm length pieces, and dried at room temperature of 25-30°C for four weeks. Afterwards, the dried leaves pieces were incinerated into a muffle furnace at 400°C until white ashes. Thereafter, one hundred grams (100 g) of ash was dissolved into one liter (1 L) of distilled water and homogenized for 2 h at room temperature (25-30°C) using a magnetic stirrer.

The homogenate has then undergone filtration two times upon a cotton wool followed by another filtration on Whatman paper. The final filtrate was evaporated in an oven at 60°C to obtain the *Wakouba* salt.

2.2 Animal Model

Adult Wistar rats (150-170 g) obtained from the laboratory animal shop of the Pasteur Research Institute in Côte d'Ivoire were used for this study. They were kept in polypropylene cages under identical animal house condition and provided with standard pellet and water ad libitum. Environmental conditions were maintained at temperature of 22±2°C and relative humidity of 60±10% [13].

2.3 Acute Toxicity Study

The acute oral toxicity study was conducted according to the method described by Bléyéré et al. [14]. Forty-two (42) Wistar rats were randomly divided into seven groups of 6 rats each according to their body weights. The rats in Group 1 were considered as controls and received 1mL of standard saline (NaCl) solution at 0.9% (m/v). From Groups 2 to 7, the rats were orally administered with *Wakouba* doses of 5,000 ; 5,500 ; 6,000 ; 6,500 ; 7,000, and 8,000 mg/kg body weight (BW) for one day, respectively, using a stainless orophageun steel. The animals were then observed continuously for 14 days, for behavioral signs, mortality and other signs of toxicity, leading to the determination of the *Wakouba* dose inducing 50% rats' mortality (50% lethal dose, LD50).

2.4 Subacute Toxicity Study

For this experiment, 36 Wistar rats were divided into a control group and five *Wakouba*-treated groups (950; 1,000; 1,500; 2,000; 2,500 mg/kg BW), each group consisting of six animals according to their body weights. Repeat-dose oral toxicity was assessed according to the Organization for Economic Cooperation and Development (OECD) guideline 407 [15]. The control animals (Group 1) received orally normal saline (0.9% NaCl) while animals from Groups 2 to 6 received *Wakouba* doses of 950; 1,000; 1,500; 2,000, and 2,500 mg/kg BW, respectively, for 28 consecutive days using a stainless orophageun cannula steel. At the day 30, the blood of the overall animals was collected according to suitable experimental protocol [16, 17] for the determination of biochemical and hematological parameters. Then, the animals were sacrificed and their vital organs (liver, heart and kidney) were removed for macroscopic examination.

2.4.1 Determination of hematological parameters

At the 29th day, all rats from various groups were anesthetized using chloroform, submitted to an overnight fasting (for 8 h), sacrificed at the day 30, and then their blood samples were collected by cardiac puncture. The blood samples of each sacrificed animal were collected into heparinized bottles (0.5 mL) to evaluate hematological parameters, namely red blood cells (RBCs), number of white blood cells (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular rate (MCR), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), and platelet count (PLT). The overall parameters were determined using an automatic hematology analyzer (Coulter KX - 21) [18,19].

2.4.2 Determination of biochemical parameters

The blood samples (1.5 mL) of each sacrificed animal were collected into dry centrifuge Huma tubes with EDTA as anticoagulant reagent. The biochemical analysis was performed using the blood serum after centrifugation at 1480 RPM for 10 min. Then, biochemical parameters such as values of urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and total proteins cholesterol (TP) were measured by standard colorimetric methods. The lipid profile in the rats' blood was also investigated, consisting in total cholesterol (TChol), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C). These parameters were measured using clinical chemistry analyzer (Vital Scientific, Netherlands).

2.4.3 Histopathological examination

The rats were dissected after blood collection and their vital tissues (liver, heart, and kidney) were carefully examined. Tissue samples were fixed into 10% formalin dehydrated in concentrated alcohol bath series (70°, 80°, and 90°) and embedded in paraffin blocks. Afterwards, ultrathin sections were de-waxed by xylene, hydrated into ethanol solutions, and

stained with hematoxylin and eosin. The histopathological examination was performed using an optical microscope (Nikon Eclipse E600, USA) at 40x magnification size. Sections were assigned grades as reported by Billingham et al. [20].

2.5 Statistical Analysis

The data were statistical analyzed using Graph Pad software (Graph Pad Prism 4 Microsoft Sendiegie, California, USA). The statistical treatment consisted in a one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple statistical comparison. The probability value was considered at P< 0.001. Results were reported as mean of variance ± SEM.

3. RESULTS AND DISCUSSION

3.1 Lethal dose 50 (LD50) Resulting from the Administration of *Wakouba*

Table I shows the percentage of rats mortality from the different groups after gavage administration of various doses of *Wakouba* between 5,000 and 8,000 mg/kg BW. These mortality percentages allow the drawing of the graph in Fig. 1 using the logarithm of *Wakouba* doses. From such a caption, the LD50 value of *Wakouba* is highlighted at 6,309±99.97 mg/kg BW.

3.2 Subacute Toxicity of *Wakouba*

3.2.1 Effect of *Wakouba* on hematological parameters of rats

The results of the hematological tests are gathered in table 2. The overall hematological parameters investigated from the *Wakouba*-treated rats, namely RBC, WBC, Hb, HCT, MCV, MCR, MCH, MCHC, LYM, and PLT values were within normal standard compared to the control rats group. They do not involve any significant differences (P > 0.05, ns) between the palm extract-treated animals and the control ones.

3.2.2 Effect of *Wakouba* on biochemical parameters of rats

The results of the various biochemical experiments on the *Wakouba*-treated rats are mentioned in table 3 against the control rats group. Oral administration of *Wakouba* at the doses of 950 to 2,500 mg/kg BW did not cause significant changes of the main serum biochemical parameters, namely aspartate aminotransferase (207 to 209 U/L), alanine aminotransferase (151 to 156 U/L), lactate dehydrogenase (264.1 to 266.9 U/L), creatine phosphokinase (155.7 to 158.4 U/L), alkaline phosphatase (292 to 294 U/L), and total proteins (64.9 to 66.9 g/L) compared to the control untreated rats. However, urea and creatinine were statistically higher (p<0.001) in rats treated with *Wakouba* at dose of 2,500 mg/kg BW (0.85 and 36.66 mg/L, respectively) compared to the control rats (0.39 and 16.20 mg/L, respectively).

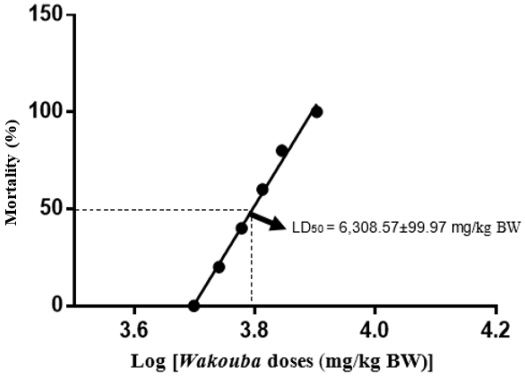

Fig. 1. Rats mortality chart drawn on the logarithm of *Wakouba* doses basis

The lipid parameters are given in Table 4. This illustration doesn't display any statistical difference (p>0.05) between the *Wakouba*-treated rats and the untreated rats for the contents in total cholesterol (1.62 to 1.68 g/L) and triglycerides (0.54 to 0.75 g/L). By contrast, HDL-cholesterol and the LDL-cholesterol were

Table 1. Rats mortality based on doses of *Wakouba* administered

Groups of rats	Doses (mg/kg BW)	Number of dead rats	Mortality (%)
1	0000	0	0
2	5,000	0	0
3	5,500	1	20
4	6,000	2	30
5	6,500	4	40
6	7,000	5	80
7	8,000	6	100

statistically higher ($p<0.001$) from the animals treated with *Wakouba* doses of 1,000 to 2,500 mg/kg BW (1.11 to 1.29 g/L and 0.23 to 0.43 g/L, respectively) than in the control rats (0.93 and 0.60 g/L, respectively). This difference emphasizes respective increase and decrease in the rates of HDL-cholesterol and LDL-cholesterol when the rats are administered with *Wakouba* doses from 1,000 to 2,500 mg/kg BW as shown in Table 4.

3.3 Histological Characteristics in Tissue Sections of *Wakouba*-treated Rats

Fig 2, 3 and 4 show the respective histopathological sections of the liver, kidney and heart in control rats and rats treated with *Wakouba* at the highest dose considered at 2,500 mg/ kg BW. The liver section of treated rat didn't present any significant damage. Hepatocytes (H) and sinusoids revealed normal architecture compared to the control rats (Fig. 2a and 2b). The higher dose of *Wakouba* also did not result in any marked deleterious effect on the kidney of treated rats.

Overall rats tissues investigated, namely Glomerulus (G), Bowman's capsule (Bc), proximal collecting tubule (P), distal collecting tubule (D), and collector tube (Tc) are with normal appearance compared to the control rats group (Fig. 3a). Fig 4 also reveals normal appearance of the heart from treated animals compared to the control. Muscle cells, as shown by arrows, form a large complex network of parallel smooth muscle fibers (SMF) and each cell remains clearly individualized.

4. DISCUSSION

The toxicological investigation of consumed plants extracts is highly important for supporting safety uses without any physiological disorder for humans. Such a study usually includes three paths, namely the determination of the LD50 by acute toxicity, the measuring of hematological and biochemical parameters, and the histological study regarding the subacute toxicity.

From the acute toxicity of *Wakouba*, the resulted LD50 is 6,308.57 mg/kg BW. According to the standards provided by the WHO [21] and Morrison and Kimble [22], *Wakouba* appears as a non-toxic substance at the tested doses since tis LD50 value is below 7,000 mg/kg BW. After treatment of healthy rats with various *Wakouba* doses (950; 1,000 1,500, and 2,000 mg/kg BW), the sub-acute toxicity investigation did not result in any change from their hematological parameters compared to the control rats. Such observations show that *Wakouba* does not affect the haematopoiesis. Indeed, the haematopoiesis, physiological process allowing the blood cells synthesis, is one of the most sensitive bio-functions targeted by toxic components [23]. Besides, the hematological parameters are appropriate traits for investigation of the physiological and pathological states from humans and animals [24]. Significant changes in these parameters from laboratory animals are predictive for the human toxicity, since rats are slightly close to humans for major biological characteristics [25].

Liver and kidney are the filtrant tissues of the organism [26] and are therefore of the main targets for some toxic medicinal plants' compounds. Oral administration of *Wakouba* to rats at doses between 950 and 2,000 mg/kg BW did not change serum values of the renal and hepatic markers compared to the control group. In fact, transaminases are suitable indicators and biomarkers [27,28] for the toxic effects assessment regarding medicinal plants; and their concentrations in blood correlate the hepatocyte damages [29,30]. For renal function, urea and creatinine are the main diagnostic parameters, while CK, LDH and AST are markers of heart failure [31-33]. These parameters are not affected by *Wakouba* doses below 2,000 mg/kg BW. However, at the dose of 2,500 mg/kg BW *Wakouba* resulted in mild dyspnea and slight increase of the urea and creatinine values in rats. The observed dyspnea could be derived from with the hypotensive effect of *Wakouba* previously reported by Doumbia et al. [34]. According to these authors, *Wakouba* could deal its hypotensive effect as a calcium antagonist and a β-blocker compared to the standard tenordate molecule known as a commercial antihypertensive. In fact, creatinine and urea are substantially eliminated from the blood by glomerular filtration, which also depends on the blood pressure in the glomerular capillaries stated at about 30 mm Hg. Therefore any drop in blood pressure may cause a decrease in glomerular pressure of about 10 mm Hg [35]. Thus, any reduction in blood pressure can cause a decrease in plasma volume filtered by the glomerulus. The high level of urea and creatinine confirmed the hypotensive effect of Wakouba and not a renal effect. Our results corroborate those reported by Masson [36] on the toxicity of *Stachytarpheta indica* in rats.

Table 2. Hematological traits of rats treated with various doses (mg/kg BW) of *Wakouba*

Parmeters	Control	Means±SD/*Wakouba* dose				
		950	1 000	1 500	2 000	2 500
WBC (x 10^3/mm^3)	8.76±0.00	8.75±0.50^{ns}	8.77±0.12^{ns}	8.76±1.12^{ns}	8.78±0.30^{ns}	8.76±0.20^{ns}
RBC (x 10^6/mm^3)	7.80±0.10	7.50±0.01^{ns}	7.40±0.01^{ns}	7.80±0.09^{ns}	7.60±0.10^{ns}	7.60±0.30^{ns}
Hb (g/dL)	12.40±0.10	12.92±0.02^{ns}	12,70±0.05^{ns}	12.50±0.08^{ns}	12.42±0.06^{ns}	12.92±0.04^{ns}
MCHC (g/dL)	30.40±1.05	30.40±0.04^{ns}	31.60±0.51^{ns}	31.80±0.47^{ns}	30.90±0.61^{ns}	31.70±0.79^{ns}
PLT (x 10^3/mm^3)	648.20±5.02	649.00±5.72^{ns}	652.00±0.41^{ns}	647.00±2.94^{ns}	657.00±3.01^{ns}	653.00±0.34^{ns}
LYM (%)	98.00±0.35	97.00±0.36^{ns}	95.00±0.93^{ns}	98.00±0.25^{ns}	96.00±0.58n^{s}	96.83±0.04^{ns}
HCT (%)	39.83±0.36	38.90±0.02^{ns}	39.40±0.99^{ns}	37.10±0.76^{ns}	39.40±0.61^{ns}	38.70±0.05^{ns}
MCV (Fl/cell)	57.82±0.01	58.08±0.01^{ns}	57.40±0.02^{ns}	56.80±0.48^{ns}	57.40±0.23^{ns}	58.20±2.33^{ns}

From the same line, values with lowercase ns script are not statistically different at p=0.05 to the control. WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; LYM, lymphocyte; HCT, hematocrit; MCV, mean corpuscular volume; SD, standard deviation

Table 3. Biochemical profile of rats treated with various doses (mg/kg BW) of *Wakouba*

Parmeters	Control	Means±SD/*Wakouba* dose				
		950	1,000	1,500	2,000	2,500
AST (U/L)	206.00±0.68	208.00±0.63^{ns}	207.00±1.06^{ns}	206.00±0.85^{ns}	209.00±0.51^{ns}	207.00±1.18^{ns}
ALT (U/L)	150.00±2.64	152.00±0.81^{ns}	151.00±2.03^{ns}	154.00±1.48^{ns}	153.00±0.93^{ns}	156.00±1.89^{ns}
Urea (mg/L)	0.39±0.01	0.41±0.01^{ns}	0.40±0.02^{ns}	0.42±0.01^{ns}	0.41±0.05^{ns}	0.85±0.03***
Creat (mg/L)	16.20±1.21	17.10±0.00^{ns}	17.80±0.72^{ns}	16.90±0.02^{ns}	16.50±0.11^{ns}	36.66±0.57***
LDH (U/L)	265.2±1.61	264.10±1.67^{ns}	266.90±0.57^{ns}	265.70±1.52^{ns}	266.40±0.13^{ns}	265.80±0.81^{ns}
CPK (U/L)	155.4±3.25	155.80±1.56^{ns}	158.40±2.78^{ns}	155.70±0.97^{ns}	156.80±1.54^{ns}	155.90±0.57^{ns}
ALP (U/L)	201.00±1.39	293.00±1.09^{ns}	292.00±1.34^{ns}	294.00±1.18^{ns}	292.00±1.73^{ns}	293.00±1.41^{ns}
TP (U/L)	65.40±0.62	66.90±0.65^{ns}	65.80±0.63^{ns}	65.90±0.89^{ns}	64.90±1.55^{ns}	66.70±0.45^{ns}

*From the same line, values with lowercase ns script are not statistically different compared to the control at p=0.05; values with *** scripts are statistically different to the control at p=0.001. SD, standard deviation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Creat, creatine; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; ALP, alkaline phosphatase; TP, total proteins cholesterol*

Table 4. Lipid profile of rats treated with various doses (mg/kg BW) of *Wakouba*

Parmeters	Control	Means±SD/*Wakouba* dose				
		950	1,000	1,500	2,000	2,500
TChol (g/L)	1.68±0.00	1.64±0.01^{ns}	1.65±0.01^{ns}	1.67±0.01^{ns}	1.69±0.05^{ns}	1.62±0.01^{ns}
TG (g/L)	0.74±0.01	0.75±0.12^{ns}	0.67±0.04^{ns}	0.63±0.01^{ns}	0.59±0.06^{ns}	0.54±0.01^{ns}
HDL-Chol (g/L)	0.93±0.11	0.98±0.01^{ns}	1.11±0.02***	1.18±0.03***	1.24±0.01***	1.29±0.05***
LDL-Chol (g/L)	0.60±0.01	0.55±0.01^{ns}	0.43±0.12***	0.36±0.05***	0.27±0.02***	0.23±0.01***

From the same line, values with lowercase ^{ns} script are not statistically different at p=0.05 to the control; values with lowercase *** scripts are statistically different to the control at p=0.001. SD, standard deviation; TChol, total cholesterol; TG, triglycerides; HDL-Chol, high density lipoprotein cholesterol; LDL-Chol, low density lipoprotein cholesterol.

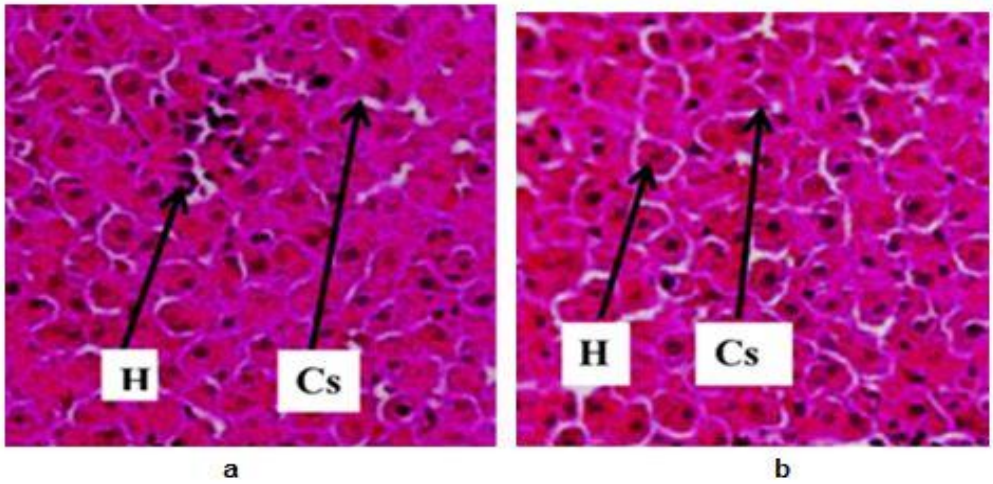

Fig. 2. Histological appearance after section of the liver from rats treated with control salt (a) and rats treated with *Wakouba* at dose of 2,500 mg/kg BW.
Colorant: Eosine-Hematoxyline; Magnification: 40x
Cs, Sinusoids; H, Hepatocyte

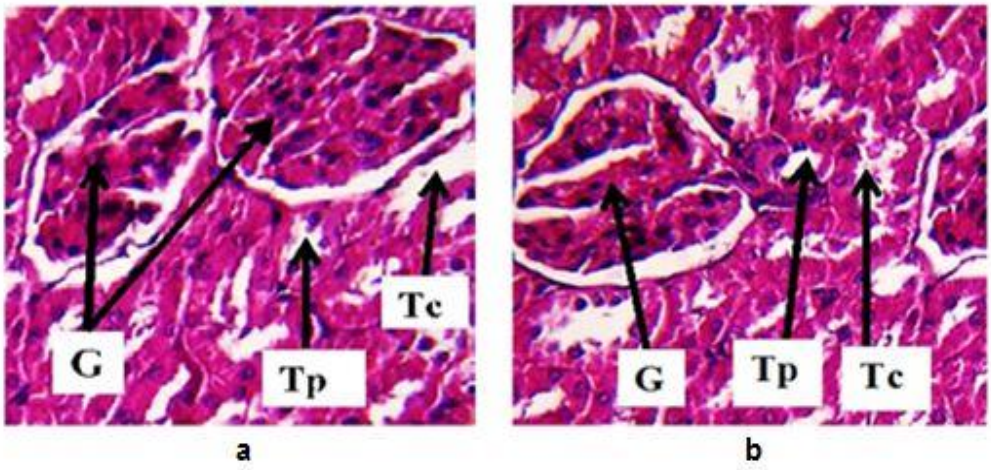

Fig. 3. Histological appearance after section of the kidney from rats treated with control salt (a) and rats treated with *Wakouba* at dose of 2,500 mg/kg BW
Colorant: Eosine-Hematoxyline; Magnification: 40x
G, Glomeruli; Bc, Bowman's capsule; Tc, distal convoluted tubules; Tp, proximal convoluted tubules

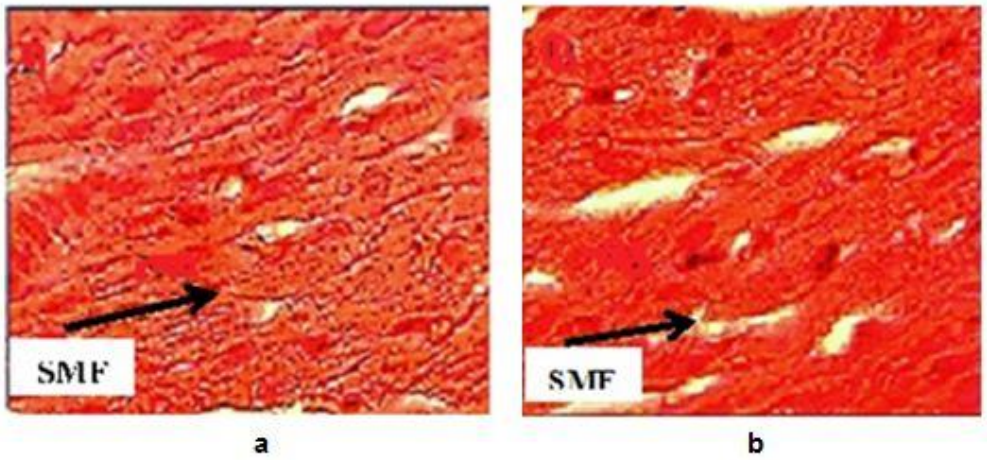

Fig. 4. Histological appearance after section of the heart from rats treated with control salt (a) and rats treated with *Wakouba* at dose of 2,500 mg/kg BW.
Colorant: Eosine-Hematoxylin; Magnification: 40x
SMF, Smooth muscle fibers

The safety of *Wakouba* is confirmed by the observation of histological section of the kidney from treated rats, showing no damage, even at higher dose of 2,500 mg/kg BW, compared to the control rats' kidney. The liver and heart from Wakouba-treated animals are also as similar as those of the controls, showing that this extract does not interfere with the metabolism of the target tissues. Regarding the lipid profile, the treatment with *Wakouba* led to an increase in the HDL-cholesterol and a decrease in LDL-cholesterol compared to the control rats group. The "good cholesterol" (HDL-cholesterol) carries the excess of organs cholesterol towards the liver where it is eliminated. They have the ability to clean the arteries from all lipid deposits due to the "bad cholesterol" (LDL-cholesterol) that gradually form real fatty plaques called atheroma. So, the HDL-cholesterol has significant contribution in reduction of the risk of atherosclerosis plackets developing. Thus, it has beneficial effect against cardiovascular concerns since atherosclerosis is known as a significant marker of these diseases [37], and its higher concentration in the serum could be protective for the myocardia [38]. Thanks to the main observations about the treated rats, *Wakouba* could be valorised as a raw antihypertensive for supporting prevention against the cardiovascular troubles.

4. CONCLUSION

The toxicity investigation about Wakouba allowed the determination of the LD50 and MTD. The values recorded indicate the safety uses of *Wakouba*. This extract also relies on hypotensive effect revealed by the higher blood value of urea and creatinine at the dose of 2,500 mg/kg BW. These statements are confirmed by the histological examination of the kidney, liver and heart without any damage in their structures. The administration of *Wakouba* also increases the blood HDL-cholesterol whereas the LDL-cholesterol level is dropped. From the overall outcomes, the *Wakouba* salt could be taken as antihypertensive and cardio-protective substance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mamadou N, Eric A, Madieye S, Diatta W, Amadou MD, Babacar F, Schini-Kerth VB. Mechanisms underlying the endothelium-dependent vasodilatory effect of an

aqueous extract of *Elaeis guineensis* jacq (Arecaceae) in porcine coronary artery rings. Afr J Tradit Complement Altern Med. 2009;7(2):118-124.

2. Victor UA, Suhaila M, Hair BB. Acute toxicity and safety assessment of oil palm (*Elaeis guineensis* Jacq.) leaf extract in rats. Journal of Medicinal Plant Research. 2003;7(16):1022-1029.
3. Irine R, Noordin MM, Radzali M, Azizah H, Hapizah N, Mahinda YA, Suhaila M. Antioxidant and hypocholesterolemic effects of *Elaeis guineensis* frond extract on hypercholesterolemic rabbits. Asian Food Journal. 2003;12:137-148.
4. Salleh MN, Runnie I, Roach PD, Mohamed S, Abeywardena MY. Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in Hep G2 cells bytropical plant extracts. J Agric Food Chem. 2002; 50:3693–3697.
5. Sasidharan S, Sharmini R, Vijayrathna S, Yoga LL, Vijenthi R, Amala R, Amutha S. Antioxidant and hepatoprotective activity of methanolic extracts of *Elaeis guineensis* (Jacq) leaf. Pharmacology Online. 2009; 3:84–90.
6. Juliana M, Jaffri, Suhaila M, Nordanial R, Intan N, Ahmad M, Mustapha N, Yazid AM. Antihypertensive and cardiovascular effects of catechin-rich oil palm (*Elaeis guineensis*) leaf extract in nitric oxide–deficient rats. J Med Food. 2011;14(7-8):775-774.
7. Chong KH, Zurainia Z, Sasidharanb S, Kalnisha PV, Devib L, Yoga LS, Ramanathan D. Antimicrobial activity of *Elaeis guineensis* leaf. Pharmacology Online. 2008;3:379-386.
8. Kweifio-OG. Anti-inflammatory activity of a Ghanaian anti-arthritic herbal preparation. Int. J. Ethnopharmacol. 1991; 33:263-267.
9. Adeneye AA, Benebo AS. Ameliorating the effects of acetaminophen-induced hepatotoxicity in rats with African red palm oil extract. Asian Journal of Traditional Medicine. 2007;2(6):244-249.
10. Selly YE, Guede Guina F, Yao BAI, Agneroh EL. Usefulness of a fixator of the oil-palm sap (1) in the revelation of a viral protein by immunofluorescence: case of rabic virus isolated from streets in Abidjan. Médecine d'Afrique Noire. 2003;47:7. French
11. Chong YH. Effects of the palm oil on the cardiovascular risks. Med J Malaysia. 1991;46(1):41-50.
12. Zirihi GN, Kra AKM, Guede-GF. Assessment of the antifungal activity of *Pyrifolia microglossa* (LAMARCK) O. KUNTZE (Asteraceae) « PYMI » on the *in-vitro* growth of *Candida albicans*. Revue de Médecine et de Pharmacopées Africaines. 2003;17:11-18. French
13. OECD. Guidelines for the Testing of Chemicals /Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. Organization for Economic Cooperation and Development. Paris, France; 2000.
14. Weiss J, Taylor GR, Zimmermann F, Nebendahl K. Collection of body fluids- In: KRINKE GJ-The laboratory Rat, The handbook of experimental Animal-Academic Press. 2000; 25:485-495.
15. OECD. Guidelines for testing of chemicals, N°407. Repeated dose oral toxicity test method. Organization for Economic Cooperation and Development. Paris, France; 2000.
16. Descat F. Hématologie du rat: hémogramme et myélogramme. Thèse médecine vétérinaire. 3.4011, 24, Toulouse, France. French; 2002.
17. Bleyere NM, Ekaza JD, Angoue YP, Datte JY, Banga NB, Cathy NMA, Vanga M, Kone M, Ehouan EE. Heterogeneity of Iron level from woman during the pregnancy in Côte d'Ivoire. Ann. Biol. Clin. 2007; 65(5):525-532. French
18. Lietchfiel JF, Wicoxon FA. A simplified method of evaluation doses-effects experiments. J. Pharmacol. Exper. Therap. 1949;96(2):99-113.
19. Bhrger C, Fischer DR, Cordenunzzi DA, Batschauer DBAP, Filho VC. Soares dos Santos AR. Acute and subacute toxicity of the hydroalcoholic extract from Wedelia paludosa (*Acmela brasilinsis*, Asteraceae) in mice. J. Pharm. Sci. 2005;8:370-373.
20. Silva EJR, Concalves ES, Aguiar FJS, Evencio LB, Lyra MMA, Coelho MCOC, Fraga MCCA, Wanderley AG. Toxicological studies on hydroalcohol extract of *Calendula officinalis* L. Phytotherapy Research. 2007;21:332-336.
21. Atangwho IJ, Ebong PE, Egbung GE, Obi AU. Extract of *Vernonia amygdalina* Del. (African bitter leaf) can reverse pancreatic cellular lesion after alloxan damage in the

rat. Australian Journal of Basic and Applied Sciences. 2010;4(5):711-716.
22. Mfon I, Akpaso, Item J, Atangwho, Amabe A, Victor A, Fischer, Anozeng O, Igiri L, Patrick E, Ebong. Effect of combined leaf extracts of *Vernonia amygdalina* (Bitter Leaf) and *Gongronema latifolium* (Utazi) on the pancreatic β-cells of Streptozotocin-Induced diabetic rats. British Journal of Medicine & Medical Research. 2011; 1(1):24-34.
23. Hodge HC, Sterner JH. Determination of substance acute toxicity by LD50. American Industrial Hygien Association. 1943;10:93.
24. WHO (World Health Organization). Traditional Medicine: Report from the Executive council secretary. 111th session. Caption 5.7 of the provisional program (EB111/9). 12-12th-2002. French
25. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature. 2006; 441:1068-1074.
26. Mukinda JT, Syce JA. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. Journal of Ethnopharmacology. 2007;112:138–144.
27. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Deun KV, Smith P, Berger B, Heller A. Concordance of toxicity of pharmaceuticals in humans and in animals. Regulatory Toxicology and Pharmacology. 2000; 32:56–67.
28. Tulsawani R. Ninety day repeated gavage administration of *Hipphophae rhamnoides* extract in rats. Food and Chemical Toxicology. 2010;48:2483–2489.
29. Rahman MF, Siddiqui MK, Jamil K. Effects of Vepacide (*Azadirachta indica*) on aspartate and alanine aminotransferase profiles in a subchronic study with rats. Human and Experimental Toxicology. 2001;20:243–249.
30. Hilaly JE, Israili ZH, Lyouss B. Acute and chronic toxicological studies of *Ajuva Iva* in experimental animals. Journal of Ethnopharmacology. 2004;91:43–50.
31. Witthawaskul P, Panthong A, Kanjanapothi D, Taesothiku IT, Lertprasertsuke N. Acute and subacute toxicities of the saponin mixture isolated from *Schefflera leucantha* Viguier. J. Ethnopharmacol. 2003;89:115-121.
32. Kumar B, Sharmila P, Vanitha PM, Sundararajan M, Rajasekara P. Hepatoprotective activity of *Trianthema portulacastrum* L. against paracetamol and thioacetamide intoxication in albino rats. J. Ethnopharmacol. 2004;92:37-40.
33. Wishart DS. Metabolomics: The principles and potential applications to transplantation. American Journal of Transplantation. 2005;5(12):2814–2820.
34. Wishart DS. Metabolomics: a complementary tool in renal transplantation. Contributions to Nephrology. 2008;160:76–87.
35. Diallo K, Eklu G, Agbonon A, Aklikokou K, Creppy EE, Gbeassor M. Acute and subchronic (28-days) oral toxicity studies of hydroalcoholic extract of *Lannea kerstingii* Engl and K. Krause (Anacardiaceae) stem bark. Journal of Pharmacology and Toxicology. 2010; 5:343-349.
36. Coulibaly FA, Djih BN, Doumbia I, Yapi HF, Djaman AJ. Enzymatic values in heart serum from rabbits treated with *Phyllanthus amarus* (Euphorbiacea). Phytotherapie. 2010;8(6):348-352. French
37. Doumbia I, Adebo IB, Coubaly FA, Djaman AJ, Guede-Guina F. Changes in serum urea and creatinine rates from female rabbits treated with *Mareya micrantha*. J. Sci. Pharm. Biol. 2007;8(2):6-13. French
38. Hobou DRAD, Fofie NBY, N'guessan K, Kone D. Assessment of the toxicity of *Stachytarpheta indica* on the rat. J. sci. Pharm. Biol. 2011;12(1):6-12. French

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27309