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Phytochemical screening, acute and subacute toxicity of aqueous extract of *Moringa oleifera* (Moringaceae) Lam 1885 on rats wistar

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Abstract
Due to the many therapeutic and nutritional properties of *Moringa oleifera*, an emphasis has been placed on its richness in secondary metabolites and its safety through an aqueous extract of its leaves. Specific reagents identified secondary metabolites with pharmaceutical effects. The acute and subacute toxicity test was conducted according to The Organization for Economic Co-operation and Development Guideline 423 and 407, respectively, on males and female rats, Wistar strain. Phytochemical screening revealed the presence of sterols and polysterols, polyphenols, flavonoids and saponosides. The lethal dose 50% of the extract is greater than 5000 mg / kg of body weight. The extract significantly reduced females to Alanine-aminotransferase at a dose of 300 mg / kg of body weight. The study showed the richness of secondary metabolites, the safety of the aqueous leaf extract of *Moringa oleifera* and its hepatoprotective effect.

Keywords: *Moringa oleifera*, triphytochemistry, toxicity, histology, rat

Introduction
Nowadays, despite the progress made in the medical field, the therapeutic use of plants is very present in some developing countries, even in the presence of a modern health system (Hafidi, 2014) [1]. Unfortunately, some plants from the pharmacopoeia are at the origin of some severe intoxications (Pinto *et al*., 2002) [2]. They are usually accidental or secondary to therapeutic use (Adejuyigbe *et al*, 2002) [3]. The severity of these intoxications is function of certain factors namely: nature of the plant, part consumed, quantity, taken fasting or not, age and circumstances. But it also depends on highly toxic substances (solanins, glycosides, aconitine, other alkaloids) (Hafidi, 2014) [1]. *Moringa oleifera* is a plant of the Ivorian pharmacopoeia used for the treatment of various pathologies. Its traditional medicinal properties have led some researchers to study its anti-inflammatory properties (Mahajan *et al*., 2007) [4], anti-hypertensive agents (Shaila *et al*., 2010) [5] and anti-hypertensive hepatoprotective agents (Pamo *et al*., 2002 [6]; (Faizi *et al*., 1995) [7] and anti-tumor (Murakami *et al*., 1998) [8] and in the treatment of many other pathologies. To secure its therapeutic use, we proposed to study its phytochemical composition, its acute toxicity and its subacute toxicity on rats of Wistar strain.

Materials
The plant material is represented by *Moringa oleifera* leaves, freshly collected during the month of June 2016. These leaves have been identified at the National Floristic Center (C.N.F.) of Félix Houphouët-Boigny University (Ivory Coast).
The animal material consists of rats *Rattus norvegicus* (Muridae) strain WISTAR. These 6- to 8-week-old rats are nulliparous and non-pregnant with a weight of between 140-160 g, used for acute and subacute oral toxicity tests. The animals coming from the animal factory of the Ecole Normale Supérieure (ENS) of Abidjan (Ivory Coast) were acclimatized at least 5 days before the beginning of the experiment. The room temperature was 26 to 30 ° C, humidity 40 to 60% and lighting 12 hours of light and 12 hours of darkness. They had free access to the food (bakery bread, maize, fish and pellets from the FACI®) and had tap water available continuously in baby bottles.

Methodology

Preparation of the extract

The harvested leaves were dried in the shade at room temperature at 26-30 ± 2 ° C for 15 days. They were then sprayed with a mini electric grinder. 50 grams of this powder were mixed with 1.25 liter of distilled water and then macerated for five times three minutes in a blender. The macerate obtained is filtered twice on square fabric, on hydrophilic cotton and once on N°1 wattman paper. The filtrate obtained is dried in an oven at 50 ° C (Zirihi *et al*., 2003) [9].

Phytochemical study of the aqueous extract of the leaves of *M. oleifera*: EAFMO

The chemical analyzes were performed by phytochemical screening. This is a qualitative analysis based on staining and / or precipitation reactions (Houghton and Raman, 1998) [10]. Table I indicates the different chemical groups sought and the specific reagents used (Lebri *et al*., 2015) [11].

Table I: Specific Reagents and Phytochemical Screening Reactions (Lebri *et al*., 2015) [11].

Chemical groups		Specific reagents	Characteristic reactions
Sterols and polyterpenes		Lieberman-Burchard reaction: Anhydrdride acetic and H2SO4	Appearance at the interface of a ring purple or purple, turning blue then green
Polyphénols		Ferric chloride reaction	Blackish blue or green color, more or less dark
Flavonoïdes		Reaction to Cyanidin	Orange-pink coloring; purple pink or red
Tanins	Gallic	Sodium-FeCl 3 Acetate Reaction	Intense blue-black color
	Catéchin	Stiasny: Formol-HCl reaction	Greenish or blackish-blue color.
Composés quinoniques		Borntraeger-UV reagent	Inflorescence intense
Saponins		Reaction to the foam index	Foam> 4 cm
Alkaloid		Dragendorff: iodobismuthate potassium	Orange coloring or a precipitate
		Bouchardat: iodine-iodide	Reddish-brown color

Acute oral toxicity

This study was conducted according to the OECD Guideline 423 by Acute Toxicity Class (2001) [12]. Two batches of three female rats were formed. On day before the beginning of the treatment, lot 1 received 1 ml / 100 g of body weight (bw) of distilled water. Due to the indication of the low toxicity of the substance, lot 2 received the initial dose of 2000 mg / kg of bw in a volume of 1ml / 100g of distilled water orally through a gastric tube. Following the single dose of EAFMO, the animals were placed in individual cages for observation. The observations were made after 30 minutes, 4 hours, 24 hours, one week and two weeks. During the treatment, the animals were weighted every other day at the same time.

Subacute oral toxicity

The subacute toxicity test is performed according to the OECD Test Guideline 407 for the testing of chemicals (OCDE, 2008) [13]. Indeed, two groups of rats were formed for this test. Group I consists of male rats and group II consists of female rats. Each group is composed of 4 lots of 5 rats / lot. Lot 1 (control) received 1 ml / 100 g of bw of distilled water while lots 2, 3 and 4 respectively received the doses 150, 300 and 600 mg / kg of bw in the same volume of water distilled regardless of the sex of the animal. By gavage, these animals received these doses daily for 28 days and weighted every other day.

Treatment of blood and organs removed

At the end of the 28 days of treatment, all the rats were sacrificed after anesthesia with ether and then a portion of the blood was collected first in the dry tubes and then centrifuged at 3000 rpm for 5 minutes. The serum obtained was used to determine the biochemical parameters (urea, creatine, Alanine-aminotransferase, Aspartate Amino Transferase). The other part of the blood was collected in EDTA (Ethylene Diamine Tetraacetic) tubes for haematological assays (red and white blood cells, platelets, etc.). Then, the animals were dissected to harvest organs such as the liver, kidneys, heart and lungs. These were rinsed in 0.9% sodium chloride and weighted. The relative weight is determined according to the formula below.

$$Pr = \frac{P_0 \, X \, 100}{PC}$$

Pr: relative weight of the organ (g / 100 g);
P_0: weight of the organ (g);
Pc: rat body weight (g).

Histopathology

After sacrifice of the rats, the organs (liver, kidneys and heart) were removed, washed with physiological saline, and preserved in 10% formalin. The completion of histological sections included tissue dehydration, toluene lightening, impregnation and paraffin embedding and organ dewaxing in toluene baths. The staining of the organ cuts is made with hematoxylin-eosin and the observation was made using an Olympus CKX41 (Germany) type microscope connected to a computer equipped with Videomet software. The magnifications (GX100) allowed us to assess the possible tissue abnormalities of the organs**.**

Statistical Analyzes

The data was entered into an Excel spreadsheet (Microsoft Office 2013, USA) and the Graph Pad Prism *version 7* software allowed us to plot the curves and histograms, then perform the statistical analyzes. The values are presented as mean ± ESM. The analysis of variance (ANOVA ONE-WAY) applied to the obtained results allowed us to appreciate the effects of the different treatments with levels of significance. Turkey's test allowed us to compare different values.

Results

The phytochemical screening of aqueous extract of leaves *M. oleifera* (EAFMO) showed the presence of some large chemical groups. Thus, this study reveals the presence of sterols and polysterols, polyphenols, flavonoids, quinonic substances and saponosides, but there is an absence of

alkaloids, gallic and catechic tannins (Table II).

Table II: Results Characterization of certain chemical compounds of the aqueous extract of *M. oleifera*

Sterol and polysterol	Phenolic groups	Flavonoids	Tannins		Quinonic substances	Saponins	Alkaloid	
			Gallic	Catechin			DRAGENDORFF	BOUCHARDAT
+	+	+	-	-	+	+	-	-

+ Presence of the phytochemical; -Absence of the phytochemical evaluated

During the 14 days of traitement with the plant extract, no mortality was detected and no clinical signs of toxicity were detected (Table III). The final weights of control and treated rats were 168.85 ± 6.721 and 169.91 ± 4.985 g respectively, an showing increase of 17.09 ± 3.18 and 18.77% ± 1.37. These values do not differ significantly (P> 0.05). According to the Globally Harmonized Classification System (GHS, 2003), the total aqueous extract of *Moringa oleifera* leaves has an LD50 greater than 5000 mg / kg of bw.

Table III: Observation of clinical signs in rats after 14 days at 2000 mg / kg BW

Observations	30 min		4h		24h		48h		1 Week		2 Weeks	
	C	T	C	T	C	T	C	T	C	T	C	T
Skin and Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Léthargy	-	-	-	-	-	-	-	-	-	-	-	-
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-
Morbidity	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-

The relative weight of the kidneys and heart showed no significant change compared to their respective controls. However, the liver weight of the rats treated, with 3,385 ± 0,078 mg showed a significant increase compared to 2,624 ± 0.1051 mg of the control (Table IV).

Table IV: Effects of EAFMO administered orally on the dry weight of the vital organs of the adult females rats after 14 days.

Organes (g/kg de BW)	Témoins	T 2000	t calculé
Reins	0,2569 ± 0,0053	0,256 ± 0,0057	t=0,1206
Foie	2,624 ± 0,1051	3,385 ± 0,0789 *	t=5,146
Cœur	0,3519 ± 0,013	0,326 ± 0,0017	t= 1,481

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Cœur 0,3519 ± 0,013 0,326 ± 0,0017 t= 1,481

The effects of daily oral administration of the aqueous leaf extract of *M. oleifera* were assessed after evaluation of behavior, weight gain, relative weight of organs, and biochemical and hematological parameters. Thus, for 28 days of treatment, no behavioral changes were observed regardless of the dose of the extract administered. Comparison of the body weight of control and treated male at doses of 100, 300, 600 mg / kg of bw showed no significant difference (p> 0.05). These values are respectively 195.2 ± 6.8 g (18.85%); 187.43 ± 4.721 g (17.89%); 173.76 ± 8.604 g (16.02%) and 192.7 ± 2.743 g (19.66%). While, the weights of females treated 183.5 ± 1.08 g (2.77%); 182.9 ± 1.378 g (4.059%); 178.3 ± 1.75 g (2.57%) at the same doses decreased significantly (p<0.01) regardless of the dose administered when compared to controls 199.1 ± 1.292 g (9.601%). The vital organ weights of male rats (Table V) and female rats (Table VI) treated with distilled water (controls) and EAFMO at doses of 150, 300 and 600 mg / kg of bw does not differ significantly (p> 0.05) except a reduction of the weight of the kidney at the dose of 600 mg / kg of bw in females.

Table VI: Effects of EAFMO administered orally on the dry weight of the vital organs of the adult females rats after 28 days

Organes (g/kg BW)	Control	150	300	600
Kidney	0,3004±0,008	0,2768±0,005	0,2934±0,006	0,2648±0,009*****
Liver	3,002±0,085	3,054±0,079	3,007±0,083	2,956±0,068
Heart	0,3274±0,004	0,3396±0,006	0,3396±0,006	0,3432±0,005
Lungs	0,5964±0,023	0,6142±0,022	0,6388±0,011	0,6072±0,011

The data is presented as mean ± Mean error **(ESM)**. Treated groups were compared to control group using one-way ANOVA followed by Turkey test was used to compare against the control. *****: significant difference P < 0,05

Table V: Effects of EAFMO administered orally on the weight of the vital organs of the adult males rats after 28 days

Organs (g/kg BW)	Control	150	300	600
Kidney	0,267±0,008	0,270±0,005	0,296±0,006	0,296±0,032
Liver	3,391±0,215	3,607±0,205	3,74±0,170	3,957±0,101
Heart	0,379±0,026	0,388±0,021	0,393±0,047	0,382±0,060
Lungs	0,784±0,056	0,701±0,098	0,877±0,089	0,712±0,130

The data is presented as mean ± Mean error **(ESM)**. Treated groups were compared to control group using one-way ANOVA followed by Turkey test was used to compare against the control. Significant difference P >0,05

In males, there was no change in both biochemical parameters (Table VII) and hematological parameters (Table VIII) compared with controls. However, a very significant reduction 31.67 ± 0.88 U/l in ALT was observed at a dose of 300 mg / kg of bw compared to 40 ± 1 U/l controls.

Table VII: EAFMO effects on biochemical parameters of adult males rats (J = 28)

Treatmen	GLU (mmol/L)	URE (mmol/l)	CR (µmol/l)	A. URIQUE (g/L)	AST (U/l)	ALT (U/l)
Control	0,86±0,028	0,1233±0,0066	6,667±0,333	13±0,577	105,3±2,333	40±1
D_{150}	0,926±0,056	0,1067±0,0033	6,33±0,333	12±0,577	111,3±2,333	39,67 ± 1,45
D_{300}	0,926±0,041	0,1033±0,0033	6,33±0,333	12,33±0,666	104,3±1,764	31,67±0,88**
D_{600}	0,905±0,025	0,105±0,005	6,5±0,5	14,67±0,333	110±2	39,67±0,88

The data is presented as mean ± Mean error **(ESM)**. Treated groups were compared to control group using one-way ANOVA followed by Turkey test was used to compare against the control. **GLU:** glucose; **URE**=urea; **CR**= creatinine; **URIC A:** Uric Acid; **AST** Aspartate aminotransferase; **ALT**: Alanine-Aminotransferase; **: significant difference P <0,05

Table VIII: EAFMO effects on hematologic parameters of male rats 28 days

Treatment	WBC×10^3/ml	RBC×10^6/ml	HGB g/dl	HCT%	MCV fl	MCH pg	MCHC g/dl	PLT×10^3/ml
Control	9,63 ± 0,96	7,983±0,26	12,73±0,43	40,1±1,4	50,47±0,97	16,03±0,088	31,53±0,48	771,7±145,8
D_{150}	9,83 ± 0,79	7,623±0,35	12,23±0,33	39,67±1,36	48,93±0,99	15,5±0,3	31±1,058	761±137
D_{300}	9,56 ± 0,84	8,003±0,38	12,23±0,18	38,7±0,47	48,2±1,62	15,27±0,448	31,53±0,6227	802±151,7
D_{600}	9,51± 1,03	7,91±0,19	12,31±0,43	40,83±1,39	49,82±0,82	15,41±0,462	31,18±0,4334	867,7±116,7

The data is presented as mean ± Mean error (ESM). Treated groups were compared to control group using one-way. ANOVA followed by Turkey test was used to compare against the Witnesses. **WBC**: white blood cell; **RBC**: red blood cells; **HGB**: hemoglobins; **HCT**: hematocrits; **MCV**: Mean Corpuscular Volume; **MCH**: Mean Corpuscular Hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **PLT**: platelet. Significant P> 0.05

Table IX: Effects of EAFMO on biochemical parameters of adult females rats (J = 28)

Treatmen	GLU (mmol/L)	URE (mmol/l)	CR (µmol/l)	A. URIQUE (g/L)	AST (U/l)	ALT (U/l)
Témoin	1,31±0,06	0,238±0,003	6,6±0,67	12,4±1,51	107,4±4,26	23,4±0,9274
D_{150}	1,18±0,04	0,24±0,004	5,8±0,48	13,8±0,37	112,6±5,21	26,8±0,7348
D_{300}	1,19±0,05	0,234±0,006	6,8±0,73	12,2±0,48	119,4±3,48	26,2±1,02
D_{600}	1,26±0,06	0,24±0,006	7,75±0,94	11,4±0,67	111,5±3,77	28±1,35*

The data is presented as mean ± Mean error **(ESM)**. Treated groups were compared to control group using one-way. ANOVA followed by Turkey test was used to compare against the control. **GLU:** glucose **URE**=urea,. **CR**= creatinine; **A.URIQUE**: Uric Acid ; **AST** Aspartate aminotransferase ; **ALT**.: Alanine-Aminotransferase; *****: significant difference P <0,05

However, in females, the biochemical analysis (Table IX) revealed a significant increase at the dose of 600 mg / kg of bw of the amount of ALT 28 ± 1.35 U/l compared to controls 23.4 ± 0, 9274 U/l. The hematological analysis (Table X) showed a very significant increase in the number of white blood cells at a dose of 150 mg / kg of bw 15.09 ×10^3± 0.46 /ml with respect to the control 10.59 ×10^3± 0.43/ml. At a dose of 600 mg / kg of bw EAFMO, the hematocrit increased very significantly 53.63 ± 1.44 % compared to the control 46.22 ± 1.705 % while the number of platelets decreases very significantly.

Table X: Effects of AFMO on hematological parameters of adult females rats

Treatent	WBC×10^3/ml	RBC×10^6 /ml	HGB g/dl	HCT%	MCV fl	MCH pg	MCHC g/dl	PLT×10^3/ml
Témon	10,59±0,4336	9,70±0,40	15,98±0,83	46,22±1,70	53,16±0,89	17,5±0,14	32,92±0,43	1156±77,31
D_{150}	15,09±0,46***	8,168±0,20	14,68±0,40	46,32±0,92	56,3±0,98	17,74±0,24	31,56±0,45	1308±93,06
D_{300}	11,9±0,69	8,768±0,45	15,34±0,88	47,82±0,94	52,34±1,22	17,46±0,31	33,42±0,49	913,8±36,82
D_{600}	9,73±0,51	9,99±0,39	17,28±1,12	53,63±1,44**	54,38±0,68	18,08±0,11	33,25±0,58	628±59,25***

The data is presented as mean ± Mean error (ESM). The Turkey test was used to compare against the Witnesses. **WBC**=White Blood Cells; **RBC**=Red Blood Cells; **HGB**=Hemoglobins; **HCT**=Hematocrits; **MCV**=Mean Corpuscular Volume; **MCH**=Mean Corpuscular Hemoglobin; **MCHC**=Mean Corpuscular Hemoglobin Concentration; **PLT**=Platelet. *** ***: significant difference P <0.01; *** * *:** significant difference P <0.001

Histological examination of the liver (Figure 1), kidney (Figure 2) and heart (Figure 3) showed no architectural changes compared to the control. On the other hand, a development of hepatocytes and centrolubular veins at the dose of 300 mg / kg of PC is observed.

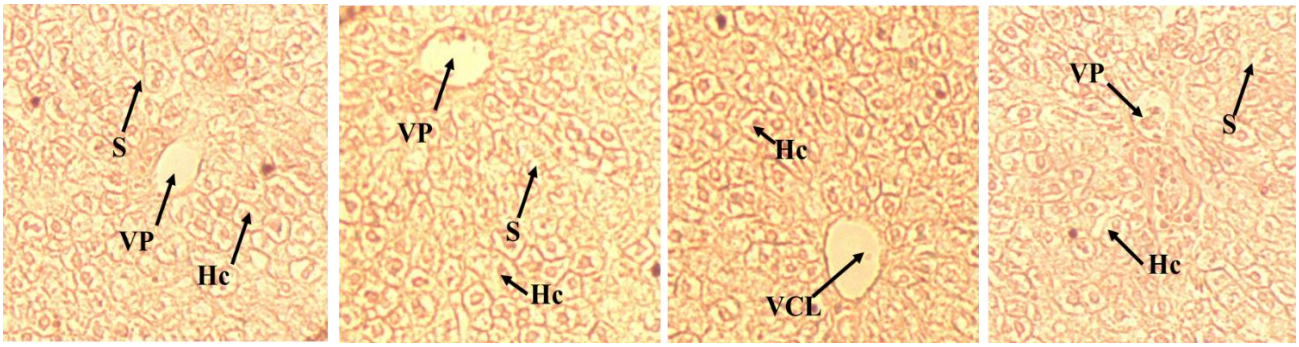

Fig 1: Photomicrographs of sections (G x 100) of rat liver in the subacute toxicity study

A1: *Witnesses****; A2****: Treated to 150 mg / kg of PC****; A3:*** *treated at 300 mg / kg of PC****; A4:*** *Treated to 600 mg / Kg of PC* ***; Hc:*** *Hepatocytes;* ***VP:*** *Vein Gate****; VCL:*** *Centrilobular Vein****; S:*** *Sinusoid*

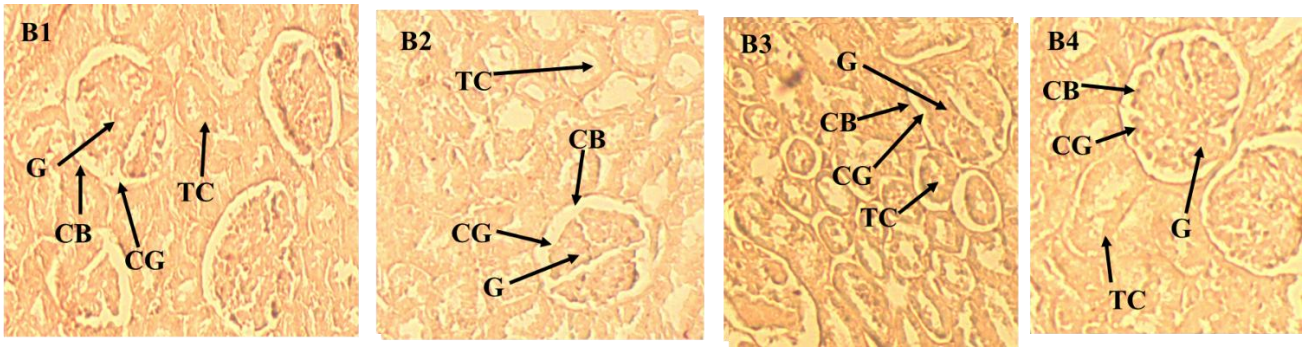

Fig 1: Photomicrographs of sections (G x 100) of rat kidney in the subacute toxicity study

B1: *Witnesses****; B2:*** *Treated to 150 mg / kg of PC****; B3:*** *treated at 300 mg / kg of PC****; B4:*** *Treated to 600 mg / Kg of PC****; G:*** *Glomerulus****; CB:*** *Bowman Capsule****; CG:*** *Glomerular* ***Canal; TC:*** *Contoured Tubule*

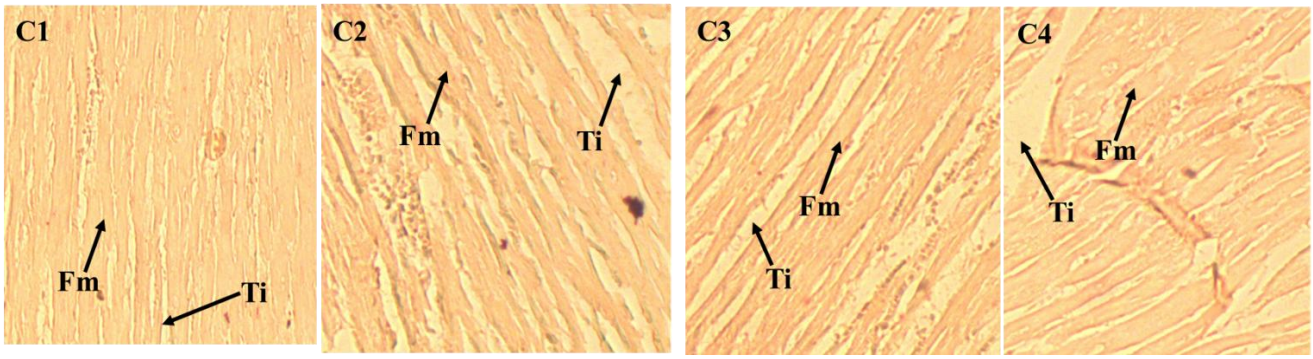

Fig 3: Photographies de sections (G X 100) du cœur de rats dans l'étude de la toxicité subaigüe

C1: *Witnesses****; C2:*** *Treated to 150 mg / kg of PC****; C3:*** *treated at 300 mg / kg of PC****; C4:*** *Treated to 600 mg / Kg of PC****; Ti:*** *interstitial tissue****; Fm:*** *muscle fiber*

Discussion

The therapeutic efficacy of a plant is perceived by its richness in secondary metabolites. Thus, the qualitative phytochemical study carried out on the EAFMO made it possible to highlight certain secondary metabolites such as sterols and polysterols, polyphenols, flavonoids, saponosides and quinone substances. These results These results are similar to those of Ibrahim and *al.* (2015) [14] ; Pinal *et al.* (2014) [15] in an aqueous extract of *M.oleifera* leaves. Some studies have shown that favonoids have antiallergic, hepatoprotective, antispasmodic, anti-inflammatory and anti-neurodegenerative properties. Terpenoids are potentially endowed with anti-inflammatory and sometimes analgesic properties (Bennett *et al.*, 2003) [16]. The presence of these active ingredients in *M. oleifera* leaves may justify their use in the prevention and treatment of certain pathologies such as hepatomegaly and gallstones.

The acute toxicity assessment of *M. oleifera* consisted of measuring and recording the various adverse effects that occurred after administration of the single dose of 2000 mg / kg bw. Indeed, at this dose, no change in behavior, signs of intoxication and mortality was recorded. The toxicity of chemical substances is based on the value of their LD50. The LD50 is the lethal dose for 50% of the tested animal population to die. It is expressed in mass of substance per bw of the animal. At the end of the acute toxicity test, the LD50 is estimated to be greater than 5000 mg / kg oral. According to the Globally Harmonized (GHS) Labeling System (2003) [17], the aqueous extract of *M. oleifera* can be classified as category 5 or unclassified products. The 2000 mg / kg dose of bw would be below the maximum tolerated dose (MTD). These results are similar to those of Indres (2017) [18] which showed that the powder of *Moringa* leaves was not toxic at a

dose of 2000 mg / kg of bw. They are also similar to those of Pierre *et al*. (2017) [19] who showed that the LD50 of the plant-based "natural" remedy was greater than 5000 mg / kg. On the other hand, they are contrary to those obtained by Abrar *et al*. (2013) [20]. Thus, these authors showed that the ethanoid extract of Tridex procumbens showed signs of toxicity (somnolence, coma, and morbidity) in Sprague Dawley rats 30 minutes after administration at 2000 mg / kg PC.

The effects of repeated daily administration for 28 days orally at repeated doses of the total aqueous extract of *M. oleifera* leaves were assessed after evaluation of behavioral parameters, weight gain, relative weight of organs and biochemical parameters. The reduction of the weight of the treated female rats could be explained by the hypoglycemic and anti-cholesteric property of the aqueous extract of *M. oleifera*. These results are similar with those of Sule and Arhoghro (2016) [21] who showed a reduction in body weight, serum glucose and serum cholesterol in rats treated with different doses of aqueous extracts of *Moringa* leaves. Ghasi *et al*. (2000) [22] also mentioned in their work that *M. oleifera* leaves have a pharmacological basis for use in India. The reduction in the relative weight of the kidney at 600 mg / kg of bw in the female rats would be due to the nephroprotective activity of EAFMO. Thus, Hanann *et al*. (2017) [23] and Sharma and Paliwal (2012) [24] showed that the aqueous extract of *M. oleifera* would have hepato-nephroprotective effects by reducing the concentration of serum urea and creatinine. Observation of biochemical parameters during the subacute toxicity study showed a decrease in ALT (Alanine-Amino-Transferase) in the rats male. In fact, transaminases are present in the liver, but also in the muscle, kidney, pancreas, and other tissues. They are synthesized at the level of the cytoplasm of the cells of these organs and discharged into the blood circulation, when these cells are damaged (Peirs, 2005) [25]. These enzymes increase in case of myopathy, rhabdomyolysis or myocardial infarction and AST, especially in case of hemolysis. ALTs are more specific for liver injury, but AST (Aspartate Amino Transferase) are somewhat more sensitive (Goddard and Warnes, 1992) [26]. In the case of this study, it is possible that the extract has a hepatoprotective effect at 300 mg / kg bw at the male level. This result confirms the hepatoprotective role of the extract which possesses secondary metabolisms such as flavonoids, one of whose properties is the protection of the liver. These data are in agreement with those obtained by Fakurazi *et al*. (2008) [27] who showed that Moringa can preserve the structural integrity of hepatocyte membrane and subsequently prevent the leakage of ALT and AST enzymes into the plasma. The high ALAT value at 600 mg / kg PC in female rats is thought to be due to a toxicity effect caused by prolonged administration of a high dose of the extract. According to OECD 423 (2001) female rats are more sensitive than males.

The hematology system presents some modifications in the female rats which have received the aqueous extract of *M. oleifera*. Blood is the main means of transportation for many drugs and xenobiotics. Certain components of the blood such as red blood cells, white blood cells, hemoglobin and globulins are very often exposed to concentrations of toxic compounds. The destruction of blood cells and the reduction of the number of circulating cells is hostile to the normal functioning of the body. In the present study, females show an increase in white blood cells and hematocrits at doses of 150 and 300 mg / kg of bw respectively. This increase may explain the traditional use of this plant in the treatment of anemia. These results are confirmed by the work of Tété-Bénissan *et al*. (2012) [28] who used *M. oleifera* leaf powder to improve the blood count profile of malnourished HIV-positive children.

Microscopic examination of the liver, kidney and heart confirms the results of the relative weight of organs and biochemical parameters.

Conclusion

This study indicates that the aqueous extract of *M.oleifera* leaves has several secondary metabolites with therapeutic effects. This plant has no toxic effects at a dose of 2000 mg / kg of bw in a single dose and orally. Repeated administration of different doses has shown signs of improvement of vital organs and blood parameters. All these results explain the name "tree of life" attributed to this plant by local population. All these results would justify the name "tree of life" attributed to this plant and its use by local populations for therapeutic purposes.

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