

Research paper

Cardiac Tolerance Study of the Ethyl Acetate Fraction of *Morinda morindoides* (Baker) Milne-Redhead (Rubiaceae) Extract in Rabbit.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IOTB and BND designed the study, performed the statistical analysis and wrote the first and final draft. Author AFC managed the biochemical markers analysis and histopathological studies. Authors AJD and JDN checked the results and managed literature and scientific searches of the study. All authors read and approved the manuscript

Abstract

Purpose: This study was conducted to assess the effect of the ethyl acetate fraction of the hydroalcohol of *Morinda morindoides* extract (AcEF1) on heart integrity and its contractility in rabbits.

Methodology: Five groups of 6 rabbits each, were injected intraperitoneally, twice a week for four weeks, with this fraction, dosed from 25 to 100 µg/kg body weight, for Groups 2 to 5 while Group 1, received 1 ml of Mac Ewen physiological fluid. Blood sampling was carried out to evaluate various parameters: aspartate aminotransferase (AST); alanine aminotransferase (ALT), Creatine phosphokinase (CPK), lactate dehydrogenase (LDH), calcium, sodium, potassium, magnesium and chloride. Histopathological studies were, also, conducted, the last week (S6), on the heart of two (2) rabbits of each group.

Results: Analysis of the parameters following administration of the extract fraction showed slight increases in AST and ALT activities as well as the concentrations of sodium and potassium, slight decrease in CPK activities and calcium concentration ($p < 0.05$) whereas LDH activities, concentrations of magnesium and chloride were unchanged ($p > 0.05$) compared to their initial values.

Histopathological studies revealed undamaged structures of the heart.

Conclusion: Overall, this fraction did not appear to exert any noxious effect on cardiac tissues or functions and should thus be well tolerated by the organism.

Keywords: Biochemical markers, Cardiac tolerance, Histopathology, *Morinda morindoides*

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1- Introduction

In African societies, the tradition of collecting, processing and applying plants and plant-based medications have been handed down from generation to generation. Traditional medicine, with medicinal plants as their most important component are sold in marketplaces or prescribed by traditional healers in their homes [1]. It has been estimated that approximately 80 % of population in the developing countries depend on traditional medicine for their primary health care [2, 3].

Morinda morindoides is one of the plants used in traditional medicine practice in Nigeria, in Democratic Republic of Congo, in Congo-Brazzaville where its leaves are frequently used against malaria, diarrhoea, amoebiasis, hemorrhoids, gonorrhea and rheumatic pains [4-8].

In Ivory Coast, leaves of this plant are traditionally used by population in the West Central against diarrhea [9, 10]. Another studies to support the traditional use of this plant showed antifungal and antibacterial activities [11, 12].

Studies performed to improve these antidiarrheal, antifungal and antibacterial activities, have allowed to isolate different extracts and fractions; from this plant. Among the different fractions tested, the ethyl acetate fraction F1 (AcEF1) of the hydroalcohol extract, offered the most promising biological activities [11, 13].

Despite several benefits to the use of plants as traditional medicine, however, it must be known that there is lack of knowledge on their undesirable induced-effect.

Therefore, to guarantee the safety use of *Morinda morindoides* by populations, early studies of the hepatic and renal tolerance of this fraction revealed that it is well tolerated by liver and kidney [14, 15]. But up now, there are no available scientific data concerning the influence of this fraction on the heart.

In fact, heart has an important role in general circulation of blood; it continuously, expanding to flow in blood and contracting to pump out blood. That role the heart on the blood flow throughout body, predisposes it to toxic drug injury [16].

It's known that a substance could affect the heart by modifying its contractility or destroying its myocardial structure [17]. This study was therefore conducted to assess the effect of the ethyl acetate fraction (F1) of *Morinda morindoides* on heart's integrity and contractility through the changes of some of its biochemical markers and tissue.

2- Materials and Methods

2-1- Plant material

The leaves of *Morinda morindoides* (Baker) Milne-Redhead (Rubiaceae) were collected in the region of Daloa, west-central Ivory Coast. The plant was authenticated by Professor Ake Assi of the Department of Botany, University Felix Houphouet Boigny,

Abidjan and a voucher specimen (no. 17710) of the plant was deposited in the herbarium of the National Floristic Center of that University.

2-2- Preparation of *Morinda morindoides* extract

The leaves of *Morinda morindoides* (Baker) Milne-Redhead (Rubiaceae) were air-dried at a room temperature (28 ± 1 °C) for 7 days and ground into fine powder. The powder was mixed with distilled water (80 g in 2 L of distilled water) for 24 h with constant stirring at 80 °C. The extract was filtered twice through cotton wool, and then through Whatman filter paper no. 1. The filtrate was evaporated to dryness in a rotary evaporator (Buchi) at 60 °C. Twenty five grams of the dry aqueous extract was added to 500 ml of ethanol and water (consisting of 356 ml of ethanol 96 % and 144 ml of distilled water) and after thorough mixing, the supernatant was evaporated in a rotary evaporator following the method of Guede-Guina et al [18]. The residue was taken as the hydro-alcohol extract.

2-3- Preparation of the chromatographic fraction of the extract

Five grams (5 g) of the hydro-alcohol extract was added to 500 ml of water/ethyl acetate mixture (consisting of 250 ml of distilled water and 250 ml of ethyl acetate) and stirred continuously for 24 h. After decantation, the supernatant was evaporated in a rotary evaporator. The residue was taken as the ethyl acetate extract of the plant leaf (AcE).

The ethyl acetate extract was subjected to separation on a chromatographic column (2 x 50 cm) with silica gel 60 (Merck, silica gel, 0.063 – 0.200 mm). The extract (0.25 g) was introduced into the column and placed on the silica gel, protected by cotton. Dichloromethane was used as eluent until total infiltration of the extract into the silica gel; thereafter, dichloromethane-methanol (95/5) mixture was used for elution until separation was complete. The four fractions obtained, based on their colour, were F1 (golden yellow), F2 (dark green), F3 (pale green) and F4 (yellow-orange) [11]. Fraction F1 (AcE F1) was evaporated in a rotary evaporator (at 60 °C) before use in the in vivo studies.

2-4- Experimental animals

Rabbits, *Oryctolagus cuniculus*, from a rabbit cattle farm in Bingerville, southern Ivory Coast were used in this study. The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Felix Houphouet Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals [19].

2-5- Administration of fraction F1

Thirty (30) rabbits (15 males and females each) were acclimatized for a month and half at ambient temperature (28 ± 1 °C) and humidity ($70 \pm 5\%$). Their age was approx. 3 months and weighed 1.2 ± 0.2 kg on the average. They were randomly divided into 5 groups of 6 rabbits each (3 males and 3 females). Animals in each group were separated according to their sex in cages (length: 79 cm, width: 50 cm and height: 38 cm). They had free access to both water and food.

Twice a week, the animals received intraperitoneally (ip) 1 ml of an injection according to their group. Group 1 (Gp1), control, received 1ml of MacEwen physiological fluid

while Groups 2 (Gp2) to 5 (Gp5) were similarly injected with 30, 60, 60 µg/ml, 90 and 120 µg/ml, respectively, of F1, equivalent to 25, 50, 75 and 100 µg/kg body weight, respectively

2-6- Collection of blood

Blood samples were obtained in the morning (from 8 to 10 am) via the marginal ear vein of the animals. Blood sampling was carried out once a week in the two weeks preceding the first application of treatment (S0), during the four weeks of treatment (S1, S2, S3 and S4), and then for two weeks after the treatment period (S5 and S6). These blood samples were collected in tubes (without anticoagulant) and centrifuged at 3000 rpm for 10 min. The serum was stored at -20 °C until analyzed for enzymatic activities and concentration of biochemical metabolites.

2-7- Assay of cardiac parameters in rabbit serum

In exception to sodium and potassium which were measured by flame photometer, SEAC *fp* 20, cardiac's parameters of the serum were measured with an automatic analyzer, Hitachi 902 (Roche), using commercial kits and certified controls (Spinreact S.A., Ctra Santa Coloma, Spain) based on the manufacturer's instructions, as summarized in Tables 1 and 2.

Table 1: Operating parameters for the quantitative determination of enzymes

Parameters	Enzyme kinetic methods	Wavelength (nm)
ALAT	Disappearance of NADH	340
ASAT	Disappearance of NADH	340
CPK	Formation of NADPH	340
LDH	Disappearance of NADPH	340

ALT= alanine aminotransferase; AST= aspartate aminotransferase; CPK=Creatine phosphokinase; LDH=lactate dehydrogenase; NAMB =2-nitro-5-aminobenzoic acid formation; NADH=nicotinamide adenine dinucleotide; NADPH=nicotinamide adenine dinucleotide phosphate

Table 2: Operating parameters for the quantitative determination of electrolytes

Parameter	Colorimetric Method	Wavelength (nm)
Calcium	formation of color complex	570
Magnesium	Formation of coloured complex	520
Chloride	formation of a red ferric thiocyanate complex	450
Sodium	Temperature (2000°K)	589
Potassium	Temperature (2000°K)	767

2-8- Preparation of tissue sections and histopathology

After the blood collection the last week (S6), two rabbits of each group were randomly chosen and sacrificed under deep anesthesia and hearts were removed and fixed with 10% buffered formalin for further analysis.

Heart tissues were cut into longitudinal blocks. An automatic processor (RH-12EP Sakura, Fine Technical Co.Ltd, Tokyo, Japan) was used for further processing blocks. About 12 hours were required for dehydration (96% alcohol for one hour x six changes, and 100% alcohol for one hour x one change).

Clearing in three changes of xylene for one hour each. Tissues were impregnated in two changes of paraffin wax with a melting point of 50° C for period of 2 hours. Embedding of tissue was done in paraffin using L-shaped metallic moulds. These blocks were put in the refrigerator for a period of 4-6 hours. Each block was cut on a rotary microtome (Microm GmbH, Waldorf, Germany). About 4-5 micro meter thick tissue section were obtained and placed in the water bath with temperature of 50°C below the melting point of paraffin wax. Cut ribbons of tissues were placed on the albumenized glass slide.

All the sections and smears (both touched and scraped) were stained with hematoxylin and eosin (H.E). These sections were examined photo microscopically for renal damage.

2-9- Statistical analysis

The results are presented as mean $\pm$ standard deviation (SD). Analysis of variance (ANOVA) with repeated measures was employed to compare the results according to the administered doses and times of treatment. Analysis of variance was considered significant when the level of probability (p) was < 0.05 . When the value of p was significant, a Post-Hoc test of Newman-Keuls was carried out. These analyses were carried out with the software, Statistica 7.1 (Statistica, Statsoft) using a general linear model (GLM).

3- RESULTS

3-1- Effect of AcEF1 on enzymes activities

Compared to their initial levels, activities of AST and ALT increased gradually after weekly administration of F1 until the third weeks when maximum values of 88.27 ± 4.42 (AST, $p = 0.000$) and 98.47 ± 6.57 (ALT ($p = 0.000$)) were obtained and no further changes were observed. LDH activities were unchanged during the study, whereas CPK activities decreased slightly ($p = 0.0311$) (Table 3 to 6).

Table 3: Effect of AcEF1 on activity of ALT (UI/ L).

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	55.17 ^a ± 3.85	71.00 ^b ± 5.14	88.67 ^c ± 10.69	98.50 ^d ± 7.48	84.33 ^c ± 4.08	71.67 ^b ± 3.33	59.17 ^a ± 2.40
Group 2	25	55.28 ^a ± 5.03	74.33 ^c ± 6.02	96.50 ^c ± 3.83	100.83 ^e ± 7.86	88.33 ^d ± 6.68	65.33 ^b ± 3.44	62.33 ^b ± 4.55
Group 3	50	57.06 ^a ± 1.29	72.83 ^b ± 5.85	99.00 ^d ± 2.53	95.33 ^d ± 4.68	89.83 ^c ± 2.71	71.00 ^b ± 3.74	53.67 ^a ± 5.05
Group 4	75	53.61 ^a ± 3.34	73.17 ^c ± 4.36	96.17 ^d ± 5.60	97.83 ^d ± 7.81	89.00 ^d ± 5.22	62.83 ^b ± 2.48	54.50 ^a ± 5.47
Group 5	100	53.67 ^a ± 4.60	70.17 ^c ± 6.31	98.33 ^e ± 6.56	99.83 ^e ± 5.38	90.83 ^d ± 4.88	61.33 ^b ± 3.93	61.00 ^b ± 4.65

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript (a-b)*and (α- γ)* respectively in row and column differ (p < 0.05). *a<b<c<d; α < β < γ; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period. ALT= alanine aminotransferase

Table 4: Effect of AcEF1 on activity of AST (UI/ L).

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	62.00 ^{ba} ± 4.82	59.50 ^{ba} ± 4.89	78.83 ^{ca} ± 12.62	87.33 ^{da} ± 3.14	74.67 ^{ca} ± 3.88	61.00 ^{ba} ± 3.63	49.50 ^{aa} ± 1.87
Group 2	25	59.28 ^{ba} ± 4.52	63.17 ^{ba} ± 4.79	88.33 ^{cb} ± 5.50	88.33 ^{ca} ± 5.75	82.83 ^{cb} ± 3.82	59.83 ^{ba} ± 2.93	54.17 ^{ab} ± 3.25
Group 3	50	60.78 ^{ba} ± 2.95	63.67 ^{ca} ± 7.84	88.67 ^{db} ± 2.58	88.50 ^{da} ± 3.56	84.50 ^{db} ± 6.44	66.83 ^{cb} ± 4.17	51.00 ^{aaβ} ± 4.56
Group 4	75	58.67 ^{ba} ± 2.95	67.33 ^{cb} ± 2.58	88.33 ^{db} ± 7.69	88.83 ^{da} ± 4.45	83.17 ^{db} ± 6.49	59.67 ^{ba} ± 1.97	53.00 ^{aaβ} ± 3.58
Group 5	100	60.94 ^{ba} ± 5.64	62.17 ^{ba} ± 6.37	89.33 ^{cb} ± 6.50	88.33 ^{ca} ± 6.02	83.17 ^{cb} ± 4.02	55.67 ^{aa} ± 4.32	54.17 ^{ab} ± 4.31

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript (a-b)*and (α- γ)* respectively in row and column differ (p < 0.05). *a<b<c<d; α < β < γ; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period. AST= aspartate aminotransferase.

Table 5: Effect of AcEF1 on activity of LDH (UI/ L).

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	106.56 ± 6.40	107.67 ± 3.72	108.67 ± 5.68	104.50 ± 4.42	107.33 ± 3.93	107.83 ± 3.97	105.50 ± 3.39
Group 2	25	105.78 ± 5.77	107.17 ± 4.40	105.00 ± 3.63	108.00 ± 4.98	108.67 ± 3.14	105.00 ± 2.90	104.83 ± 4.49
Group 3	50	112.22 ± 3.28	108.83 ± 4.07	110.00 ± 4.47	109.17 ± 1.94	107.17 ± 3.06	108.17 ± 5.23	105.17 ± 2.64
Group 4	75	104.83 ± 6.45	107.67 ± 5.92	107.83 ± 4.62	107.33 ± 3.08	107.00 ± 1.79	107.50 ± 5.09	103.33 ± 3.20
Group 5	100	105.56 ± 7.72	110.83 ± 1.94	106.50 ± 2.74	105.33 ± 3.27	107.67 ± 4.13	106.50 ± 3.83	106.83 ± 3.87

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript (a-b)*and (α- γ)* respectively in row and column differ (p < 0.05). *a<b<c<d; α < β < γ; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period. LDH=lactate dehydrogenase.

Table 6: Effect of AcEF1 on activity of CPK (UI/ L).

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	216.67 ^β ± 6.02	218.83 ^β ± 5.46	217.00 ^β ± 8.00	216.83 ^β ± 6.55	211.17 ^α ± 3.25	209.83 ^α ± 6.88	210.33 ^α ± 4.27
Group 2	25	207.67 ^α ± 7.31	206.33 ^α ± 6.62	214.33 ^β ± 5.43	211.00 ^α ± 4.73	212.33 ^α ± 2.25	211.33 ^α ± 10.78	209.83 ^α ± 4.79
Group 3	50	211.06 ^{αβ} ± 6.90	211.83 ^{αβ} ± 5.34	208.50 ^α ± 3.02	208.67 ^α ± 2.88	209.50 ^α ± 4.97	210.83 ^α ± 2.32	211.50 ^α ± 11.00
Group 4	75	210.06 ^{αβ} ± 4.91	208.83 ^α ± 2.14	208.67 ^α ± 2.25	212.50 ^{αβ} ± 3.94	211.50 ^α ± 5.89	213.17 ^α ± 11.44	207.83 ^α ± 6.11
Group 5	100	214.00 ^{αβ} ± 4.17	212.83 ^{αβ} ± 11.30	208.17 ^α ± 2.14	209.17 ^α ± 3.82	211.67 ^α ± 3.56	211.67 ^α ± 4.72	212.50 ^α ± 4.46

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript (a-b)*and (α- γ)* respectively in row and column differ (p < 0.05). *a<b<c<d; α < β < γ; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period. CPK=Creatine phosphokinase.

3-2- Effect of AcEF1 on electrolytes concentrations

Tables 7 to 11 showed that concentrations of calcium decreased ($p = 0.0000$) compared to control group, those of sodium ($p = 0.0010$) and potassium ($p = 0.0000$) increased slightly whereas concentrations of chloride and magnesium were unchanged (Chloride, $p = 0.8460$; magnesium, $p = 0.5609$) compared to their initial values.

Table 7: Effect of AcEF1 on the levels (mEq/L) of serum sodium

Groups of rabbits	Dose of AcEF1 ($\mu\text{g}/\text{Kg}$ of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	139.39 ± 4.28	139.83 ± 3.82	143.67 ± 4.72	140.50 ± 1.76	140.00 ± 2.53	139.67 ± 1.21	141.33 ± 1.86
Group 2	25	139.61 ± 1.73	140.67 ± 3.72	144.17 ± 3.71	140.33 ± 2.50	140.50 ± 1.38	142.83 ± 4.71	140.83 ± 2.48
Group 3	50	139.39 ± 2.42	143.83 ± 3.71	141.67 ± 3.88	140.83 ± 2.14	140.50 ± 1.64	140.83 ± 2.04	141.83 ± 2.40
Group 4	75	141.72 ± 1.58	140.17 ± 2.04	142.33 ± 1.63	142.00 ± 1.55	140.33 ± 1.37	140.17 ± 2.14	141.00 ± 1.67
Group 5	100	136.28 ± 4.01	139.50 ± 2.43	141.67 ± 3.20	142.50 ± 3.45	142.67 ± 2.66	141.33 ± 2.88	140.67 ± 1.63

Values are expressed as mean $\pm$ S.E.M ($n = 6$). Values without common superscript (a-b)*and (α - γ)* respectively in row and column differ ($p < 0.05$). *a<b<c<d; $\alpha < \beta < \gamma$; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

Table 8: Effect of AcEF1 on the levels (mEq/L) of serum potassium

Groups of rabbits	Dose of AcEF1 ($\mu\text{g}/\text{Kg}$ of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	4.06 ^b ± 0.25	4.00 ^b ± 0.18	4.20 ^b ± 0.28	4.12 ^b ± 0.41	4.08 ^b ± 0.29	4.10 ^b ± 0.28	4.00 ^b ± 0.09
Group 2	25	3.92 ^b ± 0.07	4.23 ^b ± 0.39	4.23 ^b ± 0.27	4.02 ^b ± 0.36	4.05 ^b ± 0.15	4.03 ^b ± 0.12	4.02 ^b ± 0.10
Group 3	50	3.85 ^b ± 0.26	4.17 ^b ± 0.39	3.78 ^b ± 0.40	4.27 ^b ± 0.42	4.23 ^b ± 0.45	4.17 ^b ± 0.36	4.03 ^b ± 0.10
Group 4	75	3.71 ^b ± 0.28	4.18 ^b ± 0.34	4.08 ^b ± 0.16	3.90 ^b ± 0.19	4.15 ^b ± 0.46	4.18 ^b ± 0.34	4.08 ^b ± 0.15
Group 5	100	3.38 ^a ± 0.31	4.02 ^b ± 0.34	4.08 ^b ± 0.27	4.10 ^b ± 0.17	4.52 ^c ± 0.45	4.18 ^b ± 0.34	4.03 ^b ± 0.15

Values are expressed as mean $\pm$ S.E.M ($n = 6$). Values without common superscript (a-b)*and (α - γ)* respectively in row and column differ ($p < 0.05$). *a<b<c<d; $\alpha < \beta < \gamma$; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

Table 9: Effect of AcEF1 on the levels (mg/ L) of serum calcium

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	109.67 ^b ± 8.89	109.00 ^b ± 4.98	111.67 ^b ± 3.08	110.83 ^b ± 6.68	113.17 ^b ± 4.07	108.50 ^{a b} ± 1.38	105.00 ^a ± 3.16
Group 2	25	122.22 ± 10.65	108.50 ^b ± 5.32	113.50 ^b ± 3.27	114.67 ^b ± 6.15	111.33 ^b ± 3.08	116.50 ^b ± 7.40	106.00 ^a ± 2.76
Group 3	50	116.44 ± 7.40	112.00 ± 5.29	114.67 ± 5.32	111.17 ± 5.19	110.83 ± 5.27	108.17 ^{a b} ± 4.62	105.00 ^a ± 3.58
Group 4	75	122.72 ^a ± 6.67	114.67 ^a ± 6.89	114.00 ^a ± 5.14	108.83 ^a ± 2.93	107.17 ^a ± 3.06	108.83 ^a ± 2.79	108.00 ^a ± 5.76
Group 5	100	121.83 ± 3.82	119.83 ^b ± 7.19	116.50 ^b ± 6.98	109.83 ^b ± 2.32	110.00 ^b ± 4.47	106.67 ^b ± 4.03	102.17 ^a ± 2.64

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript (a-b)*and (α- γ)* respectively in row and column differ (p < 0.05). *a<b<c<d; α < β < γ; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

Table 10: Effect of AcEF1 on the levels (mg/ L) of serum magnesium

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	19.94 ± 1.91	19.83 ± 2.04	19.83 ± 0.98	18.33 ± 1.51	19.33 ± 1.21	18.67 ± 1.86	19.67 ± 1.51
Group 2	25	19.44 ± 1.38	20.33 ± 1.51	19.50 ± 1.87	18.17 ± 2.04	19.00 ± 0.89	20.50 ± 1.87	18.83 ± 1.94
Group 3	50	20.44 ± 1.00	18.83 ± 2.48	19.17 ± 1.72	21.50 ± 3.27	19.33 ± 1.86	18.67 ± 1.75	19.00 ± 2.45
Group 4	75	19.33 ± 1.17	20.00 ± 1.79	19.50 ± 1.22	18.33 ± 1.75	19.67 ± 1.21	20.00 ± 2.37	20.00 ± 0.89
Group 5	100	20.72 ± 0.93	19.83 ± 1.60	19.50 ± 1.05	20.17 ± 2.23	18.67 ± 0.82	19.50 ± 2.17	19.00 ± 2.10

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript (a-b)*and (α- γ)* respectively in row and column differ (p < 0.05). *a<b<c<d; α < β < γ; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

Table 11: Effect of AcEF1 on the levels (mEq/ L) of serum chloride

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	102.06 ± 3.01	100.33 ± 3.39	103.33 ± 4.68	101.33 ± 3.50	102.00 ± 2.37	100.83 ± 2.93	104.20 ± 3.40
Group 2	25	104.22 ± 4.02	103.50 ± 3.67	103.33 ± 4.63	102.00 ± 3.29	99.67 ± 2.16	102.17 ± 2.64	104.00 ± 3.70
Group 3	50	103.33 ± 3.81	101.67 ± 3.61	104.00 ± 2.00	102.33 ± 2.94	101.33 ± 3.56	102.67 ± 4.76	105.00 ± 2.90
Group 4	75	105.94 ± 4.58	104.17 ± 3.43	101.83 ± 4.79	104.67 ± 1.97	103.17 ± 4.58	101.83 ± 3.31	99.00 ± 5.70
Group 5	100	102.17 ± 4.34	104.33 ± 3.67	101.67 ± 7.31	102.00 ± 2.00	104.67 ± 3.20	103.83 ± 4.26	102.70 ± 2.90

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript (a-b)*and (α- γ)* respectively in row and column differ (p < 0.05). *a<b<c<d; α < β < γ; S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

3-3- Histopathological study

Figure 1 revealed normal heart structure in control group (Group 1) and experimental groups showing normal appearance of myocardial fibers in longitudinal section (Group 2 to 5).

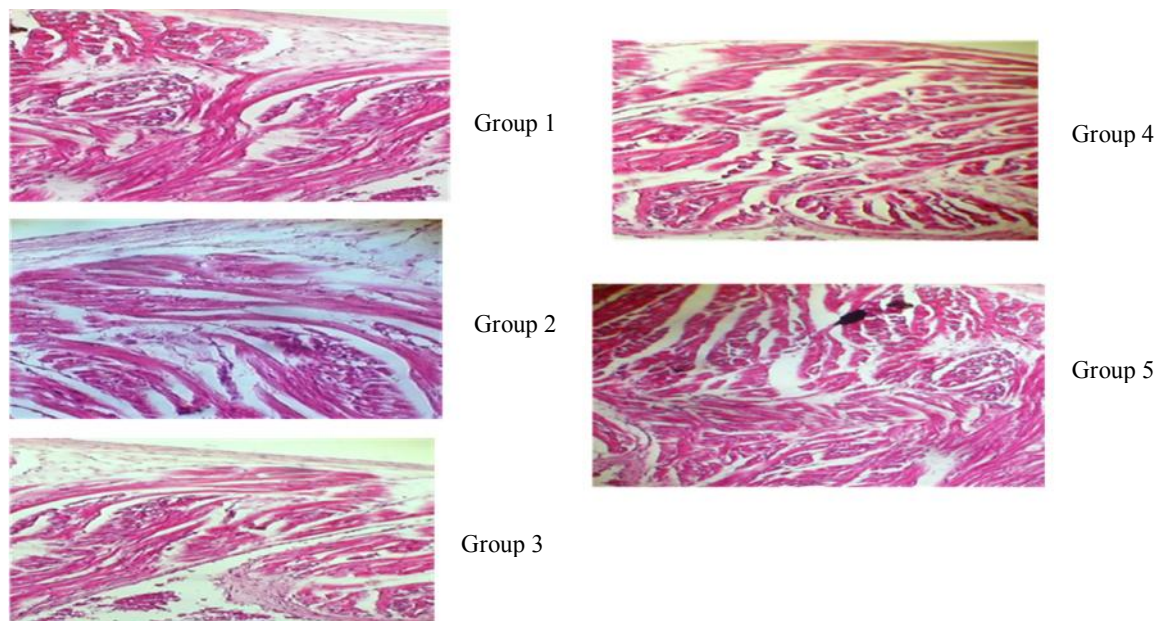


Figure 1: Photomicrograph of heart in longitudinal section in control group (Group 1) and experimental groups (2-5). Hematoxylin and eosine (H.E) stain, (x250)

4- Discussion

The effect of AcEF1 on heart has been noted on its tissue integrity and its contractility.

Influence of AcEF1 on cardiac tissue integrity

The diagnostic use of enzymes in veterinary and human clinical pathology is mostly aimed at detecting, evaluating and monitoring organ damage based on the increase in organ-specific enzymes [20].

AST, ALT, LDH and CPK were used as markers of myocardial integrity.

The initial levels of the various enzymes (AST, ALT, LDH and CPK) were in accordance with previous studies values [21, 22].

A cardiac injury induces an increase of these enzymes [23]. During this study, only transaminasic activities (AST and ALT) increased gradually the third first weeks before coming progressively to their initial values. Contrary to transaminasic activities, CPK activities, decreased, whereas, LDH activities were unchanged.

According to interpretation of Ekpo *et al.* [24], the increase in transaminasic activities found in the present study is moderate and transient and has not been induced by the myocardial damage.

In addition, histopathological studies revealed normal structure of heart in all groups (control and experimental groups). There were no features suggestive of myocyte necrosis, nuclear pyknosis, vascular proliferation, inflammatory cell infiltration, fibrosis of myocyte hypertrophy [25]. These finding indicates that, fraction F1 used within dose ranged from 25 to 100 g/kg body weight, would not have harmed cardiac tissue.

Influence of AcEF1 on the heart contractility.

Heart contracts spontaneously due to its automaticity. The spontaneous generation of cardiac electrical signals, as well as their propagation, is due to alteration in electrolyte concentrations. The major electrolytes that are involved in this process include: sodium, potassium, calcium, magnesium and chloride [26].

Despite its automaticity, heart's activity is influenced by two systems: sympathetic system for tachycardia and parasympathic system which induce bradycardia [27].

Heart's activity is therefore oriented to one of these responses according to the system stimulated. A substance, event or situation able to influence those systems modifies the heart's activity revealed by the modification of some electrolytes concentrations.

The initial concentrations of sodium, potassium, calcium, magnesium and chloride obtained in this study were in agreement with literature [21, 22].

The study showed that the concentration of calcium has decreased compared to the control group.

This decrease in calcium concentration or hypocalcaemia would be explained by inhibition, by F1, of the intracellular calcium flow. The decrease of intracellular calcium results in the inhibition of calcium releasing by the sarcoplasmic reticulum and thereby makes lack calcium available to bind to troponin-C, and then decreases contractility. This action would justify the negative inotrop and chronotrop effect of *Morinda morindoides* obtained by N'Guessan *et al.* [28] and Kamo [13].

The consequence of this action would be a low blood pressure which stimulates the rennin-angiotensin-aldosterone system for hyponatremia, hypokaliemia, hypomagnesemia and unchanged chloremia [29]. This finding is different to those obtained in this study: slight hypenatremia, hyperkaliemia and unchanged magnesemia, chloremia. This effect seems not to be action of the AcEF1 on the heart.

In addition, the changes were within their standard values in rabbits [21, 22].

Finally, AcEF1 would not disturb the cardiac contractility.

CONCLUSION

The aim of this study was to evaluate the impact of AcEF1 on cardiac tissue integrity and function.

The finding showed slight changes with certain markers but these changes, which were within their standard values, have not been induced by AcEF1.

Overall, this fraction, used in dose ranged from 25 to 100 µg/Kg of body weight, would not exert any noxious effect on cardiac tissue or contractility and thus would be well tolerated by the heart.

It would, however, be necessary to carry out feature studies including more markers and organs as well as hematological investigation in order to obtain a fuller picture of the safety profile of the extract fraction.

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ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Felix Houphouet Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

COMPETING INTERESTS

The authors report no conflict of interest in this research.

REFERENCES

- 1- **Von MHT.** Trees and Shrubs of the Sahel. Verlag Josef Margraf, Weikersheim; 1996; 562 pp
- 2- **Kirby GC.** Medicinal plants and the control of parasites. Transaction of the Royal Society of Tropical Medicine and Hygiene, 1996; 90, 605-609.
- 3- **Hostettmann K. and Marston A.** Twenty years of research into medicinal plants: results and perspectives. Phytochem Rev; 2002; 1:275-285.
- 4- **Tona L., Cimanga R.K., Mesia K., Musuamba C.T., De Bruyne T., Apres S. Et al.** *In Vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants in the Democratic Republic of Congo. *Journal of Ethnopharmacology*, (2004); 93 (1): 27-32.
- 5- **Kambu K, Tona L, Luki N, Cimanga K, Uvoya A.** Antibacterial activity of extracts from plants used in preparations as antidiarrheal at Kinshasa, Zaire. *Ann Pharm Fr*; 1990; 48 (5): 255-263.
- 6- **Cimanga K, Hermans N, Apers S, Van MS., Van Den H., Claeys M. et al.** Complement-inhibiting iridoids from *Morinda morindoides*. *J Natural Product* 2003; 66: 97-102.
- 7- **Zirihi GN, Mambu L, Guede-Guina F, Bodo B, Grellier P.** In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *J Ethnopharmacol* ; 2005; 98: 281-285. *J Ethnopharmacol* 2005; 98: 281-285.

- 8- **Cimanga K, Kambu K, Tona L, Hermans N, Apers S, Totte J et al.**
Cytotoxicity and in vitro susceptibility of *Entamoeba histolytica* to *Morinda morindoides* leaf extracts and its isolated constituents. *J Ethnopharmacol* ; 2006; 107: 83-90.
- 9- **Bahi C, N'guessan JD, Guede-Guina F.** Mise en évidence d'une action myorelaxante et cholinolytique de Bitter GG (BGG), un antidiarrhéique de source végétale. *Afr Biomed* 2000; 5(1):11-18.
- 10- **Meite S, N'Guessan JD, Bahi C, Yapi HF, Djaman AJ and Guede-Guina F.** Antidiarrheal activity of the ethyl acetate extract of *Morinda morindoides* in rats. *Trop J Pharm Res.*; 2009; 8 (3): 201-207
- 11- **Bagre, I, Bahi C, Gnahoue, G, Djaman, AJ, Guede, GF.** Composition phytochimique et évaluation in-vitro de l'activité antifongique des extraits des feuilles de *Morinda morindoides* (baker) *Milne-Redhead* (Rubiaceae) sur *Aspergillus fumigatus* et *candida albicans*. *J. Sci.Pharm. Biol.*, 2007 ; 8(1): 15-23.
- 12- **Moroh JLA, Bahi C, Dje K, Loukou YG, Guede-Guina F.** Etude de l'activité antimicrobienne de l'extrait acétatique (EAC) de *Morinda morindoides* (Baker) *Milne-Readhead* (Rubiaceae) sur la croissance in vitro des souches d'*Escherichia coli*. *Bulletin de la société royale des sciences de Liège*, 2008 ; 77 ; 44-61.
- 13- **Kamo ILBE.** Etude des effets de BGG F1 sur le Coeur isolé de rat et la pression artérielle de lapin. *DEA Biotechnologie, Université Abidjan-Cocody*, 2008, 34p.
- 14- **Tra –Bi IO, Djyh BN, Bahi C, Meite S and N'guessan JD.** A Study of the Hepatic Tolerance of the Ethyl Acetate Fraction of *Morinda morindoides* (Baker) *Milne-Redhead* (Rubiaceae) Extract in Rabbit. *Trop J Pharm Res*; 2010; 9(6): 573- 579.
- 15- **Tra-Bi IO, DJYH BN, Yeo D, Ito MP Mrs Tebele, Djaman AJ, N'guessan JD.** Renal Tolerance Study of the Ethyl Acetate Fraction of *Morinda morindoides* (Baker) *Milne-Redhead* (Rubiaceae) Extract in Rabbit. *Res. J. Pharm., Biol. Chem. Sci.*; 2011; 2 (3); 530-540.
- 16- **Wagh and Grant.** Anatomie et physiologie normales et pathologies ; Ed. Maloine, Paris-France, 2006.
- 17- **Coatmeur O., Latournerie M. and Hillon P.** Thérapeutique chez les patients atteints de maladie du foie. *Médecine thérapeutique*, 2007 ; 10 (1): 34-8.
- 18- **Guédé-Guina F, Vangah-Manda M, Harouna D, Bahi C.** Potencies of *Misca*, a plant source concentrate against fungi. *J. Ethno pharmacol.* 1993; 14 (2): 45-53
- 19- **Anonymous 1:** Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes *Official Journal L 358* , 18/12/1986; pp 0001 - 0028.

- 20- **Braun J.P., Medaille C. and Trunel C.** Clinical interpretation of enzyme activities and concentrations: A review of the main metabolic factors affecting variation. (2008); Accessed 22 July 2010. Available: <http://www.isrvma.org>.
- 21- **Anonymous 2** : Valeurs de référence de biochimie sanguine, 2004. Accessed 10 January 2011. Available: http://www.medirabbit.com/FR/Hematologie/biochimie/blood_chemistry_fr.htm.
- 22- **Coulibaly F.C., Coulibaly A., N'guessan J.D., Koffi G.K., Djaman A.J. and Guede-Guina F.** Etude des paramètres sériques biochimiques : le cas des lapins (Néozélandais-Cunistar) de Côte d'Ivoire. *Sciences et Nature*, (2007) ; 4 (1): 37-43.
- 23- **Levy S.** Augmentation de l'activité sérique des transaminases de cause non élucidée par les tests biologiques habituels. *Hepato-Gastroenterology*, 1998; 2 (5): 133-41.
- 24- **Ekpo A., Eseyin A.O., Ikpeme A.O. and Edoho E.J.** Some studies on some biochemical effects of *Vernonia amygdalina amygdalina* in rats. *Asian Journal of Biochemistry*, (2007) ; 2 (3): 193-197.
- 25- **Roe M.T., Ohman E.M., Maas A.C.P., Christenson R., Mahaffey K.W., Granger C.B. et al. (2001).** Shifting the open-artery hypothesis downstream: the quest of optimal reperfusion. *J.Am Coll. Cardiol*, 37: 9-18.
- 26- **VALDIGUIE P.** Biochimie clinique. 2^e Edition, Editions Médicales Internationales, France, (2000).
- 27- **Saoudi N. and Deharo J.C.** Physiologie des arythmies. Dans : Précis de rythmologie de la société française de cardiologie, Sauramps medical, Paris-France: 2004.
- 28- **N'guessan J.D., Trebissou J.N.D., Bahi C. and Guede Guina F.** Effets de BGG, F5 (fraction chromatographique de *Morinda morindoides*) sur l'activité contractile du cœur isolé de rat. *Rev Iv Sci Tech*, 2002; 3: 127-139.
- 29- **Nellessen E.** Troubles ioniques et insuffisance cardiaque. *La revue de Médecine générale*, 2007 ; 243: 212-217.