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Cardiac Tolerance Study 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.

Research Article

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ABSTRACT

Purpose: This study was conducted to assess the effect of the ethyl acetate fraction (AcEF1) obtained from the 96% extract of *Morinda morindoides* extract on cardiac tissue integrity and function in rabbits.
Methodology: The rabbits were divided in five groups of 6 rabbits each. Groups 2,3,4 and 5 received intraperitoneally, twice a week during four weeks, the ethyl acetate fraction at doses going from 25 to 100 µg/kg body weight while Group 1, received 1 mL of Mac Ewen fluid. Blood sampling was carried out to evaluate aspartate aminotransferase (AST), alanine aminotransferase (ALT), Creatine phosphokinase (CPK), Lactate dehydrogenase (LDH) activities and level of calcium, sodium, potassium, magnesium and chloride in serum. The eighth week of the experiments, histopathological studies were also conducted on rabbits heart.

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Results: Analysis of the serum markers showed slight increases in AST, ALT, CPK activities as well as sodium, calcium and potassium concentrations ($p<0.05$). LDH activity, magnesium and chloride concentrations were unchanged compared to their initial values. Histopathological studies had not revealed damages in the structure of the heart.
Conclusion: The ethyl acetate fraction did not exert any noxious effect on cardiac tissues and should be no toxic for cardiac tissues.

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

1. INTRODUCTION

Morinda morindoides is one of the plants used in traditional medicine practice in Ivory Coast, Nigeria, Democratic Republic of Congo, Congo-Brazzaville where its leaves are frequently claimed to treat malaria, diarrhoea, amoebiasis, hemorrhoids, gonorrhea and rheumatic pains [1-5]. In Ivory Coast, leaves of this plant are mostly used by population in the West Central region against diarrhea [6,7].

Studies performed by bioguided liquid-liquid separation with 96% ethanol, water and ethyl acetate and a subsequent silica gel fractionation had led to the obtention of different fractions. Among the different fractions tested, the ethyl acetate fraction (coded AcE F1) of the hydroalcohol extract of *M. morindoides* offered the most promising antidiarrheal activity [8,10].

A previous study of Tra Bi et al. [11,12] had shown that AcE F1 at doses comprising between 25 and 100 µg/Kg of body weight did not have significant effects on liver and kidney tissues integrity in rabbits. Until now, there are no reports of the effect of this antidiarrheal fraction on heart structure. In regard of the key role of heart for the dispatching of nutrient and drugs, such a study concerning the tolerance of this fraction might be conducted.

This study was therefore designed to assess the effect of the improved antidiarrheal fraction of *Morinda morindoides* (AcE F1) on heart integrity through serum markers and histopathological analysis of heart tissues.

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 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 96 % ethanol and after thorough mixing, the supernatant was removed and evaporated to dryness with a rotary evaporator.

2.3 Preparation of the Chromatographic Fraction of the Extract

According to the method described by Tra Bi *et al.* [10], 5 g of the 96% ethanolic extract was added to 500 mL of water/ethyl acetate mixture (1/1; V/V) and stirred continuously for 24 h. After decantation, the supernatant was removed and evaporated to dryness in a rotary evaporator. The residue (0.25g) 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 column was eluted with Dichloromethane following by dichloromethane/methanol (95/5; V/V). The elution led to 4 fractions on the basis of their color. The 4 fractions F1 (golden yellow), F2 (dark green), F3 (pale green) and F4 (yellow-orange) were evaporated and fraction F1 (AcEF1) was used for this study.

2.4 Experimental Design

Thirty rabbits 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 [13].

Rabbits (15 males and 15 females) were acclimatized for a month and half at ambient temperature (28±1°C) and humidity (70 ± 5%). The animals of 3 months approximately and weighing 1.2 ± 0.2 kg 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 with free access to water and food.

Twice a week, each animal received intraperitoneally (ip) 1 mL of AcE F1 dissolved in Mac Ewen physiological fluid. Group 1 (Gp1), control, received 1mL of Mac Ewen physiological fluid while Groups 2 (Gp2) to 5 (Gp5) received 30, 60, 90 and 120 µg/mL, respectively equivalent to 25, 50, 75 and 100 µg/kg body weight.

2.5 Collection of Blood

Blood samples (5 mL) were obtained in the morning (from 8 to 10:00 AM) via the marginal ear vein of the animals. The collection of blood was carried with intervals of 7 days, once a week during 8 weeks. The first sampling was done two weeks before the treatment (S0) following by four sampling during the 4 weeks of treatment (S1, S2, S3 and S4) and two sampling for the 2 weeks after the end of the treatment (S5 and S6). These blood samples were collected in tubes (without anticoagulant) and centrifuged at 3000g for 10 min. The serum was stored at -20°C until analysis for enzymatic activities and concentration of ions.

2.6 Biochemical Measurements

Sodium and potassium concentrations in serum were measured by flame photometer (SEAC *fp* 20). ALT, AST, CPK, LDH activities and calcium, magnesium and chloride concentrations in serum were determined with an automatic analyzer (Hitachi 902), using commercial kits (Spinreact S.A., Ctra Santa Coloma, Spain) according to manufacturer instructions.

2.7 Preparation of Tissue Sections and Histopathological Analysis

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

Hearts were dehydrated through graded solutions of alcohol (from six changes of 96% alcohol ending in one change of absolute alcohol) for one hour each. They were cleared in three changes of xylene, infiltrated in two changes of paraffin wax for one hour each using the tissue processor obtained from Sakura fine tek. Netherlands and embedded in molten paraffin wax.

Sections were cut at 4-5 micro meter with the rotary microtome (Microm, Gmbh, waldorf, Germany). All the sections and smears (both touched and scraped) were stained with hematoxylin and eosin (H.E). These sections were examined photo microscopically for cardiac damage [14].

2.9 Statistical Analysis

The results are presented as mean ± 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 for $p < 0.05$. 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 AcF1 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 1 to 4).

Table 1. Effect of AcEF1 on ALT (UI/ L) activity in rabbit serum

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 2. Effect of AcEF1 on AST (UI/ L) in rabbit serum

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight)	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	$62.00^{b\alpha}$ ± *4.82*	$59.50^{b\alpha}$ ± *4.89*	$78.83^{c\alpha}$ ± *12.62*	$87.33^{d\alpha}$ ± *3.14*	$74.67^{c\alpha}$ ± *3.88*	$61.00^{b\alpha}$ ± *3.63*	$49.50^{a\alpha}$ ± *1.87*
Group 2	25	$59.28^{b\alpha}$ ± *4.52*	$63.17^{b\alpha}$ ± *4.79*	$88.33^{c\beta}$ ± *5.50*	$88.33^{c\alpha}$ ± *5.75*	$82.83^{c\beta}$ ± *3.82*	$59.83^{b\alpha}$ ± *2.93*	$54.17^{a\beta}$ ± *3.25*
Group 3	50	$60.78^{b\alpha}$ ± *2.95*	63.67b$^{c\alpha}$ ± *7.84*	$88.67^{d\beta}$ ± *2.58*	$88.50^{d\alpha}$ ± *3.56*	$84.50^{d\beta}$ ± *6.44*	$66.83^{c\beta}$ ± *4.17*	$51.00^{a\alpha\beta}$ ± *4.56*
Group 4	75	$58.67^{b\alpha}$ ± *2.95*	$67.33^{c\beta}$ ± *2.58*	$88.33^{d\beta}$ ± *7.69*	$88.83^{d\alpha}$ ± *4.45*	$83.17^{d\beta}$ ± *6.49*	$59.67^{b\alpha}$ ± *1.97*	$53.00^{a\alpha\beta}$ ± *3.58*
Group 5	100	$60.94^{b\alpha}$ ± *5.64*	$62.17^{b\alpha}$ ± *6.37*	$89.33^{c\beta}$ ± *6.50*	$88.33^{c\alpha}$ ± *6.02*	$83.17^{c\beta}$ ± *4.02*	$55.67^{a\alpha}$ ± *4.32*	$54.17^{a\beta}$ ± *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 3. Effect of AcEF1 on LDH (UI/ L) activity in rabbit

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 4. Effect of AcEF1 on CPK (UI/ L) activity in rabbit serum

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$^{\beta}$ ± *6.02*	218.83$^{\beta}$ ± *5.46*	217.00$^{\beta}$ ± *8.00*	216.83$^{\beta}$ ± *6.55*	211.17$^{\alpha}$ ± *3.25*	209.83$^{\alpha}$ ± *6.88*	210.33$^{\alpha}$ ± *4.27*
Group 2	25	207.67$^{\alpha}$ ± *7.31*	206.33$^{\alpha}$ ± *6.62*	214.33$^{\beta}$ ± *5.43*	211.00$^{\alpha}$ ± *4.73*	212.33$^{\alpha}$ ± *2.25*	211.33$^{\alpha}$ ± *10.78*	209.83$^{\alpha}$ ± *4.79*
Group 3	50	211.06$^{\alpha\beta}$ ± *6.90*	211.83$^{\alpha\beta}$ ± *5.34*	208.50$^{\alpha}$ ±*3.02*	208.67$^{\alpha}$ ± *2.88*	209.50$^{\alpha}$ ±*4.97*	210.83$^{\alpha}$ ±*2.32*	211.50$^{\alpha}$ ±*11.00*
Group 4	75	210.06$^{\alpha\beta}$ ± *4.91*	208.83$^{\alpha}$ ± *2.14*	208.67$^{\alpha}$ ± *2.25*	212.50$^{\alpha\beta}$ ± *3.94*	211.50$^{\alpha}$ ± *5.89*	213.17$^{\alpha}$ ± *11.44*	207.83$^{\alpha}$ ± *6.11*
Group 5	100	214.00$^{\alpha\beta}$ ± *4.17*	212.83$^{\alpha\beta}$ ± *11.30*	208.17$^{\alpha}$ ± *2.14*	209.17$^{\alpha}$ ± *3.82*	211.67$^{\alpha}$ ± *3.56*	211.67$^{\alpha}$ ± *4.72*	212.50$^{\alpha}$ ± *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 5 to 9 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 5. Effect of AcEF1 on sodium concentration (mEq/L) in rabbit serum

Groups of rabbits	Dose of AcEF1 (µg/ Kg of body weight))	Time (Weeks)						
		S0	S1	S2	S3	S4	S5	S6
Group 1	0	139,39^{a} ± *4,28*	139,83^{ab} ± *3,82*	143,67^{b} ± *4,72*	140,50^{ab} ± *1,76*	140,00^{a} ± *2,53*	139,67^{a} ± *1,21*	141,33^{ab} ± *1,86*
Group 2	25	139,61^{a} ± *1,73*	140,67^{ab} ± *3,72*	144,17^{b} ± *3,71*	140,33^{a} ± *2,50*	140,50^{a} ± *1,38*	142,83^{ab} ± *4,71*	140,83^{a} ± *2,48*
Group 3	50	139,39^{a} ± *2,42*	143,83^{b} ± *3,71*	141,67^{ab} ± *3,88*	140,83^{ab} ± *2,14*	140,50^{ab} ± *1,64*	140,83^{ab} ± *2,04*	141,83^{ab} ± *2,40*
Group 4	75	141,72^{a} ± *1,58*	140,17^{a} ± *2,04*	142,33^{a} ± *1,63*	142,00^{a} ± *1,55*	140,33^{a} ± *1,37*	140,17^{a} ± *2,14*	141,00^{a} ± *1,67*
Group 5	100	136,28^{a} ± *4,01*	139,50^{ab} ± *2,43*	141,67^{b} ± *3,20*	142,50^{b} ± *3,45*	142,67^{b} ± *2,66*	141,33^{b} ± *2,88*	140,67^{b} ± *1,63*

*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 6. Effect of AcEF1 on potassium concentration (mEq/L) in rabbit serum

Groups of rabbits	Doseof AcEF1 (µg/ 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 ± 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 7. Effect of AcEF1 on calcium concentration (mg/ L) in rabbit serum

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 8. Effect of AcEF1 on magnesium concentration (mg/ L) in rabbit serum

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 9. Effect of AcEF1 on chloride concentration (mEq/ L) in rabbit serum

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

All the tissue sections obtained from the heart of rabbits in experimental groups (group 2 to 5) were not different from control animal (group 1). All sections were essentially normal without inflammatory lesion (Fig. 1).

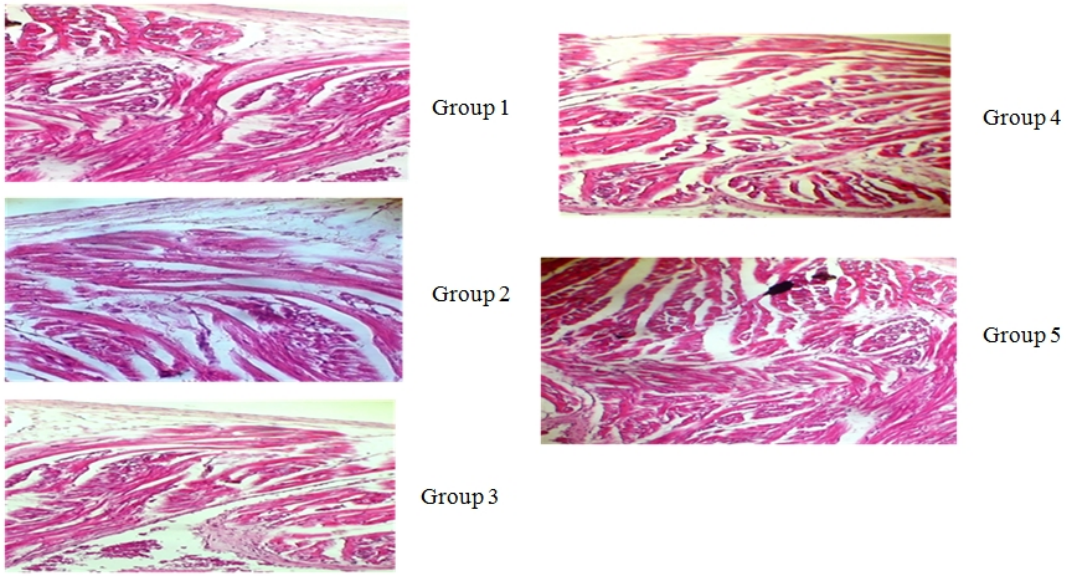

Fig. 1. Photomicrograph of heart in longitudinal section in control group (Group 1) and experimental groups (Group 2 to 5). Hematoxylin and eosine (H.E) stain, (x250)

4. DISCUSSION

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 [15]. A cardiac injury induces, in the bloodstream, the release of enzymes which are present in cardiac cells followed by the increase of their activities in the serum [16]. AST, ALT, LDH and CPK are 4 biomarkers commonly used to assess myocardial integrity [17].

In the present study, according to avalaible data concerning serum parameters in rabbits [18,19], the initial levels AST, ALT, LDH and CPK, before the treatment with AcEF1 were in the range of normal values in all the groups of animals.

During the period of treatment with AcEF1, there was a slight increase of AST and ALT activities in all the groups indicating that these variations has not been induced by the myocardial damage [20]. After the third week, AST and ALT activities decreased progressively to their initial values. On the other hand, there was no significant variation of CPK and LDH activities.

Histopathological studies revealed normal structure of heart in all groups (control and experimental groups). There were no features showing cell damage like myocyte necrosis, nuclear pyknosis, vascular proliferation, inflammatory cell infiltration, fibrosis of myocyte

hypertrophy [21].These results indicates that, fraction AcEF1 used within dose ranged from 25 to 100 µg/kg body weight, would not have harmed cardiac tissue.

Cardiac function is also under the control of electrolytes. Sodium, potassium, calcium, magnesium and chloride homeostasis are involved in cardiac function. In the present study, the initial concentrations of sodium, potassium, calcium, magnesium and chloride obtained were in the normal range reported by previous authors [17,18].

The study showed that the concentration of calcium has decreased compared to the control group. Calcium is important for heart contractions. This decrease in calcium concentration or hypocalcaemia would be explained by the inhibition of the intracellular calcium flow, by AcEF1. The decrease of intracellular calcium results in the inhibition of calcium releasing by the sarcoplasmic reticulum followed by a lack of calcium available for troponin-C, and finally the decrease of contractility. These results are in concordance with the negative inotropy and chronotropy effect of *Morinda morindoides* obtained by N'guessan *et al.* [22] and Kamo [10]. Previous study has also shown that *Morinda morindoides* lowers blood pressure [23].

It is well known that the lowering of blood pressure triggers the secretion of renin by juxtaglomerular cells in the kidneys directly into circulation. Plasma renin then carries out the conversion of angiotensinogen released by the liver to angiotensin I. Angiotensin I is subsequently converted to angiotensin II by the enzyme angiotensin converting enzyme found in the lungs. Angiotensin II is a potent vaso-active peptide that causes blood vessels to constrict, resulting in increased blood pressure. Angiotensin II also stimulates the secretion of the hormone aldosterone from the adrenal cortex. Aldosterone causes the tubules of the kidneys to increase the reabsorption of sodium and water into the blood. This increase of the volume of fluid in the body also increases blood pressure [24,25]. Our results had showed slight increase of sodium concentration in the groups receiving AcEF1 which can be correlated with the hypotension action described by N'guessan *et al.* [23]. In addition, the changes obtained, herein, were within their standard values in rabbits [17,18]. This finding shows that, AcEF1, do not deteriorate the cardiac contractility.

5. CONCLUSION

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

The results 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 specific cardiac markers such as CK-MB, C-Troponin and others 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.

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