Original Research Article

Acute and subchronic toxicities of the aqueous extract of the *Hymenocardia acida* (Euphorbiaceae) roots in rodents

ABSTRACT

The roots of Hymenocardia acida are used in traditional African medicine to treat mainly erectile dysfunction. This study aims to evaluate the lethal and sub-lethal toxicities of the aqueous extract of *Hymenocardia acida* roots in in two rodents species, namely *Mus musculus* and *Rattus norvegicus*. The acute intraperitoneal and oral toxicities of the extract were determined by the method of Miller and Tainter. Subchronic oral toxicity with doses of 500 and 1000 mg/kg body weight was assessed according to the slightly modified OECD 408 method. The results showed that the 50% intraperitoneal lethal dose was 223.87 mg/kg body weight in mice. In addition, the 50% oral lethal dose in mice was greater than 12,000 mg/kg body weight. In the subchronic study, the extract induced a significant ($P < .001$) increase in white blood cell count at 1000 mg/kg body weight after 60 days of treatment. From the thirtieth day of treatment onwards, the extract induced a significant ($P < .05$) reduction in blood glucose levels at the 500 mg/kg body weight dose and a significant ($P < .05$) increase in blood glucose levels at the 1000 mg/kg body weight dose. Aqueous extract of *Hymenocardia acida* roots is toxic by the intraperitoneal route and exerts a non-specific immunity action at high doses. It was harmless to rats at doses of 500 and 1000 mg/Kg of body weight.

Keywords: Hymenocardia acida, acute toxicity, subchronic toxicity

1. INTRODUCTION

In recent years there has been a renewed interest in herbal medicine. Indeed, more and more people are using herbal medicines to treat themselves [1]. It should be noted that the traditional use of any plant for therapeutic purposes does not guarantee its safety [2]. While the pharmacological effects of many plants have been proven in various laboratories, their toxicity is generally unknown. Therefore, toxicity assessment of herbal preparations is important in determining the safety of these remedies.

Hymenocardia acida (Euphorbiaceae) is a dioecious, deciduous shrub, reaching 6-10 m in height, with smooth, light brown or grey bark [3]. It is a savannah species, common in northern Côte d'Ivoire and Burkina Faso, whose range extends well beyond West Africa to Uganda [4]. In powder or paste form, the bark is ingested to treat abdominal pain, diarrhoea, dysentery, menstrual pain, female sterility, painful swellings, coughs and epileptic fits [5]. Chemical studies on *H. acida* have revealed the presence of alkaloid, anthocyanin, anthraquinones, carbohydrates, glycoside, cardiac, flavonoids, phenols, saponins, steroids, tannins and [6,7]

The present study aims to evaluate the acute and subchronic toxicities of the aqueous extract of *H. acida* roots in rodents.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Mice of the species *Mus musculus*, Swiss strain, 8 weeks old and weighing between 20 and 30 g were used for the acute toxicity experiments. Rats of the species *Rattus norvegicus*, Wistar strain, aged 8 weeks and weighing between 80 and 100 g were used for the subchronic toxicity test. These animals (males and females) came from the vivarium of the Ecole Normale Supérieure (Abidjan, Cote d'Ivoire). In this vivarium, the average temperature is 28 ± 2 °C with a relative humidity of 60% and a photoperiod of 12/12. The animals were fed with a standard diet for experimental animals and received water ad libitum. Animals were handled according to the guidelines of the Ethical Committee on the use and care of experimental animals of the Department of Biosciences, Université Félix Houphouët-Boigny.

2.2 Plant material

The roots of *H. acida* were collected in Korhogo in the Poro region (Cote d'Ivoire). They were chosen because they are traditionally used by the local population to treat erectile dysfunction. A sample of this plant was identified at the National Floristic Center of the University of Félix HOUPHOUËT-BOIGNY (Abidjan, Cote d'Ivoire).

2.3 Preparation of the aqueous extract

The roots of *H. acida* were cut into small pieces and dried in a room at room temperature (28-30°C). Three hundred g (300 g) of H. acida root were boiled in 1000 mL of distilled water for 30 min in a glass vessel. After cooling and filtration through cotton wool and Wattman paper, the resulting decoctate was freeze-dried. The powder obtained after freeze-drying constitutes the aqueous extract of H. acida roots (AEHA)

2.4 Acute toxicity study

Acute toxicity by intraperitoneal injection was performed with 30 mice divided into 5 groups of 6 subjects (male and female) each. Four groups were treated with increasing doses of AEHA ranging from 100 to 400 mg/kg body weight (BW). The control were given saline (NaCl 9‰). The mortality rate was recorded for each dose used. A dose-lethality curve representing the percentages of mortality versus the logarithms of the doses was plotted [8].
Acute oral toxicity was performed with 30 mice divided into 6 groups of 5 animals each. Five groups were treated with increasing doses of AEHA ranging from 2000 to 12000 mg/kg BW. The control received distilled water. Mortality, morbidity and weight change were recorded over 14 days [8].

2.5 Subchronic toxicity study

Subchronic oral toxicity was assessed according to the Organisation for Economic Co-operation and Development 408 method, slightly modified [9]. Forty-eight (48) adult rats were divided into 3 groups of 16 animals each. Each group contained an equal number of males and females and was treated as follows:
- Group 1: control, receiving distilled water;
- Group 2: treated, receiving 500 mg/kg BW of AEHA;
- Group 3: treated, receiving 1000 mg/kg BW of AEHA.

The animals received a daily volume of 1 mL of either distilled water or extract per route. The animals were weighed weekly. On days 30 and 60 of treatment, 24 subjects (4 males and 4 females) were randomly selected from each group and sacrificed by decapitation. The blood was collected for the determination of hematological and biochemical parameters. The liver, lungs, heart and kidneys were removed, weighed immediately and fixed in 10% formalin for histopathological study.

2.6 Diagnostic

Blood glucose, uric acid and triglycerides were determined by enzymatic tests [10,11]. Total cholesterol was determined by the enzymatic test of Allain et al. [12]. Urea was determined by a kinetic assay in which the onset of the reaction is linear within a defined time interval [13]. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine were determined by colorimetric methods [14,15,16]. The determination of white blood cells, red blood cells, platelets and haematocrit was performed according to the standard methods of Baker et al. [17].
Organs were preserved in 10% formalin for 48 hours and cut to a thickness of 4 µm using a microtome after embedding in paraffin. The organs were then stained with hematoxylin-eosin, fixed between slide and coverslip before being observed using a camera microscope. The histopathological evaluation was performed according to the method described by Lamb [18].

2.7 Statistical analysis

Statistical analysis of the experimental results was performed using GraphPad Prism 5.01 (USA). The values are presented as mean ± standard error (M ± SEM). The data were evaluated by the one-way ANOVA method of analysis followed by Tukey's multiple comparison test at the 5% threshold to assess the significance of the observed differences.

3. RESULTS

3.1 Acute intraperitoneal toxicity

Intraperitoneal administration of AEHA caused a decrease in mobility and an increase in drowsiness time in mice. The first deaths occurred 1 hour after treatment. The 50 % Lethal Dose (LD50) value of AEHA in mice by the intraperitoneal route is 223.87 mg/kg BW (Fig 1)

3.2 Acute oral toxicity

Fig 1 Oral administration of AEHA did not result in death in mice. The oral LD50 of AEHA in mice is therefore greater than 12,000 mg/kg BW.

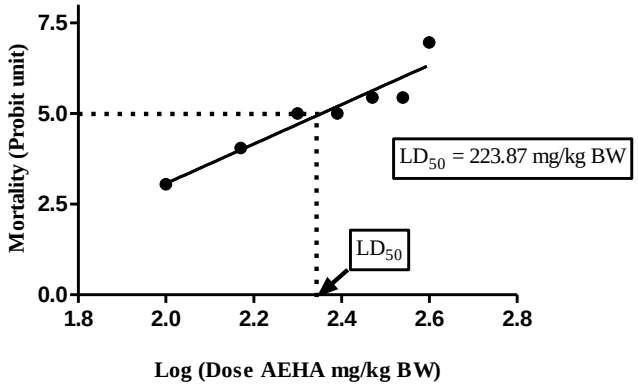

Figure 1: Dose-fatality curve after intraperitoneal injection of AEHA to mice

3.3 Subchronic toxicity

3.3.1 Effect of AEHA on the evolution of the body weight of animals

During treatment, the body weights of the rats increased progressively with time. The body weight gain of animals treated with 500 and 1000 mg/kg BW of AEHA was approximately the same and there was no significant difference between the treated and the control (Figs 2 and 3).

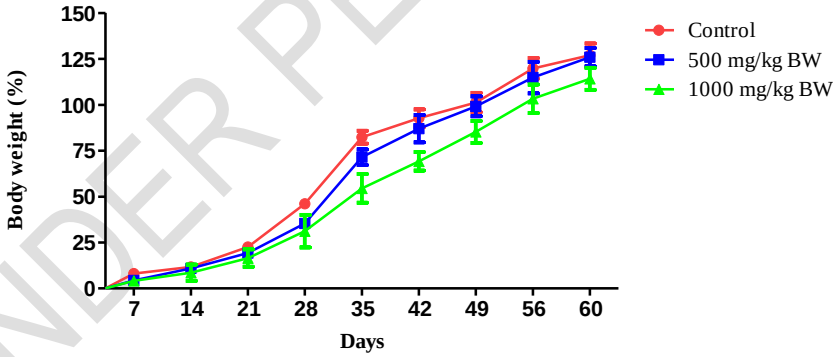

Figure 2. Effect of different doses of AEHA administration on the body weight of males after 60 days treatment.
Data are presented as mean +/- SEM (n = 8)

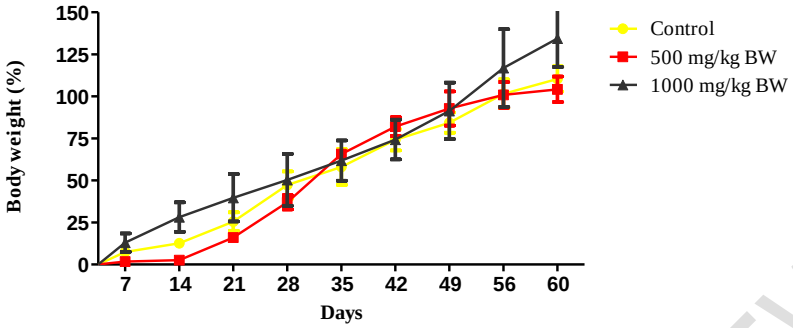

Figure 3. Effect of different doses of AEHA administration on the body weight of females after 60 days treatment.
Data are presented as mean +/- SEM (n = 8*).*

3.3.2 Effect of AEHA on vital organ weights of animals

After 30 days of treatment with AEHA, the results showed no significant change in the weights of kidney, heart, liver and lung in the animals compared to the controls. However, after 60 days of treatment with AEHA, there was a significant (*P < .05*) increase in heart weight in females at 500 and 1000 mg/kg BW compared to control. Also there was a significant increase (*P < .05)* in kidney weight in females at 500 mg/kg BW compared to control. Then, the results showed respectively a significant increase in lung weight in females at 500 (*P < .05*) and 1000 mg/kg BW (*P < .01*) compared to control and no significant variation in treated males compared to control. Finally no significant variation in liver weight in males and females compared to controls on day 60 of AEHA treatment was revealed. (Table I).

Table I. Effect of different doses of AEHA administration on vital organs of animals after 60 days treatment.

		Treatment (60 days)		
Vital organs (g/100g BW)		Control	$AEHA_{500}$	$AEHA_{1000}$
Heart	Male	0,35 ± 0,01	0,31 ± 0,01	0,33 ± 0,01
	Female	0,30 ± 0,02	0,38 ± 0,01 *	0,37 ± 0,01 *
Liver	Male	2,82 ± 0,13	2,76 ± 0,11	3,12 ± 0,12
	Female	3,01 ± 0,08	3,47 ± 0,15	3,35 ± 0,15
Kidney	Male	0,94 ± 0,04	0,96 ± 0,04	0,94 ± 0,06
	Female	0,45 ± 0,02	0,55 ± 0,02 *	0,56 ± 0,03
Lung	Male	0,97 ± 0,12	0,76 ± 0,08	0,95 ± 0,05
	Female	0,60 ± 0,02	0,73 ± 0,05 *	0,85 ± 0,01 **

Data are presented as mean ± SEM (n = 8*). * P < .05, ** P < .01*

3.3.3 Effects of AEHA on hematological parameters in animals

Administration of AEHA induced a significant *(P < .05*) increase in white blood cell count at 500 mg/kg BW in females and at 1000 mg/kg BW (*P < .001*) in males and females after 30 days of treatment. For blood platelets, AEHA treatment resulted in a significant decrease (*P < .05*) at 1000 mg/kg BW after 30 days of treatment. Red blood cells, hematocrit, and hemoglobin did not change significantly in animals after 30 and 60 days of AEHA treatment compared to controls (Table II and III**).**

Table II. Effect of different doses of AEHA administration on the white blood cell, red blood cell, hemoglobin, hematocrit, blood platelet of animals after 30 days treatment.

		Treatment (30 days)		
		Control	$AEHA_{500}$	$AEHA_{1000}$
WBC (×103 µL-1)	Male	21,98 ± 1,41	23,64 ± 0,7	40 ± 0,9 ***
	Female	5,48 ± 0,54	7,84 ± 0,76 *	13,4± 0,5 ***
RBC (×106 µL-1)	Male	6,61 ± 0,11	6,94 ± 0,2	7,15 ± 0,13
	Female	6,38 ± 0,20	6,94 ± 0,3	6,38 ± 0,59
HGB (g/dL)	Male	10,93± 0,55	11,60 ± 0,15	12,27 ± 0,29
	Female	11,58 ± 0,46	11,97 ± 0,52	11,13 ± 0,67
HCT (%)	Male	36,20 ± 2,85	39,43 ± 0,90	41,13 ± 1,88
	Female	42,57 ± 2,17	40,17 ± 1,91	37,33 ± 2,26
PLT (×103 µL-1)	Male	857 ± 57	1323 ± 149	785 ± 91
	Female	662 ± 12	661 ± 6	616 ± 3 *

Data are presented as mean ± SEM (n = 8*). * P < .05, ** P < .01, *** P < .001*

Table III. Effect of different doses of AEHA administration on the white blood cell, red blood cell, hemoglobin, hematocrit, blood platelet of animals after 60 days treatment.

		Treatment (60 days)		
		Control	$AEHA_{500}$	$AEHA_{1000}$
WBC (×103 µL-1)	Male	24,00 ± 2,08	21,30 ± 0,64	23,73 ± 0,86
	Female	12,87 ± 0,8	14,4 ± 0,8	11,67 ± 0,29
RBC (×106 µL-1)	Male	8,32 ± 0,10	8,44 ± 0,22	8,42 ± 0,41
	Female	7,66 ± 0,33	8,05 ± 0,08	8,01 ± 0,15
HGB (g/dL)	Male	13,77 ± 0,12	14,23± 0,32	13,73 ± 0,52
	Female	13,40 ± 0,96	13,90 ± 0,32	13,17 ± 0,41
HCT (%)	Male	45,73 ± 0,03	46,20 ± 1,51	45,17 ± 2,17
	Female	45,90 ± 1,00	45,97 ± 0,28	41,93 ± 2,04
PLT (×103 µL-1)	Male	996 ± 84	950 ± 82	875 ± 47
	Female	795 ± 2	826 ± 20	762 ± 5

Data are presented as mean ± SEM (n = 8*). * P < .05*

3.3.4 Effects of AEHA on serum glucose and lipid concentration

The blood glucose and lipid values of the animals after 30 and 60 days of treatment with AEHA are shown in Table IV. The extract induced after 30 and 60 days of treatment a significant decrease *(P < .05*) in glucose level at 500 mg/kg BW and a significant increase (*P < .05*) in glucose level at 1000 mg/kg BW in males compared to control; On the other hand in females no significant variation was observed compared to controls. On the 30th day of treatment there was a significant increase (*P < .01*) at 500 and 1000 mg/kg BW in triglycerid level in males compared to control. Also a significant (*P < .05*) increase in triglycerid level is observed at 500 mg/kg BW in females compared to control. At day 60, a significant (*P < .05*) increase in triglyceride level at 1000 mg/kg BW was observed in females compared to control. After 60 days of treatment there was a significant (*P < .01*) decrease in cholesterol level at 1000 mg/kg BW in males compared to control.

Table IV. Effect of different doses of AEHA administration on serum concentrations of Glucose, Triglycerid and total cholesterol of animals

Duration of treatment	**Doses (mg/Kg BW)**		**Glucose (g/L)**	**Triglycerid g/L)**	**Total cholesterol (g/L)**
30 days	Control	Male	1,15 ± 0,04	0,80 ± 0,10	0,76 ± 0,01
		Female	1,01 ± 0,061	0,71 ± 0,02	0,74 ± 0,04
	$AEHA_{500}$	Male	0,97 ± 0,01**	0,83 ± 0,03	0,75 ± 0,01
		Female	0,87 ± 0,09	0,85 ± 0,02*	0,85 ± 0,02
	$AEHA_{1000}$	Male	1,27 ± 0,03*	0,79 ± 0,04	0,75 ± 0,01
		Female	0,91 ± 0,04	0,76 ± 0,03	0,82 ± 0,01
60 days	Control	Male	1,09 ± 0,014	0,74 ± 0,02	0,77 ± 0,01
		Female	0,90 ± 0,06	0,58 ± 0,07	0,66 ± 0,07
	$AEHA_{500}$	Male	0,87 ± 0,02***	0,73 ± 0,01	0,75 ± 0,03
		Female	0,89 ± 0,05	0,45 ± 0,01	0,78 ± 0,01
	$AEHA_{1000}$	Male	1,19 ± 0,03**	0,75 ± 0,02	0,61 ± 0,03**
		Female	0,96 ± 0,05	0,77 ± 0,03*	0,74 ± 0,03

Data are presented as mean ± SEM (n = 8*). * P < .05, ** P < .01, *** P < .001*

3.3.5 Effects of AEHA on serum concentration of renal parameters

There were no significant changes in serum urea, uric acid and creatinine at 500 and 1000 mg/kg BW in males and females compared to controls (Table V).

3.3.6 Effects of AEHA on serum concentration of liver parameters

The results of liver parameter analyses after 30 and 60 days of treatment with AEHA are shown in Table VI. A significant increase (*P < .01*) in total protein level at 500 mg/kg BW in males after 60 days of treatment compared to controls was observed. The other hepatic parameters (AST, ALT) did not change significantly at 500 and 1000 mg/kg BW in males and females.

Table V. Effect of different doses of AEHA administration on serum concentrations of Urea, Uric acid and Creatinin of animals

Duration of treatment	Doses (mg/Kg BW)		Urea (g/L)	Uric Acid (mg/L)	Creatinin (mg/L)
30 days	Control	Male	0,34 ± 0,01	11,33 ± 0,66	2,83 ± 0,17
		Female	0,37 ± 0,03	11,33 ± 0,88	3,00 ± 0,57
	$AEHA_{500}$	Male	0,33 ± 0,01	13,00 ± 0,57	2,50 ± 0,29
		Female	0,33 ± 0,01	12,67 ± 0,88	2,66 ± 0,33
	$AEHA_{1000}$	Male	0,34 ± 0,01	11,00 ± 0,57	2,50 ± 0,29
		Female	0,39 ± 0,02	12,33 ± 0,33	2,33 ± 0,33
60 days	Control	Male	0,31 ± 0,01	11,33 ± 0,88	4,33 ± 0,33
		Female	0,25 ± 0,02	13,00 ± 0,57	6,67 ± 1,45
	$AEHA_{500}$	Male	0,35 ± 0,01	11,67 ± 0,66	4,66 ± 0,33
		Female	0,35 ± 0,01	14,67 ± 0,67	4,67 ± 0,67
	$AEHA_{1000}$	Male	0,31 ± 0,02	12,67 ± 0,67	5,00 ± 0,58
		Female	0,25 ± 0,02	14,33 ± 0,88	5,67 ± 0,88

Data are presented as mean ± SEM (n = 8*). * P < .05*

Table VI. Effect of different doses of AEHA administration on serum concentrations of AST, ALT and total protein of animals

Duration of treatment	Doses (mg/Kg BW)		AST (UI/L)	ALT (UI/L)	Total Protein (g/L)
30 days	Control	Male	256,0 ± 2,08	71,00 ± 6,11	66,00 ± 2,52
		Female	219,3 ± 27,8	60,33 ± 6,01	68,67 ± 1,20
	$AEHA_{500}$	Male	244,7 ± 12,91	60,67 ± 2,18	67,67 ± 0,88
		Female	280,3 ± 12,5	63,33 ± 4,09	68,00 ± 2,51
	$AEHA_{1000}$	Male	275,0 ± 17,56	65,00 ± 2,51	68,00 ± 0,58
		Female	195,0 ± 14,5	59,33 ± 1,85	68,67 ± 0,88
60 days	Control	Male	241,3 ± 16,48	36,00 ± 1,53	71,33 ± 0,33
		Female	247,3 ± 12,3	41,33 ± 0,33	74,33 ± 2,33
	$AEHA_{500}$	Male	257,7 ± 11,78	39,00 ± 1,53	77,00 ± 0,58**
		Female	254,3 ± 37,5	43,67 ± 1,85	76,00 ± 0,57
	$AEHA_{1000}$	Male	294,3 ± 53,25	41,67 ± 3,28	72,67 ± 0,88
		Female	191,3 ± 10,8	37,00 ± 1,00	75,00 ± 2,08

Data are presented as mean ± SEM (n = 8*). * P < .05*

3.3.7 Effects of AEHA on the histological structure of the liver and kidney

Histopathological examination of the liver revealed no apparent signs of inflammation or hepatic cell necrosis in either control or treated rats (Fig 4). Furthermore, there was no significant difference between the kidneys of treated and control rats (Fig 5).

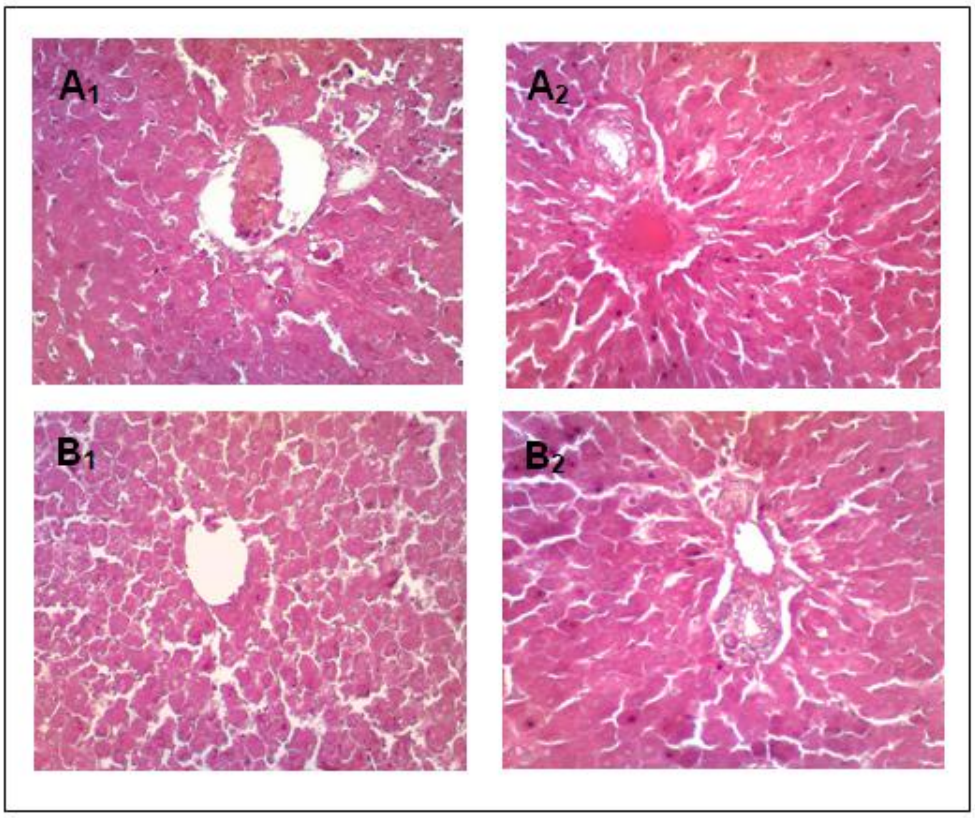

Figure 4: Micrographs of liver of control and 1000 mg/Kg BW of AEHA-treated rats after 60 days treatment.

$A_{1:}$ Female control; A_2: Male control; B_1: Female Treated 1000 mg/kg BW; B_2: Male Treated 1000 mg/kg BW; Magnification: ×400; Hematoxylin and Eosin staining

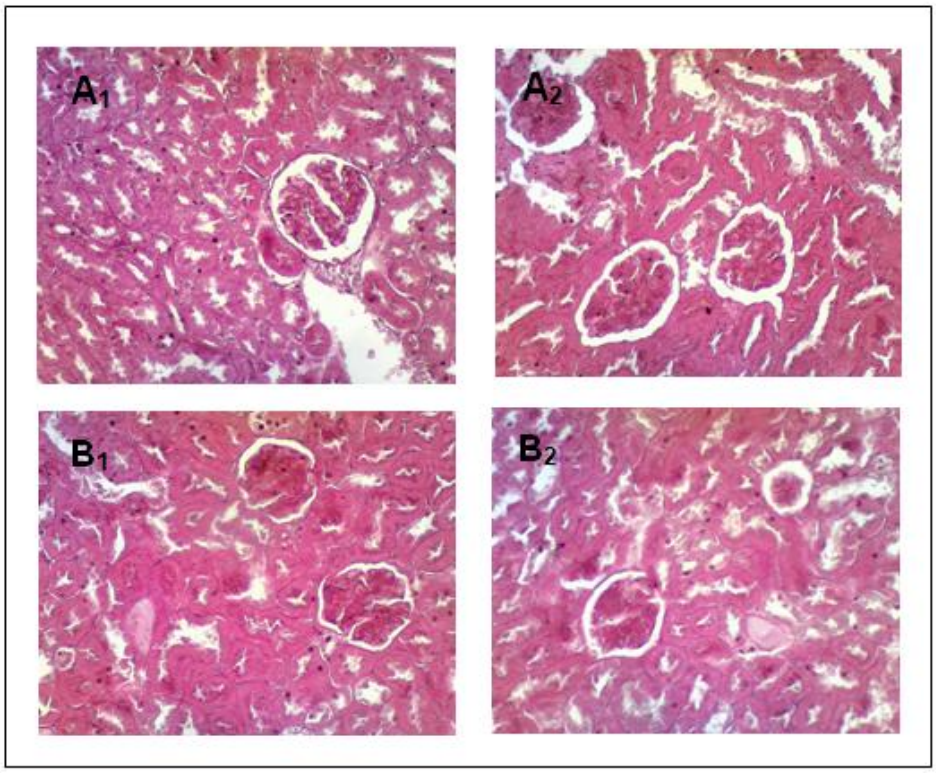

Figure 5: Micrographs of kidney of control and 1000 mg/Kg BW of AEHA-treated rats after 60 days treatment.
$A_{1:}$ Female control; A_2: Male control; B_1: Female Treated 1000 mg/kg BW; B_2: Male Treated 1000 mg/kg BW; Magnification: ×400; Hematoxylin and Eosin staining

4. DISCUSSION

Substances with an LD50 between 50 and 500 mg/kg BW are toxic and those with an LD50 above 5000 mg/kg BW are practically non-toxic [19]. According to this classification, AEHA is intraperitoneally toxic and not orally toxic. It is known from the literature that the toxicity of a substance varies according to the species and the route of administration [20]. The LD50 values of *H. acida* roots obtained in mice show that they vary according to the route of administration. Variations in LD50 depending on the route of administration and the species have already been reported with the lyophilized aqueous extract of *Tanacetum vulgare* [21]. Changes in body weight have been used as an indicator of adverse effects of drugs, chemicals and bioactive substances [22].

Since no significant changes in body weight were observed in rats in the treated groups compared to the control after daily treatment for 60 days, it is suggested that oral administration of AEHA has no effect on the normal growth of rats. Loss of appetite often leads to weight loss due to disturbances in carbohydrate, protein or fat metabolism [23]. Similarly, no significant changes in vital organ weights have been observed. Relative organ weights are considered a relatively sensitive indicator in toxicity studies [24].

At the hematological level, an increase in white blood cell count was observed in animals at different treatment doses on day 30 of AEHA treatment. This could be the result of the rats' response to the subchronic toxicity of the extract [25]. It could also be a stimulation of the non-specific immune defence by the plant extract. Indeed, the tannins present in this extract have immunostimulant activities [26]. Other authors have observed similar results. This is the case of Gupta et al. with the aqueous extract of *Clerodendrum phlomidis* leaves [27].

Glucose determination after 60 days of treatment with AEHA revealed a significant decrease in its concentration at 500 mg/kg BW to in males. This result could be related to the alkaloids present in the plant extract. Indeed, alkaloids have hypoglycemic properties [28]. This blood glucose lowering activity is in agreement with the results obtained by Adisa et al. [29]. A similar result was obtained by Gupta et al. with the aqueous extract of *C. phlomidis* leaves [28]. The increase in blood glucose levels induced by the aqueous extract at the dose of 1000 mg/kg BW could be explained by an inhibition of insulin production via the □ cells of the islets of Langerhans by its bioactive compounds constituting AEHA. These bioactive substances could also stimulate the production of glucagon, adrenaline and glucocorticoids, which are hyperglycemic hormones at the origin of the phenomenon of neoglucogenesis [30].

There was an increase in triglyceride levels after 60 days of treatment with different doses in females compared to controls. This result could be explained by the richness of the extract of this plant in chemical compounds such as alkaloids. Indeed, these compounds have the capacity to positively influence the cardiovascular system by reducing, for example, body fat [31]. These results corroborate the work of Gupta et al. who found an increase in the level of this parameter with the aqueous extract of *C. phlomidis* leaves [27]. In males, however, there was no significant variation in this parameter in the experiment. These results are similar to those obtained by Bleu et al. who after administration of *Passiflora foetida* extract observed no significant variation in serum triglycerid concentration [32]. For cholesterol, there was a significant decrease in its blood level on day 60 of treatment in males at 1000 mg/kg BW compared to the control. This decrease in cholesterol levels indicates that AEHA has a hypolipidemic property [33]. This result is in agreement with that obtained by Bleu et al. [32]. Indeed, these authors administered a dose of 800 mg/kg BW of aqueous extract of *P. foetida* to rats and observed a decrease in blood cholesterol level.

5. CONCLUSION

The aqueous extract of *Hymenocardia acida* roots is toxic by intraperitoneal administration and non-toxic by oral administration in single dose. . It was harmless to rats at doses of 500 and 1000 mg/Kg of body weight in the subacute toxicity study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animals were handled according to the guidelines of the Ethical Committee on the use and care of experimental animals of the Department of Biosciences, Université Félix Houphouët-Boigny.

REFERENCES

1. WHO. Guidelines for Assessing Quality of Herbal Medicines With Reference to Contaminants and Residues. World Health Organization, Geneva. 2007; 105 p. https://apps.who.int/iris/handle/10665/43510
2. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Toxicological studies of hydromethanolic leaves extract of Grewia crenata. International Journal of Pharmaceutical Science and Drug Research. 2012; 4: 245–249.
3. Arbonnier M. Trees, shrubs and lianas of West African dry zones. CIRAD, Margraf Publishers Gmbh, MNHN, Paris, France. 2004; 573 pp.
4. Turiaux L. Tanins végétaux du Katanga. Bull. Agr.Congo belge. 1943 ; 33, 245-54, 1942 et 34, 160. DOI : https://doi.org/10.4000/chs.1292
5. Koné WM, Kamanzi AK, Traoré D, Bruno B.. Anthelmintic activity of medicinal plants used in northern Côte d'Ivoire against intestinal helminthiasis. Pharmaceutical Biology. 2005 ; 43(1): 72–78. DOI: https://doi.org/10.1080/13880200590903408
6. Sofidiya MO, Odukoya, OA, Familoni OB, Inya-Agha SI. Free radical scavenging activity of some Nigerian medicinal plant extracts. Pakistan Journal of Biological Sciences. 2009; 9 (8):1438–1441. DOI : 10.3923/pjbs.2006.1438.1441
7. Olotu NP, Ibrahim H, Ilyas N, Ajima U, Olotu AI.Depistagephytochimique et etude analgesiques de l'ecorce de la racine de Hymenocardia acida Tul. (Euphorbiaceae).International Journal of Drug Developpement et Research. 2011; 3,219-223.
8. Miller LC, Tainter MC. Estimation of LD50 and its error by means of logarithmicprobit graph paper. Proc. Soc. Exp. Boil. Med. 1944; 57: 261-264. DOI: https://doi.org/10.3181/00379727-57-14776
9. OCDE. Ligne directrice de l'OCDE pour les essais de produits chimiques : Étude de toxicité orale à dose répétée pendant 90 jours sur les rongeurs. OCDE 408; 2018: 16p. DOI: https://doi.org/10.1787/20745788
10. Dingeon B, Ferry JP, Roullet A. Automatic essay of blood sugar by Trinder'smethod., Ann. Biol. Clin. 1975; 33: 3-13.
11. Young DS, Pestaner L, Gibber man V. Effects of drugs on clinical laboratory test.Clin. Chem., 1975; 21: ID-432D. PMID: 1091375
12. Allain CC, Poom LS, Chan CS, Richmonal WS, Fu PC. Enzymatic determination of total serum cholesterol. Clin. Chem. 1974; 20: 470-475. PMID : 4818200
13. Kaplan LA, Szabo LL, Opherin EK. Clinical chemistry: interpretation and techniques, 3rd ed. lea and febliger, Philadelphia. 1988; 112-231.
14. Reitman SN, Frankel S. A colorimetric method for the determination of SGPT and SGPOT. Am. J. Clin. Pathol. 1957; 25: 56- 62. DOI: 10.1093/ajcp/28.1.56

15. Henry RJ. Clinical chemistry, principles and techniques. Edition, Harper and Row. 1974; 543p.
16. Tietz NW. Clinical Guide for Laboratory Tests. W. B. Saunders Company: Philadelphi: USA. 1976; pp.723
17. Baker FJ, Silverton RE, Kilshaw D, Shannon R, Guthrie DL, Egglestone S, et al,. Introduction to haematology. In Introduction to Medical Laboratory Technology (6th edn). Butterworths: London and Boston. 1985; 147-334.
18. Lamb GM. Manual of Veterinary Laboratory Techniques in Kenya. Ministry of livestock development/CIBAGEIGY, Basale: Switzerland, 1981; 93-107.
19. Diezi J. Toxicology: Basic principles and chemical impact. In Pharmacology: Fundamental Principles and Pratice, Slatkine M. (ed). Academic Press: Genève. 1989; 33-44.
20. Morrisson JK, Quinton RM, Reinerth. The Purpose and Value of LD50 Determination. Modern Trends in Toxicology. Butterworths: London. 1968; 1-17.
21. Lahlou S, Israili Z, Lyoussi B. Acute and chronic toxicity of a lyophilised aqueous extract of Tanacetum vulgare leaves in rodents. J. Ethnopharmacol. 2008; 117: 221-227. DOI: 10.1016/j.jep.2008.01.024
22. Theo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90day oral gavage toxicity study of D-methylphenidate and D,Lmethylphenidate in spragueDawly rats. Toxicology. 2002; 179:183-196. DOI: 10.1016/s0300-483x(02)00338-4
23. Fzeonwumelu JOC, Julius AK, Muhoho CN, Ajayi AM, Oyewale AA, Tanayen JK, et al,. Biochemical and histological studies of aqueous extract of Bidens pilosaleaves from Ugandan Rift Valley in Rats. British Journal of Pharmacologyand Toxicology. 2011; 2(6): 302–309.
24. Lullmann-Rauch R. Histologie. De Boeck Supérieur, Bruxelles, Belgique. 2008; 704p.
25. Kelly WR. Veterinary clinic diagnosis, Balliere Tindal, London. 1977; 271-282.
26. Feldman KS, Sahasrabudhe K, Smith RS, Scheuchenzuber WJ. Immunostimulation by plant polyphenols. A relationship between tumor necrosis factoralpha production and tannin structure. Bioorg. Med. Chem. Lett. 1999; 9(7): 985-990.
27. Gupta RK, Hussain T, Panigrahi G, Das A, Singh GN, Sweety K, et al,. Hepatoprotective effect of Solanum xanthocarpum fruit extract against CCl4 induced acute liver toxicity in experimental animals. Asian Pac. J. Trop. Med. 2012; 4: 964-968. DOI: 10.1016/S1995-7645(11)60227-7
28. Kinghorn AD, Balandrin MF. Quinolizidine alkaloids of the Leguuminosae: structural types, analysis, chemotaxonomy and biology activities. In: Pelletier W. S. (ed) Alkaloids: chemical and biology perspectives. Wiley, New York. 1984; 2: 105-148.
29. Adisa RA, Choudhary MI, Adewoye EO, Olorunsogo OO. Hypoglycaemic and biochemical properties of cnestis ferruginea. Afr. J. Trad. CAM. 2010; 7(3): 185. 194.
30. Labroussse-Lhermine. Diabete et corticoïdes. Service de Diabétologie-maladie. Métabolique et Nutrition CHU Rangueil. 2008 ; 2p.
31. Schmeda-Hirschmann G, Rodriguez JA, Loyola JI, Astudillo L, Bastida J, Viladomat F, et al,. Activity of Amaryllidaceae alkaloids on the blood pressure of normotensive rats. Pharmacol. Comm. 2000; 6: 309-312. DOI: https://doi.org/10.1211/146080800128736105
32. Bleu GM, Kouakou K, Zahoui OS, Touré A, Traoré F. Oral acute toxicity and estrogenic effects of the extracts of Passiflora foetida Linn.(Passifloraceae) leaves in

female Wistar albino rats. Ann Biol Res. 2011; 3(9): 4609-4616. DOI: http://dx.doi.org/10.4314/ijbcs.v5i5.1

33. Nwozo SO, Kasuma TF, Oyinloye BE. Eugenia caryophyllus extract exerts hypocholesterolemic and antioxidant effects in high-cholesterol-fed rats. Avicenna Journal of Medical Biochemistry. 2015; 3(2):301-47. DOI: 10.17795/ajmb-30147