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Acute and Subacute Toxicity of an Aqueous and Ethanolic Extract of *Harungana madagascariensis* LAM (Hypericaceae) Stem Bark in Wistar Rats

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Abstract **Original Research Article**

Harungana madagascariensis is widely used for treatment of various ailments in traditional medicine. The aim of this study was to investigate potential toxic effect of the aqueous and ethanolic extracts of *H. madagascariensis* stem bark in rats. OECD Guidelines 423 and 407 were used for acute and sub-acute toxicity study. The acute toxicity study of both extracts revealed no lethal effects and behavioural signs of toxicity at the tested doses indicating that LD_{50} is greater than 5000 mg/kg. In the sub-acute toxicity study, both extracts induced significant reduction of the body weight ($p < 0.05$). The EE*Hm* treatments at the dose of 1000 mg/kg increased significantly the relative heart weight in female rats. The EA*Hm* treatment at the dose of 300 mg/kg, caused significant increase of AST level in male rats ($p < 0.05$). Administration of EA*Hm* at all doses increase significantly WBC counts ($p < 0.05$) and the levels of platelets at dose of 1000 mg/kg in the male rats ($p < 0.05$). However, it induced an decrease in HDL-C in female and an increase in HDL-C in male ($p < 0.05$). EA*Hm* increased significantly the levels of creatinine at 1000 mg/kg dose, and electrolytes level at doses 600 mg/kg and 1000 mg/kg in the male rats compare to untreated group ($p < 0.05$). The EE*Hm* treatments cause significant decrease in urea level of female rats at all doses ($p < 0.05$) and significant increase of Na^+ level at the dose of 300 mg/kg. The EE*Hm* decreased K^+ level at the dose of 1000 mg/kg in female rats ($p < 0.05$). In male rats, it has favorized the increasing of K^+ level at the dose of 1000 mg/kg and Cl^- levels at all doses ($p < 0.05$; $p < 0.01$). Results indicate that oral doses of aqueous extract of *Harungana madagascariensis* is relatively safe in rats in acute use. But in long term use, it could causes consequences on heart weight, lipid profile and electrolyte homeostasis.

Keywords: *H. madagascariensis*, *Acute and Subacute toxicity*, haematological and biochemical parameters.

INTRODUCTION

Herbal plant is use for treatment of various ailments in traditional medicine, about eighty percent of the world's population depend on traditional medicine for primary health care (Ugwah *et al.*, 2013; Ekor, 2014). The medicinal plants contain active molecules that are at the origin of the therapy. But this molecules can lead to vitals organ functions impairment (Cristavao *et al.*, 2007). *Harungana madagascariensis* of Hypencaceae family, is a small to a medium tropical shrub (up to 1.65 m high) with fine stellate hairs and ovate lateral leaves (Irvine, 1961). A plant whose leaves have been shown by previous studies to have antibacterial, antifungal, anti-hepatotoxic activity and can be used in the treatment of otitis externa in dogs and cats (Geotilini *et al.*, 1983; Bourée, 1987; Basex and Loche 1996) also that the bark of the trunk has an anti-protozoan, antimalarial and antibacterial activity (Toty *et al*, 2013) caught our attention. It turns out that natural substances of plant origin are endowed with several biological activities such as antioxidant, anti-inflammatory, anticancer, antimicrobial activity, (Strobel and Daisy, 2003 ; Patwardhan, 2005; Rad *et al.*, 2014)

There are few studies evaluating the safety use of *Harungana madagascariensis* stem bark. The current study described an acute toxicity in single oral dose and subacute txicity by repeate dose by oral route on hematological, liver and kidney biochimical parameters in Wistar rats.

MATERIAL AND METHODS

Plant material and extraction method

The plant material used consisted of trunk bark of *Harungana madagascariensis* harvested in Abidjan (Ivory Coast) and identified by an expert in Botany of the

Centre National de Floristique (UFR-Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire).

Aqueous extract

The stem bark of *Harungana madagascariensis* was harvested, sorted, washed and dried at room temperature at the Microbiology Laboratory of the Higher Teacher Training School (*Ecole Normale Supérieure, ENS*) and then crushed to obtain a vegetable powder which was used to prepare the various extracts. The aqueous extract was prepared according to the following method. One hundred grams (100g) of plant powder was macerated in one litre of distilled water by homogenisation in a blender. The homogenate obtained was successively filtered twice on cotton wool and then on Whatman 3 mm filter paper. The filtrate obtained was dehydrated using a "Prolabo" type oven at a temperature of 50°C (Zirihi *et al.*, 2003)**.**

Ethanolic extract

The method of (Zirihi *et al.*, 2003) was used to obtain the various 70% hydroethanol extracts of *H. madagascariensis*. A 70% hydroethanol solution (ethanol/water 70:30) was used for the preparation of hydroethanol extracts of *H. madagascariensis* in vials. One litre of the hydroethanolic solution and 100 g of *H. madagascariensis* powder were used for this purpose. The resulting mixtures were homogenised using a magnetic stirrer for 24 hours. The homogenate obtained was successively filtered twice on cotton wool and then on Whatman 3 mm filter paper. The filtrate obtained was dehydrated using a "Prolabo" type oven at a temperature of 50°C (Zirihi *et al.*, 2003).

Animals

Rats (*Rattus norvegicus*, Muridae, L.1753) of Wistar strain were used to carry out this work. They are reproduced at the vivarium of the *Ecole Normale Supérieur* (ENS, Abidjan). The resulting litters are fed and watered *ad libitum* to reach a weight between 160 and 180 g under standard environmental conditions, temperature 25° C, with a light-dark cycle of 12 hours.

Experimental design

Acute oral toxicity study

An acute and sub-acute oral toxicity studies were conducted in accordance with the Organization for Economic Co-operation and Development (OECD) Guideline 423 and 407 respectively (OECD, 2008). The five parameters of the Hippocratic screening were analyzed: conscious state, general activity ; reflexes, activities on the central nervous system and activities on the autonomic nervous system (Malone and Robichaud, 1962 ; Neyres *et al.*, 2012). The body weight were recorded for 14 days.

Sub-acute oral toxicity study

The sub-acute toxicity study was carried out according to Organisation for Economic Co-operation and Development, test guidelines 407 for testing chemicals (OECD, 2008). A total of 48 male and female Wistar rats weighing between 160 and 180 g were randomly divided into four groups (n = 6 males and 6 females /group). Rats in treatment groups orally received *Harungana madagascariensis* stem bark aqueous or ethanolic extract at doses of 300, 600 and 1000 mg/kg every day. The extract was administered *per os* on a daily basis for 28 days. Rats in control groups were administrated NaCl 0.9 % (vehicle).

During the experimental period, the body weights of all groups were measured twice a week.

Hematological, biochemical analyzer and histopathological examination

At the end of the treatment period, all rats fasted all night (12h). Blood samples are collected for measurement of hematological parameters (EDTA-2K coated tubes) and biochemical (dry tubes). Hematological analyses were performed using an automated hematological analyzer (MYDRAY BC 30S). The following parameters such as red blood cell (RBC), hemoglobin (HGB), Hematocrit, white blood cell (WBC) and platelet counts (PQT) were determined. Blood samples were collected and centrifuged at 3000 rpm for 10 minutes. Serum samples were removed, kept in Eppendorf tubes and stored at -20°C. Serums were further analysed using Semi-automatic biochemical analyzer (spectrophotometer Rayto RT-9200) to determine the level of alanine amino transferase (ALT), aspartate amino transferase (AST), triglycerides (TG), total cholesterol (TC) and cholesterol HDL. After euthanasia, the rats were sacrificed and the organs were removed for autopsy, measurement of organ weight.

Statistical analysis

The values are expressed as mean ± standard error of mean of six experiment (Mean ±. SEM). GraphPad Prism 7 software, (Microsoft, San Diego California, USA) is used for statistical analysis of data and graphical representations. The statistical significance of the data has been determined using one way Analyse of Variance (ANOVA) and post hoc Turkey test. The level of significant was taken as $p < 0.05$.

RESULTS

Acute toxicity study

Aqueous or ethanolic extract of *H. madagascariensis* at a dose of 1000 ; 2000 and 4000 mg/kg oral treatment with only dose induced no abnormal signs of toxicity in behavioral patterns and mortality in treated group compare to control (Table 1 and 2). In addition, produced no significant body weight and relative organs weight variation in treated group compare to control ($P > 0.05$; Figure 1 A - B).

Table 1: Behavioural and clinical effects of aqueous extract of *Harungana madagascariensis* stem bark in experimental rats

		Observed clinical signs				
Observation durations	**Doses (mg/kg)**	**Isolation**	**Vomiting**	**Diarrhea**	**Bleeding**	**Mortality**
30 min to 1 hour	**Control**	*No*	*No*	*No*	*No*	*No*
	1000	*No*	*No*	*No*	*No*	*No*
	2000	*No*	*No*	*No*	*No*	*No*
	4000	*No*	*No*	*No*	*No*	*No*
24 hours	**Control**	*No*	*No*	*No*	*No*	*No*
	1000	*No*	*No*	*No*	*No*	*No*
	2000	*No*	*No*	*No*	*No*	*No*
	4000	*No*	*No*	*No*	*No*	*No*
14 days	**Control**	*No*	*No*	*No*	*No*	*No*
	1000	*No*	*No*	*No*	*No*	*No*
	2000	*No*	*No*	*No*	*No*	*No*
	4000	*No*	*No*	*No*	*No*	*No*

No: Absence of clinical signs assessed

Table 2: Behavioural and clinical effects of ethanolic extract of *Harungana madagascariensis* stem bark in experimental rats

		Observed clinical signs				
Observation durations	**Doses (mg/kg)**	**Isolation**	**Vomiting**	**Diarrhea**	**Bleeding**	**Mortality**
30 min to 1 hour	**Control**	*No*	*No*	*No*	*No*	*No*
	1000	*No*	*No*	*No*	*No*	*No*
	2000	*No*	*No*	*No*	*No*	*No*
	4000	*No*	*No*	*No*	*No*	*No*
24 hours	**Control**	*No*	*No*	*No*	*No*	*No*
	1000	*No*	*No*	*No*	*No*	*No*
	2000	*No*	*No*	*No*	*No*	*No*
	4000	*No*	*No*	*No*	*No*	*No*
14 days	**Control**	*No*	*No*	*No*	*No*	*No*
	1000	*No*	*No*	*No*	*No*	*No*
	2000	*No*	*No*	*No*	*No*	*No*
	4000	*No*	*No*	*No*	*No*	*No*

No: Absence of clinical signs assessed

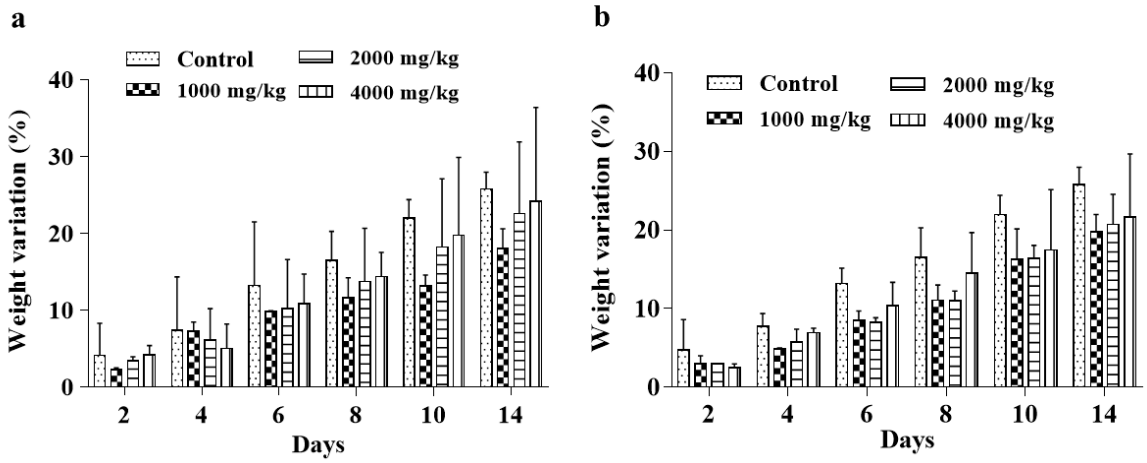

Figure 1: Changes in the body weights in females rat adminstered *H. madagascariensis* stem bark aqueous (a) or ethanolic (b) extract in the acute toxicity study. *Values are expressed as mean ± SEM, n = 6. (p > 0.05 ; as compared to the control group)*

Sub-acute toxicity study

Body weight of rats

The aqueous extract of *H. madagascariensis* at a dose of 300 mg/kg decreased significantly the body weight of the treated male rats for 28 days ($p < 0.05$). Indeed, as summarized in Figure 3, the body weight of the rats decreased relatively during the study when compared with the control groups. However, treatments at the doses of 300 at 1000 mg/kg cause significant change in the body weight not dose dependent maner of female rats for 28 days ($p < 0.05$).

H. madagascariensis ethanolic extract at a doses of 600 and 1000 mg/kg decreased the body weight of the treated male rats for 28 days when compared with the control groups ($p < 0.05$). The female rats treated with extract at a doses of 300; 600 and 1000 mg/kg don't showed weight gain throughout the entire experimental period. The increase of body was the similar in treated and control groups ($p > 0.05$; Figure 3).

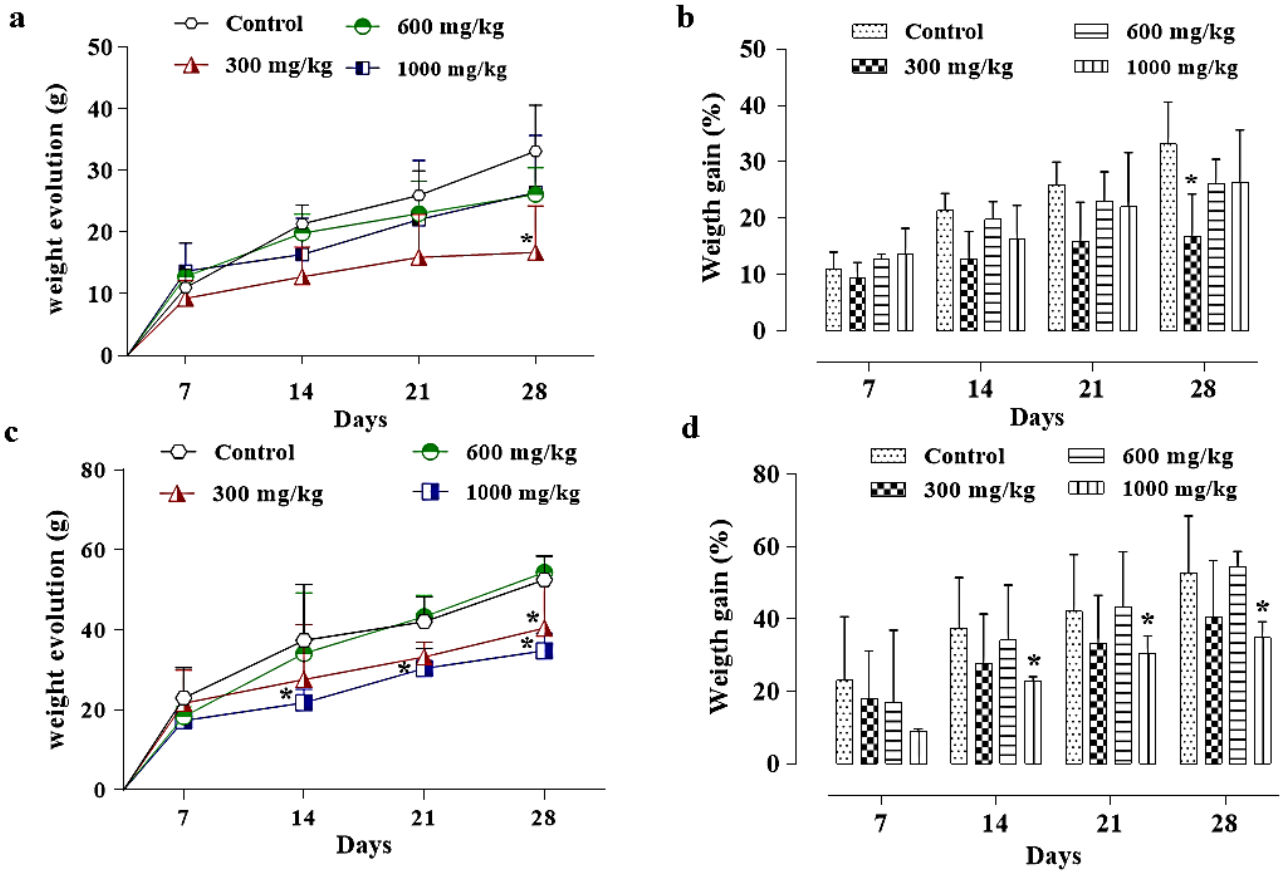

Figure 2: Changes in the body weights in males (a-b) and females (c-d) rat treated with aqueous extract of *H. madagascariensis* stem bark in the subacute toxicity study. Values are expressed as mean ± SEM, n=6. (*p < 0,05 ; compared to the control group)

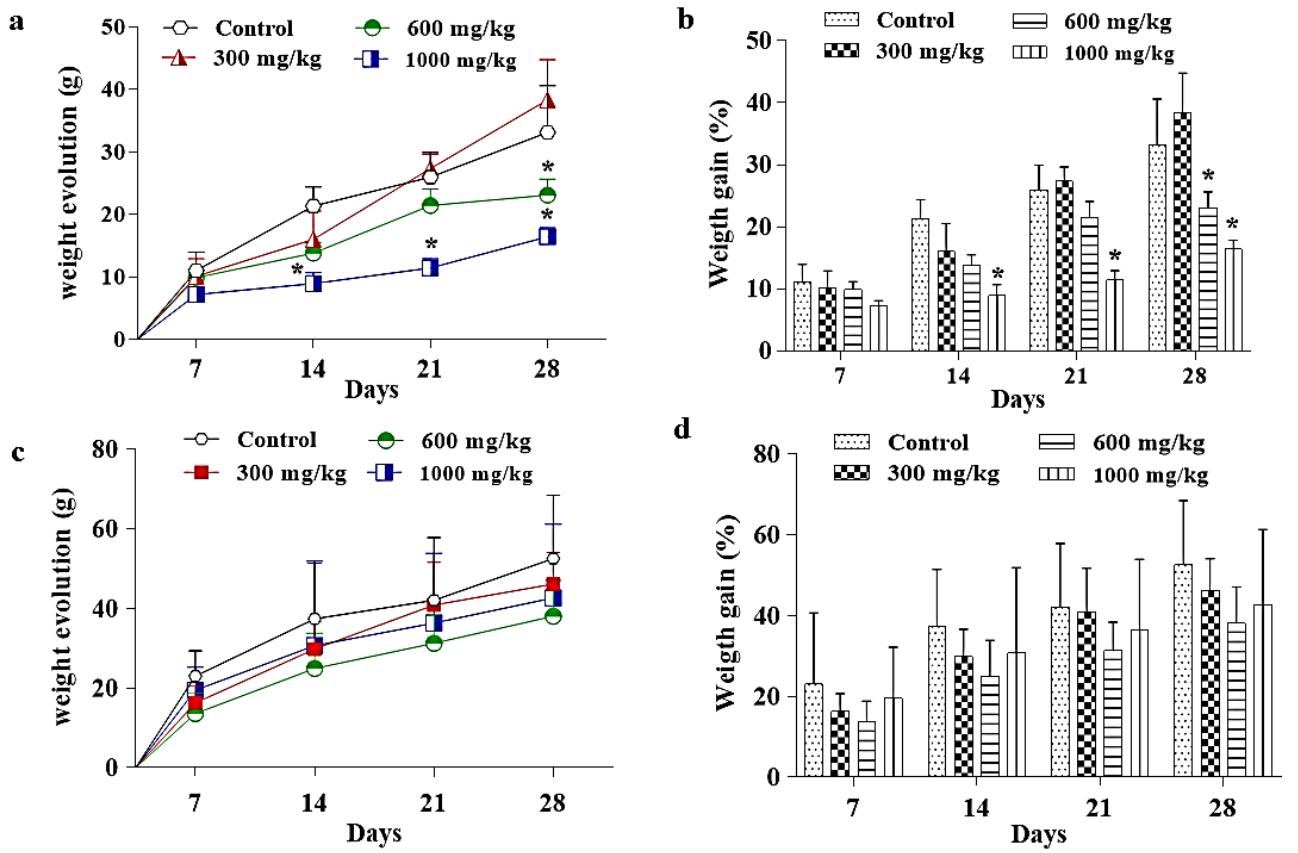

Figure 3: Changes in the body weights in males (a-b) and females (c-d) rat treated with ethanolic extract of *H. madagascariensis* stem bark in the subacute toxicity study. Values are expressed as mean ± SEM, n=6. (* p < 0,05 ; compared to the control group)

Relative organ weights

Data related to relative organ weights for both male and female rats treated with aqueous extract of *H. madagascariensis* are summarized in Table 3 and 4. Similarly, gross examination of internal organs of both the control and treated groups including, heart, liver, and kidneys, did not reveal any abnormal findings related to the administration of the extract ($p > 0.05$). However, ethanolic extract of *H. madagascariensis* treatments at the doses of 1000 mg/kg cause significant increase in relative heart weight of female rats for 28 days ($p < 0.05$). The mean absolute weights of the heart were 0.40 ± 0.02 and 0.53 ± 0.03 g/100 g body weight respectively in control group and female rats treated.

Table 3: Relative organs weight of female and male rats (g/100 g body weight) after 28 days of treatment with aqueous extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	Heart	Kidney	Liver	Lung
Female	Control	0.40 ± 0.02	0.58 ± 0.03	3.56 ± 0.21	0.85 ± 0.04
	EEHm_{300}	0.40 ± 0.01	0.59 ± 0.03	3.92 ± 0.19	0.95 ± 0.07
	EEHm_{600}	0.38 ± 0.03	0.59 ± 0.04	3.83 ± 0.39	1.06 ± 0.07
	EEHm_{1000}	0.45 ± 0.03	0.61 ± 0.03	3.59 ± 0.15	0.88 ± 0.07
Male	Control	0.31 ± 0.02	0.52 ± 0.05	3.81 ± 0.28	0.86 ± 0.06
	EEHm_{300}	0.39 ± 0.03	0.67 ± 0.11	4.37 ± 0.43	1.05 ± 0.17
	EEHm_{600}	0.40 ± 0.03	0.61 ± 0.05	4.56 ± 0.36	0.91 ± 0.16
	EEHm_{1000}	0.41 ± 0.01	0.62 ± 0.03	4.09 ± 0.15	0.81 ± 0.05

Each value represents the mean ± Standard deviation ; (n = 6); values are statistically no different from control at ( ) p > 0.05. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.

Table 4: Relative organs weight of female and male rats (g/100 g body weight) after 28 days of treatment with ethanolic extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	Heart	Kidney	Liver	Lung
Female	Témoin	0.40 ± 0.02	0.58 ± 0.03	3.56 ± 0.21	0.85 ± 0.04
	EEHm_{300}	0.41 ± 0.01	0.53 ± 0.02	3.20 ± 0.01	0.75 ± 0.03
	EEHm_{600}	0.41 ± 0.03	0.63 ± 0.01	3.61 ± 0.16	1.02 ± 0.09
	EEHm_{1000}	0.53 ± 0.03*****	0.53 ± 0.02	3.17 ± 0.05	1.00 ± 0.06
Male	Témoin	0.31 ± 0.02	0.52 ± 0.05	3.81 ± 0.28	0.86 ± 0.06
	EEHm_{300}	0.34 ±0.00	0.58 ± 0.06	4.36 ± 0.15	1.01 ± 0.01
	EEHm_{600}	0.35 ± 0.01	0.55 ± 0.02	3.98 ± 0.38	0.97 ± 0.09
	EEHm_{1000}	0.42 ± 0.01	0.61 ± 0.06	4.13 ± 0.26	0.86 ± 0.04

*Each value represents the mean ± Standard deviation ; (n = 6); values are statistically different or not from control at *p < 0.05 or( ) p > 0.05 ; One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

Biochemistry analysis

Liver parameters and lipid profile

Tables 5 and 6 summarize the levels or activities of liver parameters and lipid profile in female and male rats.

Administration of the aqueous extract of *H. madagascariensis* at 300, 600 and 1000 mg/kg doses, has non significant effect on levels of ALT, AST, TG, TC, and HDL-C in female groups of rats compared to the control groups (p > 0,05; Table 5). Hovewer, this treatment at the dose of 300 mg/kg, caused significant increase of AST level of 230 ± 11 to 379 ± 24 UI/L in male rats compared to the control groups (p < 0,05 Table 5).

Daily oral sub-acute treatment with *H. madagascariensis* ethanolic extract at 300, 600 and 1000 mg/kg doses, caused any significant effect on ALT, AST, TG and TC levels in both sex of rats compared to thoses of the control groups (p > 0,05; Table 6). However, the extract induced an decrease in HDL-C in female (of 0.27 ± 0.02 to 0.18 ± 0.01 g/L) and an increase in HDL-C in male (of 0.20 ± 0.02 to 0.29 ± 0.02g/L) rats from 1000 mg/kg (p < 0,05; p < 0,01 Table 6).

Table 5 : Biochimical liver parameters and lipid profile of female and male rats after 28 days of treatment with aqueous extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	ALAT (UI/L)	ASAT (UI/L)	T. CHOL (g/L)	C-HDL (g/L)	TG (g/L)
Female	Control	42.33 ± 4.70	223 ± 7	0.51 ± 0.04	0.28 ± 0.05	0.82 ± 0.16
	EAHm_{300}	44.67 ± 2.19	239 ± 16	0.44 ± 0.04	0.28 ± 0.04	0.78 ± 0.12
	EAHm_{600}	40.67 ± 4.26	219 ± 33	0.39 ± 0.04	0.27 ± 0.07	0.71 ± 0.07
	EAHm_{1000}	53.33 ± 6.36	263 ± 28	0.47 ± 0.04	0.24 ± 0.05	0.69 ± 0.07
Male	Témoin	46.67 ± 5.46	230 ± 11	0.44 ± 0.07	0.20 ± 0.02	0.76 ± 0.02
	EAHm_{300}	58.33 ± 7.00	379 ± 24*****	0.49 ± 0.06	0.27 ±0.04	0.59 ± 0.08
	EAHm_{600}	29.00 ± 4.73	237 ± 22	0.57 ± 0.05	0.29 ± 0.05	0.50 ± 0.09
	EAHm_{1000}	39.67 ± 4.10	241 ± 38	0.57 ± 0.05	0.31 ± 0.02	0.52 ± 0.05

*Each value represents the mean ± Standard deviation; (n = 6); values are statistically different or not from control at *p < 0.05 or( ) p > 0.05 ; One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

Table 6: Biochimical liver parameters and lipid profile of female and male rats after 28 days of treatment with ethanolic extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	ALAT (UI/L)	ASAT (UI/L)	T. CHOL (g/L)	C-HDL (g/L)	TG (g/L)
Female	Control	42.33 ± 4.70	223 ± 7	0.51 ± 0.04	0.28 ± 0.05	0.82 ± 0.16
	EEHm_{300}	32.33 ± 7.31	190 ± 12	0.46 ± 0.03	0.27 ± 0.02	0.75 ± 0.01
	EEHm_{600}	41.00 ± 2.08	223 ± 9	0.47 ± 0.01	0.26 ± 0.01	0.72 ± 0.06
	EEHm_{1000}	35.33 ± 2.03	204 ± 12	0.44 ± 0.05	0.18 ± 0.01******	0.64 ± 0.09
Male	Témoin	46.67 ± 5.46	230 ± 11	0.44 ± 0.07	0.20 ± 0.02	0.76 ± 0.02
	EEHm_{300}	26.00 ± 5.03	163 ± 20	0.40 ± 0.04	0.21 ± 0.01	0.45 ± 0.12
	EEHm_{600}	34.67 ± 6.01	210 ± 34	0.47 ± 0.09	0.25 ± 0.01	0.52 ± 0.06
	EEHm_{1000}	52.00 ± 9.24	282 ± 30	0.57 ± 0.05	0.29 ± 0.02*****	0.88 ± 0.05

*Each value represents the mean ± Standard deviation ; (n = 6); values are statistically different or not from control at *p < 0.05 or( ) p > 0.05 ; One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

Renal parameters and electrolytes level

Tables 7 and 8 summarize the levels or activities of renal parameters and electrolytes level in female and male rats.

Daily oral treatment with aqueous extract of *H. madagascariensis* stem bark at 300, 600 and 1000 mg/kg doses, for 28 days has non significant effect on renal parameters (urea and creatinine) and, electrolytes (Na^+, K^+ and Cl^-) level in female rats compare to untreated group ($p > 0.05$; Table 7). However, this treatment increased significantly the levels of creatinine at 1000 mg/kg dose, and electrolytes (Na^+ and Cl^-) level at doses 600 mg/kg and 1000 mg/L in the male rats compare to untreated group ($p < 0.05$; Table 7).

Ethanolic extract of *H. madagascariensis* treatments at the doses of 300, 600 and 1000 mg/kg cause significant decrease in urea level of female rats for 28 days ($p < 0.05$; Table 8). The mean of this parameters were 0.38 ± 0.04, 0.26 ± 0.01, 0.25 ± 0.03 and 0.19 ± 0.01 g/L respectively in control group and, 300, 600 and 1000 mg/kg treated rats ($p < 0,05$;). This treatments, did not affected the urea level of male rats, and creatinine levels in female and male for 28 days ($p > 0.05$). However, ethanolic extract cause significant increase of Na^+ level (139 ± 1.20 mEq/L vs 145 ± 0.88 mEq/L) at the dose of 300 mg/kg and decreased K^+ level (5.33 ± 0.19 mEq/L vs 4.80 ± 0.06 mEq/L) at the dose of 1000 mg/kg in female rats for 28 days ($p < 0.05$; Table 8).

In male treated rats, *H. madagascariensis* ethanolic extract has favorized the increasing of K^+ level at the dose of 1000 mg/kg and Cl^- levels at doses of 300, 600 and 1000 mg/kg treated rats ($p < 0.05$; $p < 0.01$; Table 8).

Table 7: Biochimical renal parameters and electrolytes levels of female and male rats after 28 days of treatment with aqueous extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	Urea (g/L)	Creatinine (mg/L)	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)
Female	Control	0.38 ± 0.04	4.00 ± 0.57	139 ± 1.20	5.33 ± 0.19	98.7 ± 0.33
	EAHm_{300}	0.39 ± 0.01	4.67 ± 0.67	142 ± 3.71	4.63 ± 0.22	104.0 ± 2.08
	EAHm_{600}	0.42 ± 0.06	4.33 ± 0.33	140 ± 2.52	4.87 ± 0.24	102.3 ± 3.33
	EAHm_{1000}	0.46 ± 0.03	6.00 ± 0.57	140 ± 1.00	5.10 ± 0.40	103.0 ± 1.73
Male	Témoin	0.37 ± 0.04	3.00 ± 0.57	138 ± 1.16	5.13 ± 0.45	99.0 ± 0.67
	EAHm_{300}	0.33 ±0.06	4.00 ± 0.57	137 ± 0.67	4.47± 0.35	99.33 ± 0.67
	EAHm_{600}	0.43 ± 0.02	5.00 ± 0.57	148 ± 1.00*****	4.40 ± 0.31	107.7 ± 1.20******
	EAHm_{1000}	0.46 ± 0.11	6.67 ± 0.67*****	147 ± 3.18*****	5.73 ± 0.15	104.3 ± 1.20*****

*Each value represents the mean ± Standard deviation; (n = 6); values are statistically different or not from control at *p < 0.05 or( ) p > 0.05 ; One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

Table 8: Biochimical renal parameters and electrolytes levels of female and male rats after 28 days of treatment with ethanolic extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	Urea (g/L)	Creatinine (mg/L)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
Female	Control	0.38 ± 0.04	4.00 ± 0.57	139 ± 1.20	5.33 ± 0.19	98.7 ± 0.33
	EEHm_{300}	0.26 ± 0.01*****	4.33 ± 0.33	145 ± 0.88******	5.00 ± 0.12	103.3 ± 1.45
	EEHm_{600}	0.25 ± 0.03*****	3.67 ± 0.33	141 ± 0.57	4.90 ± 0.06	101.3 ± 0.88
	EEHm_{1000}	0.19 ± 0.01*****	3.00 ± 0.57	140 ± 0.88	4.80 ± 0.06*****	102.0 ± 1.15
Male	Témoin	0.37 ± 0.04	3.00 ± 0.57	138 ± 1.16	5.13 ± 0.45	99.0 ± 0.67
	EEHm_{300}	0.33 ± 0.06	2.67 ± 0.33	145 ± 2.08	4.63 ± 0.09	105.3 ± 1.20*****
	EEHm_{600}	0.25 ± 0.02	3.67 ± 0.33	146 ± 4.04	5.77 ± 0.18	106.3 ± 1.20******
	EEHm_{1000}	0.29 ± 0.04	4.00 ± 0.00	142 ± 1.20	6.37 ± 0.09*****	105.0 ± 1.00*****

*Each value represents the mean ± Standard deviation; (n = 6); values are statistically different or not from control at *p < 0.05 or( ) p > 0.05 ; One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

Hematological study

The effect of aqueous and ethanolic *H. madagascariensis* extracts on hematological indices was examined at the end of treatment (Table 9; Table 10).

Treatment for 28 days has non significant effect on WBC, RBC, platelet count, hemoglobin and hematocrit in female treated rats ($p > 0.05$; Table 9). Administration of aqueous extract of *H. madagascariensis* increase WBC counts only in male rats compare to untreated group ($p < 0.05$; Table 9). The WBC counts means are 6.67 ± 1.24 ; 10.60 ± 0.35; 10.80 ± 0.21 ; 11.32 ± 1.06 (x 10/mm^3) respectively for control group and treated rats at doses of 300, 600 and 1000 mg/kg

Furthermore, treatment with ethanolic extract increased significantly the levels of platelets of 1013 ± 10 to 1361 ± 122 (x 10^3cells/mm^3) at dose of 1000 mg/kg

in the male rats compare to untreated group ($p < 0.05$; Table 10).

Table 9: Hematological parameters of female and male rats after 28 days of treatment with aqueous extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	RBC ($10/mm^3$)	WBC ($10/mm^3$)	Hematocrit (%)	Platelets (10^3cells/mm^3)	Hemoglobin (g/dL)
Female	Control	7.47 ± 0.26	9.77 ± 2.46	40.70 ± 1.29	805 ± 79	13.00 ± 0.45
	EEHm_{300}	7.39 ± 0.25	10.66 ± 1.84	39.83 ± 1.34	871 ± 86	12.90 ± 0.36
	EEHm_{600}	6.70 ± 0.81	9.60 ± 1.59	38.27 ± 2.47	842 ± 104	11.96 ± 0.94
	EEHm_{1000}	7.52 ± 0.25	10.32 ± 1.67	40.73 ± 1.64	928 ± 37	13.00 ± 0.50
Male	Control	7.35 ± 0.43	6.67 ± 1.24	39.97 ± 2.01	1013 ± 10	12.47 ± 0.53
	EEHm_{300}	7.06 ± 0.28	10.60 ± 0.35*****	38.20 ± 2.41	1130 ± 137	12.17 ± 0.60
	EEHm_{600}	6.91 ± 0.38	10.80 ± 0.21*****	39.37 ± 2.41	1031 ± 41	13.17 ± 0.64
	EEHm_{1000}	7.96 ± 0.15	11.32 ± 1.06*****	43.23 ± 0.53	1271 ± 95	13.67 ± 0.07

*Each value represents the mean ± Standard deviation ; (n = 6); values are statistically different or not from control at *p < 0.05 or( ) p > 0.05 ; One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

Table 10: Hematological parameters of female and male rats after 28 days of treatment with ethanolic extract of *H. madagascariensis* stem bark in the subacute toxicity study

	Groups	RBC ($10/mm^3$)	WBC ($10/mm^3$)	Hematocrit (%)	Platelets (10^3cells/mm^3)	Hemoglobin (g/dL)
Female	Control	7.47 ± 0.26	9.77 ± 2.46	40.70 ± 1.29	805 ± 79	13.00 ± 0.45
	EEHm_{300}	7.79 ± 0.10	11.97 ± 1.50	41.27 ± 0.43	962 ± 32	13.10 ± 0.06
	EEHm_{600}	6.93 ± 0.13	11.45 ± 0.37	35.67 ± 2.39	848 ± 144	11.40 ± 0.61
	EEHm_{1000}	7.36 ± 0.37	10.97 ± 1.26	41.00 ± 1.21	858 ± 6	13.17 ± 0.44
Male	Control	7.35 ± 0.43	6.67 ± 1.24	39.97 ± 2.01	1013 ± 10	12.47 ± 0.53
	EEHm_{300}	7.40 ± 0.15	6.38 ± 0.54	39.47 ± 0.47	1006 ± 61	12.43 ± 0.12
	EEHm_{600}	7.16 ± 0.04	8.00 ± 0.57	40.20 ± 0.47	997 ± 41	12.47 ± 0.12
	EEHm_{1000}	7.85 ± 0.09	7.02 ± 1.19	42.50 ± 0.23	1361 ± 122*****	13.57± 0.23

*Each value represents the mean ± Standard deviation; (n = 6); values are statistically different or not from control at *p < 0.05 or ( ) p > 0.05; One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

DISCUSSION

Medicinal plants are being used since centuries to treat different diseases and contain many metabolites (Ridtitid, 2008; Teoh, 2016). These metabolites have interressant biological activities but they can also be related to toxic effects. For example, the phenolic compounds can be hematotoxic and hepatotoxic, and may provoke mutagenesis and carcinogenesis (Michalowicz and Duda, 2007). Therefore, the evaluation of *H. madagascariensis* toxicity is indispensable. Hence, the present study was conducted to assess its toxicological profile by performing acute oral toxicity in female rats for 14 days and sub-acute oral toxicity in rats of both sexe for 28 days.

Aqueous or ethanolic extract of *H. madagascariensis* at a dose of 1000; 2000 and 4000 mg/kg oral treatment with only dose induced no abnormal signs of toxicity in behavioral patterns and mortality in treated group. All substances with LD_{50} > 2000 at 5000 mg/kg are categorised as unclassified or category 5 according to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, (OECD, 2008). This suggests that the oral LD_{50} of the plant being greater than 5000 mg/kg day be safe. Kanga *et al.* (2019) showed, the single oral doses of 1000, 2000 and 5000 mg/kg of aqueous extract of *Piper umbellatum* leaves was unable to induce mortality or toxic effects in wistar rats.

During the subacute toxicity study, ethanolic extract of *H. madagascariensis* treatments at the dose of 1000 mg/kg cause significant increase in relative heart weight of female rats for 28 days. The increase in relative organ weight could be caused by the induction of xenobiotic enzymes leading to increased proteins synthesis (Ijeh and Chukwunonso, 2006).

The ethanolic extract of *H. madagascariensis* at dose of 1000 mg/kg, induced an decrease in HDL-C in female and an increase in HDL-C in male rats. The present study data are partialy in agreement with findings of Etame *et al.* (2017) who showed that administration of methanol extract of *H. madagascariensis* in sub-acute treatment, decreased HDL cholesterol concentrations from 200 mg/kg in animals of both sexes. These findings indicate, extract provoke disturbance of lipid metabolism and could therefore have hyperlipidemic property at high-dose. This could lead to heart disease risk, and can promotes the development of fatty deposits and atheroma on the arteries leading to cardiovascular disease (Hewing and *al.*, 2012).

Daily oral treatment with aqueous extract of *H. madagascariensis* increased significantly the levels of creatinine at 1000 mg/kg dose, and electrolytes (Na^+ and Cl^-) level at doses 600 mg/kg and 1000 mg/L in the male rats. Creatinine clearance, an indicator of glomerular filtration rate is used for assessing kidney function. An increase in blood creatinine level may indicate kidney dysfunction. Any medication that interferes with the normal actions of the kidneys can lead to elevations in blood creatinine (Ezeugwunne *et al.*, 2017). The changes in this parameters has not observed in the female rats. This may be because of hormonal differences in the animals.

The ethanolic extract at the doses of 300, 600 and 1000 mg/kg cause significant decrease in urea only in female rats and an important disturbance of electrolytes on both sexes. These decrease in the concentration of urea in the treatment group show that the ethanol extract *H. madagascariensis* did not affect the renal function of the rats. If the kidneys were damaged, urea would accumulate in blood and increased urea in plasma indicated kidney failure in carrying out its filtration function (Guyton and Hall, 2006). However, these protective effects must be considered carefully, because the electrolytes appear to be disturbed.

According, Mukinda and Syce, (2007) and Jain *et al.*, (2009) the hematological parameters are sensitive markers of the physiological changes in response to any environmental pollutant or toxic stress, and an important index of physiological and pathological state, both in man and in animal. The aqueous extract of *H. madagascariensis* increase WBC counts only in male rats and treatment with ethanolic extract increased significantly the levels of platelets. So, Etame *et al.*, (2017) showed that *H. madagascariensis* methanolic extract resulted in animals of both sexes an increase in white blood cell counts at 200 mg/kg and decrease of platelets. This study supports the potential immunomodulatory role of *H. madagascariensis* methanolic extract (Razak *et al.*, 2021). Moreover, the data showed remarkable elevated levels of platelet count indicating hemostatic activity of ethanolic extract of *H. madagascariensis* (Li *et al.*, 2008). In opposition to the results of Etame *et al.* (2017). Difference certainly related to the types of extract. Blood platelets have a vital role in the process of blood coagulation. Increased coagulation is associated with several cardiovascular diseases (Mekhfi *et al.*, 2004). While decreased coagulation leads to prolonged bleeding time (Vane and Botting, 2003). Nevertheless, these immunostimulatory and hemostatics properties could be very useful in the fight against microbial infection.

CONCLUSION

The oral LD_{50} of aqueous extract of *H. madagascariensis* has been shown to be greater than 5000 mg/kg and is generally considered safe. In long term admistration, *H. madagascariensis* has caused reduction of body weight, increase of heart weight and modulate lipid profile. It also, disturbanced kidney and electrolyte homeostasis, and shown immunostimulatory and hemostatics properties.

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