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ACUTE AND SUB-ACUTE TOXICITY OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF THE RECIPE FOR THE LEAVES OF *ERIOSEMA ERICI*-ROSENII RE FR. AND *NEOBOUTONIA MELLERI* MÜLL.ARG. PLAIN

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SUMMARY

Eriosima erici and Neoboutonia melleri are two plants used in the form of a recipe in traditional Congolese medicine to treat various pathologies. However, the use of this recipe does not guarantee its harmlessness. It is in this perspective that we estimated the acute and subacute toxicities of the aqueous extracts and the hydroethanolic of the recipe according to the protocols of the OECD n° 425 and 407. The effects of these extracts were evaluated on the general behavior, mortality, weight change as well as haematological and biochemical parameters. Concerning the acute toxicity, the administration of a single dose of 5000 mg/Kg, with the hydroethanolic extract led to a reduction in mobility and polyuria from the first hour following administration with a significant reduction in weight. However, no mortality was observed. The LD50 is estimated to be greater than 5000 mg/Kg. Regarding subacute toxicity, daily administration of a dose of 600 mg/kg of the two extracts for 28 days to rats was well tolerated. However, there are some changes in certain hematopoietic (VGM and white blood

cells) and biochemical (AST, ALT, urea, uric acid and tryglycerides) parameters with reversibility of the effects observed with the aqueous extract after stopping treatment.

KEYWORDS: acute toxicity, subacute toxicity, recipe, *Eriosema erici* and *Neoboutonia melleri.*

INTRODUCTION

Traditional medicine is a major source of cultural heritage in Africa to which the population remains attached in terms of primary health care.[1] The WHO estimates that 80 % of the African population uses traditional medicine for their health needs.[2] If the pharmacological effects of many plants have been proven in several laboratories, their toxicity seems to be unknown. However, for a drug with pharmacological effects to possibly be used as a medicine, its activity must first appear at doses for which the toxicity is negligible.[3] Because its absorption can have the effect of disrupting the functioning of the body, causing physiological disorders that can go as far as the death of exposed individuals. Thus, toxicity tests must accompany biological activity tests during the selection of these new molecules. Therefore, the evaluation of the toxicity of herbal preparations is important to determine the margin of safety of these remedies.[4] Eriosema erici and Neoboutonia mulleri are two plants used in traditional Congolese medicine separately, or in recipes to treat various diseases, including epilepsy, inflammation, stomach aches... On the other hand, if many pharmacological studies are carried out on euphorbiaceae and fabaceae in general, those on the genus *Ereosima* and *Néoboutonia* are rather rare. Nevertheless, the acute toxicity, hepathoprotective and antioxidant effect of *Neoboutonia* bark have been evaluated.[5] To this end, estimating the safety margin of the recipe based on the leaves of *E. erici* and *N. mulleri* by carrying out acute and subacute toxicity studies would be an asset for the populations who use it because it has been reported that several Studies of traditional herbal treatments have reported toxicity issues.[6]

MATERIAL AND METHODS

Plant material

The plant material consisted of the leaves of Eriosema erici and Neoboutonia mülleri collected in Mossendjo in the Department of Niari (Republic of Congo) in September 2020. Botanical identification of the plant material was done by Mousamboté, botanist systematistof Higher Normal School of Agronomy and Forestry (HNSAF) and confirmed at the Herbarium of the National Institute for Research in Exact and Natural Sciences

(NIRENS) witch a collected sample was compared to a reference sample number 5640 and 1900 respectively for *E. erici* and *N. melleri*. After harvest, these leaves were dried separately at the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE) for two weeks at room temperature (26 ± 1°C). After drying, they were pulverized separately using a mortar. The powders obtained were mixed to prepare the aqueous extract and the hydroethanolic extract of the recipe. The aqueous extract of the recipe was prepared by decoction. 12.5 g of *Eriosema erici* powder and 12.5 g of *Neoboutonia melleri* powder were mixed with 500 mL of distilled water in a heating balloon and boiled for 15 minutes. After cooling and filtration, the aqueous decoction obtained was evaporated at a temperature of 55°C using an evaporator (Thermosi SR 1000). The hydroethanolic extract of the recipe was prepared by maceration. 12.5 g of *Eriosema erici* powder from and 12.5 g of powder from *Neoboutonia Melleri* were mixed with 500 ml of the 50 % hydroethanolic solution. The mixture was left for maceration with magnetic agitator for 48 hours. After filtration, the hydroethanolic maceration was evaporated at a temperature of 55°C using an evaporator (Thermosi SR 1000). The aqueous and hydroethanolic extracts of the recipe obtained was kept to assess acute and subacute toxicity.

Animals materiel

Albino rats (100 - 150 g) and albino mices (25 - 30 g) of either sex these three-month-old obtained from the Faculty of Science and Technical of Marien NGOUABI-University were used. They were fed with a standard feed and water ad libitum. They were acclimatized during one week before experimentation and were housed under standard conditions (12 hours light and 12 hours dark) and at the temperature of 27˚C ± 1˚C. The rules of ethics published by the International Association for the Study of Pain[7] have been considered.

Evaluation of acute toxicity of recipe extracts in mice

The acute toxicity of the aqueous extract and hydroethanolic extracts of was evaluated according to OECD 2002 no. 423 guideline.[8] Mice were fasted for 24 h before the treatment and then divided into three groups of three mice each and treated with different doses of distilled water (negative control, 5 ml/kg), aqueous extract (5000 mg/kg) and hydroethanolic extract (5000 mg/kg). After oral administration, macroscopic observations including the general behavior such as mobility, ptosis, alertness, piloerection, aggressiveness, stool status, vomiting was evaluated at five hours. Mortality was evaluated in 48 h and the weight evolution was noted every 2 days for 14 days.

Evaluation of subacute toxicity of recipe extracts in rat

The subacute toxicity study was carried out according to OECD 2008 no. 407 guideline.[9] 5 groups of 5 rats each were made up. Before the treatment, the blood of the animals of the different groups was taken from the retro-orbital vein to evaluate the hematological and biochemical parameters. After that, the animals were treated orally every morning for 28 days with different doses of distilled water (control group, 5 mL/kg), aqueous extract of the recipe (satelite group, 600 mg/ kg), the aqueous extract of the recipe (additional group, 600 mg/kg), the hydroethanolic extract (satelite group, 600 mg/kg) and the hydroethanolic extract (additional group, 600 mg/kg). 28 days after treatment, the animals of the control groups and the satellite groups for the two extracts were anesthetized with diethyl ether and their blood was taken from the retro orbital vein as before for the analyses. They were then sacrificed after anesthesia with diethyl ether. Noble organs such as the heart, liver, spleen and kidneys were removed for macroscopic observations (color and shape) and weight assessment. Finally, to see the persistence or reversibility of the toxic effects, the animals of the additional groups respectively for the aqueous and hydroethanolic extract were observed 14 days after stopping the treatment. The same analyzes were carried out after these 14 days of observation.

Biochemical and hematological analyzes

Blood samples were taken by puncture at the level of the retro orbital sinus of rats previously anesthetized with diethyl ether. The blood sample is collected in two tubes. The first tube containing EDTA was used for hematological analyzes using an automaton (Yumizen H550 type). These analyzes focused on the number of red blood cells, white blood cells, platelets, etc.). The second dry tube was centrifuged using a brand centrifuge (TD4A-WS DEESK) at 3000 rpm for 15 minutes. The serum obtained is stored at -20°C for the analyzes of the biochemical parameters (ASAT, ALAT, LDH, LDL, Creatinine, etc.). Measurements are made at a characteristic wavelength for each assay. The assay of the analyzed parameters is accomplished by special ready-to-use kits using a spectrophotometer (Sinothinker type).

Statistical Analysis

The Excel 2016 software was used to process the data. All values were expressed as mean ± standard error of mean (SEM). Analysis of variance followed by Student-Fischer t test “t” was performed. The significance level was set at $p < 0.05$.

RESULTS

Acute toxicity

After administration of a high dose of the two extracts to the animals, the results obtained show no change in the general behavior of the mice was observed in the groups treated with the aqueous extract of the recipe at a dose of 5000 mg/kg. However, a change in mobility and polyuria were observed in animals treated with the hydroethanolic extract of the recipe compared to control group treated with distilled water. Also, no mortality was recorded during the duration of the experiment. Moreover, a significant reduction in the relative weight ($p<0.05$) of the mice treated with the hydroethanolic extract on days 2 is noted compared to the control with respective values of 107.37 g and 124.99 g (figure 1). On the other hand, no significant difference in weight ($p > 0.05$) was observed for mice treated with the aqueous extract of the recipe for 14 days compared to the control (figure 1).

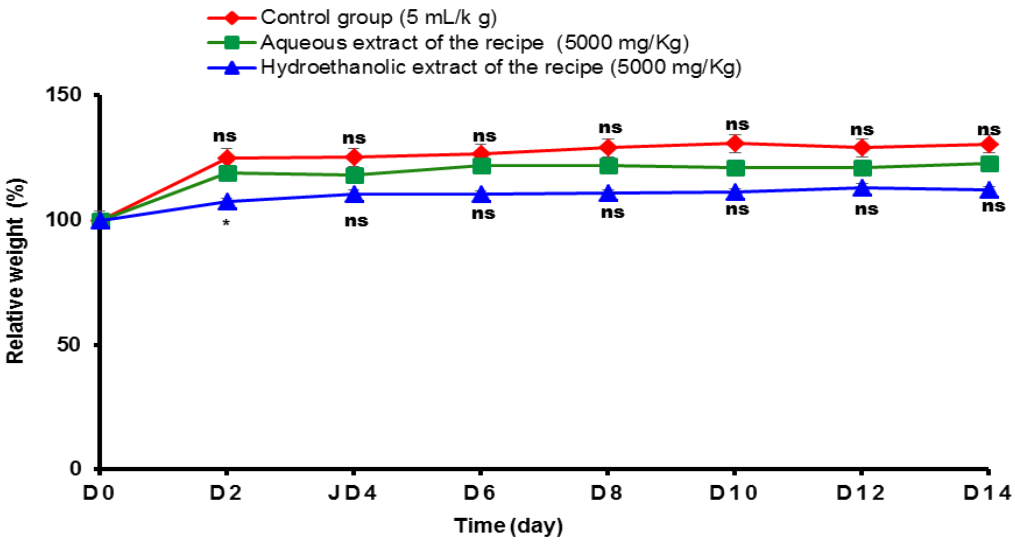

Figure 1: Effect of the aqueous extracts and the hydroethanolic of the recipe on the evolution of relative weight in swiss mice. Each value represents the mean ± ESM. *p < 0.05; Significant different (Student t-test) versus control; ns (p>0.05; no signicant different (Student t-test) versus control.

Subacute toxicity

The evolution of the relative weight of the rats during the 28 days of the subacute toxicity study is shown in Figure 2. It emerges from this figure that the rats treated with the hydroethanolic extract of the recipe at a dose of 600 mg /kg show a significant weight gain ($p < 0.05$) from the second week to the fourth week compared to the control group. The relative

weight of the rats treated with the hydroethanolic extract at the second week is 210.42; 221.28 for W3 and 232.70 % for W4. On the other hand, they are 195.96, 203.86 and 216.22 for the control group respectively at W2, W3 and W4. Moreover, during the 28 days of experimentation, no significant increase in weight ($p > 0.05$) was observed for the rats treated with the aqueous extract of the recipe at the same dose compared to the control. Also, during the 28 days of experimentation, no mortality was recorded.

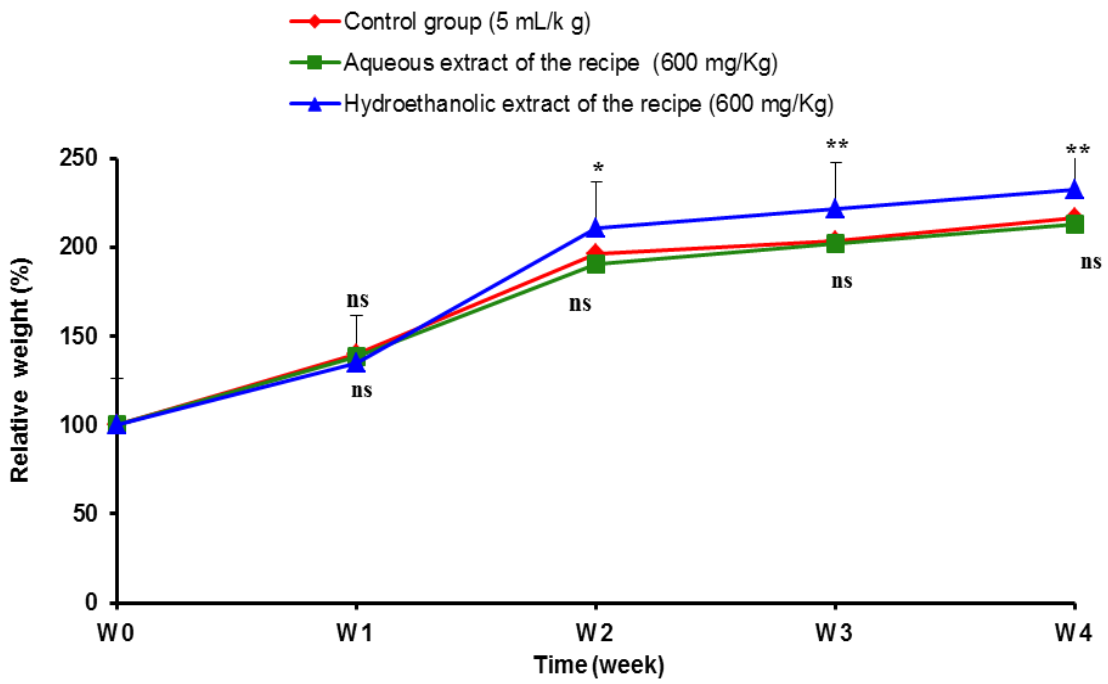

Figure 1: Effect of the aqueous extracts and the hydroethanolic of the recipe on the evolution of relative weight after 28 days of treatment in rat. Each value represents the mean ± ESM. *p < 0.05; **p<0.01 Significant different (Student t-test) versus control; ns (p>0.05; no signicant different (Student t-test) versus control.

Effect of the aqueous and hydroethanolic extracts of the recipe on the weight of the noble organs

Table 1 presents the weights of the organs taken from the animals. the results show that after 28 days of treatment, the aqueous extract of the recipe at a dose of 600 mg/kg causes a significant increase ($p < 0.05$) in the weight of the liver of the rats compared to the control rats. 14 days after discontinuation of treatment, there is a persistence of the increase in the liver ($p < 0.05$) to which is added a significant increase in the spleen ($p < 0.01$) compared to the control. Concerning the hydroethanolic extract, we also note a significant increase (p

<0.01) in the liver after 28 days of treatment compared to the control animals. Nevertheless, two weeks after stopping treatment, no change in the organs analyzed (liver, spleen, heart and kidneys) was observed in comparison with the control group. The weight of the liver goes from 6.03±0.017 to 5.75±0.056 respectively during and after discontinuation of treatment. Also, the macroscopic observations showed no variation in texture and coloring of the organs of the animals treated with the type of extracts of the recipe compared to the control group.

Table 1: Effect of the aqueous and the hydroethanolic extracts of the recipe on the weight of the noble organs.

Organs	Treatment (28 days)			14 days after treatment	
	Control (5mL/Kg)	Aqueous extract (600 mg/Kg)	Hydroethanolic extract (600 mg/Kg)	Aqueous extract (600 mg/Kg)	Hydroethanolic extract (600 mg/Kg)
Heart	0.78±0.026	0.807±0.052 ^{ns}	0.808±0.043 ^{ns}	0.8005±0.044 ^{ns}	0.832±0.041 ^{ns}
liver	5.67±0.132	6.07±0.099*	6.03±0.017*	6.4±0.001*	5.75±0.056 ^{ns}
spleen	0.584±0.013	0.615±0.037 ^{ns}	0.577±0.013 ^{ns}	0.695±0.002**	0.554±0.022 ^{ns}
Right kidney	0.545±0.094	0.595±0.044 ^{ns}	0.551±0.005 ^{ns}	0.575±0.009 ^{ns}	0.563±0.039 ^{ns}
Left kidney	0.339±0.022	0.385±0.022 ^{ns}	0.390±0.045 ^{ns}	0.342±0.019 ^{ns}	0.324±0.011 ^{ns}

Each value represents the mean ± ESM. *p < 0.05; **p<0.01 Significant different (Student t-test) versus control group; ns (p>0.05; no signicant different (Student t-test) versus control group.

Effect of aqueous and hydroethanolic extracts of recipe on hematological parameters in rats

The effect of the aqueous and hydroethanolic extracts of the recipe from the leaves of *E. erici* and *N. melleri* on the hematological parameters of the rats is presented in Table 2. After 28 days of treatment, a significant increase (p <0.001) of the mean corpuscular volume (MCV) is observed for the rats treated with the aqueous and hydroethanolic extracts of the recipe compared to D0, with respective values of 49.49±0.6 and 50.17±0.25 µm3. Added to this is an increase in the number of white blood cells (p <0.01) for the two extracts of the recipe compared to D0. Fourteen (14) days after discontinuation of treatment, there is a persistence of the increase in MCV (p <0.001) for the two extracts of the recipe. On the other hand, the number of white blood cells decreased insignificantly (p >0.05) for the aqueous and hydroethanolic extracts of the recipe compared to at D0. These values go from 9.71±0.45 and 7.98±0.08 103/µL for the aqueous extract and 9.44±0.64 and 7.61±03103/µL for the hydroethanolic extract.

Table 2: Effect of aqueous extracts and the hydroethanolic of the recipe on hematological parameters in wistar rats.

Parameters	Treatement							
	Control group (5mL/kg)		Aqueous extract of recipe recette (600 mg/Kg)			Hydroethanolic extract of recipe (600 mg/Kg)		
	D0	D28	D0	D28	D14	D0	D28	D14
RBC (10^6/µL)	8.74±0.16	8.09±0.25^{ns}	8.31±0.24	7.89±0.30^{ns}	7.26±0.02^{ns}	8.34±0.23	7.97±0.20^{ns}	8.01±0.11^{ns}
HB (g/dL)	15.23±0.23	15.06±0.77^{ns}	14.70±0.42	14.35±0.46^{ns}	14.1±0.76^{ns}	14.76±0.36	14.93±0.47^{ns}	14.76±0.37^{ns}
Ht (%)	40.06±0.84	40.05±0.79^{ns}	39.06±0.45	41.40±0.74^{ns}	40.11±0.74^{ns}	39.51±0.99	40.58±0.62^{ns}	41.37±0.6^{ns}
MCV (µm^3)	46.13±0.39	48.5-±0.78^{ns}	46.98±0.46	49.49±0.6***	50.16±0.34***	47.30±0.36	50.17±0.25***	51.63±0.08***
Plq (10^3/µL)	1036.27±7.38	931.53±5.93^{ns}	987.76±19.9	998.30±18.69^{ns}	974.59±6.55^{ns}	1024.03±65.51	904.77±42.30^{ns}	1098.02±54.7^{ns}
WBC (10^3/µL)	7.06±0.01	7.13±1.11^{ns}	7.80±0.67	9.71±0.45**	7.98±0.08^{ns}	7.54±1.11	9.44±0.64**	7.61±03^{ns}

Each value represents the mean ± ESM. **p < 0.01; ***p<0.001 Significant different (Student t-test) versus D0; ns (p>0.05 ; no signicant different (Student t-test) versus D0 ; RBC: red blood cell; WBC: white blood cell; HB: hemoglobin; Ht: hematocrit; MCV: mean corpuscular volume; Plq: wafer.

Effect of aqueous and hydroethanolic extracts of the recipe on the biochemical parameters in rats

The results of the effect of the aqueous and hydroethanolic extracts of the recipe for the leaves of *E. erici* and *N. melleri* at a single dose of 600 mg/kg on the biochemical parameters of the rats are presented in Tables 3 and 4. These results show for hepatic biomarkers a significant increase ($p<0.01$) in transaminases (AST and ALT) for the hydroethanolic extract during 28 days of treatment compared to the initial day (table 3). On the other hand, after 14 days of discontinuation of treatment, a reversibility of the concentration of transaminases is noted. Also, the aqueous extract of the recipe showed a significant increase ($p <0.05$) in ALT during the 28 days of treatment. 14 days after discontinuation of treatment, there is a persistence of this increase compared to D0. These values are 39.25±0.403 (J0); 41.91±0.682 (D28) and 42.62±0.554 (D14 after stopping treatment). With regard to the lipid balance (Table 3), the aqueous extract of the recipe (600 mg/kg) showed no modification of the parameters lipids compared to D0. On the other hand, the hydroethanolic extract of the recipe showed a significant increase in triglycerides ($p <0.05$) and HDL ($p <0.01$) during the 28 days of treatment compared to D0. 14 days after stopping the treatment, there is a reversibility of the concentration of these two parameters which goes from 1.71±0.184 to 0.985±0.030 mg/L for triglycerides and 1.06±0.014 to 0.93±0.043 mg/L for HDL respectively at Day 28 and Day 14 after treatment discontinuation. Concerning the myocardial parameters (CK-MB, LDH and Trp Ic), no significant difference ($p >0.05$) was observed during 28 days of treatment then 14 days after treatment discontinuation for two extracts from the recipe compared to the initial day (Table 4).Assessment of biomarkers kidneys showed some changes (Table 4). For the aqueous extract of the recipe at a dose of 600 mg/kg, there is an increase in the protein concentration ($p <0.01$) 28 days after treatment compared to D0. These values are 56.41±0.231 (D0) and 61.52±0.210 (D28). However, 14 days after discontinuation of treatment, reversibility of this effect is noted. Furthermore, treatment with the hydroethanolic extract for 28 days caused a significant decrease in the concentration of uric acid ($p <0.01$) and urea ($p <0.001$) compared to the initial day (D0). These values decrease from 38.56±0.611 to 32.29±0.596 mg/L and 0.41±0.049 to 0.35±0.00 mg/L respectively for uric acid and urea. After discontinuation of treatment followed by a rest period of 14 days, there is a reversibility of these concentrations which return to normal.

Table 3: Effect of the aqueous extracts and the hydroethanolic of the recipe on the hepatic and lipid biomarkers of the different groups of rats.

Organs	Parameters	Treatment							
		28 days (four weeks)						14 days after treatment (two weeks off)	
		Distilled water (5mL/kg)		Aqueous extract (600 mg/Kg)		Hydroethanolic extract (600 mg/Kg)		Aqueous extract (600 mg/Kg)	Hydroethanolic extract (600 mg/Kg)
		D0	D28	D0	D28	D0	D28	D14	D14
Liver	Glycemia (g/L)	1.15±0.13	0.98±0.09^{ns}	1.00±0.34^{ns}	0.91±0.01^{ns}	0.98 ±0.01^{ns}	0.91 ±0.04^{ns}	0.90 ±0.02^{ns}	0,89 ±0,03^{ns}
	ASAT (UI/L)	66.81±0.452	66.74±0.343^{ns}	66.6±0.802^{ns}	65.91±0.180^{ns}	65.87±0.235^{ns}	67.46±0.272**	64.33±0.257^{ns}	65,80±0,785^{ns}
	ALAT (UI/L)	39.33±0.421	39.86±0.326^{ns}	39.25±0.403^{ns}	41.91±0.682*	39.10±0.318^{ns}	41.66±0.954*	42.62±0.554*	38,96±0,170^{ns}
	DB (mg/mL)	0.81±0.025	0.79±0.014^{ns}	0.81±0.197^{ns}	0.80±0.028^{ns}	0.83±0.048^{ns}	0.80±0.072^{ns}	0.81±0.003^{ns}	0,82±0,082^{ns}
	BT (mg/mL)	0.55±0.030	0.56±0.010^{ns}	0.55±0.059^{ns}	0.56±0.053^{ns}	0.56±0.015^{ns}	0.57±0.007^{ns}	0.56±0.022^{ns}	0,56±0,006^{ns}
	Trigl (g/L)	1.02±0.102	1.14±0.03^{ns}	0.89±0.021^{ns}	1.06±0.119^{ns}	1.16±0.057^{ns}	1.71±0.184*	1.17±0.038^{ns}	0.985±0.030^{ns}
Lipids	TC (g/L)	1.43±0.006	1.09±0.082^{ns}	1.03±0.238^{ns}	1.05±0.006^{ns}	1.05±0.069^{ns}	1.00±0.028^{ns}	1.00±0.038^{ns}	1.03±0.076^{ns}
	HDL (g/L)	1.05 ±0.070	1.07±0.069^{ns}	0.93±0.022^{ns}	0.91±0.039^{ns}	0.92±0.012^{ns}	1.06±0.014**	0.89±0.001^{ns}	0.93±0.043^{ns}
	LDL (g/L)	0.32±0.032	0.32±0.026^{ns}	0.32±0.045^{ns}	0.33±0.062^{ns}	0.34±0.060^{ns}	0.31±0.098^{ns}	0.31±0.016^{ns}	0.35±0.014^{ns}

Each value represents the mean ± ESM. *p < 0.05; **p<0.01 Significant different (Student t-test) versus D0; ns (p>0.05 ; no signicant different (Student t-test) versus D0. AST: aspartate aminotransferase; ALT: alanine-amino-transferase; DB: direct bilirubin; TB: total bilirubin; Trigl: triglyceride; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein;

Table 4: Effect of the aqueous extracts and the hydroethanolic of the recipe on the myocardial and renal biomarkers in rats.

Organs	Parameters	Treatment							
		28 days (four weeks)						14 days after treatment (two weeks off)	
		Distilled water (5mL/Kg)		Aqueous extract (600 mg/Kg)		Hydroethanolic extract (600 mg/Kg)		Aqueous extract (600 mg/Kg)	Hydroethanolic extract (600 mg/Kg)
		D0	D28	D0	D28	D0	D28	D14	D14
	Ck-MB (UI/L)	225.45± 5.82	230.56± 1.77^{ns}	215.67± 1.11^{ns}	212.08± 1.87^{ns}	228.49± 6.65^{ns}	255.63± 22.79^{ns}	215.50± 4.97^{ns}	243.81± 26.71^{ns}
Heart	LDH (UI/L)	1382.12 ±113.98	1401.73±93.80 ns	1393.58±118.38 ns	1317.29±121.63^{ns}	1370,24±143,6^{ns}	1375.86±140.6^{ns}	1364.84±185.6^{ns}	1374.41± 63.78^{ns}
	Tpr Ic (ng/mL)	0.06±0.002	0.071±0.002^{ns}	0.071±0.012^{ns}	0.076±0.029^{ns}	0.06±0.004^{ns}	0.061±0.006^{ns}	0.075±0.01^{ns}	0.062±0.004^{ns}
kidneys	Creat (mg/L)	7.80± 0.179	7.72±0.099^{ns}	8.23±0,170^{ns}	7.80±0.795^{ns}	7.76±0.177^{ns}	8.22±0.187^{ns}	8.14±0.418^{ns}	7.50±0.404^{ns}
	Uric A(mg/L)	37.38±2.574	38.06±0.179^{ns}	36.48±3.235^{ns}	38.02±0.321^{ns}	38.56±0.611^{ns}	32.29±0.596**	37.12±2.429^{ns}	37.6±0.564^{ns}
	Urea (mg/L)	0.39±0.060	0.40±0.007^{ns}	0.41±0.041^{ns}	0.43±0.016^{ns}	0.41±0.049^{ns}	0.35±0.009**	0.39±0.024^{ns}	0.38±0.041^{ns}
	Albumin (g/L)	40.42±0.149	40.87±0.596^{ns}	38.99±0.140^{ns}	39.52±0.218^{ns}	40.10±0.492^{ns}	40.97±0.707^{ns}	38.54±0.813^{ns}	41.77±0.944^{ns}
	Protein (g/L)	56.07±0.181	57.69±0.901^{ns}	56.41±0.231^{ns}	61.52±0.210**	59.69±0.932^{ns}	59.78±0.877^{ns}	57.65±0.711^{ns}	59.80±0.158^{ns}

Each value represents the mean ± ESM.**p<0.01 Significant different (Student t-test) versus D0; ns (p>0.05 ; no signicant different (Student t-test) versus D0. CK-MB: creatin kinase MB; LDH: lactate dehydrogenase; Tpr Ic: troponin I cardiac; Uric A : urique acid ; Creat : creatine

DISCUSSION

This work was carried out to evaluate the acute and subacute toxicities of aqueous and hydroethanolic extracts of the recipe of the leaves of *Eriosema erici* and *Neoboutonia melleri*. According to Siwe et al, (2015)[10] it is possible that a medicinal plant when administered in vivo shows some toxic signs. It appears that the hydroethanolic extract of the recipe administered acutely at a single dose of 5000 mg/Kg causes a reduction in mobility and polyuria in mice during the first hour following administration compared to the control (distilled water). This would suppose a sedative and diuretic effect of this high dose extract. Also, no mortality was recorded during the 14 days of experimentation. This could be explained by the fact that the compounds contained in this organ of the plant would not be toxic orally or the association of these two plants would attenuate the toxic effect. Therefore, the lethal dose 50 (LD50) of is estimated to be greater than 5000 mg/Kg and according to the globally harmonized classification system (GHS), these extracts are classified in category 5 and considered as non-toxic substances by the oral route.[11] In addition, the analysis of the evolution of weight in acute tests showed a non-significant reduction in the body weight of the animals treated with the aqueous extract and a significant reduction ($P<0.05$) on D2 for the hydroethanolic extract of the recipe compared to the control. Change in body weight is used as an indicator of adverse effects of chemical compounds.[12] Also, weight loss is correlated to the physiological state of the animal. The results observed could be explained by a decrease in food consumption due to the anorexic effect probably exerted by the aqueous extract. Similar results are obtained by by Etamé et al, (2018)[13] who worked on the study of the acute and subacute toxicity of the palm wine extract of the rhizomes of *Curcuma longa*; also Kunyima et al, (2018)[14] on the toxicity and Antimalarial effect of 80% ethanolic extracts from the stems of *Olivacea nantia*, *Punctata garcinia* and *Acuminate massularia*. The acute use of medicinal plants is a rather rare practice in traditional medicine, treatments generally take several days, this is how the evaluation of the effects of these two extracts linked to repeated use of medicinal plants proves to be judicious. Indeed, the subacute treatment of rats for 28 days (4 weeks) with the two extracts at a dose of 600 mg/kg showed a significant increase in liver weight with persistence 14 days after stopping treatment for the aqueous extract. The remarkable increase in the liver can be attributed to increased circulation due to increased demands for detoxifying compounds, or inflammation of the liver caused by liver tissue damage which would be explained by the leakage of enzymes from the tissue towards the plasma caused by the alteration of the membrane permeability.[15] When the liver cell is damaged by hepatotoxic substances, there is a decrease or an increase in

enzymes such as transaminases (ASAT and ALAT). This finding is confirmed in this study by the results of hepatic biochemical parameters. Indeed, the administration of the extracts of the recipe caused an increase in ALT for the aqueous extract and, for the hydroethanolic extract, an increase in the two enzymes (ASAT and ALT) compared to the initial day. Indeed, ALT is more specific for liver damage, but AST is mainly present in the liver, muscles, heart, kidneys, brain and pancreas.[16] These results corroborate certain authors who worked respectively on the hepatoprotective effect of the aqueous extract of *Crossopteryx febrifuga* in the wistar rat and on the evaluation of the Acute and sub-chronic toxicity of *Piper guineense* extracts in laboratory rodents.[17-18] In addition, we noted in this study an increase in the volume of the spleen with the hydroethanolic extract 14 days after stopping the treatment. This increase could be explained by a late onset of signs of toxicity; but also by an immunomodulatory effect of this extract on the body's immune system because the spleen is an organ of the immune system involved in the maturation of cells that fight against infections.[19] This observation could be confirmed by the variations in hematological parameters observed; because it is important to know that the spleen and bone marrow are erythropoietic organs that are often the target of drugs[20], especially in bone and marrow where red blood cell production takes place.[21-22] Indeed, the daily administration of the extracts of the recipe for 28 days (4 weeks) reveals a considerable increase ($p < 0.01$) of the VGM and the white blood cells compared to the controls. White blood cells are the body's protective cells against infection.[23] An increase in white blood cells suggests an immunostimulatory effect of these extracts.[24] The high rate of VGM could be explained by an action of the two extracts of the recipe on the erythrocyte line. Similar results were obtained by Kouadio et al, (2022)[25] who worked on the effect of Ficus sycomorus leaf extracts on the haematological and biochemical parameters of Wistar rats. In addition, in this study we did not note a significant increase in the volume of the heart and kidneys. Nevertheless, the total absence of toxicity cannot be justified by a variation in the volume of the organ because the manifestations can be limited to the molecular level before reaching whole tissues and organs.[26] Therefore, the compounds contained in these extracts could cause changes in the hematopoietic system and some biochemical markers (cardiac, renal and lipid). Despite this, this study shows no significant change in myocardial markers (LDH, Ck MB, Troponin Ic) for the two extracts of the recipe compared to the initial day. This would assume that these two extracts would not cause myocardial disturbances. On the other hand, the renal parameters showed a decrease in uric acid and urea for the hydroethanolic extract. Urea is the primary form of nitrogenous waste removal from proteins and amino acids. These

results suggest that the hydroethanolic extract would not disturb nucleic acids (DNA and RNA) and proteins by causing their catabolism. This finding is important for the protection of renal function because it is known that a decrease of at least 50 % in glomerular filtration can lead to hypercreatinine.[27]

The lipid panel is a blood test that targets the lipid compounds in the blood: cholesterol, triglycerides, LDL and HDL. In this study, no modification of these parameters was observed with the aqueous extract of the recipe compared to the initial day. However, a non-significant ($p>0.05$) increase in HDL and a significant ($p<0.05$) increase in triglycerides compared to the initial day were observed with the hydroethanolic extract of the recipe. The increase in HDL levels is a protective factor for the heart muscle due to its beneficial effect against cardiovascular complications, in particular atherosclerosis.[28] This suggests that this extract could therefore prevent cardiovascular complications.[16] Similar results were obtained by Amadi et al., (2019)[29] on the biochemical effect of *Piper guineense* in diabetic females and Mikolo et al., (2020)[30] on the evaluation of acute and subacute toxicity of the extract watery leaves of *Tetracera potatoria*. Although glucose is the primary source of energy, triglycerides are also considered an energy store. These come from food and the liver.[31] Therefore, the increase in triglycerides observed may be correlated with the increase in liver and weight gain observed in this study.

CONCLUSION

The study of the acute toxicity of the aqueous and hydroethanolic extracts of the recipe of the leaves of E. erici and N. mülleri at 5000 mg/kg made it possible to affirm that these extracts are not toxic with an LD50 greater than 5000 mg/ kg. However, administered subacutely for 4 weeks orally at a dose of 600 mg/kg, the two extracts increase the weight of the organs (liver and spleen) with reversibility two weeks after stopping treatment for the hydroethanolic extract. Despite their good margin of safety in the traditional environment, they should be taken with caution in people with liver problems.

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Conflicts of interest

The author declares no conflict of interest.

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