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Evaluation of acute toxicity and heavy metals levels of aqueous and ethanolic extracts of *Euphorbia hirta, Citrus aurantifolia* and *Heterotis rotundifolia*

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Key words: Acute toxicity, Heavy metals, *Euphorbia hirta, Citrus aurantifolia, Heterotis rotundifolia*.

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Abstract

Euphorbia hirta, *Citrus aurantifolia* and *Heterotis rotundifolia* are plants used in traditional Beninese medicine to treat various diseases and the data on their toxicity are almost non-existent. This study was undertaken to evaluate their toxicity. The acute oral toxicity of aqueous and ethanolic extracts of these plants was assessed according to OECD guideline 423 with Wistar rats at a single dose of 2000 mg/kg body weight. In addition, the contents of heavy metals such as Lead (Pb), Cadmium (Cd), chromium (Cr), Arsenic (As), Selenium (Se), Aluminum (Al), Manganese (Mn), Zinc (Zn), Copper (Cu) and Iron (Fe) were determined using the optical emission mass spectrophotometry coupled to induced plasma. Single dose administration of plant extracts did not cause any clinical signs in rats during the 14 days of observation. Moreover, no significant differences ($p > 0.05$) were observed between the hematological and biochemical parameters of the rats treated with the extracts and those of the normal control rats, whatever the day of levy. The extracts showed no toxicological effects at the administered dose. This result was confirmed by histological examination of the kidneys and liver of rats which showed no architectural deformation. Otherwise, the heavy metal content sought is below the tolerable level according to WHO/FAO standards. It is therefore established that, *Euphorbia hirta, Citrus aurantifolia* and *Heterotis rotundifolia* do not exhibit toxicity at the studied dose (2000 mg/kg of b.w.) and do not present any risk of toxicity to the heavy metals sought.

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Introduction

Nowadays, treatments by a plants are a prominent place and are experiencing a new craze given the increasing share of use of medicinal plants in the world (Chikhi, 2013). World Health Organization (WHO) estimates about 80% of people in developing countries use medicinal plants for primary health care (WHO, 2002). Moreover, in traditional medicine, there are many plants that successfully treat diseases against which modern medicine sometimes finds it difficult to find a cure (Millogo-Koné, 2008). Thereby, traditional medicine can be considered an integral part of primary health care to improve access to care. But medicinal plants are often used without a satisfactory demonstration of their pharmacological activities (Sreemoy *et al.,* 2015). The therapeutic power of plants is therefore always appreciated empirically, that is to say without any scientific proof (Nacoulma, 1996).

In Benin, as everywhere in Africa, people are seeing the problem of the cost and/or accessibility of conventional drugs in rural areas, and are increasingly turning to traditional pharmacies and traditional healers. However, traditional medicine, despite its strengths, must face obstacles, such as the doses used in the preparation and administration of drugs. Traditionally, the doses to be administered are expressed in different ways: a few drops of the product in the form of instillations auricular, oral, nasal, ocular or vaginal, a cup, a ladle, a shot glass liquor or beer, a calabash, a teaspoon, a tablespoon, a pinch of 2, 3 or 4 fingers, a handle, a liter or a bucket (N'guessan, 2008).

The doses used are really inaccurate and this inaccuracy is a real problem for traditional medicine (Barry, 1999; Adjoungoua *et al.,* 2006).

Otherwise, the constantly recourse of populations to plants is due, among other things, to the fact that the plants are of natural origin and that the use of their extracts for therapeutic purposes would not pose any danger to the organisms (Sreemoy *et al.,* 2015). But in these last years, several studies carried out on traditional herbal treatments have revealed toxicity problems or interactions that could cause therapeutic failures or accidents (Hmamouchi, 1999). However, adverse effects of plants have also been reported in several studies.

These are hepatotoxic and allergic effects (Saad *et al.,* 2006), nephrotoxic (Colson et De Broe, 2005; Kwan *et al.,* 2006), Cardio-toxic (Moritz *et al.,* 2005), neurotoxic (Benjamin *et al.,* 2001; Ernst, 2003) and even deadly effects (Jensen and Allen, 1981).

In addition, the toxic elements of plants are little known, mainly because of their natural complexity (Zeggwagh, 2013). Nevertheless, some plants are considered like toxic because of the presence in these, some inclusions such as calcium oxalates. These substances cause irritation, vomiting and stomach upset.

The prospection of the extracts administered empirically then requires a posology monitoring, to avoid the real risks of therapeutic accidents that can sometimes prove tragic (Adjoungoua *et al.,* 2006).

Besides, the human body needs the metallic and non-metallic elements for its operation. However, the presence of these elements in some dose can lead to toxic effects. Otherwise, plants can accumulate heavy metals during their evolution.

Thus, in view of the disadvantages of the accumulation of these heavy metals in the body, the evaluation of their contents in medicinal plant extracts is of great importance (Khan *et al.,* 2008; Sharma *et al.,* 2009; Jabeen *et al.,* 2010).

Euphorbia hirta, Citrus aurantifolia and *Heterotis rotundifolia* are used traditionally in Benin to treat various diseases. Previous studies have shown that the aqueous and ethanolic extracts of these plants have antibacterial activity. If these species were also valued for their bioactive substance composition and larval toxicity (Dougnon *et al.,* 2017), data on their acute toxicity and heavy metal content are almost

non-existent or insufficient. Indeed, the aim of this study is to evaluate some toxicological effects of the aqueous and ethanolic extracts of these plants. This through the acute oral toxicity and the dosage of heavy metals in these extracts. This will give an overall idea of their toxicological effect to improve and increase their use.

Materials and methods

Plants collection and identification

The plants were collected at Kpomassè (*Euphorbia hirta*) and Abomey-Calavi (*Citrus aurantifolia and Heterotis rotundifolia*) in south of Benin then identify to the national herbarium of the University of Abomey-Calavi.

They were then dried in the laboratory at 25°C for two weeks. After drying, they were made into powder and the powders are used to make the aqueous and ethanolic extracts.

Aqueous extracts preparation

50 g of powder of the plant were macerated in 500 ml of distilled water with frequent agitation for 48 hours at room temperature. After two filtrations on hydrophilic cotton and one filtration on Whatman paper No1, the filtrate was then dried at 50°C and the powder thus obtained constitutes the total aqueous extract (Guede-Guina *et al.,* 1995)

Ethanolic extracts preparation

At this level, 50 g of powder of the plant are macerated in 500 ml of 96% ethanol, and stirred for 48 hours. The homogenate obtained was filtered twice on hydrophilic cotton and once on Whatman No1 paper. After concentration with rotavapor, the filtrates were deposited in an oven at 50°C until obtained a dry mass (Sanogo *et al.,* (2006; N'Guessan *et al.,* 2007)

Animals used

Wistar adult rats having 10 to 12 weeks old and weighing between 100 and 200 g were used in this study. The rats were obtained from the Institute of Applied Biomedical Sciences (ISBA) of the University of Abomey-Calavi. They were placed in aerated metal cages containing litters of wood chips regularly renewed. The animals thus placed are acclimated to the conditions of the animal house for a week before the treatment. They were fed from the standard pellets with access to water at will.

Evaluation of acute toxicity at single-dose of each extracts

The acute toxicity assessment was carried out in accordance with Organization for Economic Cooperation and Development Guideline 423 (OECD, 2002). Thus, after acclimation, the animals are divided into 7 groups of 3 animals each depending on the weight. The animals in the first group served as controls and received only physiological water.

The other six groups received respectively the aqueous extract of *Euphorbia hirta* (EA-Eh); ethanolic extract of *Euphorbia hirta* (EE-Eh); aqueous extract of *Citrus aurantifolia* (EA-Ca); ethanolic extract of *Citrus aurantifolia* (EE-Ca); aqueous extract of *Heterotis rotundifolia* (EA-Hr) and the ethanolic extract of *Heterotis rotundifolia* (EE-Hr). The dose administered was 2000 mg/kg body weight for all groups.

Observation

After the different treatments, the animals were observed every 30 minutes for 8 hours on the first day and every day for fourteen days. During this period, symptomatic disorders (agitation, lack of appetite, eye color, motor difficulties, diarrhea, lethargy and dyspnea) were observed.

Blood processing

Blood samples were collected from each rat by orbital puncture on seventh day (day 7) and fourteenth day (day 14) after treatment. These samples were used for biochemical and hematological analyzes. Biochemical analyzes consisted of determining biochemical parameters like, the rates of urea, creatinine and transaminases (ASAT: Aspartate Amino Transferase and ALAT: Alanine Amino Transferase).

Hematological analyzes have focused on Hematological parameters like, Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Hemoglobin (Hb), Hematocrit (Ht), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean corpuscular hemoglobin (MCH) and Mean corpuscular volume (MCV).

Histopathological examination

After fourteenth day observations and levying, one rat per group was dissected using chloroform as anesthetic. For each dissected rat, the organs such as kidney and liver were removed, rinsed with physiological water and then fixed immediately in 10% buffered formalin for 24 h at 4°C.

The each organ were dehydrated with alcohol, embedded in paraffin, cut into 3-4 µm thick sections by microtom and stained with haematoxylin-eosin dye for photomicroscopic observation (Chang et Chi-Rei, 2015). Microscopic observations were made to examine possible cells damage or tissues changes.

Determination of heavy metals level in the different extracts

To evaluate the heavy metals content of the various extracts, the method of optical emission mass spectrophotometry coupled to induced plasma were used. The main heavy metals and metalloids sought in the extracts are: Lead (Pb), Cadmium (Cd), Chromium (Cr), Arsenic (As), Selenium (Se), Aluminum (Al), Manganese (Mn), Zinc(Zn), Copper (Cu) and Iron (Fe).

Principle

Hot digestion of the extracts was carried out in an acidic medium. To do this, in a test tube containing 10 mg of extract, were added 10 ml of a mixture of nitric acid, hydrochloric acid and hydrogen peroxide (HNO_3-HCl-H_2O_2 (8 :1 :1, v/v/v)).

The whole was heated to 120°C for about 3h or until the solutions were completely digested. Due to the corrosive nature of the acids used, 1 ml of this solution was diluted in 20 ml of distilled water and then filtered thrice successively. The filtrate was used for Spectrophotometer reading with adequate wavelengths (Pb220,353; Cd228,802; Cr205,56; As188,979; Se196,026; Al396,153; Mn257,610; Zn213,857; Cu327,393; Fe238,204) (Choi *et al.*, 2014).

Statistical analysis

The Microsoft Excel version 2016 spreadsheet was used for data entry and coding. The GraphPad Prism software (version 7.0) was used to make graphs and statistical analyzes. Each experiment was repeated thrice and a structuring of means was done. This allowed us to compare the differences with an analysis of variance (ANOVA) followed by Dunnett's multiple comparison test with 5% significance.

Results

Acute toxicity

Tables 1 and 2 show, the effects of the different types of extracts on the hematological parameters respectively at day 7 and day 14 after administration of the extracts of the three plants studied. At day 7, there was a significant ($p<0.05$) difference between the hematocrit (Ht) and the Mean corpuscular volume (MCV) of normal control rats and those treated with aqueous (MCV) and ethanolic (Ht) extracts of *Heterotis rotundifolia*.

The difference is very significant ($p <0.01$) in the rats treated with aqueous (MCHC) and ethanolic (Ht and MCHC) extracts of *Citrus aurantifolia* (Table 1). At day 14, there was a significant difference ($p<0.05$) between the hemoglobin rates of the normal control rats and the rats treated with the aqueous extracts of *Heterotis rotundifolia*. For the rats that have been treated with this same extract, the difference is very significant at the rates of hematocrit (Ht) and Mean corpuscular volume (MCV). For rats treated with the other types of extract, there was no significant difference ($p>0.05$) compared to normal control rats (Table 2). Analysis of variance showed that no significant difference ($p>0.05$) between Day 7 and Day 14 results of hematological parameters (Table 1 and 2).

Table 1. Effects of different types of extracts on hematological parameters at Day 7.

	RBC (10^9/L)	WBC (10^9/L)	Hb (g/dl)	Ht (%)	MCHC (g/dl)	MCH (pg)	MCV (fl)
Control	6.73±0.25	13.35±1.05	12.80±0.10	43.00±1.40	29.80±1.00	19.00±0.70	63.85±0.25
EA-Eh	7.29±0.30	14.80±3.87	12.87± 0.51	41.83± 1.36	30.73±0.59	17.70±1.28	57,47±3.35
EE-Eh	7.50±0.13	11.70±0.30	14.63±0.15	48.23±0.80	30.33±0.42	19.53±0.47	64.33±1.43
EA-Ca	6.99±0.40	10.63±0.47	12.10±2.48	40.60±2.55	33.00±0.30**	20.60±2.12	67.20±3.06
EE-Ca	5.40±0.49	10.53±1.06	10.95±0.75	32.75±1.55**	32.90±0.10**	20.35±0.45	69.35±2.35
EA-Hr	5.39±1.78	10.07±2.91	12.31±1.73	38.00±6.08	30.33±1.53	19.20±1.59	72.67±5.69*
EE-Hr	6.66±0.51	8.63±2.58	11.47±0.74	35.00±2.65*	30.67±1.53	19.77±1.76	67.00±2.00

Data was expressed as Mean ± S.E.M

Table 1 & 2 legends : *= significant difference; **= very significant difference; EA-Eh=aqueous extract of *Euphorbia hirta*; EE-Eh=ethanolic extract of *Euphorbia hirta*; EA-Ca= aqueous extract of *Citrus aurantifolia* ; EE-Ca=ethanolic extract of *Citrus aurantifolia*; EA-Hr= aqueous extract of *Heterotis rotundifolia*; EE-Hr ethanolic extract of *Heterotis rotundifolia* ; RBC=Red Blood Cell Count, WBC=White Blood Cell Count, Hb=Hemoglobin, Ht=Hematocrit, MCHC=Mean Corpuscular Haemoglobin Concentration, MCH=Mean corpuscular hemoglobin and MCV=Mean corpuscular volume.

Table 2. Effects of different types of extracts on hematological parameters at Day 14.

	RBC (10^9/L)	WBC (10^9/L)	Hb (g/dl)	Ht (%)	MCHC (g/dl)	MCH (pg)	MCV (fl)
Control	6.56±1.49	13.30±2.44	11.35±3.05	39.70±1.22	31.80±3.12	23.80±1.40	62.00±2.00
EA-Eh	5.53±1.28	15.20±2.46	12.50±0.35	35.07±4.52	34.73±2.16	23.50±5.94	61.67±5.86
EE-Eh	6.54±0.34	8,7±0,30	14.05±0.15	42.1±1.00	33.45±0.35	21.55±0.85	64.5±1.50
EA-Ca	6.18±0.68	8.07±2.30	13.27±1.45	35.90±3.49	35.07±0.42	21.50±0.75	61.33±2.08
EE-Ca	5.73±0.34	8.67±0.46	12.19±0.29	36,37±0,64	34.17±0.40	22.00±0.73	65.67±2.08
EA-Hr	7.87±0.60	7.53±2.72	15.30±1.10*	49.33±4.93**	31.00±1.73	23.67±2.08	80.33±10.50**
EE-Hr	5.93±0.26	12.20±5.15	13.00±1.06	39.00±2.00	31.33±1.15	22.33±3.06	71.00±2.65

In view of these results, it can be said that the aqueous and ethanolic extracts of the plants studied (*Euphorbia hirta, Citrus aurantifolia, Heterotis rotundifolia*) have no effect on the hematological parameters.

Figs 1, 2, 3 and 4 show the effect of the various extracts on the biochemical parameters namely, respectively, uremia, creatinemia, aspartate amino transferase (ASAT) and alanine amino transferase (ALAT).

There are no significant difference ($p>0.05$) between the urea, creatinine and transaminase (ASAT and ALAT) rates in the normal control rats and those the rats treated with the different extracts, whatever the day of the levy.

At day 7, the urea rate was 0.36±0.09 g/L in normal control rats. This rate varies between 0.32±0.11 and 0.40±0.03 g/L in the rats that have been treated with the various extracts. At day 14, it is 0.38±0.11 g/L in normal control rats and varies between 0.34±0.12 and 0.42±0.01 g/L in the rats treated with the different extracts (Fig. 1).

The creatinine rate is 9.15±0.25 g/L on day 7 and 9.05±0.15 g/L on day 14 in normal control rats. This rate varies between 7.37±3.01 and 11.07±1.45 g/L on day 7 and between 7.77±1.10 and 10.70±5.87 g/L on day 14 among treated rats (Fig. 2).

In addition, aspartate amino transferase (ASAT) varies between 24.33±7.64 and 35.33±8.33 IU/L at day 7 and between 19.40±9.40 and 28.00±10, 44 IU/L at day 14; this between normal control and experimental rats (Fig. 3). Finally, alanine amino transferase (ALAT) varies between 17.33±2.08 and 25.67±6.11 IU/L at day 7 and between 16.47±5.90 and 31.33±4.51 IU/L at day 14 taking into analysis, normal control and experimental rats (Fig. 4).

Table 3. The means contents in mg/kg of heavy metals sought in extract of the studied plants.

	EA-Eh	EE-Eh	EA-Ca	EE-Ca	EA-Hr	EE-Hr
Pb	1.249±3.214	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	2.371±1.154	UDL (<0.001)
Cd	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	0.025±0.165	UDL (<0.001)
Cr	0.768±0.26	0.463±0.342	0.242±0.453	0.654±0.170	1.233±0.164	0.204±0.436
As	UDL (<0.001)	0.013±0.290	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)
Se	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)
Al	1.171±0.406	0.810±0.152	1.660±0.203	2.288±0.252	2.106±0.142	2.931±0.276
Mn	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)
Zn	2.169±0.106	1.179±0.112	2.333±0.063	3.210±0.095	2.843±0.177	2.427±0.071
Cu	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)
Fe	UDL (<0.001)	UDL (<0.001)	UDL (<0.001)	0.009±0.323	0.022±0.717	UDL (<0.001)

Table 3 legend: Pb=Lead, Cd=Cadmium, Cr=Chromium, As=Arsenic, Se=Selenium, Al=Aluminum, Mn=Manganese, Zn=Zinc, Cu=Copper, Fe=Iron; EA-Eh= aqueous extract of *Euphorbia hirta*; EE-Eh= ethanolic extract of *Euphorbia hirta*; EA-Ca= aqueous extract of *Citrus aurantifolia*; EE-Ca= ethanolic extract of *Citrus aurantifolia*; EA-Hr= aqueous extract of *Heterotis rotundifolia*; EE-Hr ethanolic extract of *Heterotis rotundifolia*

From the analysis of Figures 1, 2, 3 and 4, it appears that the extracts have no effect on the biochemical parameters.

Fig. 5 and 6 show the histological sections of the normal control rats and those of the rats treated with the extracts of the plants studied. It's found that the hepatocytes (Hp) have normal appearance and are arranged in cords, separated by the sinusoids (S) which in their turn flow into the centrilobular vein (V).

Hepatic architecture remained unanimous in normal control and experimental rats (Figure 5 A and B). Likewise, the experimental rats have a typical renal architecture to that of the rats normal control rats.

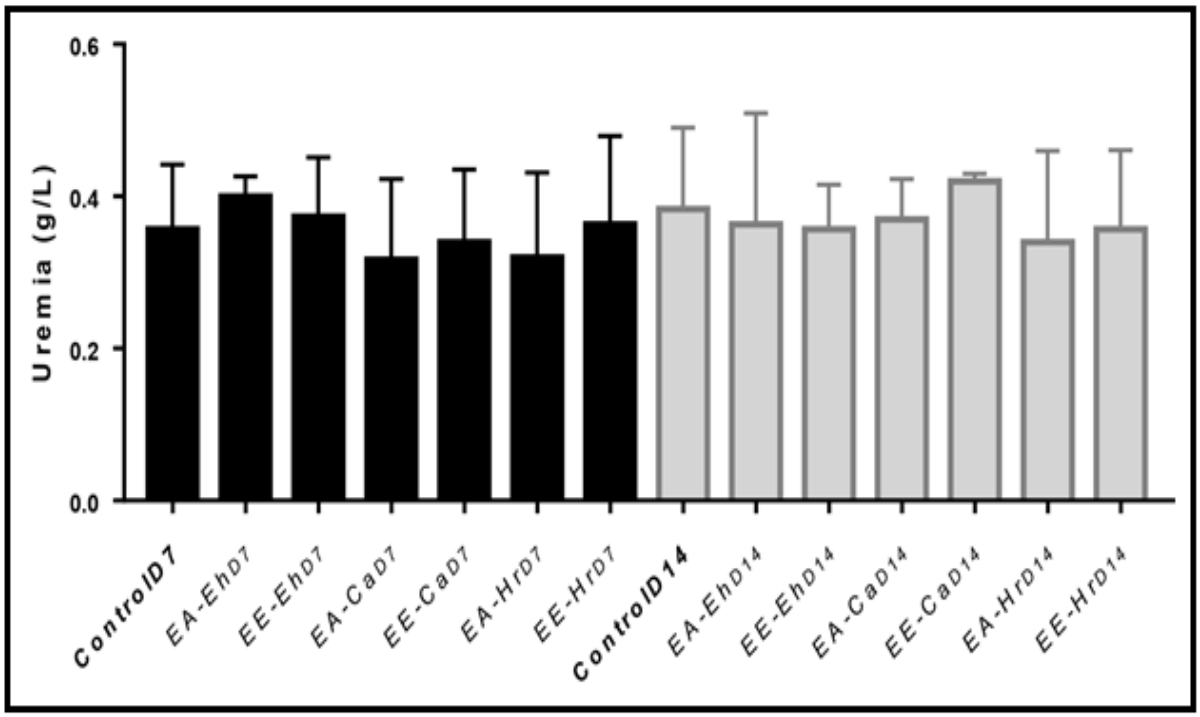

Fig. 1. Effects of extracts on Uremia.

Figs 1,2,3 and 4 legends: EA-Eh= aqueous extract of *Euphorbia hirta*; EE-Eh= ethanolic extract of *Euphorbia hirta*; EA-Ca= aqueous extract of *Citrus aurantifolia*; EE-Ca= ethanolic extract of *Citrus aurantifolia*; EA-Hr= aqueous extract of *Heterotis rotundifolia*; EE-Hr ethanolic extract of *Heterotis rotundifolia*, D7=Day seven; D14=Day fourteen.

The glomeruli (G), proximal tubes (TP), distal tubes (TD) and collecting ducts (CC) are all normal and well identifiable (Figure 6 A and B). These histological examinations show that the aqueous and ethanolic extracts of *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* at the administered dose (2000 mg/kg of body weight) did not induce any architectural changes in organs (Liver and Kidney).

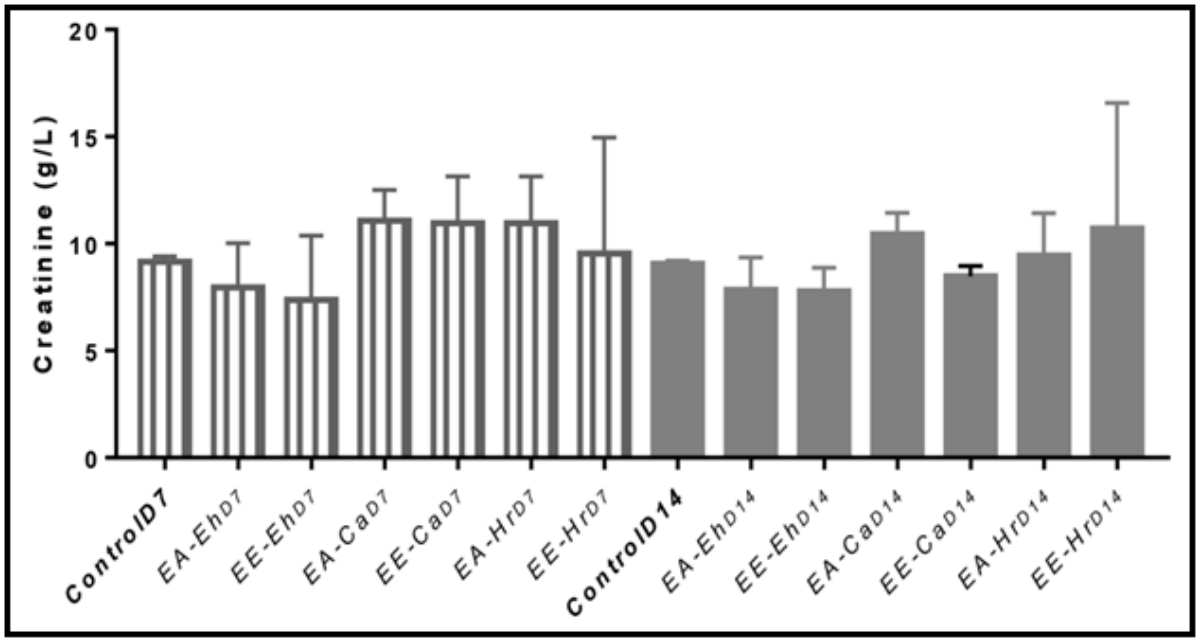

Fig. 2. Effects of extracts on Creatinine.

Heavy metals level determination

Table 3 presents the contents of heavy metals sought in the various extracts. It's found that the heavy metal contents vary according to the extracts and the plants. In addition, all heavy metals are below tolerable levels according to FAO/WHO standards. Manganese and copper were not detected in any extracts of the plants studied.

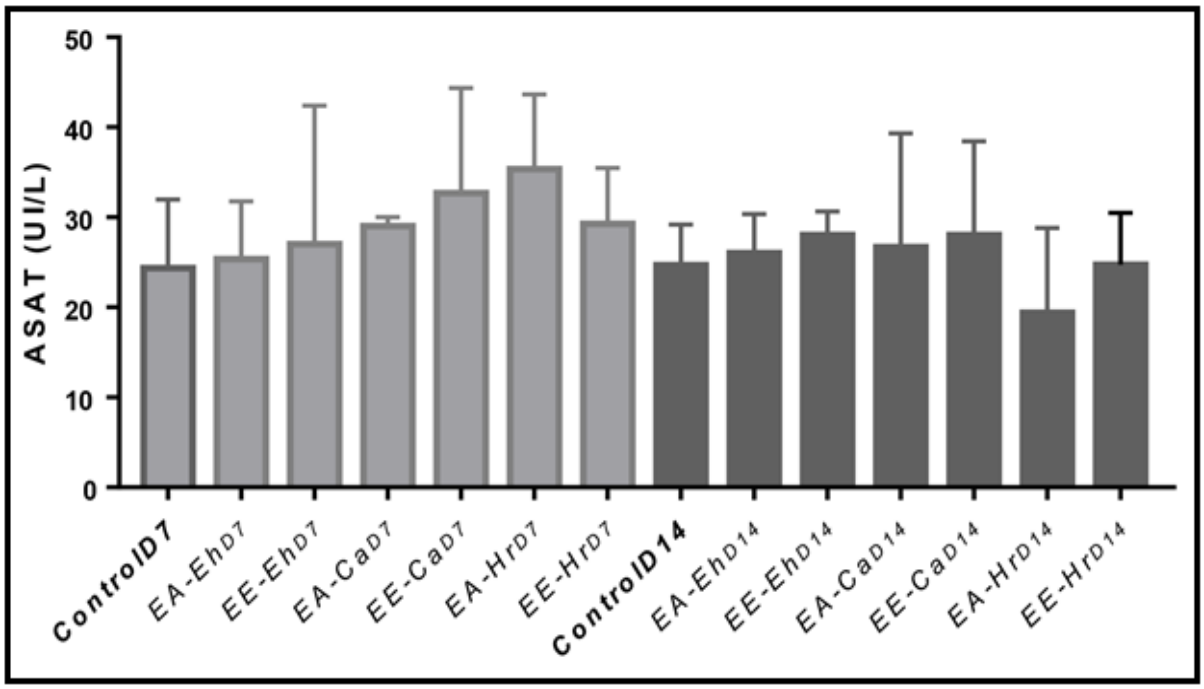

Fig. 3. Effects of extracts on ASAT.

That's, their value is less than 0.001 mg/kg of extract; value below which machine (spectrophotometer) can't detect the content (UDL). Considering *Euphorbia hirta* extracts, Zinc has a higher content (2.169 ± 0.106 mg / kg) with the aqueous extract whereas the lowest metal content is Arsenic (0.013 ± 0.290 mg / kg) with the ethanolic extract. For *Citrus aurantifolia* extracts, only Chromium, Aluminum, Zinc and Iron (ethanolic extract) contents were detected. Other metals have a value below the limit of detection by the spectrophotometer. In *Heterotis rotundifolia* extracts, it's only Arsenic, Selenium, Manganese and Copper that could not be detected in any extract of this plant.

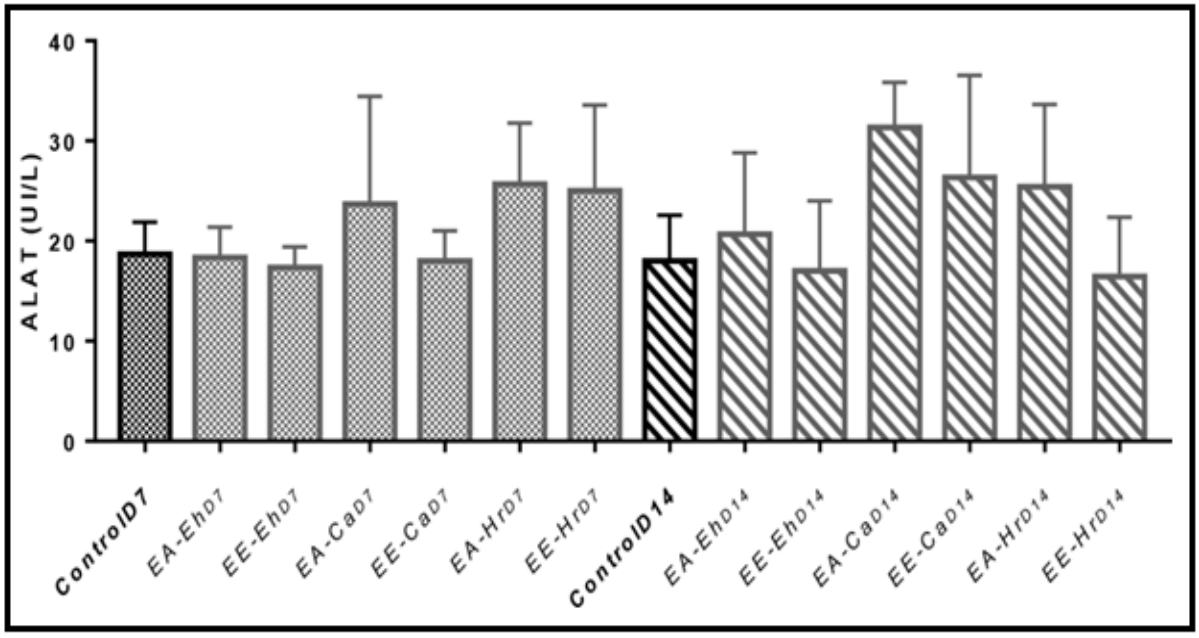

Fig. 4. Effects of extracts on ALAT.

Discussion

In this study, the acute oral toxicity of aqueous and ethanolic extracts of three plants (*Euphorbia hirta*, *Citrus aurantifolia*, *Heterotis rotundifolia*) was carried out at a single dose of 2000 mg/kg body weight using the Wistar rat model. Oral administration of the extracts was not caused any significant changes in rats. Likewise, any signs of toxicity such as decreased sensitivity to pain, sound or locomotion was not observed. At the end of the 14 days of observation, there are no deaths in the treated rats, which did not make it possible to determine the LD50s.

In general, there are no difference between the hematological (Table 1 and 2) and biochemical (Figs. 1, 2, 3 and 4) parameters of the control and experimental rats, whatever the day of levying. In addition, the analysis of variance also showed that there are no variation between these parameters according of levying day. Otherwise, the few differences observed in the hematological parameters in places may be related to the diet and/or to the physiological conditions of the rats during levying. Analysis of uremia and creatinemia revealed that the administration of the extracts did not cause any significant changes (Figs 1 and 2). Urea and creatinine are considered as major markers of nephrotoxicity, although serum urea is often considered to be a more reliable predictor of renal function than serum creatinine (Palani *et al.,* 2009). Transaminases or amino transferases (ASAT and ALAT) are tissue enzymes usually found in the liver, but also in the muscle, kidneys, pancreas, and other tissues. ALTs are more specific for liver damage, but ASATs are a bit more sensitive (Goddard and Warnes, 1992). These enzymes are synthesized in the cytoplasm where it plays an important role and are discharged into the bloodstream when the cells are damaged (Peirs, 2005).

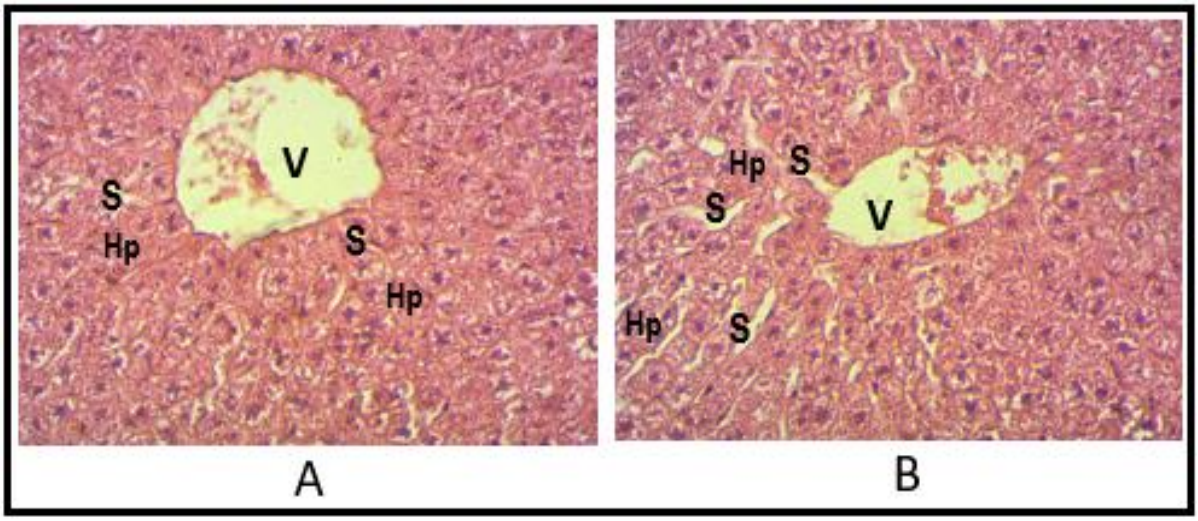

Fig. 5. Hepatic Histology of the Normal Control rat (A) and the rat Treated with the Extract (B).

Figs 5 and 6 legends: Hp= hepatocytes, S=sinusoids, V=centrilobular vein, G=glomeruli, TP=proximal tubes, TD=distal tubes, CC=collecting ducts.

In this study the extracts did not induce any significant effect on these enzymes (Figs 3 and 4). As a result, the extracts have no toxic effect at the administered dose. This result was confirmed by histological examination of the liver and kidneys. These organs have not showed any structural abnormalities in the treated rats (Figs 5 and 6). However, Ping *et al.* (2013) in their study on the acute and subacute toxicity of the methanolic extract of *Euphorbia hirta* had reached the same conclusion with higher doses.

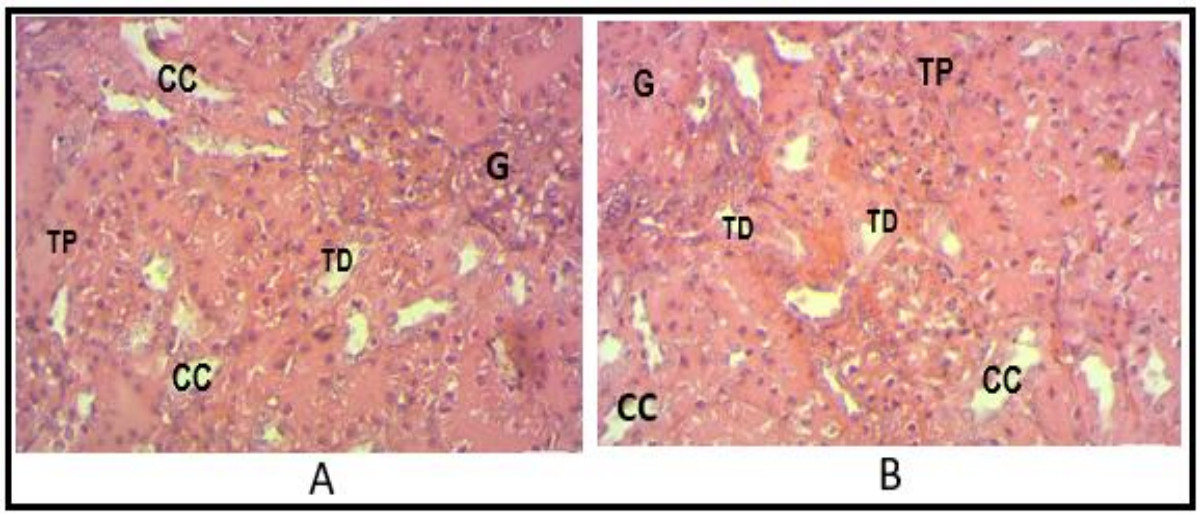

Fig. 6. Renal histology of the normal control rat (A) and of the rat treated with the extract (B).

The same observations were made also in Onyeka *et al.* (2018) studies. Also, Chunlaratthanaphorn *et al.* (2007) in their study on the acute and subacute toxicity of the aqueous extract of *Citrus aurantifolia* root found no toxic effects of this plant. These results are also in correlation with those of Sheik *et al.* (2013) in their study on the toxicity of several species of *Citrus* genus. It's the same for *Heterotis rotundifolia* with among others studies like that of Abere *et al.* (2010).

Moreover, the contents of heavy metals were evaluated in the extracts of these plants. This will provide a global view of the toxicological aspect of

these plants for a better valuation. Thus, several observations related to the presence of heavy metals in plants stem from the analyze results of our extracts. These results show that the contents of heavy metals are based on extracts and plants. It can be said that plants do not have the same potential for metal accumulation. The presence of these metallic elements in the extracts of our plants may be the consequence of a contamination of these plant materials in their environment caused by the soil composition and the pesticides use (Zuin et Vilegas, 2000). However, our results show that the values found for the sought heavy metal contents are within tolerable limits, thus excluding any possible danger for consumers (FAO/WHO, 1984; Jabeen *et al.,* 2010).

Certain metals such as Cadmium, Selenite, Manganese and Copper have not been detected in the *Euphorbia hirta* extracts. The same observations have been made in the Kntapo *et al.* (2018) study. Besides, iron, zinc and chromium were detected in the extracts of this plant in the Olayiwola (2013) and Guessan *et al.* (2015) studies with higher levels than ours. Otherwise, several heavy metals (Cadmium, Nickel, Lead, Cobalt, Iron, Chromium, Zinc etc.) were detected in Guerra *et al.* (2012) and Thummajitsakul *et al.* (2018) studies, in their research's on *Citrus* genus plants with levels significantly different from ours. Also, Manganese and Copper were determined with varying levels in the Ajayi *et al.,* (2013) study, whereas their determination was not possible in the present study with our methodology. Thus, the differences observed in the heavy metals contents of *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* extracts in the present study and those of the above-mentioned authors, can be due, among other things, to the soils nature and the methodologies used. It should be noted that several factors can influence, the metallic trace elements concentration in the plants. These include the nature of the element, its content and chemical form in the soil, the type of plant, the nature and pH of the soil, its proximity to industrial zones and several other factors (Femenia *et al.,* 1995; Adeleke et Abiodun, 2010).

The present study carried out, the acute toxicity of the aqueous and ethanol extracts of *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* on Wistar rats. The administration of these crude extracts at 2000 mg/kg body weight dose to each rat did not cause any clinical signs and any deaths were recorded during the 14 days of observation. In these extracts, the contents of heavy metals are variable from plant to another, but all below the tolerable level. These results are reassuring for the medicinal use that the population makes of these plants. However, this study should be supplemented by subacute and chronic toxicity tests to determine the therapeutic margin. But the fact remains that the results are favorable to the production of an improved traditional drug, after preclinical and clinical tests.

Conflicts of Interest

Authors declare no conflict of interests.

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