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Research Article

ASSESSMENT OF HEAVY METALS CONTENT AND THEIR EFFECTS ON TOXICITY OF *Acmella uliginosa Sw.*

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ABSTRACT

The use of medicinal plants has increased in recent decades due to their affordability and especially because of the assumption that natural remedies are safe. Some medicinal plants are used as leafy vegetable. In Bйnin, *Acmella uliginosa* is one of these medicinal plants used as green leafy vegetable. Unfortunately, they are contaminated by environmental and industrial waste such as heavy metals. Thus, the aim of this study was to evaluate the heavy metals (As, Pb, Cd, Hg, Cu, Mn and Zn) contents of *Acmella uliginosa*, harvested in Cotonou (CAU) and Pobи (PAU) and to assess and compare the oral acute toxicity of the two samples.

The heavy metals content was assessed by anodic and cathodic stripping voltammetry method. The oral acute toxicity of aqueous extracts was evaluated following the guidelines N° 423 of the Organization of Cooperation for Economic Development. The phytochemical study was assessed by thin layer chromatography (TLC) method.

The heavy metal analysis revealed the presence of arsenic, lead, mercury, copper, manganese, and zinc in Cotonou sample while only zinc, manganese and copper are present in sample collected in Pobи. The heavy metals concentrations range from 0.028 ± 0.007 to 0.108 ± 0.052. Cadmium is absent in studied samples. At a dose of 2000mg/kg body weight, no adverse effects were recorded in Pobи sample in contrary to sample collected at Cotonou. The results showed that *Acmella uliginosa* harvested in Pobй (PAU) does not contained toxic heavy metals while sample from Cotonou (CAU) contained various heavy metals. Aqueous extract of PAU has no toxicity effect in contrary to Cotonou sample. The toxicity of *A. ulignosa* collected in Cotonou could be due the presence of heavy metals.

KEYWORDS: heavy metals, *Acmella uliginosa*, acute toxicity

INTRODUCTION

Medicinal plants are an important source of health care in the world and global demand is increasingly growing [1]. The World Health Organization estimated that about 80 % of the populations in developing countries are unable to afford drugs and rely on traditional medicines especially those that are plant-based [2]. Apart from their medicinal properties, population uses medicinal plants as leafy vegetables which dominated the traditional African cuisine today. They are important in human nutrition as sources of nutrients and non-nutritive food constituents as well as for the reduction in diseases risks. In a WHO/FAO Global strategy on diet, physical activity and health, it have been reported that nutrition and prevention of chronic diseases, sets population nutrient goals. Intake of a minimum of 400 g of fruits and vegetables per day was recommended for the prevention of chronic diseases such as heart diseases,

cancer, diabetes and obesity [3]. In Africa, leafy vegetable occur as cultivated, semi-cultivated, weedy and wild plants, with ecological, social and cultural values, playing a significant role in the day to day food and nutritional requirements of local people mainly in rural areas [4, 5]. In Benin, garden crops, mainly green leafy vegetables, ensures quantitatively and qualitatively the food security of rural and urban populations. Green Leafy Vegetables are predominantly known for their high nutritional content and are mostly consumed for their health and nutritional benefits. In south of Bйnin, green leafy vegetable, *Acmella uliginosa*, is produced by gardeners in urban areas (Cotonou) and domesticated in rural areas (Pobй) and the sauce is highly appreciated for its anthelmintic property. It is used as an antibiotic, which stimulates milk production and facilitates the elimination of blood clots in women after delivery [6, 7]. Despite their medicinal and nutritional properties, green leafy vegetables consumption poses serious problems because of their contamination with heavy metals that are one of the most significant aspects of food quality assurance [8, 9]. In our previous study, *Acmella uliginosa* extract was found to be active against standard bacteria but unfortunately toxic by showing significant modifications of biochemical, hematological parameters and alterations of liver and kidney [10]. In this study, we assessed some heavy metals contents (As, Pb, Cd, Hg, Cu, Mn and Zn) in A*cmella uliginosa* collected in urban (Cotonou) and rural (Pobи) areas and evaluate the effect of heavy metals on the toxicity of this green leafy vegetable.

MATERIAL AND METHODS

Study area

The study was conducted in two regions separated by about 100 km.

Cotonou situated in department of Littoral, between latitudes 6°21' - 6°36' North and longitudes 2°26' - 2°47' East. It covers a total land area of 79 km^2 with a population estimated to about 679 012 [11]. Cotonou alone concentrates more than 2/3 industries of the country and transport are provided mainly by Taxi Moto called *Zemidjan*. Agricultural activities are mainly reduced to gardening that provides leafy vegetables to the entire population [12]. Unfortunately, industrial, port, road traffic and airport activities generate an important air and soil pollution.

Pobe Located 80 km from Cotonou in department of Plateau, between latitudes 6°95' - 7°13' North and longitudes 2°35' et 2°47' East. It covers a total land area of 400 km^2 with a population estimated to about 123 740 [11]. Pobи is a rural area whose main activity is agriculture. Gardening supplies tomatoes, okra, peppers and Green leafy vegetables.

Vegetal material

Acmella uliginosa leaves were harvested in April 2015 in Cotonou (CAU) and Pobй (PAU). They were authenticated by botanists from University of Abomey-Calavi. A specimen has been deposited at the National Herbarium of Abomey-Calavi University (AA6624/HNB). The collected samples were washed separately with de-ionized water, dried in the laboratory (22°C ± 3) and then powdered.

Extracts preparation

A powdered leaves (150 g) of CAU and PAU were extracted by decoction with 750 ml of distilled water for 1 h using a heating mantle (Electromantle MA Solid State Stirrer, 60°C). The mixture obtained after decoction was filtered through Whatman paper (Qualitative Circles 150 mm Cat No. 1001 150). The same extraction was repeated two times for 30 min to obtain the aqueous filtrate which was concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor R II) to obtain aqueous extracts.

Determination of heavy metals

The heavy metals concentrations were determined in aqueous extracts of CAU and PAU. Heavy metals were determined using portable heavy metals analyzer system (Metalyser HM 3000). Heavy metals determination was based on anodic and cathodic stripping voltammetry using disc working electrodes. The techniques usually consist of a pre-concentration of the metals in the electrode surface, followed by a potential sweep (voltammetry method) to dissolve pre-concentrated species of interest, making the quantification of them. In this study, arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), Manganese (Mn) and Zinc (Zn) were determined. The extracts were prepared at 1 mg/ml in ultra-pure deionized water. Heavy metals concentrations were determined automatically by the metalyser.

Oral acute toxicity

Female wistar rats, nulliparous, nonpregnant with body weight of 210 ± 10 g were used. The acute toxicity of plants was assessed according to guidelines n° 423 of the Organization for Economic Cooperation and Development [13].

The rats were divided into two groups of three animals. The tested group was fed with a single dose of 2000 mg/kg body weight (bw) of the aqueous extract of PAU while the control group received 1 ml of distilled water. Experiment was then performed according to the method described previously [14]. After feeding, the animals were observed carefully during the first four hours and daily during thirteen days for behaviors or toxicity symptoms. Animals were also weighed on days 1, 4, 7, 11 and 14.

Haematological and Biochemical parameters analyses

In our previous study, the haematological and biochemical parameters of CAU were analyzed. The present study analyzed the haematological and biochemical parameters of PAU. These analyses were done according to method described previously [10]. Briefly, the animals were anesthetized with thiopental (0.5 ml/Kg bw) and sacrificed at the end of the experience. The blood was collected into

two tubes (dry and EDTA) for biochemical and haematological tests. An automatic hematological analyzer (Sysmex, XP-300, Japan) was used for hematological parameters assessment while spectrophotometers (ErbaChem 7, Germany) was used for biochemical parameters.

Histopathological examination

The histopathological study of CAU has been done previously. The histopathological study of PAU was done in this study according to the methods described [14]. Kidney and liver were removed from treated and untreated rats, weighed and immediately stored in buffered formalin 10% for histological examination.

Phytochemical

Phytochemical screening was carried out according to the described methods [15]. In our previous study, the phytochemical constituents of CAU have been determined. In this study, phytochemical constituents of PAU were determined. 10 µl of extract (10 mg/ml) and silica gel plate (silica gel 60 F254, support rigide en aluminium, Merck) was used. Fourteen (14) secondary metabolites groups include alkaloids, coumarins glycosylated, anthracene derivatives, glycosylated flavonoids, cardiac glycosides, essential oils, lignans, naphtoquinones aglycone, anthocyanin, bitter principles, saponins, tannins, terpene and glycosylated triterpenes, were investigated.

Statistical analysis

Data collected from the biochemical and hematological analyses were expressed as mean ± SEM (n = 3). One-way ANOVA was used to test the means. Results were considered significant when p value less than 0.05.

RESULTS

In our previous work, aqueous extract of *Acmella uliginosa* collected in Cotonou (CAU) was found to have significant toxicity by promoting significant modifications of biochemical, hematological parameters and alterations of kidney and liver [10]. In the present study, concentrations of heavy metals such as Cadmium (Cd), Lead (Pb), arsenic (As), mercury (Hg), Copper (Cu), Manganese (Mn) and Zinc (Zn) were determined in aqueous extracts of *A. uliginosa* harvested in Cotonou (CAU) and Pobи (PAU, 80 Km from Cotonou). The oral acute toxicity of aqueous extracts of PAU and phytochemical contents were also evaluated in order to determine the impact of heavy metals on the quality of this medicinal leafy vegetable.

Phytochemical screening

Phytochemical constituents of leaves of PAU are showed in Table 1. The phytochemical analysis of PAU revealed the presence of alkaloids, coumarins glycosylated, anthracene derivatives, lignans, triterpenes, glycosylated flavonoid, anthocyanin pigments, terpenes glycosylated, essential oils, bitter principles, tannins and saponins. Our previous work on *Acmella uliginosa* harvested in Cotonou (CAU) showed similar results to those obtained in the present study, unlike alkaloids and naphthoquinones. Other studies have also shown the presence of alkaloids, glycosides, polyphenols, saponins, flavonoids and tannins in the same species [16]. The difference in the chemical composition could be linked to the plant phenology [17].

Content of heavy metals of CAU and PAU

The results are compiled in Table 2. The results showed that metals concentrations range from 0.028 ± 0.007 to 0.108 ± 0.052. Cadmium is absent in both samples. The aqueous extract of *CAU* contains Lead (Pb), arsenic (As), mercury (Hg), Copper (Cu), Manganese (Mn) and Zinc (Zn) with concentrations ranging from 0.028 ± 0.007 to 0.108 ± 0.052. The highest concentration of heavy metal in CAU is arsenic (0.340 ± 0.036) followed by manganese (0.108 ± 0.052) and mercury (0.084 ± 0.054). The most toxic heavy metals such as Arsenic, lead, mercury were not found in the sample harvested in Pobи (PAU). The sample of *A. uliginosa* collected in Pobй contains only copper (Cu), zinc (Zn) and manganese (Mn).

ORAL ACUTE TOXICITY

To confirm or refute the safety of the aqueous extract of *A. uliginosa* harvested in Pobи, physical, biochemical, hematological and histological parameters were evaluated fourteen days after administration of the aqueous extract to Wistar rats.

Effect of aqueous extract of PAU on body weight

Evaluation of oral acute toxicity showed that the animals tolerated the aqueous extract of PAU at a dose of 2000 mg/kg body weight. After fourteen (14) days of general observation, no clinical signs of toxicity were recorded on tested rats. During this period, a constant and significant increased ($p < 0.05$) of body weight of treated animals and control was observed (Figure 1). The increase in body weight of rats could be due to feeding which rats were submitted.

Effect of aqueous extract of PAU on the relative weight of organs

No significant change was observed in the relative body weight of the kidneys and liver after administration of PAU (Figure 2). These observations indicated that the aqueous extract of *A. uliginosa* had no effects on liver and kidneys weight.

Effect of PAU extract on biochemical parameters

Biochemical parameters were evaluated on the tested and control rats fourteen days after administration of the aqueous extract of PAU. The results are summarized in Table 3. No significant change was induced in plasma glucose, creatinine, alanine aminotransferase and aspartate aminotransferase on experimental rats in comparison to control ($p > 0.05$).

Effect of PAU aqueous extract on hematological parameters

The results of blood examinations are summarized in Table 4. No significant differences ($p > 0.05$) of the studied blood parameters were observed in tested rats in comparison with control. The analysis of hematological parameters is important for assessing the risk of alteration of the hematopoietic system in toxicity studies [18]. The results obtained in this study suggested that the aqueous extract of PAU presented no risk of alteration of the hematopoietic system.

Effect of PAU on kidneys and liver histology

Figures 3 show histological sections of the liver and kidneys of control and rats treated with the aqueous extract of PAU at a single dose of 2000 mg/kg body weight. The histological sections of the liver and kidney showed no change in the tissue architecture of treated rats compared to control. Glomeruli (G), renal tubules, hepatocytes (H) and the central veins (VC) of all animals are normal. These results indicate that PAU has no toxicity effect on rats at a single dose of 2000 mg/kg of body weight.

Table 1: Phytochemical analysis of Acmella uliginosa collected in Pobe (PAU)

Phytochemicals	**Extracts**		
	CH_2Cl_2	**CH_3OH**	**H_2O**
Alaloıds	-	+	-
Coumarin	-	+	+
Anthracene derivative	-	+	+
Flavonoıd	-	+	+
Cardiac glycosides	-	-	-
Essential oils	+	-	-
Lignan	-	-	+
Naphtoquinones aglycones	-	-	-
Anthocyanic pigments	-	-	+
Saponin	-	-	+
Tannin	+	-	-
Terpenes glycosylated	-	-	+
Triterpene	+	-	-

(+) present, (-) absence; CH_2Cl_2: dichloromethane extract, CH_3OH: Methanol extract, H_2O: aqueous extract

Table 2: heavy metals content of aqueous extracts of PAU and CAU

Parameters	***Concentrations of heavy metals (ppm)***	
	Cotonou sample	**Pobe Sample**
[Zn]	0.034 ± 0.006	0.087 ± 0.020
[Cd]	Nd	Nd
[Pb]	0.028 ± 0.007	Nd
[As]	0.340 ± 0.036	Nd
[Hg]	0.084 ± 0.054	Nd
[Mn]	0.108 ± 0.052	0.042 ± 0.027
[Cu]	0.054 ± 0.004	0.013 ± 0.00063

Zinc (Zn), Cadmium (Cd), Lead (Pb), arsenic (As), mercury (Hg), Manganese (Mn), Copper (Cu)

Table 3: Effect of aqueous extract of PAU on biochemical parameters

Biochemical parameters	Control	Tests	*P value*
Glycémie	1.27 ± 0.40	1.25 ± 0.07	0.8711
Créatinine	6.62 ± 0.42	6.42 ± 0.3	0.5451
ALAT	114.33 ± 11.84	96.67 ± 17.21	0.2256
ASAT	144.33 ± 32.04	160.33 ± 35.16	0.5917

ALAT: alanine aminotransferase, ASAT: aspartate transaminase. Values are Mean ± SEM (n = 3), differences were considered significant when p-values less than 0.05 ($p < 0.05$)

Table 4: Effect of aqueous extract of PAU on hematological parameters

Hematological parameters	Control	Tests	P-value
Hb (g/dl)	14.26 ± 0.61	13.83 ± 0.71	0.4686
Htc %	46.54 ± 3.02	43.85 ± 3.05	0.3399
VGM (fl.)	53.66 ± 2.51	53 ± 00	0.691
TCMH (Pg)	16.46 ± 0.81	16.7 ± 0.3	0.6785
CCMH%	30.66 ± 0.75	31.53 ± 0.67	0.2099
GR(T/L)	8.65 ± 0.47	8.26 ± 0.52	0.4017
GB (G/L)	16.22 ± 5.62	14.42 ± 2.39	0.6494
L%	68.73 ± 10.38	65.13 ± 4.47	0.6234
NEUT%	18.03 ± 13.7	15.16 ± 2.2	0.7542
M%	13.23 ± 3.91	19.67 ± 6.60	0.2354
PLT (G/l)	719 ± 80.06	726.33 ± 49.33	0.9003

Hb: hйmoglobine ,**Hte :** hйmatocrite; **GR :** globules rouges ; **CCMH :** concentration corpusculaire moyenne en hйmoglobine ; **VGM :** volume globulaire moyen ; **TCMH :** taux corpusculaire moyen en hйmoglobine ; **GB :** globules blancs ; **NEUT:** neutrophiles; **PLT :** plaquette; **L :** lymphocytes ; **M :** monocytes, **P:** valeur de la Probabilitй associйe

Figure 1: Effect of aqueous extract of PAU on body weight of rats

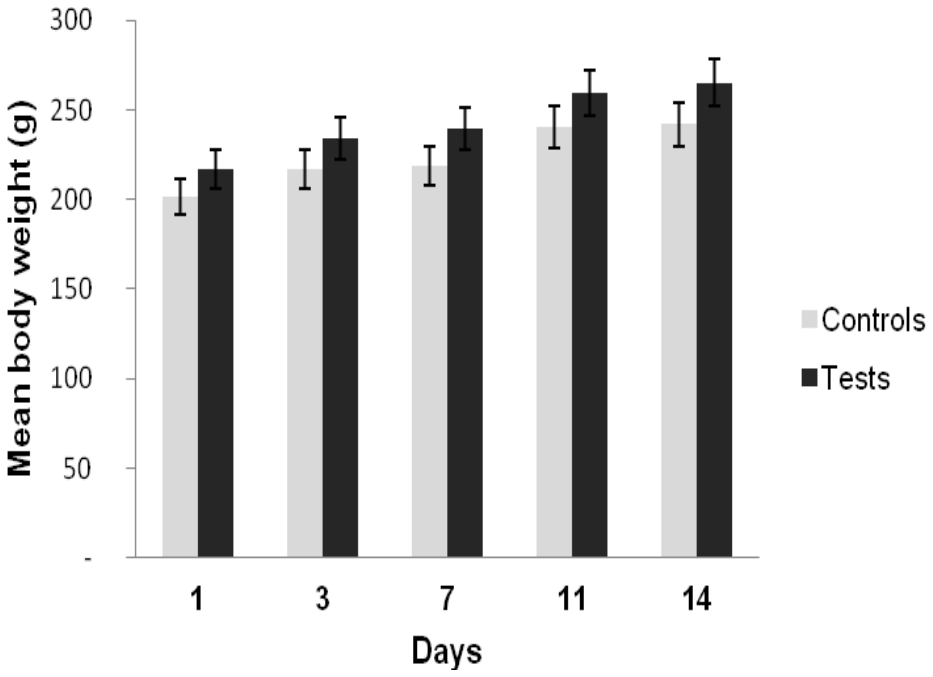


Figure 2: PAU effect on the mean relative weight of kidney and liver of tested rats

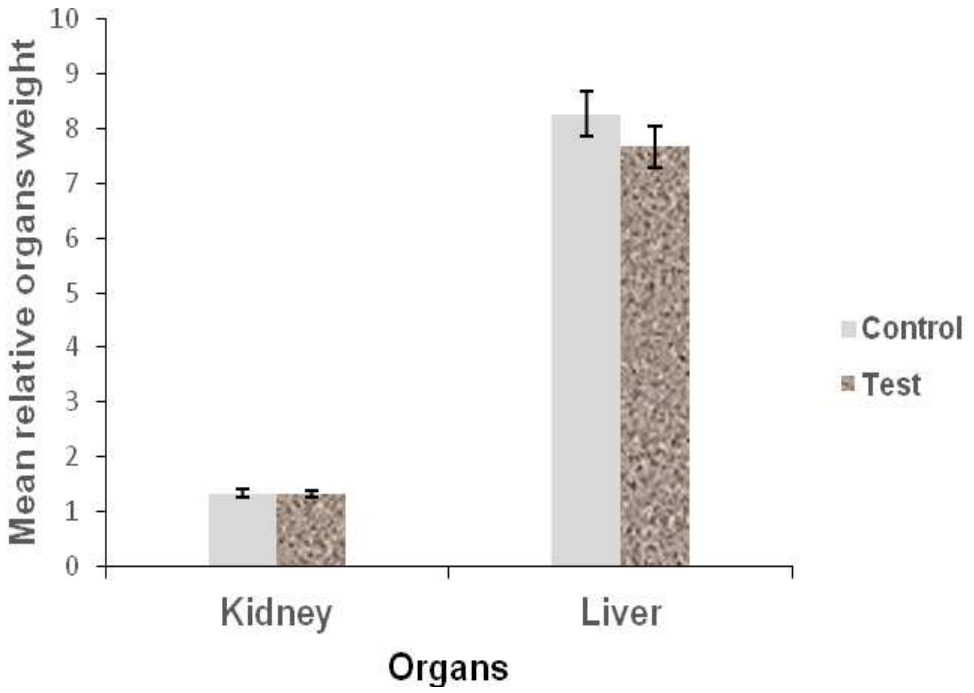
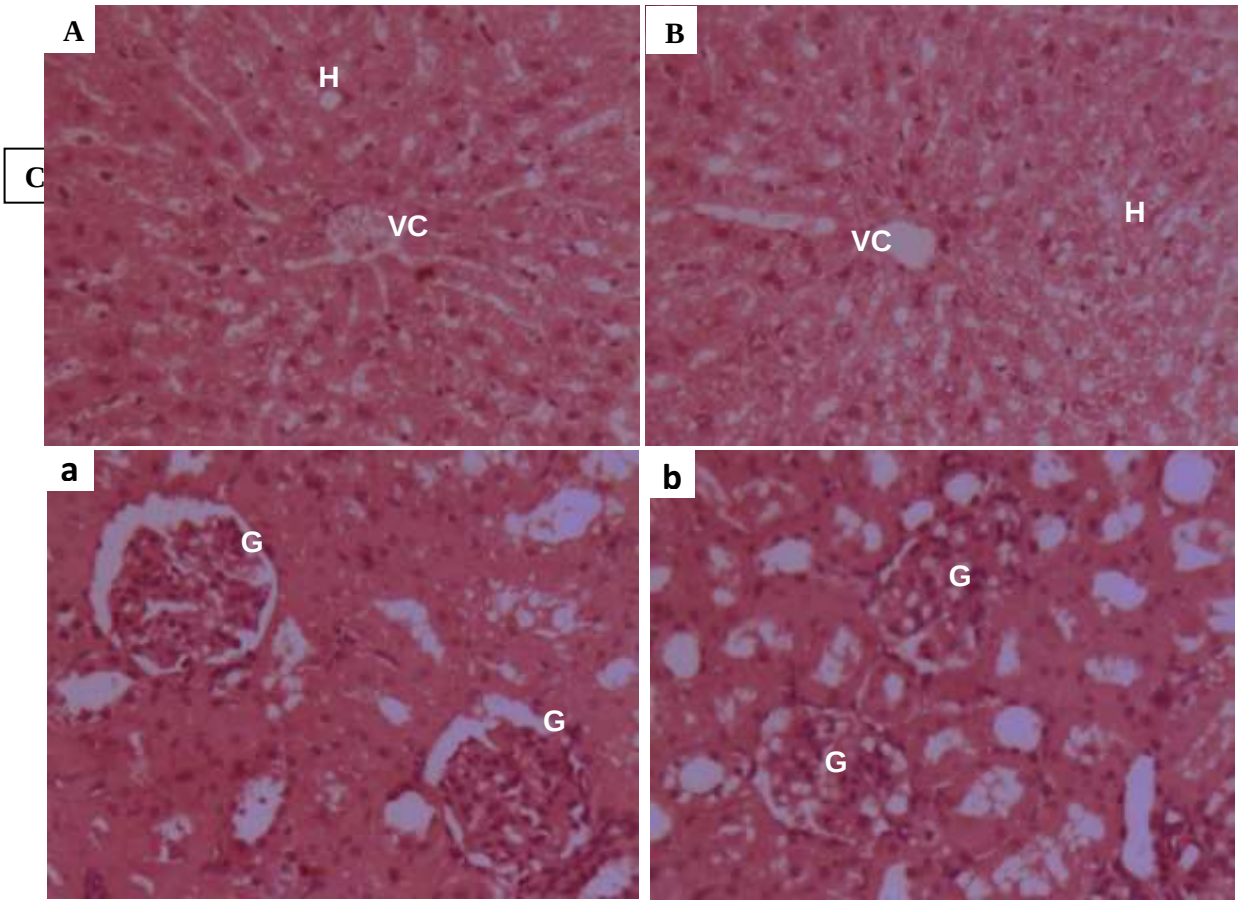


Figure 3: Histological observations of kidney and liver A : Control liver B : liver of treated rats a : kidney of control ; b : kidney of treated rats ; G : cortical glomeruli VC : central veins; H: hepatocytes



DISCUSSION

Creatinine is used for diagnosis of renal function and transaminases provide information on the liver and kidneys functions [19, 20]. The non-significant difference in the level of creatinine and transaminases could suggested that PAU aqueous extract does not affect liver and kidney after administration of a single dose of 2000 mg/kg bw. These results differ from our previous study in which significant changes in biochemical parameters were observed in rats after administration of the aqueous extract of CAU. Significant changes were also observed in all the hematological parameters of treated rats. These results indicated potential liver injury with a significant increase in creatinine levels [10]. This change in CAU and PAU oral toxicity could be due to the collected area of the two samples. In fact, CAU was collected in an industrial area while PAU was harvested in a rural area. The assessment of heavy metals contents of CAU and PAU showed significant toxic heavy metals in CAU while three essential metals were found in the extract of PAU. Previous study has already showed the impact of the collection area on the presence or absence of heavy metals in plants [21]. Previous study also reported hepatocellular effects in mice exposed to mixtures of Pb + Hg + Cd and Pb + Hg + As + Cd at low concentrations than those recommended by World Health Organization (WHO). A significant increase was observed in the activity of transaminase and alkaline phosphatase in the treated mice. A significant decrease in liver weight was also observed for the groups of mice exposed to Pb + Hg mixture [22]. Our previous work on CAU, have reported liver and kidney damage in rats treated with a single dose of 2000 mg/kg body weight. The lesions observed in the kidneys and liver could be due to the presence of residues of heavy metals and/or pesticides derived from processing vegetables, road traffic, exhaust gases from the airport and industries.

The simultaneous accumulation of heavy metals such as As, Pb, Hg in the aqueous extract of CAU could justify hepatic and renal lesions observed during previous study. These results indicate that *A. uliginosa* harvested in Pobи (PAU) is devoid of harmful metals and could be considered safe for consumption.

ETHICAL APPROUVAL

The toxicity assay was performed according to Organization for Economic Cooperation and Development, guidelines n° 423. The protocol was approved by the scientific committee of research protocols in the Laboratory.

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