

## Original Research Article

# Acute and sub-acute oral toxicity studies of an aqueous extract of *Chrysanthellum americanum* (L.) Vatke (Asteraceae) in rodents

### Abstract

*Chrysanthellum americanum* is a medicinal plant widely used in the North region of Cameroon for the treatment of epilepsy and convulsion. This study aimed to investigate the acute and subacute toxicity of the aqueous extract of *C. americanum* using experimental rodent models. For the acute toxicity test, a single dose of 27.69, 69.22, 138.45, 276.9 and 5000 mg/kg of extract was administered orally to mice. In the subacute study, the extract was administered orally to rats at the doses of 100, 250, 500 and 1000 mg/kg/day for 28 days; and finally, biochemical, haematological, and histological analysis were performed. The highest dose of the aqueous extract (5000 mg/kg) administered orally in the acute toxicity did not induce mortality. However, at the dose of 1000 mg/kg in the sub-acute treatment, an increase in white blood cell and platelet levels were observed in the haematological parameters. A slight decrease in the cholesterol and hepatic enzyme (ALAT, ASAT) levels for the biochemical parameters was noticed compared to the control group. From this study, it can be concluded that *C. americanum* can be considered safe in oral administration at the dose tested since it did not cause lethality or undesirable effects in the general behaviour.

**Keywords:** *Chrysanthellum americanum*, acute toxicity, sub-acute toxicity, aqueous extract

### 1. Introduction

Many years back, medicinal plants have been used in different regions of African countries for the treatment of several diseases [1]. The therapeutic use of plants for human diseases treatment is a very old practice and has evolved with the history of humanity. This use was mainly based on the idea that plants are natural, easily accessible and risk-free way of treatment. Despite the widespread use of herbal medicine, very few scientific studies have been provided information on the safety of *Chrysanthellum americanum* (L.) Vatke [2]. Consumers often believed that natural is synonymous of harmlessness. However, a plant can be both useful and toxic; it is a matter of dose: "Potion and poison have the same Latin root" [3]. Recently, in developed countries, interest in plants as a source of new drugs has increased. Toxicological study of any new drug is necessary before it is placed on the market. The interest of such study is to determine the degree of toxicity of the drug with the aim of better clinical prescription. The measurement of toxicity is also complex. Toxicity can be acute or

chronic and can vary from one organ to another depending on several factors such as age, genetics, sex, diet, physiological state or state of health of the organism [4]. “*Chrysantellum americanum* (L.) Vatke is a plant that belongs to the Asteraceae family. It is an herbaceous that grows in the tropical zone of Africa from Senegal to Nigeria and tropical America from southern Mexico to northern Brazil preferring the wastelands, dry and rocky” [5]. It has long been used in traditional medicine as hepatoprotector [6, 7, 8, 9]. “It is also used in the treatment of biliary drainage, **rheumatic and kidney pains as an analgesic**, vasoprotector capillary and venous endothelium” [10]. **The plant** is useful for cystic lithiasis, venous insufficiency, **arthritis, fever**, hepatitis, jaundice, and dysentery [11, 12, 13]. In Cameroon, studies on its anticonvulsant and anti-amnesic effects have been carried out [14]. Total flavonoids and total saponins present in the different parts of ***C. americanum*** induced a protective action against venous endothelium and relieve the liver after certain food poisoning [15, 16, 17]. “It also reduces the fragility and permeability of capillaries and veins and is therefore suitable for the treatment of venous diseases of the lower limbs” [14, 15]. “Most of the therapeutic properties of *C. americanum* extracts are attributed to saponins (chrysanthellin A and B) and flavonoids” [18, 19, 20, 15]. **The plant extract has been shown to possess** pharmacological activity, especially analgesic, anti-inflammatory, anti-cholesterolemic and anti-ulcer activity [5]. Despite the variety of uses and the widely recognized efficacy of the preparation of ***C. americanum***, the safety profile of this important medicinal plant has not been elucidated. The aim of this present study is to evaluate the toxicological profile of the decoction of whole plant of ***C. americanum***.

## **2. MATERIALS AND METHODS**

### **2.1. Chemicals**

All chemicals’ reagents used in the tests of the biochemical and haematological parameters come from Bio-Africa (Yaoundé, Cameroon).

### **2.2. Plant collection**

The whole fresh plant of ***C. americanum*** was harvested in April 2018 in Garoua, North region of Cameroon. **The plant** was identified at the National Herbarium of Cameroon (Yaoundé) by comparison with the sample recorded under the number 7728 / SRF / Cam. The whole plant **was** washed with tap water, dried at room temperature, crushed and sifted **with a 0.5 mm diameter mesh size sieve** to obtain fine a powder. The powder was kept in a tightly sealed glass for the preparation of the aqueous extract.

### 2.3. Plant aqueous extraction

To obtain aqueous extract, 10g of the plant powder was introduced into a beaker containing 100 mL of distilled water. The mixture was heated till boiling for 20 minutes on a hot plate at 100°C. After cooling, the mixture was filtered using Whatman paper number 1. The resulting filtrate was evaporated in the ventilated oven set at 60°C to obtain 1.8 g of dry extract of *C. americanum*, which allowed us to calculate the extraction yield of 18% following the formula below. Then the extract was kept at 4°C.

$$\text{Yield (\%)} = \frac{\text{Mass of dry extract (g)}}{\text{Mass of dry powder (g)}} \times 100$$

### 2.4. Experimental Animal

Rats (*Rattus norvegicus*) and mice (*Mus musculus*) used were obtained from the animal housing of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaounde, Cameroon. Sixty Swiss albinos mice of 2 to 3 months old (20-30 g) and thirty rats (150-160 g) of both sexes were used respectively for the assessment of acute and subacute toxicity. The animals grouped per sex, were maintained in the plexiglass cages under standard laboratory environmental conditions (ventilated room, 24°C, 75% relative humidity, 12 hours of light/dark cycle) and had free access to tap water and food.

### 2.5. Acute toxicity for the evaluation of LD<sub>50</sub>

Acute oral toxicity of *C. americanum* aqueous extract was assessed using OECD standard protocol [21]. Mice were randomly divided into six groups of 10 animals each per sex (5 females and 5 males). After the fasting period of 14 hours, all animals were weighed and the different doses of *C. americanum* administered were calculated based on the body weight of each animal. The plant extracts were prepared in distilled water. Animals were then treated as follows: The first group A (control group) received distilled water (10 ml/kg) while the other groups (B, C, D, E) received orally, respective doses of 27.69, 69.22, 138.45, 276.9 and 5000 mg/kg of aqueous extract in a single administration. Food was provided to the mice about one hour after the different treatments. Doses of aqueous extracts of *C. americanum* administrated to the rodents were those obtained from our previous studies on the anticonvulsant and anti-amnesic effect in mice models [14] and effective doses were considered for rodent toxicity tests. Toxicity symptoms displayed by animals were observed 30 minutes after administration,

then after every 1 hour for 8 first hours and then once a day for 14 days. All observations were systematically recorded individually for each animal. Mortality, changes in physical appearance, behaviour, injuries, pain and signs of illness were monitored daily during the whole study period [1].

## **2.6. Sub-acute toxicity**

Sub-acute toxicity of the aqueous extract of *C. americanum* was assessed in Wistar rats of 4-6 months old. At the beginning of the experiment, rats were divided into 5 groups of 6 female rats each (n = 6). The extract, dissolved in distilled water, was daily administered to the rats by oral gavage for 28 days according to OECD at the different doses of 100, 250, 500 and 1000 mg/kg, respectively for the test groups B, C, D and E. The control group (group A) received distilled water. The effects of oral administration of *C. americanum* aqueous extract on food and water intake were assessed daily and the body weight weekly (D7, D14, D21 and D28). After 28 days, the rats were sacrificed after an overnight fast, under ethyl ether anaesthesia. Blood collected from the jugular vein and serum (obtained after centrifugations of the blood) were submitted respectively, to haematological and biochemical analysis. Liver and kidneys were quickly removed and rinsed with a 0.9% saline solution. Each organ was weighted and then fixed in 10% formaldehyde solution for histopathological examinations.

## **2.7. Haematological and biochemical analyses**

The blood samples obtained at the end of the 28 days treatment period were analysed immediately using an automatic haematology analyser. Parameters analysed included white blood cells (WBC), red blood cells (RBC), leukocyte and platelet counts, haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). For the biochemical analysis, blood sample of each animal was allowed to clot and then centrifuged at 4000 rpm for 10 minutes. The serum collected was used for biochemical analyses such as total proteins (TP), transaminase aspartate (ASAT), transaminase alanine (ALAT), uric acid (URIC), creatinine (CRE), total cholesterol (CHOL) and glucose (GLU) levels using standard analysis kits from Fortress Diagnostics Ltd, UK.

## **2.7. Histopathological studies of liver and kidneys**

All histopathological studies on liver and kidneys were performed according to the following procedures. The organs were dehydrated with 100% ethanol solution and preserved in paraffin. They were then treated in thick sections of 4 to 5 µm, then stained with haematoxylin-eosin and observed under a microscope.

## 2.8. Statistical analysis

Results are expressed as mean  $\pm$  standard error of mean (S.E.M.). Significance differences between control and experimental groups were assessed by Tukey's post hoc test for multiple comparisons using Graph Pad Prism software. *P*-values less than 0.05 were considered to be significant.

## 3. RESULTS AND DISCUSSION

“Since ancient times, in African, Chinese and Ayurvedic medicines, plants have been used as important source of natural chemical compounds with enormous therapeutic potential” [22, 23]. “Plant medicine is the oldest form of health care known to humanity and, about 80% of the world's population use it to manage health problems” [24]. “Almost any substance can be harmful at certain doses but, at the same time, can have benefit effects at a lower dose. Between these two limits, there is a range of possible effects, from subtle long-term chronic toxicity to immediate lethality” [25]. During the acute toxicity assessment period, no deaths or signs of severe toxicity were observed after single oral administration of the different doses of *C. americanum* aqueous extract up to the highest dose (5000 mg/kg). At the highest dose of 5000 mg/kg, 1 hour after the oral administration of *C. americanum*, signs and symptoms responses occurred in both males and females mice and these included a decrease in motor activity, heaving breathing and fur erected (Table 1).

**Table 1.** Acute toxicity of *Chrysanthellum americanum* aqueous extract administered orally to different groups of mice

Dose (mg/kg)	Sex	D/T	Mortality latency (h)	Toxic symptoms
10ml/kg (distilled water)	Male	0/5	–	None
	Female	0/5	–	None
27.69 mg/kg	Male	0/5	–	None
	Female	0/5	–	None
69.22 mg/kg	Male	0/5	–	None
	Female	0/5	–	None
138.45 mg/kg	Male	0/5	–	None
	Female	0/5	–	None
276.90 mg/kg	Male	0/5	–	None
	Female	0/5	–	None

5000 mg/kg	Male	0/5	–	motor activity, heavy breathing and fur erected
	Female	0/5	–	motor activity, heavy breathing and fur erected

D/T = Dead/Treated mice; None = No toxic symptoms observed; mortality latency = time to death after oral administration.

The aqueous extract of *C. americanum* was administered to different groups of 10 mice (5 males, 5 females) per os in a single dose taken at different doses. The animals in each group were carefully examined for any signs of toxicity (behavioural changes and mortality) for 14 days. The control group received distilled water (10 ml/kg, *p.o.*).

A practical way to characterize the toxicity of a substance is to determine its lethal dose 50 (DL<sub>50</sub>). This dose identifies the symptoms of intoxication and compares the substances with each other as to their toxic potential. The acute toxicity test assesses adverse events that occur in a short period of time after the administration of a single dose of a test substance. The results of the acute toxicity study indicate that the aqueous extract of *C. americanum* administered orally at different doses (27.69, 69.22, 138.45, 276.9 and 5000 mg/kg) induced no signs of severe toxicity or death in mice, and consequently, the DL<sub>50</sub> value of the extract could be greater than 5000 mg/kg. However, laboured breathing, reduced motor activity and poor hair-coat were observed in animals that received the dose of 5000mg/kg. These changes can be explained by the fact that, an analysis of the protein fraction of *C. americanum* revealed a raw protein content determined by the Kjeldahl method equal to 10%, after the acid hydrolysis of proteins, by GC-MS, fifteen amino acids were identified: among which GABA [10] an inhibitory neurotransmitter that is generally present in anti-epileptic drugs and has sedative properties. This sedation property may be explained by the present of flavonoids in the extract which are recognized for their strong power of antidepressive and anxiolytic [26, 27]. “The aqueous extract of *C. americanum* can therefore be considered to be safe during oral acute administration up to dose 5000mg/kg. The current results of the acute toxicity are consistent with the study identified in other reviews where the aqueous extract of *C. americanum* administered orally, showed a lethal dose of 50% (DL<sub>50</sub>) greater than 3000 mg/kg of body weight” [9].

A sub-acute toxicity study examined the toxicity caused by repeated doses over an extended period of 28 days of oral administration in rats. In this subacute toxicity study, rats treated with the aqueous extract of *C. americanum* orally at the doses of 100, 250, 500 and 1000 mg/kg showed no signs of morbidity and mortality. During the experimental period, no apparent changes in behaviour were observed compared to the control group. The effects of daily administration of *C. americanum* aqueous extracts on body weight variation; food and volume of water consumed during the treatment period are shown in Table 2. In addition, a significant decrease ( $P < 0.05$ ) in the amount of food consumption was observed in animals of group E (1000mg/kg) compared to the control group (distilled water). Concerning the volume of water consumed by the rodents during these 28 days, no significant difference is noted in treated group compared to the control group. There were no significant difference also in body weight gain or loss was noted between the control group (A) and the treated groups (B, C, D and E) which is respectively of  $187.8 \pm 4.8$  g,  $135.3 \pm 3.76$  g,  $206.7 \pm 1.86$  g,  $175.3 \pm 5.01$  g and  $183.8.3.1 \pm 4$  g. Based on these observations, we can therefore conclude that the normal increase in body weight determined the health status of the animals.

**Table 2.** Effects of *Chrysanthellum americanum* aqueous extract administration on water and food consumption and weight gain

Weekly treatment	Control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Food (g/day/rat)					
W1	$157.71 \pm 3.5$	$147.14 \pm 1.56$	$157.85 \pm 1.87$	$154.71 \pm 2.34$	$139.71 \pm 4.87^*$
W2	$160 \pm 2.40$	$159 \pm 1.98$	$158 \pm 3.9$	$159.71 \pm 3.76$	$140 \pm 3.98^*$
W3	$168 \pm 1.96$	$167.71 \pm 2.08$	$166.71 \pm 2.97$	$164.85 \pm 4.02$	$143 \pm 4.35^*$
W4	$172.71 \pm 3.76$	$178.85 \pm 1.54$	$169.14 \pm 1.23$	$173.57 \pm 2.87$	$147.57 \pm 3.64^*$
Water (ml/day/rat)					
W1	$50 \pm 3.55$	$44.28 \pm 16.93$	$52.14 \pm 15.89$	$50 \pm 15.56$	$54.28 \pm 9.90$
W2	$55.85 \pm 3.37$	$56.42 \pm 20.74$	$69.28 \pm 18.82$	$51.71 \pm 12.62$	$52.14 \pm 12.06$
W3	$55 \pm 6.12$	$66.71 \pm 12.12$	$63.85 \pm 10.22$	$57.142 \pm 14.12$	$54.42 \pm 9.27$
W4	$57.14 \pm 7.78$	$57.85 \pm 9.22$	$67.85 \pm 16.72$	$63.57 \pm 18.90$	$55.14 \pm 9.34$
Weight (g)					
W1	$164.5 \pm 4.76$	$113.1 \pm 2.6$	$184.7 \pm 1.79$	$153.7 \pm 4.38$	$175.8 \pm 3.07$
W2	$169.3 \pm 3.89$	$121.1 \pm 3.45$	$192.8 \pm 1.83$	$158.7 \pm 5.06$	$178.7 \pm 4.07$
W3	$173.2 \pm 3.82$	$131.9 \pm 2.03$	$198.2 \pm 2.13$	$162.7 \pm 4.96$	$180.5 \pm 3.12$
W4	$187.8 \pm 4.8$	$135.3 \pm 3.76$	$206.7 \pm 1.86$	$175.3 \pm 5.01$	$182.8 \pm 3.14$

The results are expressed in mean  $\pm$  S.E.M. n = 6 animals; the significant difference compared to the control group were: \*P < 0.05. W: week of treatment. The aqueous extract of the plant were administered daily orally to groups of rats (n=6) at the

following doses: Distilled water (control), 100 mg/kg, 250 mg/kg and 500 mg/kg, 1000 mg/kg for 28 days.

The biochemical profile of rats treated with the aqueous extracts of *C. americanum* (100, 250, 500 and 1000 mg/kg) are presented in Table 3. The biochemical analysis is a common test that measures certain parameters in blood and urine to diagnose diseases or treatments. Basically, it involves determining the concentration and comparison of biochemical indices in blood components. In this case the results show no significant changes in the level of total proteins, creatinine, glucose, urea in animals that received the aqueous extract of *C. americanum* for 28 days compared to the control group. During this period, there was a significant ( $P < 0.05$ ) decrease in liver marker enzymes ALAT (20.76 U/I), ASAT (60.85 U/I) and cholesterol (39.55 mg/dl) in treated animals at the dose of 1000 mg/kg compared to control group (25.6 U/I for ALAT, 63.39 U/I for ASAT and 51.28 mg/dl for cholesterol). However, no significant variation of glucose, protein, urea and creatinine was registered in test groups compared to the control. These observations of significant decrease in liver enzyme levels and total cholesterol may indicate that the aqueous extracts *C. americanum* has respectively hepatoprotective effects and contain hypolipemic agents in the extract [28, 29, 30].

**Table 3.** Effect of sub-acute oral administration of the aqueous extract of *Chrysanthellum americanum* on the biochemical parameters of Wistar rats

Biochemical Parameters	Control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Glucose (mg/dl)	78.51± 1.09	65.27±4.36	67.07±3.80	81.94±2.94	82.57±1.76
Protein (mg/dl)	10.94±0.72	10.09±1.15	10.18±1.91	9.34±0.75	8.34±0.75
Cholesterol (mg/dl)	51.28±0.09	45.77±0.09	43.39±0.44	43.65±0.07	39.55±0.04*
Urea (mg/dl)	36.06±3.90	37.02±1.18	36.22±5.39	35.70±5.34	33.84±2.83
Creatinine (mg/dl)	5.11±0.53	5.26±2.46	5.09±2.61	4.09±3.52	4.17±5.41
ALAT (U/I)	25.6±0.32	24.9±0.43	23.92±0.46	23.82±0.39	20.76±0.29*
ASAT (U/I)	63.39±0.27	61.93±0.40	61.25±0.32	62.11±0.33	60.85±0.38*

The results are expressed in mean ± S.E.M. n = 6 animals. The significant difference compared to the control group was: \*P < 0.05. The aqueous extracts of the plant were administered daily orally to groups of rats (n=6) at the following doses: Distilled water (control), 100 mg/kg, 250 mg/kg and 500 mg/kg, 1000 mg/kg for 28 days.

The assessment of haematological parameters can be used to determine the extent of the harmful effects of foreign compounds, including plant matter, on blood [31]

The evaluation of the effects of subacute oral administration of the aqueous extracts of *C. americanum* (100, 250, 500 and 1000 mg/kg) on haematological parameters is summarized in Tables 4. Only the number of white blood cells and platelets was significantly ( $P < 0.05$ ) higher in the treated groups at the dose of 500 mg/kg ( $17.13 \times 10^3/\mu\text{l}$ ) and 1000 mg/kg ( $19.93 \times 10^3/\mu\text{l}$ ) compared to the control group ( $12.75 \times 10^3/\mu\text{l}$ ). Platelets were also high in the rat groups treated with plant extract at doses of 250 mg/kg ( $973.00 \times 10^3/\mu$ ) and 1000 mg/kg ( $927.17 \times 10^3/\mu$ ) compared to the control group ( $855.83 \times 10^3/\mu$ ). However, no significant variation on RBC, HGB, MCV, MCH, MCHC and HCT counts or levels was registered. No significant changes in the haematological parameters of treated rats could be attributed to plant extract. This increase in white blood cells directly indicates the strengthening of the body's immune defence [31]. So, suggests that the plant extracts contain biologically active ingredients that can strengthen the immune system by increasing the number of defensive white blood cells.

**Table 4.** Effect of sub-acute oral administration of aqueous extract of *Chrysanthellum americanum* on haematological parameters of Wistar rats.

Parameters	Control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
WBC ( $\times 10^3/\mu\text{l}$ )	12.75 $\pm$ 3.23	12.77 $\pm$ 2.01	13.03 $\pm$ 0.69	17.13 $\pm$ 4.16*	19.93 $\pm$ 3.07*
RBC ( $\times 10^6/\mu$ )	7.75 $\pm$ 0.35	8.10 $\pm$ 0.67	7.98 $\pm$ 0.36	8.08 $\pm$ 0.36	8.18 $\pm$ 0.65
HGB (g/dl)	14.07 $\pm$ 0.68	13.60 $\pm$ 1.50	14.00 $\pm$ 0.41	14.12 $\pm$ 0.51	14.18 $\pm$ 0.89
MCV (fL)	52.97 $\pm$ 1.13	52.73 $\pm$ 0.69	52.90 $\pm$ 1.29	53.48 $\pm$ 1.13	53.02 $\pm$ 2.0
MCH (pg)	18.02 $\pm$ 0.72	16.78 $\pm$ 0.92	17.57 $\pm$ 0.54	17.47 $\pm$ 0.50	17.37 $\pm$ 0.82
MCHC(g/dl)	34.00 $\pm$ 0.86	31.72 $\pm$ 1.82	33.18 $\pm$ 0.46	32.68 $\pm$ 0.47	32.77 $\pm$ 0.60
HCT (%)	41.35 $\pm$ 0.01	42.67 $\pm$ 0.03	42.18 $\pm$ 0.01	43.2 $\pm$ 0.02	43.28 $\pm$ 0.03
PLT( $10^3/\mu$ )	855.83 $\pm$ 127.88	886.5 $\pm$ 369.13	973 $\pm$ 198.80**	881 $\pm$ 137.29	927.17 $\pm$ 187.5**

**Results** are expressed in mean  $\pm$  S.E.M. n = 6 animals. The significant difference compared to the control was: \* $P < 0.05$  and \*\* $P < 0.01$ . The aqueous extracts of the whole plant were administered daily orally to groups of rats (n = 6) at the following doses: Distilled water (control), 100 mg/kg, 250 mg/kg and 500 mg/kg, 1000 mg/kg for 28 days. Number of white blood cells (WBC), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), platelets (PLT), average globular volume (MCV), medium corpuscular haemoglobin (MCH), mean haemoglobin concentration (MCHC).

Table 5 shows the effects of sub-acute oral administration of the aqueous extracts of *C. americanum* on the organ weights of Wistar rats. In this study, there was no significant change in the weight of the liver, kidneys, spleen, heart and brain of the animals of

groups treated with extract at the different doses over the 28 consecutive days compared to animals in the control group.

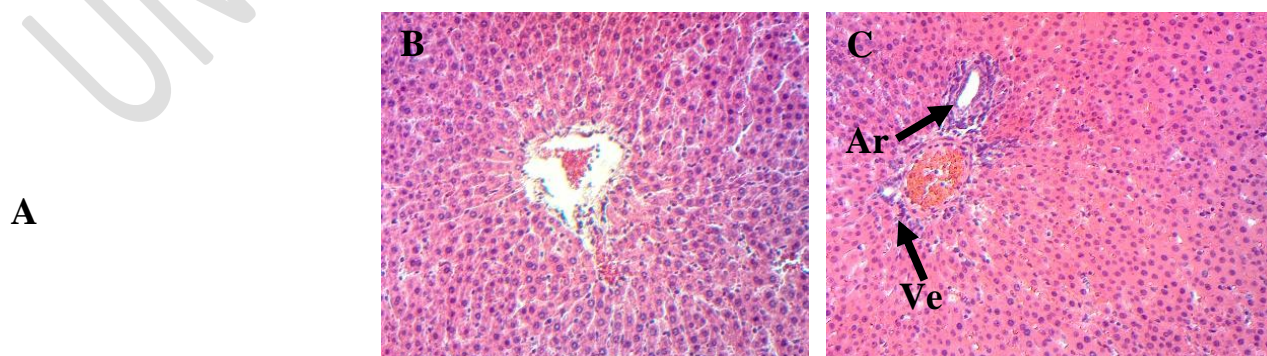
**Table 5.** Effect of sub-acute oral administration of the aqueous extract of *Chrysanthellum americanum* on the organ weights of Wistar rats

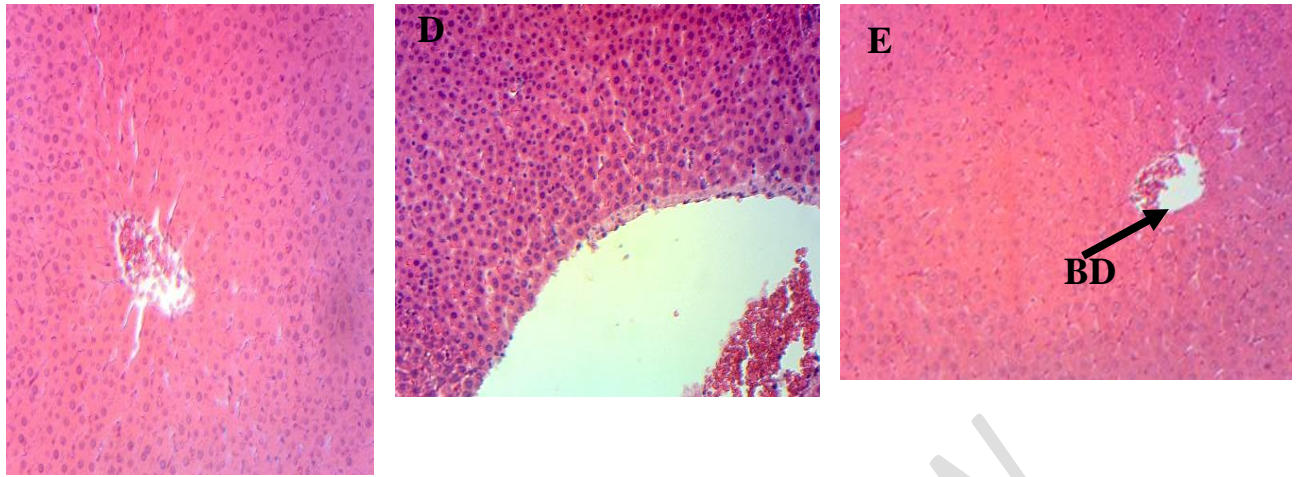
Organs	Control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	6.37±0.33	5.83±1.69	6.01±0.41	5.36±0.21	5.92±0.46
Kidney	1.49±0.26	1.40±0.28	1.27±0.08	1.14±0,05	1,22±0.05
Brain	2.11±0.08	2.10±0.08	2.05±0.12	1.97±0.07	2.02±0.11
Spleen	0.71±0.07	0.79±0.13	0.74±0.25	0.71±0.07	0.75±0.10
Heart	0.83±0.05	0.79±0.05	0.80±0.06	0.80±0.06	0.79±0.05

The results are expressed in mean ± S.E.M. n = 6 animals. There is no significant difference compared to the control. The aqueous extracts of the whole plant were administered daily orally to groups of rats (n = 6) at the following doses: Distilled water (control), 100 mg/kg, 250 mg/kg and 500 mg/kg, 1000 mg/kg for 28 days.

“The Evaluation of liver and kidney function is of primary importance in assessing the toxic properties of extracts and medications” [31]. Histological examination of some visceral organs (liver and kidney,) of animals who received daily the different doses of *C. americanum* aqueous extracts for 28 days are presented in the figure 1 and 2. The results revealed no signs of vascular or inflammatory changes.

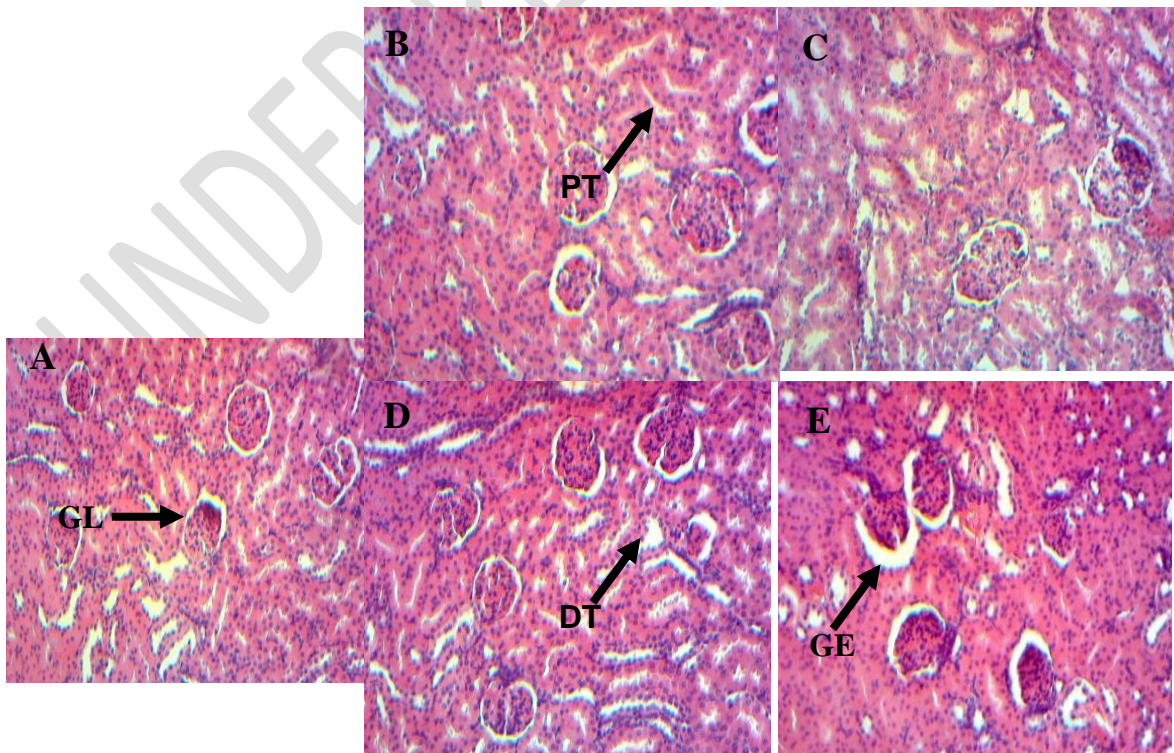
Figure 1 shows the histopathology of the hepatic tissue of animals of the control group and test groups treated with the different doses of extracts. We observed a normal vein and artery structure and a clearly visible of bile duct in this organ. We observed a leukocyte infiltration on the sections of rats of group E treated with 1000 mg/kg of aqueous extract of *C. americanum*. The biochemical, haematological and histological analysis did not show any abnormality, it could be explained by poor rinsing of the section before reading under the microscope.





**Figure 1.** Photomicrographs of stained liver sections of rats treated with aqueous extract of *C. americanum*. A: distilled water (10 ml/kg), B: 100 mg/kg, C: 300 mg/kg, D: 500 mg/kg, E: 1000 mg/kg, Ve: Vein, Ar: Artery, BD: Bile Duct, Magnifications, all  $\times 100$ .

Figure 2 shows the histopathology of the renal tissues of rats. In this present study, we observed a normal structure of the glomerulus, Proximal Tube, Distal Tube, Glomerular space in kidney sections of the animals. No abnormality was also observed on the organs of animals receiving the different dose of the aqueous extract.



**Figure 2.** Photomicrographs of stained Kidney sections of rats treated with aqueous extract of *C. americanum*. A: distilled water (10 ml/kg), B: 100 mg/kg, C: 300 mg/kg, D: 500 mg/kg, E: 1000 mg/kg, GL : Glomerulus, PT : Proximale Tube, DT : Distal Tube, GE : Glomerular space Magnifications, all  $\times 100$ .

Macroscopic and microscopic examination of some vital organs show that the aqueous extract of *C. americanum* at the dose 1000 mg/kg which is the highest dose used in this subacute toxicity study produced no alteration of the cell structure or harmful effect on organs.

## 5. Conclusion

The oral administration of different doses of *C. americanum* aqueous extract in the acute toxicity test did not cause any lethality or adverse changes in the general behaviour. Nevertheless, some sedative effects were recorded in mice treated with the high dose of 5000 mg/kg of *C. americanum* extract. The sub-acute oral administration of the plant extract did not induce significant alterations in almost all biochemical and haematological parameters in rats. However, a significant increase of white blood cell and platelet was registered in rats treated. Besides, slight decreases of cholesterol and hepatic enzyme (ALAT, ASAT) levels in rats treated with the plant extracts were also recorded. Thus, we can conclude that *C. americanum* aqueous extract might be considered as less or non-toxic since  $LD_{50} > 5000$  mg/kg and no significant variation was noticed in biochemical and haematological parameters of the rodents and also without toxicity symptoms in the rat organs.

## Ethical Approval

The study was carried out in accordance with the Cameroon National Ethical Committee (Ref No. FW-IRB00001954, 22 October 1987). The authorization number (UB-IACUC N°06/2022) was given and the study was done also in conformity with the international regulation, minimizing the number of rodents used and their suffering.

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