GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Phytochemical composition and toxicity study of the aqueous extract of the leaves of *Vernonia colorata* (Willd.) Drake in Wistar rats

SAWADOGO Paténéma [1,*], SAWADOGO Touwindséda Aimée [1], DA Filkpièrè Léonard [2], TINDANO Basile [1], OUEDRAOGO Youssoufou [1] and BELEMTOUGRI Gourounga Raymond [1]

[1] *Laboratory of Animal Physiology, Sciences, Training and Research Unit of Life and Earth Sciences, University Joseph Ki-Zerbo, 03 BP 7021, Ouagadougou 03, Burkina Faso.*
[2] *Laboratory of Life and Earth Sciences, Training and Research Unit of Sciences and Technology, University Norbert Zongo of Koudougou, BP: 376, Burkina Faso.*

GSC Biological and Pharmaceutical Sciences, 2022, 18(03), 155–163

Publication history: Received on 11 February 2022; revised on 16 March 2022; accepted on 18 March 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.18.3.0103

Abstract

The aim of the work is to study phytochemical composition and acute and subacute toxicities of the aqueous extract of leaves of *Vernonia colorata* (AEVC). The acute toxicity study was performed by administering a single oral dose of AEVC of 5000 mg/kg body weight (bw) to female Wistar rats. For toxicity study, the treatments were administered orally for 28 consecutive days. The control group received distilled water (10 mg/kg bw). The test groups received doses of 100, 500 and 1000 mg/kg bw respectively and the satellite groups received distilled water (satellite control) and extract at the maximum dose of 1000 mg/kg bw (satellite). Acute toxicity assessment showed that the LD_{50} is greater than 5000 mg/kg bw. The study of the biochemical parameters showed a very significant decrease ($p < 0.01$) in ALAT at a dose of 100 mg/kg bw, thus reflecting the extract hepatoprotective effect. The results also showed an arise of phosphatemia ($p < 0.01$) at doses of 500 and 1000 mg/kg bw. For the hematological parameters, the red blood cell count increased significantly ($p < 0.05$) at the dose of 100 and 500 mg/kg bw and very significantly ($p < 0.01$) at the dose of 1000 mg/kg bw. On the other hand, a highly significant ($p < 0.001$) decrease in platelets was observed. From these results, we conclude that in therapy, the use of *Vernonia colorata* extract in the medium term would not present a risk of toxicit.

Keywords: *Vernonia colorata*; Acute toxicity; Subacute toxicity; Phytochemical; Rats

1. Introduction

The medicinal use of the leaves and roots of plants in the control and treatment of disease has been common practice for a long time. The genus *Vernonia* belongs to the Asteraceae family. Some of its species are used in food and in traditional medicine. It is distributed in the tropical and subtropical region of Africa, Asia and America [1]. One of these species, *Vernonia colorata* (Willd.) Drake, a shrubby plant of 3 to 4 meters high, is widespread in countries such as Burkina, Mali, Togo, Benin, Senegal and Cameroon [2]. It is used there for the treatment of fever, gastritis, jaundice, arterial hypertension, general tiredness, yellow fever [3, 4] and in the human food.

Furthermore, the leaves of *Vernonia colorata* are used locally in decoction for the treatment of malaria and as an antibiotic in disease of the liver, stomach, gastrointestinal disorders and skin rashes [5]. In addition the decoction of stem, root and trunk barks of *Vernonia colorata* are used in the treatment of schistosomiasis, sterility and frigidity [6]. In the Center region of Burkina Faso, for example, ethnobotanical surveys showed that *Vernonia colorata* is among the species most used for the traditional treatment of certain infectious diseases such as malaria, stomach ailments, and

* Corresponding author: SAWADOGO Paténéma
Laboratory of Animal Physiology, Sciences, Training and Research Unit of Life and Earth Sciences, University Joseph Ki-Zerbo, 03 BP 7021, Ouagadougou 03, Burkina Faso.

Copyright © 2022 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

diseases of the skin [3, 7].Others studies have shown that *Vernonia colorata* macerates leaf have various pharmacological activities such as antidiabetic, antioxidant, antitumor, anti-inflammatory, diuretic, antihypertensive, ant plasmodium, hypoglycemic and analgesic [2, 3, 7, 8, 9]

However, few scientific data exists on the pharmacological properties of *Vernonia colorata*, particularly on the toxicity of the aqueous extract. The aim of this study is to highlight the phytochemical constituents and to study the acute and subacute toxicities of the aqueous extract of leaves of *Vernonia colorata* (AEVC) in Wistar rats.

2. Material and methods

2.1. Plants

The leaves of *Vernonia colorata* were collected at University of Joseph KI-ZERBO in Ouagadougou (12° 22' 42.05" N; 1° 29' 59.87" W) in October 2018 between 7 a. m. and 11 a.m. The plant sample has been authenticated by the team of the Herbarium of the University Joseph KI-ZERBO (Burkina Faso). A sample of the plant n° 6964, was preserved with the identification number, 17964.

2.2. Animal

Female Wistar rats of eight-week old provided by Laboratory of Animal Physiology of University Joseph KI-ZERBO were used. These animals were subjected to conventional animal husbandry conditions. They had free access to drinking water and food (granules enriched with 29% protein provided by AFAB "*Atelier de Fabrication d'Aliments pour Bétail de* Bobo Dioulasso"). The handling and treatment of the animals were done in accordance with the ethical standards of animal research of Burkina Faso. The experimental protocol approval number is CE-UJKZ/2020-04.

2.3. Preparation of extract

The fresh leaves of *Vernonia colorata* were dried in the laboratory under artificial ventilation and without sunlight. Five hundred grams (500 g) of leaf powder of *Vernonia colorata* were macerated in a stainless steel beaker at 30°C in 3000 mL of distilled water for 24 hours. The macerated obtained was filtered through a fine-mesh nylon cloth. The residue is taken up in a volume of 1000 mL of distilled water, mixed and filtered again. The filtrates obtained were then centrifuged at 2000 rpm for 10 min. The supernatants were recovered and lyophilized to obtain the aqueous extract of *Vernonia colorata* (AEVC).

2.4. Phytochemical screening

Phytochemical screening consisted of looking for important chemical groups in the total extract and in the hydrolyzed extract: the most phytochemical compounds checked were steroids and triterpene glycosides, flavonoids, anthracenosides, coumarins and derivatives, cardenolides, polyphenols (tannins), alkaloid salts, anthocyanosides, saponins and reducing compounds. The classical methods and techniques were used as described by Ciulei [10].

2.5. Acute and subacute toxicities of the aqueous extract of *Vernonia colorata*

2.5.1. Acute toxicity

The acute toxicity study was conducted according to guideline 423 of OECD [11]. Six (06) nulliparous rats of 8 weeks old were divided into 2 groups of 3 rats: a control group and a test groups. They are fasted for 24 hours before any administration of AEVC. The control group received distilled water at a rate of 10 mL/kg of body weight (bw). The test groups received orally a single dose of 5000 mg/kg bw of the extract solution in a volume of 10 mL/kg bw. After treatment, the rats were observed for one hour. All the groups were then resupplied (food and water) one hour after administration. The observations were achieved one hour, then after 24 hours, 48 hours, 72 hours, and 14 days and were focused on mortality, drowsiness, food intake, bristling hair, muzzle color, lethargy and mobility, eye color, type of defecation (diarrheal or not), convulsion, salivation.

2.5.2. Subacute toxicity

Subacute toxicity was performed according to OECD Guideline 407 for the Testing of Chemicals [12]. Thirty-six (36) rats of 8 weeks old, were divided into groups of 6 rats. The control group received distilled water (10 mL/kg bw). The three test groups received the extract at the doses of 100, 500, and 1000 mg/kg body weight repectively and in volumes of 10 mL/kg bw. The two satellite groups received distilled water at 10 mL/kg bw (satellite control), and the extract at the maximum dose of 1000 mg/kg bw (satellite), respectively. Administrations of the extract were made orally for 28

consecutive days. The satellite groups were observed 14 days after stopping treatment to assess any reversibility. Each week, the rats' body weights as well as the amount of food consumed were weighed. After 28 days of treatment, the control and test rats were anesthetized by intraperitoneal injection of a ketamine/xylazine solution corresponding to 1/10 of the body weight. The ketamine/xelazine solution was prepared respecting the proportions of 1 mL of ketamine (50 mg/mL) for 0.7 mL of xylazine (20 mg/mL). Blood from each rat was collected by cardiac puncture. Anesthesia and blood sampling of the satellite groups were done after 14 days of observation. The blood samples were collected in two types of tubes: a first type with anticoagulant (EDTA) for hematological analyzes and a second type without anticoagulant (dry tubes) for biochemical analyses. The blood collected in the dry tubes was left to settle for sedimentation at room temperature and then centrifuged at 3600 rpm for 10 minutes. The aliquots of collected serum were stored in a freezer at -20°C for the various biochemical assays.

After blood collection, rats were autopsied, and lungs, kidneys, spleen, liver, and hearts were taken and weighed and related to the whole animal's weight (relative organ weight).

2.6. Statistical analyses

The data was entered with the Excel 2016 software package. The GraphPad Prism 5.03 software (GraphPad Software Inc, San Diego, USA) was used for the calculations of means and standard errors, statistical tests and graphical representations. One-way analysis of variance (ANOVA I) followed by Tukey-Kramer post-hoc test of GraphPad in SAT software were used to compare the data. The difference between the values was considered statistically significant, very significant and highly significant if, $p < 0.05$; $p< 0.0l$; and $p< 0.00l$, respectively.

3. Results and discussion

3.1. Phytochemical composition

Phytochemical screening tests revealed the presence of saponosides and polyphenols (tannins) in the total aqueous extract. After hydrolysis of AEVC to isolate organic fraction, characterization revealed the presence of steroidal and triterpene glycosides, anthracenosides and coumarins and derivatives (Table 1)

Table 1 Phytochemical compounds of *Vernonia colorata*

Total aqueous extract	
Polyphenols (tanins)	Presence
Saponosides	Presence
Alkaloids salts	No detected
Anthocyanosides	No detected
Reducing compounds	No detected
Hydrolyzed aqueous extract	
Steroidal and triterpenes glycosides	Presence
Anthracenosides	Presence
Coumarins and its derivatives	Presence
Flavonoids	No detected
Cardenolides	No detected

3.2. Acute toxicity

Oral administration of EAVC to rats at dose of 5000 mg/kg bw did not cause abnormal behavior or animal death during the first 72 hours. The effects observed during the 14 consecutive days following administration did not differ from those of the first 72 hours. The LD_{50} is therefore greater than 5000 mg/kg bw.

3.3. Subacute toxicity

Subacute toxicity was assessed from food intake, morphometric, biochemical and hematological parameters.

3.3.1. Effect of aqueous extract of Vernonia colorata on food intake

The aqueous extract of *Vernonia colorata* did not induce any significant variation in the food intake of the treated rats compared to the control (Figure 1). The extract did not influence the palatability of rats.

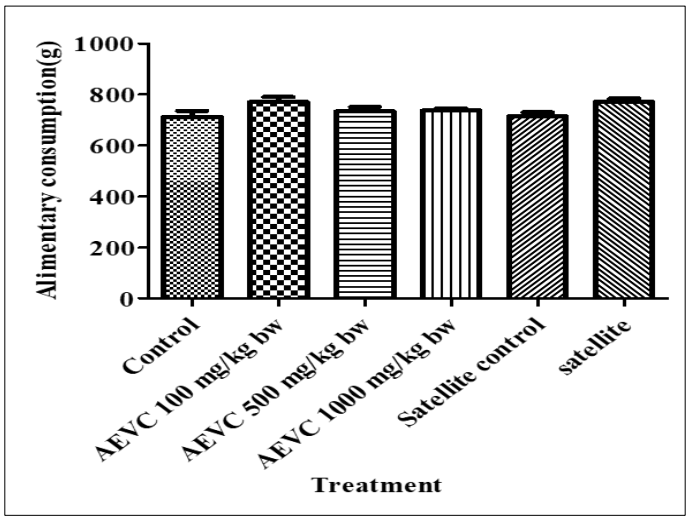

Figure 1 Mean food intake of the rats during the treatment. Values are expressed as mean ± standard error to the mean (SEM); n = 6

3.3.2. Body weight gain

Aqueous extract of *Vernonia colorata* did not induce significant difference in weight gains between the different groups of animals (control, tests and satellites). However, with the exception of the satellite control, a little peak ($p > 0.05$) in weight gain was observed during the first week. But the differences are not significant, compared to the weight gains of the following weeks (figure 2).

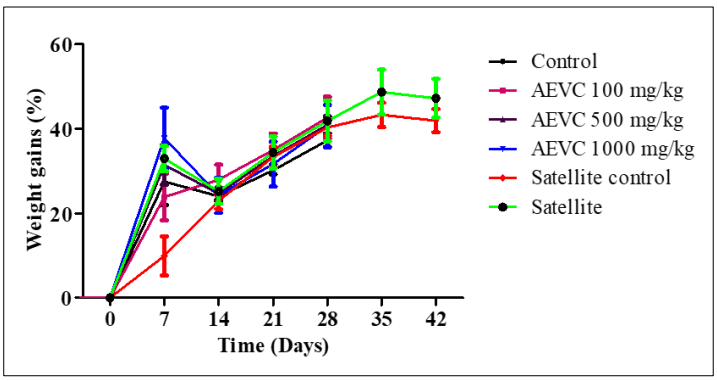

Figure 2 Evolution of weight gains of female rats treated with AEVC and controls during the treatment. Values are expressed as mean ± standard error to the mean (SEM); n = 6

3.3.3. Relative weights organs

The AEVC did not induce significant variation in the relative weights of the organs examined at the doses of 100, 500 and 1000 mg/kg bw compared to the control. Similar results were observed compared to the satellite and satellite control groups (Table 2).

Table 2 Effect of aqueous extract of *Vernonia colorata* on relative weights of rat organs

Organs	Treatment (mg/kg bw)					
	Control	**AEVC 100**	**AEVC 500**	**AEVC 1000**	**Control satellite**	**Satellite**
Lungs	0.51 ± 0.01	0.50 ± 0.02	0.52 ± 0.02	0.56 ± 0.03	0.52 ± 0.03	0.50 ± 0.03
Heart	0.37 ± 0.03	0.33 ± 0.03	0.38 ± 0.07	0.37 ± 0.01	0.29 ± 0.01	0.41 ± 0.00
Liver	2.26 ± 0.09	2.67 ± 0.09	2.87 ± 0.17	2.94 ± 0.07	3.51 ± 0.12	3.39 ± 0.15
Kidneys	0.67 ± 0.04	0.67 ± 0.04	0.70 ± 0.04	0.71 ± 0.04	0.58 ± 0.01	0.63 ± 0.03
Spleen	0.23 ± 0.00	0.23 ± 0.00	0.22 ± 0.01	0.33 ± 0.07	0.22 ± 0.01	0.27 ± 0.00

Values are expressed as mean ± standard error to the mean (SEM); n = 6.

3.3.4. Hematological parameters

The significant variations induced by AEVC were observed for the concentration of platelets (decrease) ($p < 0.001$), and those of red blood cells and hematocrit (increase of both).

For platelet concentrations, a highly significant decrease ($p < 0.001$) was noted at all the test doses used (100, 500 and 1000 mg/kg bw). For red blood cell concentrations, administration of AEVC induced a significant ($p < 0.05$) increase at the doses of 100 and 500 mg/kg bw and very significant ($p < 0.01$) at the dose of 1000 mg/kg bw. For the hematocrit, significant and very significant increases were observed, at the doses of 100 mg/kg bw and 1000 mg/kg bw, respectively.

For the other parameters, concentrations of hemoglobin, white blood cells, lymphocytes, monocytes and granulocytes, no significant variation was observed at the doses of AEVC used.

Between the rats of the satellite groups and those of the satellite control group, for all the hematological parameters, no significant variation was observed.

All of these results are presented in Table 3.

Table 3 Effect of aqueous extract of *Vernonia colorata* on hematological parameters

Hematological parameters	Treatments (mg/kg bw)					
	Control	**AEVC 100**	**AEVC 500**	**AEVC 1000**	**Control satellite**	**Satellite 1000**
WBC (10^3/µL)	8.05 ± 0.66	6.81 ± 0.73	9.35 ± 1.19	9.43 ± 0.61	5.58 ± 0.50	6.06 ± 0.14
RBC (10^6/µL)	3.63 ± 0.89	7.48 ± 0.08 *	6.37 ± 0.58 *	8.01 ± 0.27 **	2.81 ± 1.32	3.19 ± 1.35
Hb (g/dl)	14.7 ± 0,40	14.8 ± 0.21	14.76 ± 0.14	15.33 ± 0.23	14.13 ± 0.43	14.30 ± 0.27
Hct (%)	20.7 ± 4,65	41.22 ± 0.62 *	36.18 ± 2.68	43.97 ± 0.48 **	17.48 ± 6.98	15.66 ± 6.67
Plt (10^3/µl)	1631 ± 91.15	676.8 ± 8.5 ***	908.7 ± 74.09 ***	748 ± 27.75 ***	894.20 ± 67	892.5 ± 38.97
Lym (%)	91.03 ± 0.96	88.2 ± 0.14	89.98 ± 0.73	86.23 ± 1.19	80.90 ± 2.33	75.35 ± 1.92
Mon (%)	6.50 ± 0.79	5.38 ± 0.16	5.21 ± 0.36	7.13 ± 0.24	7.8 ± 0.79	9.63 ± 1.19
Gra (%)	2.45 ± 0.42	6.41 ± 0.07	4.64 ± 0.54	6.63 ± 1.11	11.30 ± 1.61	13.28 ± 1.92

GB: White blood cells, RBC: Red blood cells, Hb: Hemoglobin, Lym: Lymphocytes, Gra: Granulocytes, Mon: Monocytes, Plt: Platelets, Hct: Hematocrits. Values are expressed as mean ± standard error to the mean (SEM); n = 6. Significant difference compared to the control: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.01$ (***)

3.3.5. Biochemical parameters

The significant variations observed are a drop in blood ALAT and chloride levels, and an increase in protein and phosphorus levels.

The decrease in ALAT levels is very significant at the dose of 100 mg/kg bw. That of chlorides is significant, and is observed at a dose of 500 mg/kg bw. The AEVC induced a very significant increase in phosphorus levels at 500 and 1000 mg/kg bw and another significant increase for protein levels at a dose of 1000 mg/kg bw. For all the other parameters (blood levels of ASAT, creatinine and calcium), the AEVC, at all the doses used, did not lead to significant modification.

No significant variation was observed between the satellite and satellite control group, all biochemical parameters combined.

All of these results are presented in Table 4.

Table 4 Effect of aqueous extract of *Vernonia colorata* on biochemical parameters

Biochemical parameters	Treatments (mg/kg bw)					
	Control	AEVC 100	AEVC 500	AEVC 1000	Satellite control	Satellite 1000
ASAT (UI/L)	103.5 ± 2.48	107.2 ± 8.38	114.3 ± 6.99	116.7 ± 4.38	108.7 ± 2.97	119.8 ± 5.35
ALAT (UI/L)	35.33 ± 2.98	25.48 ± 0.52 **	37.73 ± 3.64	29.28 ± 1.05	32.08 ± 1.27	30.45 ± 0.83
Protein (g/dL)	61.96 ± 1.19	63.34 ± 1.22	66.24 ± 1.77	67.29 ± 0.87 *	62.76 ± 1.78	65.98 ± 0.35
Creatinine (µmo/L)	58.27 ± 1.39	62.51 ± 4.01	54.15 ± 4.53	46.83 ± 3.35	53.59 ± 2.02	42.98 ± 2.13
Calcium (mmol/L)	2.38 ± 0.16	2.57 ± 0.15	2.41 ± 0.13	2.55 ± 0.20	2.58 ± 0.19	2.46 ± 0.04
Phosphorus (mmol/L)	2.66 ± 0.07	2.87 ± 0.14	3.30 ± 0.17 **	3.27 ± 0.09 **	2.62 ± 0.08	2.86 ± 0.14
Chloride (mmol/L)	108.2 ± 1.16	107.2 ± 1.01	102.5 ± 1.28 *	103.5 ± 0.99	105 ± 2.20	102.7 ± 1.02

ASAT: Aspartate Aminotransferase, ALAT: Alanine Aminotransferase. Values are expressed as mean ± standard error to the mean (SEM); n = 6. Significant difference compared to the control: $p < 0.05$ (*); $p < 0.01$ (**).

4. Discussion

Previous work on *Vernonia colorata* showed an LD50 greater than 5000 mg/kg bw [6, 13]. With this LD50 which is also found in the currently study, *Vernonia colorata* extract is practically non-toxic [14]. In addition, according to the Globally Harmonized System of Classification and Labeling of Chemicals of the OECD [15], AEVC would be classified in category 5, that is to say, non-toxic.

The phytochemical screening results of the present study showed the presence of saponosides and polyphenols (tannins), steroidal and triterpene glycosides, anthracenosides and coumarins and derivatives in the leaves of *Vernonia colorata*. These results are in agreement with those of Siaka [16] except for the flavonoids which was not found in our study. Studies have shown that methanolic and ethanolic extracts of *Vernonia colorata* leaves contain alkaloids and flavonoids [5, 7, 13]. The absence of alkaloids and flavonoids in the aqueous extract could be explained by the type of extraction used and also by the existence of chemotype within the species of *Vernonia colorara* [16, 17]. The presence in AEVC of the compounds such as, phenols, triterpenes, saponoids, glycosides, steroids, whose main properties are antiplasmodial, antioxidant, antibacterial and anti-inflammatory and hepatoprotective and antimicrobial, would justify its traditional use for the treatment of certain diseases [8, 9, 13]. Saponosides are also known to help lower blood cholesterol levels and reduce the incidence of cardiovascular disease [5]. Triterpenoids exhibit cytotoxicity against a variety of tumor cells as well as anticancer properties [1]. Our extract could be useful in the fight against tumors. Coumarins have anti-inflammatory, anticancer, non-toxic anticoagulant properties by inhibiting the function of vitamin K necessary for prothrombin biosynthesis, against renal failure [18, 19]. In addition, tannins have anti-inflammatory, anti-allergic, anti-hemoroidal and anti-diarrheal properties [20].

The AEVC did not have a difference of the variations of the weight gains. The decrease in weight gains in the second week is probably not due to the extract since the weight gains of the control group also decreased during this same period.

Aqueous extract of *Vernonia colorata* induced an increase in red blood cell count and hematocrit level. Erythropoietin is the main stimulator of erythropoiesis. This hormone is mainly synthesized by the kidneys in epithelial cells, hepatic cells and bone marrow and thus leads to an increase in the number of red blood cells in the blood [21, 22]. The stimulus for erythropoietin secretion is a decrease in oxygen transport in the capillaries [23]. The increase in red blood cells and

hematocrit due to the extract indicates that AEVC could be used for the fight against anemia, thus justifying its traditional use in the fight against malaria.

A decrease in the platelet count was noted during this study. Thrombocytopenia is a decrease in platelets (< 150,000/mm3) and occurs in the event of drug treatment (heparin, quinine, quinidine, rifampicin, cotrimoxazole, penicillin, oral antidiabetics, aspirin, etc.) [24, 25]. Thrombocytopenia can be caused by bone marrow aplasia affecting all lineages or only platelets [26]. Bone marrow depression is caused by chemotherapy in the fight against cancer. Our extract would be beneficial in the fight against cancer cells, therefore an antitumor property. However, this decrease deserves to be deepened by other studies in order to confirm or invalidate these results.

The extract caused a significant decrease in ALAT levels. Other work done by Gomé [27] and Sawadogo [28] showed a similar decrease in ALAT levels respectively with aqueous extracts of *Passiflora foetida* Linn. and *Celosia trigyna* in rats. ALAT is a cytosolic enzyme secreted in hepatic cells from where it is released into the blood in the event of hepatic cell necrosis [27, 29]. It is a liver-specific enzyme, used as the best indicators of liver function and serve as biomarkers predicting possible toxicity [30, 31]. Its plasma level rises rapidly when the liver is damaged to various reasons including hepatic cell necrosis, hepatitis, cirrhosis as well as the hepatotoxicity of certain drugs [27]. The decrease in ALAT levels in our study shows that AEVC could have a hepatoprotective effect in these animals.

In addition, hyperphosphatemia was observed. Hyperphosphatemia is usually caused by a mobilization of intracellular phosphorus. It is caused either by metabolic acidosis caused by renal failure, or by the release of phosphorus during rhabdomyolysis [32]. Subsequent studies have shown that hyperphosphatemia is due to reduced filtration and excretion of phosphorus during progression of kidney disease [33, 34]. They also argued that the kidneys play a central role in maintaining phosphorus homeostasis through proper excretion of phosphorus in the urine. Thus, a decrease in the glomerular filtration rate will cause serum phosphorus levels to rise because its excretion will be reduced. Further studies on the histopathology of the kidneys will elucidate this aspect.

Furthermore, no significant difference was found between the satellite control data and the satellite data. Thus, the effects of AEVC would be reversible.

5. Conclusion

Our study corroborated the safety of consumption and therapeutic use of *Vernonia colorata* leaves. AEVC does not induce mortality, hypertrophy or atrophy on noble organs. In addition, it does not stimulate the appetence of rats and did not influence their weight. Phytochemical screening confirmed the presence of phenols, steroids and terpenoid. The presence of these metabolites forms the scientific basis for the traditional use of *Vernonia colorata*. Analysis of hematological parameters indicates that the extract stimulates the production of red blood cells. It would reduce anemia and would be effective in the treatment of malaria. Biochemical analysis indicates that the extract would be hepatoprotective. Studies on the histopathology of the kidneys and liver would be necessary in order to elucidate the cause of the hyperphosphatemia and hepatoprotective effect.

Compliance with ethical standards

Acknowledgments

We would like to thank the Animal Physiology Laboratory at University Joseph KI-ZERBO and the institute for Research in Health Sciences. Also, the authors appreciate everyone that contributed to the success of this research work.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

All experiments were validated by the ethics committee of Joseph KI-ZERBO University. The experimental protocol approval number is CE-UJKZ/2020-04.

References

[1] Kiplimo JJ. A Review on the Biological Activity and the Triterpenoids from the Genus *Vernonia* (Asteraceae Family). In Res J Pur & Appl Chem. 2016; 11(3): 1-14.

[2] Faye O, Sall C, Kane O. Antidiabetic Activity of 40 Plants of the Senegalese Flora, an Important Therapeutic Diversity for Populations. A J Appl Chem Res. 2020; 7(2): 15-32.

[3] Cioffi G, Sanogo R, Diallo D, Romussi G, De Tommasi N. New Compounds from an Extract of *Vernonia colorata* Leaves with Anti-inflammatory Activity. J Nat Prod. 2004; 67(3): 389-394.

[4] Yapi AB, Kassi N'D J, Fofie N'GBY, Zirihi GN. Ethnobotanical study of medicinal Asteraceae sold on the markets of the autonomous district of Abidjan (Côte d'Ivoire). *Int. J. Biol. Chem. Sci*. 2015; 9(6): 2633-2647.

[5] Adu JK, Twum K, Brobbey A, Amengor C, Duah Y. Resistance modulation studies of vernolide from *Vernonia colorata* (Drake) on ciprofloxacin, amoxicillin, tetracycline and erythromycin. The J Phytopharmacol. 2018; 7(5): 425-430.

[6] Fane S. Study of the toxicity of certain plants sold on the markets of the district of Bamako [State Diploma in Pharmacy] Fac Méd Pharma et d'Odonto-Stomato, Univ Bamako. 2003.

[7] Samson G, Adama H, Nabere O, Odile GN. Anti- bacterial activity and phytochemical composition of extracts of three Asteraceae species from Burkina Faso. A J Pharma Clin Res. 2012; 5(2): 37-44.

[8] Asante DB, Henneh IT, Acheampong DO, Kyei F, Adokoh CK, Ofori EG, Domey NK, Adakudugu E, Tangella LP, Ameyaw EO. Anti-inflammatory, anti-nociceptive and antipyretic activity of young and old leaves of *Vernonia amygdalina.* Biomedicine & Pharmacotherapy. 2019; 111: 1187–1203.

[9] Akoto CO, Acheampong A, Boakye YD, Asante B, Ohene S Amankwah F. Anthelminthic, Anti-Inflammatory, Antioxidant, and Antimicrobial Activities and FTIR Analyses of *Vernonia camporum* Stem-Bark. Journal of Chemistry. 2021; Vol 2021: 1-15.

[10] Ciulei I. Methodology of Analysis of Vegetal Drug. Pratical Manual on Industrial Utilization of Medicinal and Aromatic Plants. Bucharest (Faculty of pharmacy); 1982.

[11] OECD (Organization for Economic Cooperation and Development). OECD guidelines for the testing of chemicals. Acute Oral Toxicity - Acute Toxicity Class Method. Test n °. 2001; 423: 14.

[12] OECD (Organisation for Economic Cooperation and Development), OECD Guidelines for the Testing of Chemicals. Subacute oral toxicity; Test no °. 2008; 407: 14.

[13] Idris MH, Mann A, Kabiru AY, Busari MB. In vivo Antiplasmodial Activity and GC-MS Analysis of *Vernonia colorata* (Willd) Drake Leaf. Euro J Med Plants. 2016; 14(3): 1-11.

[14] Diezi J. Toxicology: Basic principle and clinical implications. Pharmacology from fundamental concepts to therapeutic applications. Edition Frison-roche Paris and Edition Slatkine, Published by Schorderet, Genève ; 1989.

[15] OECD (Organization for Economic Cooperation and Development). Globally Harmonized System of Classification and Labeling of Chemicals. ST/SG/AC.10/30/Rev. 2013; 5: 579.

[16] Siaka S, Golly KJ, Guessennd N, Soro Y, Djama AJ, Dosso M. Phytochemical assessment and antimicrobial activity of leaves extract of *Vernonia colorata* (Wild.) Drake on Resistant Germs of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J Chem Pharma Res. 2012; 4(5): 2490-2494.

[17] Dabire AP, Ouedraogo Y, Belemtougri RG, Stanislas S, Martin T. Phytochemical and toxicity study of *Excoecaria grahamii Stapf* aqueous extract on female mice. In J Pharm Sci Res. 2017; 2(2): 12-18.

[18] Akkol EK, Genç Y, Karpuz B, Sobarzo-Sánchez E, Capasso R. Coumarins and Coumarin-Related Compounds in Pharmacotherapy of Cancer. Cancers. 2020; 12: 1959.

[19] Rohini K, Srikumar PS. Therapeutic Role of Coumarins and Coumarin-Related Compounds. J Thermodynamics and Catalysis. 2014; 5: 130.

[20] Sharma K, Kumar V, Kaur J, Tanwar B, Goyal A, Sharma R, Gat Y, Kumar A. Health effects, sources, utilization and safety of tannins: a critical review. *Toxin Review*. 2019; 40(4): 432-444.

[21] Lacombe C, Mayeux. Erythropoietin. *Medicine / Sciences*. 1995; 11: 947-955.

[22] Da FL, Ouedraogo Y, Somé AA, Sawadogo T A, Sawadogo P, Bayala B. Toxicity studies on the leaves of *Senna alata*, a medicinal plant from Burkina Faso, in mice and rats. W J Pharm Pharm Sci. 2020; 9 (11): 85-95.

[23] Rosencher N, Ozier Y. Perioperative erythropoietin. Clinical and Biological Transfusion. 2003; 10: 159–164.

[24] Suciu O, Le Hello C, Maïza D, Gautier. Does antiaggregation have a place in late heparin-induced thrombocytopenia? J Mal Vasc (Paris) © Masson. 2005; 30(2): 94-97.

[25] Serraj K, Mecili M, Aouni M, Maaouni A, Andrès E. Idiosyncratic drug thrombocytopenia. The Journal of Internal Medicin. 2009; 30: 866–871.

[26] Berthélémy S. The hemogram or blood count. Pharmaceutical news. 2014; n° 538.

[27] Gomé MB, Kouakou K, Touré A, Traoré F. Study of the acute and subchronic toxicity of the aqueous extract of *Passiflora foetida Linn.* (*Passifloraceae*) in rats and mice. In J Bio Chem Sci. 2011; 5(5): 1777-1789.

[28] Sawadogo TA. Ouédraogo Y, Da F L, Ilboudo S, Sawadogo P, Bayala B. Acute and subacute toxicities of the aqueous extract of the leaves of *Celosia trigyna (L.)*. W J Pharma Res. 2020; 9(15): 95-109.

[29] Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury II. Recommandation for use of laboratory tests in screening, diagnosis and monitoring. Clinique Chemical. 2000; 46: 2050-2068.

[30] Al-Habori M, Al-Aghbari A, Al-Mamary M, Baker M. Toxicological evaluation of Catha edulis leaves: a long term feeding experiment in animals. J Ethnopharmacol. 2002; 83: 209-217.

[31] Mukinda JT, Eagles PFK. Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Polygala fruticosa* in female mice and rats. J Ethnopharmacol. 2010; 128: 236–240.

[32] Dieval F, Strozyk L., Vanderlinden T, Forzy G, Dhondt JL. Major hyperphosphatemia. Annals of Clinical Biology. 2005; 63(4): 433-4.

[33] Ospina CAG, Holguín MC, Escobar DC, Valencia CAR. Importance of hyperphosphatemia in chronic kidney disease, how to avoid it and treat it by nutritional measures. *Rev. Colomb. Nefrol*. 2017; 4(1): 24-41.

[34] Singh S, Bhatta S. Biochemical and hematological parameters in chronic kidney disease. Mmihs. 2018; 4(1): 4-11.