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Acute and subacute toxicity of the aqueous extract of *Amaranthus viridis* (Amaranthaceae) leaves in rats

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ABSTRACT

Amaranthus viridis is a plant of the family Amaranthaceae, used by traditional medicine in the treatment of several diseases. The aim of this study was to evaluate the toxicological activities of *A. viridis*. To assess these activities, two types of test were performed: Acute and subacute toxicity test. Phytochemical analysis performed on the aqueous extract of *A. viridis* revealed the presence of polyphenol, flavonoids, tannins, sterol-polyterpenes, Saponosids, cardiac glycosides, traces of alkaloids and leucoanthocyanines. The aqueous extract of *Amaranthus viridis* showed no evidence of single dose toxicity (2000 mg/kg) when studying acute toxicity. The subacute toxicity study of the aqueous extract of *A. viridis* at doses of 200, 400 and 600 mg/kg showed no signs of toxicity on biochemical, hematological or histological parameters. The results showed that *A. viridis* does not cause toxicity at the doses studied.

Keywords: *Amaranthus viridis*, toxicity, Histopathology examination.

INTRODUCTION

Medicinal plants have long been used for the treatment of certain diseases. According to **Samuelson**, the use of plants as a source of medicine dates back more than 1000 years [1]. The World Health Organization, estimates populations using medicinal plants to more than 80% [2]. The medicinal plants contain active molecules that are at the origin of the therapy. Researchs have shown that medicinal plants have fertilizing properties, hypotensive activity, anti-candidiasis activities [3-5]. However, although medicinal plants have several therapeutic virtues, they are not free from any danger of intoxication. Several researchers have pointed out the potential toxicity, as well as the risks associated with the use of certain species of plants and vegetables [6]. The work of Peyrin-Biroulet *et al*. revealed that some plant species have hepatotoxic effects [7]. Seen, the risks of toxicity, studies on the safety and effectiveness of medicinal plants has become one of the main concerns to guarantee the use [8].

Amaranthaceae family is widely used by the population. The importance towards this family result from the fact that it has several properties namely: activities anti diabetic and anti-cholesterol, which reduces the pains, regulates the fertility [9-11].

Our study focuses on *Amaranthus viridis*, plant of the family Amaranthaceae to evaluate the toxicity of this plant on certain parameters (heart, liver and kidney).

MATERIALS AND METHODS

Plant material

The leaves of *Amaranthus viridis* have been collected in March 2016 from Abobo area district of Abidjan (Côte d'Ivoire) among herbalists. The plant was authenticated by Dr. Boraud N'takpé Maxime, of the Université Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire).

Preparation of extract

The leaves of plant were washed, dried in ambient air in a room, sheltered from the sun and coarsely powdered. 50g of the leaves powder were macerated in 1 L of distilled water, with micro-vortex stirring at 350 rpm for 24 h, then macerated in 0.5 L of distilled water for other 24h. The macerate is then filtered on poplin cloth, then on Wattman paper No. 1, before being dried in an oven at 40 °C, until you get powder.

Phytochemical study

The qualitative phytochemical study was performed to identify the main phytoconstituents present in the leaves of *A. viridis.*

Animals

The animal model used was adult male and female rats (120- 150g), from the vivarium of the ENS (Ecole Normale Supérieure) of Abidjan, have been used. They were raised at ambient temperature of 22 ± 3 °C with 40 to 60% moisture and a photoperiod of 12 hours light and 12 hours darkness. The animals were fed on diet of fish, bread, corn and water *ad libitum.*

Acute toxicity study

Acute toxicity study was conducted under the guideline OECD N° 423 [12]. Two groups (control and test group) of three healthy wistar female rats (130-160g) have received a limit dose of 2000 mg/kg of the AEAV. The observations focused on mortality, convulsions, salivations, sleep and coma each day for 14 days.

Subacute toxicity study

For this Study, the rats, numbering forty, were divided into four groups of ten (five males and five females), according to the OECD 407 guideline [13]. Three of these lots were treated with the different doses of the extract (200, 400 and 600 mg/kg) and the fourth was administered with the vehicle (control). The animals were administered by gavage, daily, for 28 consecutive days. At the end of the experiment, the animals were sacrificed to collect their blood and organs (heart, liver and kidney) for biochemical, hematological and histological analyzes.

Blood analysis

Biochemical analyzes included liver function markers (AST, ALT), bilirubins (total and direct), total cholesterol, triglycerides and nephrotic markers (urea, creatinine and uric acid), using Cobas C 311. Hematologic parameters included red and white blood cells, hemoglobin, hematocrit, MCV, MCH, MCHC, platelets, lymphocytes, monocytes, eosinophils, neutrophils and basophils, with the unit XN-1000 (Sysmex).

Histopatholigical examinations

The organs, previously fixed in 10% concentrated formalin, underwent several liquor baths of increasing degree (80°, 90° and 100°), before passing into toluene and being included in the liquid paraffin. The paraffin blocks are mounted on a microtome to make the cuts. Soaked in aqueous dyes (Hematoxylin and Eosin) and observed under microscopes.

Statistical analysis

The software used are: EXCEL and Graph Pad. All data are expressed on average ± SEM. Analysis of variances (ANOVA) was applied to the different results. The Newman-Keul test was used to compare the different columns. The value $p < 0.05$ is considered significant.

RESULTS

Phytochemical study

Results of phytochemical screening are presented in the table 1.

Table 1: Phytochemical screening of extract of *Amaranthus viridis* leaves

Phytochemical Compounds		Test used	AEAV
Polyphenols		Ferric chloride FeCl3 (2%)	+++
Flavonoids		Hydrochloric alcohol & Isoamyl alcohol	++
Tannins	Catechin	Stiasny test (Formaldehyde & Hydrochloric acid concentrated)	++
	Gallic	Sodium acetate & Ferric chloride	+++
Leucoanthocyanins		hydrochloric acid & isoamyl alcohol	+
Sterols-polyterpenes		Acetic anhydride & Concentrated Sulfuric acid	++
Saponosides		Foam test	++
Cardiotonic glycosides		chloroform (CHCl3)	++
coumarins		ammonium hydroxide NH4OH (25 %)	-
Quinones		Borntraeger test (Ammonia)	-
Alkaloids	Dragendorff	Potassium iodobismuthate solution	+
	Bourchardat	odured iodine reaction	-

+++(high presence); ++ (Low presence); + (Trace); - (Absence)
AEAV: Aqueous Extract of *Amaranthus viridis*

Acute toxicity test

The acute toxicity results showed no evidence of toxicity of the aqueous extract of *A. viridis* in animals administered at the 2000 mg/kg dose limit.

Subacute toxicity test

- Effects of Treatment on male Body Weight Variation

Administration of various doses of aqueous extract of *A. viridis* (200, 400 and 600 mg/kg bw) during 28 days of treatment had no

significant change (p> 0.05) on the body weight of male rats compared to controls. However, there is an increase of 9% and 12.43%, 400 and 600 mg/kg respectively compared to control group weight (Figure 1).

- Effects of Treatment on Female Body Weight Variation

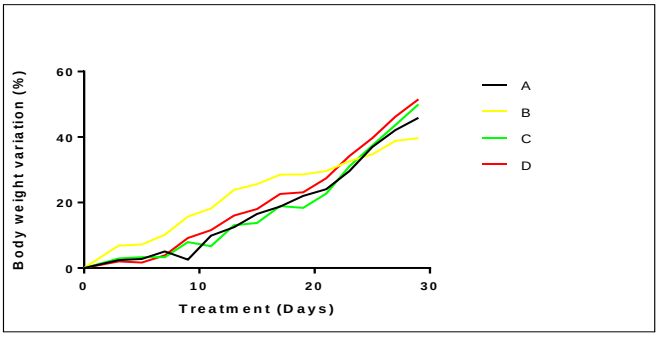
Administration of 400 mg/kg aqueous extract of *A. viridis* during 28 days of treatment showed significant difference (P<0.05) compared to control. This difference reflected into a decrease in body weight of 23.66% compared to control. However, there is also a non-significant decrease of 1.58% and 14.48% body weight at respective doses of 200 and 600 mg/kg compared to control group weight (Figure 2).

- Effect of treatment on relative weight of Heart, Liver and Kidney

The results on the relative weight of organs (heart, liver, and kidney) are shown in Table 2. These results showed non-significant difference (P> 0.05) between the different doses administered and the control on the heart in both sexes. On the other hand, on the liver and the kidney, the results showed significant differences at certain doses.

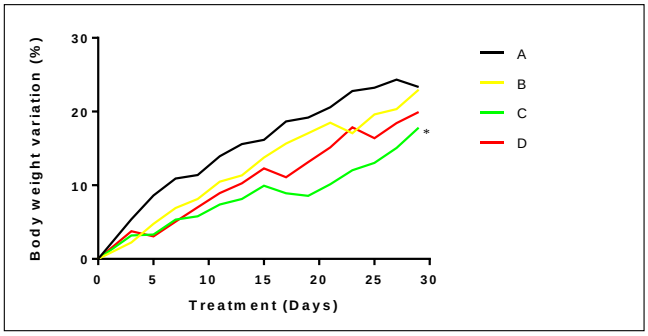
At the liver level, analysis of variance found a significant difference (P<0.05) resulting in a weight increase of 6.69 % with the dose of 600 mg/kg compared to the control in the male. The other doses (200 and 400 mg/kg) of the extract did not cause any significant change in the relative weight of the liver in the male and no significant difference with all doses of AEAV in the female.

On kidney, the analyses reveal a significant difference (P<0.01) at the dose of 200 mg/kg in the female. This difference results in a reduction in kidney weight of 9.45% compared to the control. However, there was a non-significant decrease in kidney weight at all treatment doses in males (200, 400 and 600 mg / kg) and at doses of 400 and 600 mg/kg in females.



A: Control; **B**: 200 mg/kg bw; **C:** 400 mg/kg bw; **D**: 600 mg/kg bw

Figure 1: Effect of the treatment with the aqueous extract of *Amaranthus viridis* on the variation of the body weight of male wistar rat



*: p<0,05; significant difference
A: Control; **B**: 200 mg/kg bw; **C:** 400 mg/kg bw; **D**: 600 mg/kg bw

Figure 2: Effect of the treatment with the aqueous extract of *Amaranthus viridis* on the variation of the body weight of female wistar rat

Table 2: Effect of aqueous extract of *Amaranthus viridis* on organ weights rats in the subacute toxicity study

Organ (g/100g bw)	Sex	Control	AEAV 200	AEAV 400	AEAV 600
Heart	Male	0,362±3,474	0,345±5,028	0,347±5,256	0,360±3,777
	Female	0,382±11,23	0,339±10,81	0,376±10,62	0,379±11,11
Liver	Male	3,407±56,9	3,411±62,59	3,549±36,46	3,635±26,33*
	Female	3,185±87,24	3,091±87,37	3,039±85,53	2,925±85,24
Kidney	Male	0,275±2,877	0,260±4,316	0,269±5,231	0,264±3,576
	Female	0,268±4,03	0,243±4,637**	0,262±2,953	0,256±3,305

*: p<0,05; **:p<0.01 significant difference
AEAV 200: Aqueous extract of *Amaranthus viridis* to 200 mg/kg
AEAV 400: Aqueous extract of *Amaranthus viridis* to 400 mg/kg
AEAV 600: Aqueous extract of *Amaranthus viridis* to 600 mg/kg

- Effect on hematological parameters male

The effect of the extract *A. viridis* on hematological indices was examined at the end of treatment (table 3). Variances analysis showed no significant difference on several parameters (WBC, RBC, HBG, HCT, MCV, MCH, MCHC, Platelet count, Lymphocyte, Basophil). On monocyte and eosinophil, the results showed significant differences at all treatment doses. On the neutrophil, analyses showed significant differences at doses 200 and 400 mg/kg (P<0.01) compared to control.

- Effect on hematological parameters female

The effect of extract on hematological indices was examined at the end of treatment. (table 4) Variances analysis showed no significant difference on several parameters (WBC, RBC, HBG, HCT, MCV, MCHC, Platelet count, Lymphocyte, Monocyte, Eosinophil and Basophil). On the other hand, on MCH (P<0.001) and neutrophils (P<0.01; P<0.05)), the analyses showed significant differences at all treatment doses.

- Effect on serum biochemical parameters

The results of the biochemical parameters are recorded in Table 5. The results showed no significant difference in some parameters (ALT, Uric acid, triglycerides, urea and creatinine) at all treatment doses in both sexes compared to the control On the other hand, the parameters such as AST, total bilirubin, direct bilirubin and total cholesterol recorded significant differences, depending on the dose administered and the sex.

At the AST level, the analyses showed significant differences at doses 200 (P<0.001) and 400 (P<0.01) mg/kg in the male compared to control group. These differences result in a decrease of 21.87% and 13.69 at the respective doses of 200 and 400 mg / kg. This same parameter showed no significant difference in females at any dose.

On total bilirubin, the analyses showed a significant difference in the male at the dose of 200 mg / kg. it results in a decrease of 46.84% compared to the control.

On of direct bilirubin, the results showed significant differences in the male at doses 200 (P<0.05) and 400 (P<0.001) mg / kg compared with the control group. There is a decrease of 30.18% and 60.59% respectively at doses 200 and 400 mg / kg. However, no significant changes were recorded in the female.

On cholesterol, the results showed significant differences, at all treatment doses in the male and at the dose 400mg / kg in the female.

- Histology of heart, liver and kidney

Optical microscope examination of the heart, liver and kidney sections showed normal histology of these organs at all treatment doses compared to the control group. No deleterious effects on the histological characteristics of the heart, liver and kidneys of the treated rats (Figure 3).

Table 3: Effect of aqueous extract of *Amaranthus viridis* on hematological parameters of male rats in the subacute toxicity

Parameters	Control	AEAV 200	AEAV 400	AEAV 600
WBC (10^3/µL)	25,17±2,133	27,86±1,345	21,22±1,242	21,07±1,623
RBC (10^6/µL)	8,603±0,2662	8,197±0,3048	8,217±0,02603	8,443±0,06386
HB (g/dL)	13,97±0,3383	13,73±0,2404	13,5±0,2082	13,67±0,3333
Hematocrit (%)	50,7±0,5568	47,3±1,102	47,4±1,35	47,73±1,172
MCV (fL)	55,33±0,348	53,23±53,23	55,8±0,1155	53,8±0,2082
MCH (pg)	16,33±0,1202	16,1±0,3512	16,03±0,1202	16,03±0,2333
MCHC (g/dL)	29±0,2309	28,67±0,1453	28,43±0,1453	28,6±0,05774
Platelets (10^3/dL)	726,7±104,7	835,3±69,19	933,7±52,36	549±85,28
Lymphocyte (%)	85,33±1,467	80,03±2,397	81,77±2,256	79,63±2,146
Monocyte (%)	7,867±1,049	12,07±0,809**	10,53±0,348*	5,5±0,4359*
Eosinophil (%)	1,333±0,1453	2,44±0,2663**	2,967±0,2028**	3,333±0,2603**
Neutrophil (%)	12,9±0,7211	6,4±1,137**	8,033±0,4842**	14,63±0,6173
Basophil (%)	0,3625±0,0375	0,375±0,025	0,375±0,025	0,345±0,03202

*: p<0,05; **: p<0,01; significant difference).

Table 4: Effect of aqueous extract of *Amaranthus viridis* on hematological parameters of female rats in the subacute toxicity study

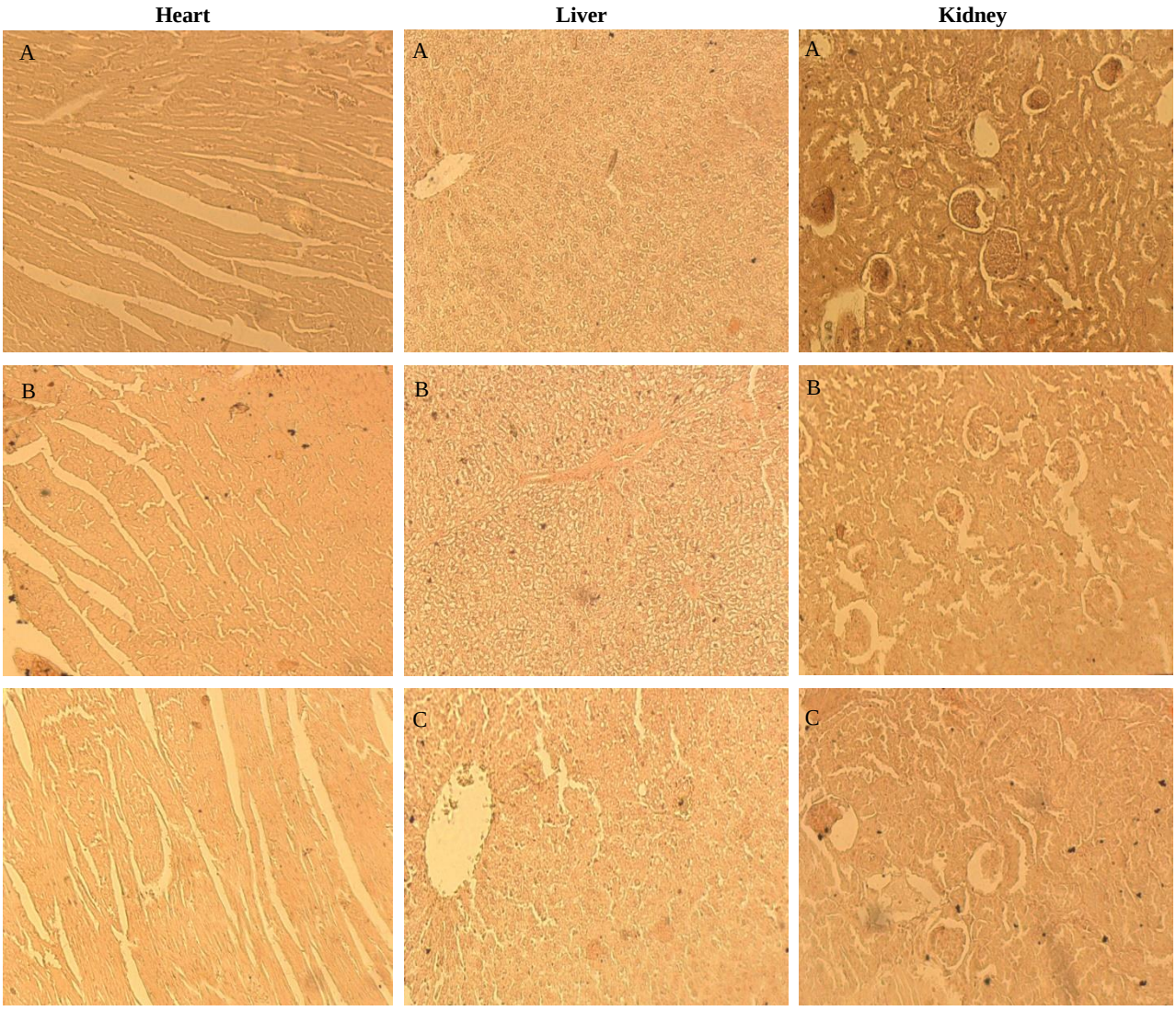
Parameters	Control	AEAV 200	AEAV 400	AEAV 600
WBC (10^3/µL)	17,77±1,475	23,12±2,005	27,4±2,085	25,13±1,175
RBC (10^6/µL)	8,4±0,18	7,895±0,135	7,775±0,185	8,795±0,185
HB (g/dL)	14,1±0,3	12,6±0,3	12,95±0,25	14,45±0,35
Hematocrit (%)	48,75±1,05	44,4±1,1	44,5±0,8	50,25±0,95
MCV (fL)	58,95±0,85	56,15±0,95	56,9±1,4	55,85±0,95
MCH (pg)	17,55±0,35	15,85±0,35***	16,7±0,3***	16,5±0,4***
MCHC (g/dL)	29,42±0,32	28,4±0,3	29,43±0,325	28,45±0,35
Platelet (10^3/dL)	889,5±46,5	1137±46,5	960,5±48,5	902±47
Lymphocytes (%)	83,65±2,45	75,85±2,35	74,4±2,2	74,15±2,25
Monocytes (%)	11,72±1,585	9,2±1,7	10,13±1,775	8,5±1,6
Eosinophil (%)	1,4±0,3	2,45±0,35	2,15±0,25	2,95±0,25
Neutrophil (%)	19,4±1,2	16,25±1,05**	16,25±1,45*	17,85±1,65*
Basophil (%)	0,3667±0,06667	0,2667±0,06667	0,2333±0,06667	0,3±0,05774

*: p<0,05; **: p<0,01; ***: p<0.001; significant difference

Table 5: Effect of aqueous extract of *Amaranthus viridis* on the biochemical parameters rats in the subacute toxicity study

Parameters	Sex	Control	AEAV 200	AEAV 400	AEAV 600
AST(UI/L)	M	168,7±3,523	131,8±4,153***	145,6±4,756**	173±3,703
	F	124,2±2,603	133,1±2,648	134,5±1,86	123,7±2,642
ALT (UI/L)	M	28,77±1,588	32,97±1,059	27,7±1,115	30,53±1,139
	F	36,43±3,171	51,07±3,563	43,8±3,635	39,37±4,431
Total Bilirubin (mg/l)	M	0,5747±0,06333	0,3055±0,01652*	0,5573±0,06971	0,464±0,03349
	F	0,2475±0,01268	0,2527±0,01446	0,2686±0,01629	0,2784±0,01207
Direct bilirubin (mg/l)	M	0,2216±0,01036	0,1547±0,02352*	0,08733±0,01675***	0,2027±0,01675
	F	0,1492±0,01	0,1545±0,0079	0,1392±0,0116	0,1342±0,0124
Uric acid (mg/l)	M	18,93±1,011	16,13±1,798	15,87±1,02	15,6± 0,6928
	F	12,93±1,33	17,27±1,452	17,07±1,676	10,47±1,354
Total Cholesterol (g/l)	M	0,4067±0,01453	0,5267±0,02848**	0,56±0,005774**	0,47±0,02082*
	F	0,4267±0,02186	0,4433±0,01764	0,5133±0,02963*	0,48±0,02082
Triglyceride (g/l)	M	0,7467±0,05783	0,7033±0,05239	0,8633±0,07172	0,7867±0,0786
	F	0,9567±0,07839	1,17±0,07	1±0,08888	0,9567±0,0584
Urea (g/l)	M	0,3633±0,02963	0,2667±0,04055	0,26±0,02646	0,3067±0,01202
	F	0,2633±0,01856	0,2933±0,01856	0,2633±0,02028	0,2433±0,01764
Creatinine (mg/l)	M	3,75±0,4787	3,75±0,25	3,25±0,25	3,25±0,25
	F	4,333±0,3333	4,667±0,3333	4,667±0,3333	4,667±0,3333

*: p<0,05; **: p<0,01; ***: p<0.001; significant difference
M: Male **F:** Female



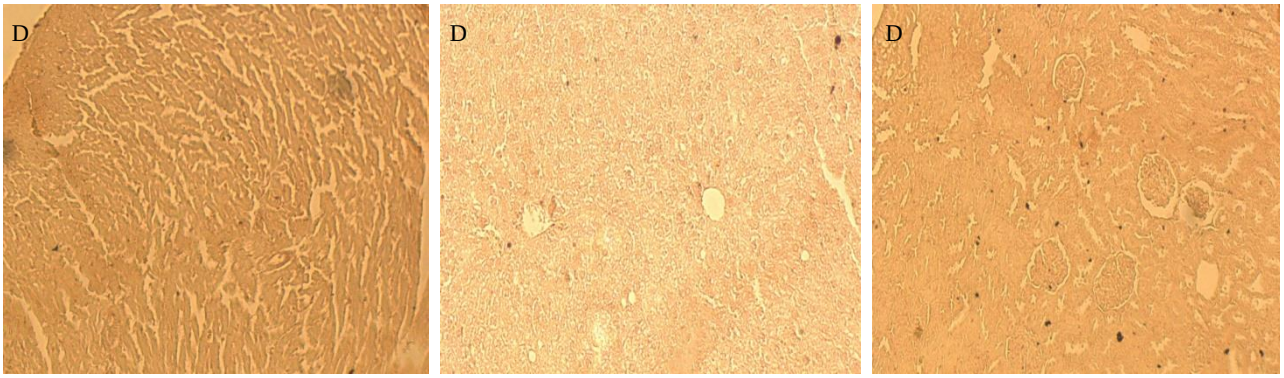


Figure 3: Photomicrograph of tissue sections of rats following 28 day treatment. A: Control; B: *A. viridis* (200 mg/Kg per day); C: *A. viridis* (400 mg/Kg per day); D: *A. viridis* (600 mg/Kg per day)

DISCUSSION

Medicinal plants are used a lot because all that is natural is free of toxic effects [14]. However, research has identified the toxic nature of some plants. The scientific knowledge of the toxicity study is very necessary to unveil the clinical potential of *A. viridis*.

Phytochemical studies have revealed the presence of Polyphenols, Flavonoids, Tanins, Stérols-polyterpens, Saponosids, Cardiotonic glycosides and traces of alkaloids and leucoanthocyanins. The results are similar to those obtained by Adetutu *et al.*, Ashok *et al.*, which showed the presence of these compounds in their studies [15, 16]. These results show that *A. viridis* possess some molecules with known biological activity. Indeed, flavonoids and Saponin have anti-inflammatory, antioxidant, bone properties and hepatoprotective effect [17-20]. Polyphenols have cardiovascular properties and fight against degenerative diseases [21, 22].

No mortality and signs of toxicity were observed after administration of the dose limit (2000 mg/kg). This shows that the lethal dose is above this dose limit.

28 days of treatment with the aqueous extract of *A. viridis*, has shown no significant difference in body weight of the male rats compared to the control. However, there was a significant decrease in body weight of female rats at 400 mg/kg. The administration of AEAV showed no significant difference between the rats (male and female) in the relative weight of the heart and a significant difference at the dose of 200 mg / kg in the kidneys in the female. However, there is a decrease in the weight of these organs in both sexes at all doses compared to control.

Changes in body weight and relative weight vital organs are indicators of the effect of an administered substance [23]. These results confirm the work of Saravanan *et al.*, [24]. Indeed the work of Saravanan showed that *A. viridis* has cardioprotective activities. This cardioprotective activity could be explained by the presence of polyphenols in this extract. The dose of 600 mg/kg AEAV showed a significant difference ($P<0.05$) in relative liver weight in males. However, the histologic results revealed a normal liver architecture at this dose.

The evaluation of the hematological parameters is very important in the determination of the anomalies induced by a plant extract [25]. The hematological analysis of MCH, neutrophils and monocytes showed a statistical difference in the groups treated with the AEAV compared to controls. The other hematological parameters such as WBC, RBC, HBG, HCT, MCV, MCHC, Platelet count, Lymphocyte, Monocyte, Eosinophil and Basophils haven't shown a significant difference between treated and control. However, the differences obtained in this study do not show a hematological change, since they are in the normal range of health for this animal species [26].

The study of biochemical parameters are indicators of toxicity, raising the effectiveness or the installation of a toxicity on the vital organs. In this study, parameters AST, Total Bilirubin, Direct bilirubin and cholesterol showed difference between treated and controls. Doses of 200 and 400 mg/kg in males significantly reduced serum AST and direct bilirubin. The decrease of these parameters would show the hepatoprotective action of AEAV at these doses. This hepatoprotective activity could be explained by the presence in this extract of flavonoids. Indeed, Lin Wan and Jian-Guo showed that flavonoids have a protective activity on the liver [20]. Nevertheless, these observed differences are normal for this species, so there is no toxic effect [26].

The histological inspection on the treated and control shows the aqueous extract of *Amaranthus viridis* does not cause any toxic effect.

These results are similar to histological studies of Menagati *et al.* on the repeated administration of the aqueous extract of *Alibertia edulis* [27]. These studies have shown that the aqueous extract of *Alibertia edulis* induces no histological changes on brain, liver, lung and kidney.

CONCLUSION

The aqueous extract of *Amaranthus viridis* study does not cause any apparent toxicity in an animal model. The different doses of the extract did not cause any mortality and apparent toxicity during the 28 days of treatment. Histological examination showed no change in the architecture of the internal organs of the heart, liver and kidneys, in both control and treated groups. However, complementary studies are necessary in order to enter information on this plant.

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