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Evaluation of acute and subacute toxicity induced by methanol extract of *Amaranthus viridis* (Amaranthaceae) leaves in wistar rats (*Rattus norvegicus*)

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Abstract
Objective: to study the acute and subacute toxicity of the methanolic extract of *Amaranthus viridis* leaves on an animal model (Rat).
Methods: Acute toxicity was performed according to OECD Guideline 423, by a dose limit of 2000 mg / kg body weight. The observations (during 14 days) concerned the number of deaths, convulsions, sleep and coma. Subacute toxicity was achieved according to the OECD guideline 407. Four groups of 10 rats (5 males and 5 females) received either the dose differences of the methanolic extract (200, 400 and 600 mg/kg) or the vehicle (olive oil). At the end of the treatment, the biochemical, hematological and histological parameters were studied in order to evaluate the toxicity over a long duration of treatment.
Conclusion: The results showed that the methanolic extract of *Amaranthus viridis* administered at the dose 200 and 400 mg/kg is safe at this treatment time on the parameters studied. In contrast, administered at a dose of 600 mg/kg, the methanolic extract revealed signs of toxicity on organ weights, liver and histological markers. Administration at dose of 600 mg/kg is not safe.

Keywords: Subacute toxicity, *Amaranthus viridis*, methanolic extract

Introduction
Amaranthus viridis (Linn) is a plant of the family Amaranthaceae, Commonly known Amaranthus green. It is a cosmopolitan plant widely distributed in tropical and subtropical regions and remains the most widespread species in the Pacific, specifically in Southeast Asia, Africa and America and also in parts of the world temperate [1-2].
Green Amaranth is commonly used as an ingredient in some sauces and has different traditional uses [2]. In India and Nepal, this plant is cultivated and used to reduce pain due to intense activity, in Pakistan, like some plants, it is used to treat respiratory diseases, to regulate bleeding, excessive menstruation and diarrhea, in Côte d'Ivoire this plant is used to help the pregnancy to progress [3-6]. The work of other researchers has also shown that *A. viridis* has anti-inflammatory properties, fighting against eye infections, seizures and epilepsy, anti-hyperglycemic properties and antipyretic [7-10]. It is therefore a medicinal plant with many therapeutic virtues. However, like medicinal plants, Green Amaranth is not exempt from the risks of intoxication. The work of Peyrin-Biroulet et *al*. revealed that some plant species have hepatotoxic effects [11]. Seen, the risks of toxicity, studies on the safety and effectiveness of medicinal plants have become one of the main concerns to guarantee the use [12].
The objective of this study is to evaluate the toxicity of the methanolic extract of *Amaranthus viridis* on the biochemical, hematological and histological parameters of the rats during a few days.

Materials and Methods
Plant material
Plant specimen of *Amaranthus viridis* were collected during March from Abobo (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 1L of methanol (95°), with micro-vortex stirring at 350 rpm for 48 h. The macerate is then filtered on poplin cloth, then on Wattman paper No. 1. The filtrate was concentrated using a rotary evaporator at 40 °C, until a more or less dry dough is obtained.

Phytochemical study
This study consisted of a qualitative assay to determine the phytoconstituents present in the methanolic extract of *Amaranthus viridis*.

Animals
Healthy adult male and female wistar rats weighing 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
The acute oral toxicity study was conducted in accordance to the method recommended by Organization for Economic Cooperation and Development guidelines (OECD) N° 423 for the assessment of acute toxicity of medicinal plants [13]. Two groups (control and test group) of three healthy wistar female rats were administered a limit dose of 2000 mg/kg of the methanol extract of *Amaranthus viridis* (MEAV) and animals were observed for mortality and clinical signs for the first hour and monitored each day for 14 days.

Subacute toxicity study
Sub-acute toxicity test was performed according to the Organization of Economic Co-operation and Development guideline 407 for testing of chemicals [14]. For this study, forty rats were used. These rats were distributed 4 batches of 10 rats, due to 5 males and 5 females in batches. The first batch group (control) was administered with olive oil and the other three batches received respectively the doses 200, 400 and 600 mg/kg of the methanolic extract. The animals were administered by gavage, daily, for 28 consecutive days and observed daily for any adverse effects or toxic signs and behavioral changes, mortality and morbidity until the end of the experiment. The animals were sacrificed on the 29th day. Their blood collected in different tubes, allowed to study the biochemical and hematological parameters. Organs such as heart, liver and kidney were removed, weighed and kept in a fixator for histopathological examination.

Blood analysis
Biochemical parameters such as total and direct bilirubin, cholesterol, triglycerides, markers of renal (urea, uric acid and creatinine) and hepatic (alanine aminotransferase, aspartate aminotransferase) function were assayed using Cobas C 311. Hematologic analysis measured total and differential leukocytes, erythrocytes, platelets, hemoglobin, hematocrit and red blood cell counts with the XN-1000 (Sysmex) automated device.

Histopatholigical examinations
Organs such as heart, liver and kidneys were collected from the animals for histopathology. The organs were removed, weighed and fixed in 10% neutral buffered formalin. After fixation, the organ fragments were cleaved, dehydrated with increasing absolute ethanol concentrations then Toluene and embedded in paraffin. Sections were cut at a thickness of 5 µm and stained with hematoxylin and eosin (H & E) for evaluation through light microscopy.

Statistical analysis
All data are expressed on average ± SEM. Graphical representation and data processing were performed using Graph Pad Prism and EXCEL software. The statistical analysis was performed by analyzing the variances (ANOVA One-Way). The differences between the averages were determined according to the Newman-Keuls test. $P < 0.05$ is considered significant, $P < 0.01$ very significant and $P < 0.001$ highly significant.

Results and Discussion

- **Phytochemical study**

The results of the phytochemical study carried out on the methanolic extract of *Amaranthus viridis* showed a strong presence of Polyphenols, Flavonoids and Tannins. A presence of Alkaloides, Cardiotonic glycosides, Sterols-Polyterpenes, Leucoanthocyanines and traces of Saponosides (Table 1).

- **Toxicity acute**

The acute toxicity test showed the normal behaviour of the treated rats. No deaths or any toxic symptoms were observed animals treated with a single dose of the MEAV (2000 mg/kg).

The subacute toxicity on Body and organ weight, hematology and biochemical parameters, histopathology
Administration of the methanolic *Amaranthus viridis* extract during the 28-day treatment period in the subacute toxicity study, recorded changes in body weight in male (figure 1) and female rats (figure 2). The results obtained show that the methanolic extract induces an increase in body weight at all treatment doses (200, 400 and 600 mg/kg) in both males and females. This increase is pronounced in the male, resulting in an increase of 38.05% at the dose of 200 mg/kg ($P<0.05$) and 65.23% at the dose of 400 mg/kg ($P<0.05$) compared to control.
The results on the relative weight of organs (heart, liver, and kidney) are shown in Table 2. The results showed non-significant difference ($P>0.05$) in the heart between the treated and control groups regardless of sex. In contrast, in the liver, the results show significant differences at doses 400 and 600 mg / kg in both sexes. These differences result in an increase in liver weight of 15.16% ($P<0.05$) and 15.27% ($P<0.05$) respectively at doses 400 and 600 mg/kg compared to control in the male. In females the differences result in an increase of 20.98% ($P<0.01$) and 16.74% ($P<0.01$) respectively at doses 400 and 600 mg/kg compared to control group. In the kidney, there was a significant difference ($P<0.05$), resulting in an increase of 8.85% at the 600 mg/kg dose in the male gender.

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

Phytochemical compounds		Test used	MEAV
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)
Meav: Methanol Extract of *Amaranthus viridis*

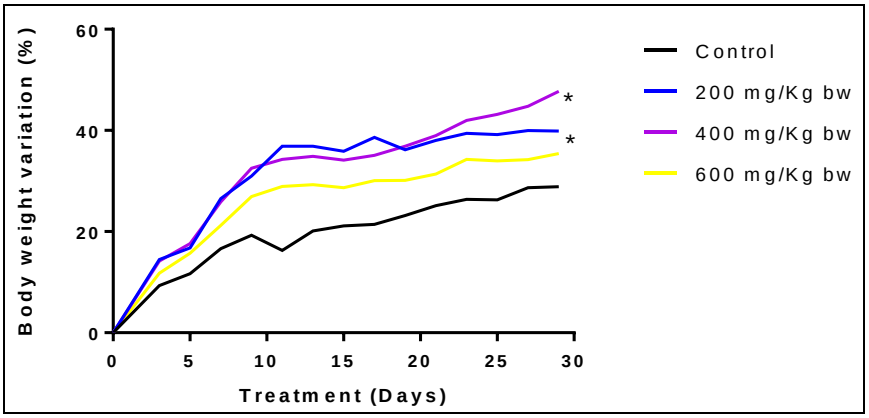

Fig 1: Effect of the treatment with methanol extract of *Amaranthus viridis* on the variation of the body weight of male wistar rat
*: p<0,05; significant difference with regard to control (olive oil).

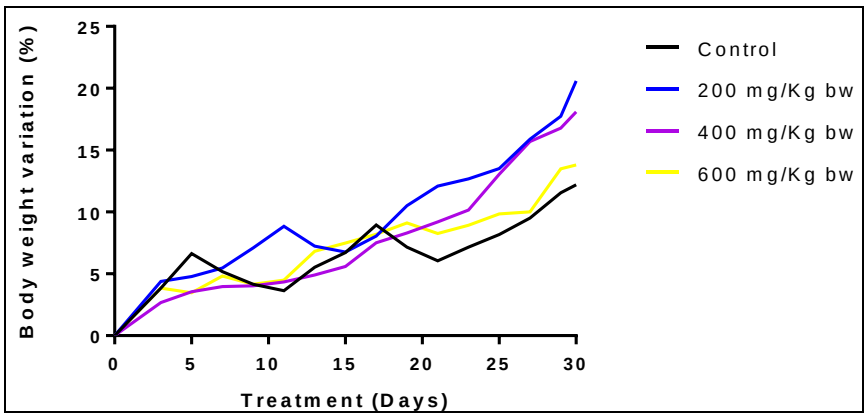

Fig 2: Effect of the treatment with the methanol extract of *Amaranthus viridis* on the variation of the body weight of female wistar rat

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

Organ (g/100g B.C)	Sex	Control	MEAV 200	MEAV 400	MEAV 600
Heart	Male	0,366±8,59	0,363±8,548	0,356±8,614	0,364±8,476
	Femelle	0,357±2,998	0,353±4,255	0,355±3,967	0,362±3,679
Liver	Male	3,496±107,1	3,824±107,7	4,026±106,4*	4,030±107,8*
	Femelle	3,369±94,55	3,662±94,69	4,076±98,01**	3,933±102,5**
Kidney	Male	0,271±3,719	0,279±4,312	0,281±4,603	0,295±4,384*
	Femelle	0,272±7,303	0,277±7,607	0,286±7,175	0,266±7,166

*: p<0,05; **: p<0,01; significant difference with regard to control (olive oil)

The hematological and biochemical parameters were studied. The results obtained with regard to the hematological parameter are recorded in Tables 3 according to gender. The results showed significant differences in some hematological parameters by sex. Thus, at the level of the hematological values males, significant decreases compared to control were found on the platelets (the dose 200 mg/kg), the Lymphocytes (200 and 600 mg/kg), the Monocytes (600 mg/kg) and the neutrophils (the dose 400 mg/kg). On female hematological parameters, the results showed significant increases compared

to control on WBC (400 mg/kg), hematocrit and monocytes (400 and 600 mg/kg). Significant decreases in HB and Eosinophils (200 mg/kg), MCV and MCH (200 and 400 mg/kg), Lymphocyte (600 mg/kg).

Results on biochemical parameters (Table 4) by sex showed significant differences on some markers. Thus, on AST, there were significant differences in the female resulting in a decrease (P <0,05) of this parameter at doses 200 (11,88%) and 400 mg/kg (9,93%) and an increase of this marker at 600 mg/kg (8.63%) compared to controls. In males, there was a non-significant increase in AST at doses of 400 and 600 mg/kg. On ALT, a significant decrease was observed at the 200 mg/kg dose (P <0,05). Total bilirubin in males showed a significant decrease at all treatment doses. This decrease translates to 55,28% (200 mg/kg), 25,32% (400 mg/kg) and 17,66 (600 mg/kg) relative to the control groups. In contrast, in the female, total bilirubin increased significantly at a dose of 600 mg/kg (P<0.05). On direct bilirubin, the results showed a significant decrease in this marker at 200 mg/kg (P <0.05) compared with controls in both gender. In contrast, this marker increased significantly by 24.30% (400 mg/kg) and 67.60% (600 mg/kg) relative to control group.

Histological examination of the heart, liver and kidney is shown in Figure 3. Histological results showed a normal architecture of the heart between the treated and control groups. On the other hand, in the liver and kidneys, histological examination revealed hepatic and glomerular necrosis, especially at the 600 mg/kg dose compared to control.

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

Parameters	Sex	Control	MEAV 200	MEAV 400	MEAV 600
WBC (10^3/µL)	Male	16,16±0,7358	15,97±0,7288	14,55±0,7304	12,75±0,731
	Femelle	8,817±0,8235	9,657±0,5794	13,07±0,7522*	11,33±1,103
RBC (10^6/µL)	Male	9,357±0,1965	9,067±0,1978	9,137±0,1994	9,09±0,2003
	Femelle	8,043±0,08838	7,757±0,09025	8,123±0,08988	7,94±0,08718
HB (g/dL)	Male	14,47±0,1856	14,53±0,2186	14,07±0,1856	14,33±0,2186
	Femelle	12,7±0,1155	12,23±0,06667	12,83±0,08819	12,53±0,08819*
Hematocrit (%)	Male	51,2±0,9074	50,53±0,8762	50,07±0,8876	50,17±0,8838
	Femelle	41,53±0,5457	42,97±0,5207	44,53±0,5364*	43,93±0,5239*
MCV (fL)	Male	54,03±0,809	54,53±0,5457	54,6±0,755	54,3±0,3606
	Femelle	56,43±0,2404	55,27±0,2728*	55,07±0,1856*	55,8±0,2082
MCH (pg)	Male	15,53±0,2906	16,03±0,3844	15,4±0,2517	15,67±0,1764
	Femelle	16,43±0,1856	15,53±0,2186*	15,57±0,2028*	15,93±0,1202
MCHC (g/dL)	Male	28,47±0,2728	28,57±0,2404	28,8±0,3512	28,9±0,4041
	Femelle	28,57±0,1856	28,37±0,2186	28,3±0,1528	28,77±0,1764
Platelets (10^3/dL)	Male	1033±57,5	770±57,07*	915,3±54,93	949,7±56,96
	Femelle	1026±51,55	808±49,49	1006±46,97	954,7±46,63
Lymphocyte (%)	Male	69,93±2,27	63,07±2,278*	73,57±1,924	64,13±2,724*
	Femelle	68,74±3,798	62,7±2,669	59,27±3,852	53,2±2,946*
Monocyte (%)	Male	2,533±0,5897	3,7±0,6351	2,7±0,6	5,733±0,584*
	Femelle	6,9±0,7767	10,17±0,7219*	7,967±0,7265	12,23±0,6173**
Eosinophil (%)	Male	1,7±0,3215	2,5±0,3512	2,433±0,3383	2,667±0,3528
	Femelle	2,5±0,1732	0,8333±0,1856***	2,133±0,2028	2,233±0,1764
Neutrophil (%)	Male	24,57±0,5364	27,47±0,8087	21,33±0,9615*	27,81±0,9011
	Femelle	25,36±2,252	24,37±1,96	32,4±2,25	30,63±2,092
Basophil (%)	Male	0,3333±0,03333	0,3333±0,03333	0,2667±0,03333	0,2667±0,03333
	Femelle	0,3±0,05774	0,3667±0,1202	0,4±0,1155	0,2±0,05774

*: p<0,05; **: p<0,01; ***: p<0.001; significant difference with regard to control (olive oil)

Table 4: Effect of methanol extract of *Amaranthus viridis* on the biochemical parameters rats in the subacute toxicity study

Parameters	Sex	Control	MEAV 200	MEAV 400	MEAV 600
AST (UI/L)	Male	149,1±6,951	142,9±5,921	160,7±6,104	161,5±5,774
	Femelle	154±3,616	135,7±3,504*	138,7±4,157*	167,3±2,466*
ALT (UI/L)	Male	64,77±2,685	51,87±3,391*	72,07±1,855	65,83±3,891
	Femelle	41,23±4,359	34,4±3,349	29,23±2,423	36,7±2,065
Total Bilirubin (mg/l)	Male	1,291±0,06695	0,5773±0,5773***	0,964±0,06712**	1,063±0,06758**
	Femelle	0,6007±0,06741	0,6647±0,06817	0,484±0,06451	0,944±0,06886*
Direct bilirubin (mg/l)	Male	0,816±0,053	0,5407±0,05203*	0,6593±0,05305	0,7133±0,7133
	Femelle	0,2633±0,01453	0,262±0,01677	0,3273±0,01272*	0,4413±0,01462***
Uric acid (mg/l)	Male	9,2±0,8505	6,467±0,809	10,37±0,8192	8,167±0,8511
	Femelle	14,1±1,582	12,57±1,445	12,93±1,507	12,23±1,538
Total Cholesterol (g/l)	Male	0,5233±0,02728	0,46±0,46*	0,5433±0,02404	0,5533±0,02404
	Femelle	0,5867±0,05487	0,61±0,05508	0,5467±0,05364	0,5±0,05196
Triglyceride (g/l)	Male	0,4333±0,02848	0,49±0,02646	0,46±0,03215	0,39±0,03055
	Femelle	0,7533±0,03383	0,5367±0,0318*	0,5767±0,03756*	0,66±0,03786
Urea (g/l)	Male	0,2367±0,01856	0,28±0,01732	0,3067±0,01856*	0,2733±0,01764
	Femelle	0,3033±0,02906	0,27±0,03055	0,3367±0,0318	0,2433±0,02963
Creatinine (mg/l)	Male	4,333±0,3333	3,333±0,3333	3,667±0,6667	3±0,5774
	Femelle	4,333±0,3333	4,667±0,3333	3,667±0,3333	3,667±0,3333

*: p<0, 05; **: p<0, 01; ***: p<0.001; significant difference with regard to control (olive oil)

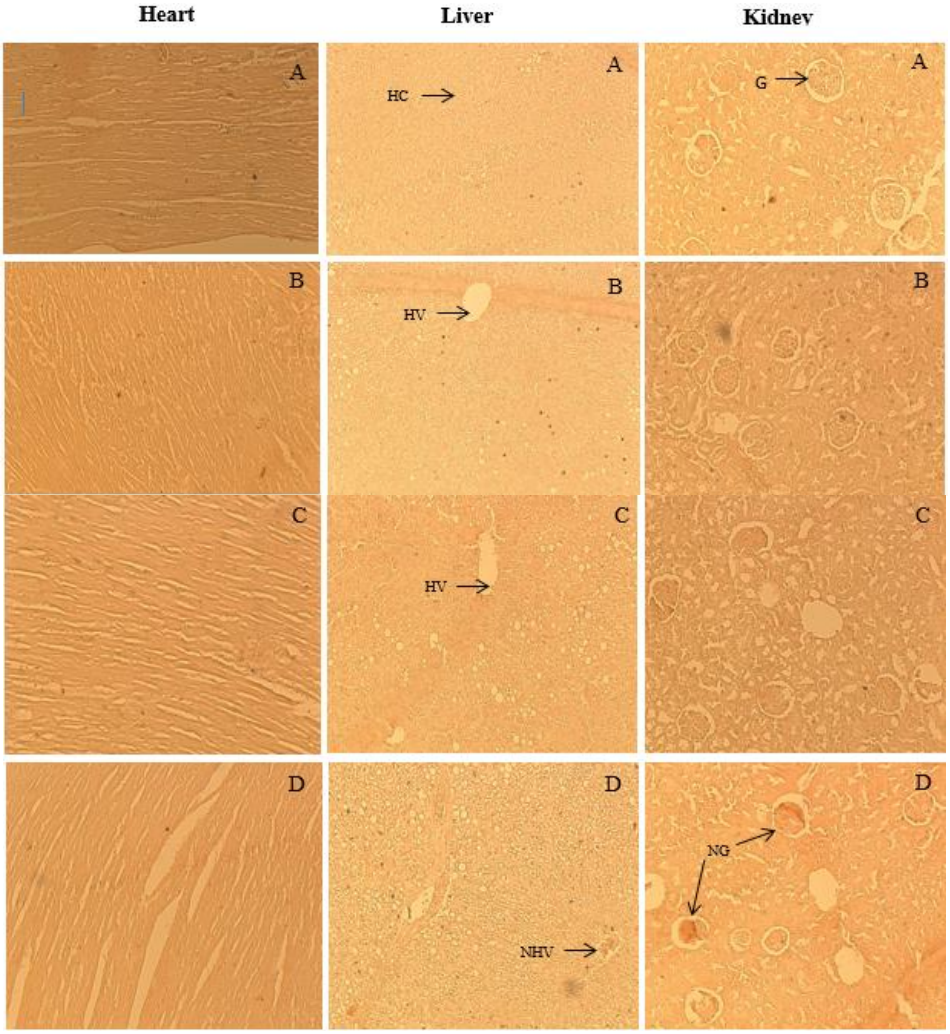

Fig 3: Photomicrograph of tissue sections of rats following 28 day treatment
A: Control; B: *A. viridis* (200 mg/Kg per day); C: *A. viridis* (400 mg/Kg per day);
D: *A. viridis* (600 mg/Kg per day)
G (Glomerulus), HV (hepatic vein), HC (cell hepatic), NG (necrotic glomerulus), NHV (necrotic hepatic vein)

Discussion

Phytochemical results revealed the presence of several secondary metabolites described in Table 1. The results obtained are similar to those obtained by Saravanan et *al.* which showed the presence of these compounds in their study [15]. The metabolites found in the methanolic extract have well-known properties. Indeed, Polyphenols have cardiovascular properties and fight against degenerative diseases [16-17]. The tannins have antibacterial and anti-inflammatory properties, flavonoids have antioxidant activity [18-20].

These results on the acute toxicity of the methanolic extract of *Amaranthus viridis* have shown that the lethal dose of this plant is greater than the 2000 mg/kg limit dose. These results confirm the work of Girija et *al.* which locates the toxicity of the extract of this plant beyond 5000 mg/kg [21].

Changes in body and organ weights are a clear indicative of damage caused by the substance test [22]. The increase in liver volume in the treated groups (400 and 600 mg/kg) and kidneys at a dose of 600 mg/kg in the sex male, compared to the control group could be explained by the installation of toxicity at these doses.

Blood is the main carrier of substances in the body; its components are very sensitive to toxins. Hematological parameters therefore represent an important clinical response to toxic compounds [23]. The results found on the hematological data showed variations in both sexes. However, these recorded differences compared to controls are within the physiological limit of this species [24].

The serum enzymes AST and ALT are considered sensitive markers of hepatocellular toxicity and its increased activity indicate liver damage [25]. The increase in AST and direct bilirubin, especially at the 600 mg / kg dose, could result in the installation of hepatic toxicity caused by the administration of the methanolic extract of *Amaranthus viridis* at this dose. On the other hand, the reduction of the serum levels of AST, ALT and direct bilirubin at a dose of 200 mg/kg can be explained by a hepatoprotective effect of the extract at this dose. These results confirm the work of Ashok et *al.*, which has shown that the methanolic extract administered at doses of 200 and 400 mg/kg have a hepatoprotective effect [26]. This hepatoprotective activity at a dose of 200 mg/kg would be due by the presence in this extract of flavonoids. Indeed, Lin Wan and Jian-Guo (2018) showed that flavonoids have a protective activity on the liver [20]. This hepatoprotective effect is similar to the studies conducted by Pallara and Velikkakathu who found these same effects by studying the acute and subacute toxicity of the extract *Lygodium flexuosum* in rat [27].

Hepatic and nephrotic necrosis at the 600 mg/kg dose shows that administration is not safe and may induce toxicity.

Conclusion

The present results show that the methanol extract of *Amaranthus viridis* shows no signs of toxicity when administered in a single dose. On the other hand, for a 28-day treatment in different doses, the methanolic extract caused biochemical, histological and relative weight variations of the liver, especially at a dose of 600 mg / kg. This justifies that repeated administration of the methanolic extract of *Amaranthus viridis* is not safe from 600 mg/kg. It could be toxic at this dose. Therefore, further assessments are required to conduct clinical studies of this plant.

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