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Subacute toxicity of an aqueous extract of *Alternanthera pungens* (Amaranthaceae) in wistar rats

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Abstract
Alternanthera pungens (Amaranthaceae) is an African pharmacopoeia plant known for its antidiabetic properties. The aim of this study was to evluate the oral toxicity of the aqueous extract of the whole plant of *Alternanthera pungens* in Wistar rats. A qualitative phytochemical test was carried out. The toxicity tests were carried out in accordance with guidelines 407 of the Organisation for Economic Co-operation and Development (OECD). Oral administration of doses ranging of 500 to 2000 mg/kg bw of the aqueous extract of *Alternanthera pungens* in continuous doses for 28 days allowed a series of hematological and biochemical analyses. This aqueous extract contains sterols and polyterpenes, saponosides, alkaloids, catechic tannins, anthocyanins. The results of the toxicity test and the hematological and biochemical analyses indicate that this extract is non-toxic. This absence of toxicity by oral route would support its use in various therapies in traditional African medicine.

Keywords: *Alternanthera pungens, Subacute toxicity*

1. Introduction

Medicinal plants have been used since the dawn of time to relieve ailments and cure diseases. According to WHO, almost 80% of the world's population depends on traditional medicine for primary health care [1]**.** *Alternanthera pungens* (Amaranthaceae) (Syn. *Alternanthera repens*), commonly known as 'khaki-weed', is a creeping plant with small, paired leaves bearing flowers with small cylindrical or almost round-headed whitish bracts [2]. This plant is native to South America and has been cited as a weed in various parts of the world such as Germany, France, Italy, Australia, Africa etc. [3]. It is a plant widely used in traditional medicine. In Ghana, the plant is used to combat abdominal pain, and also has abortive and galactagogue properties [4]. In Côte d'Ivoire, it is used to treat jaundice, dysentery, malaria and infections in general [5]. The work of Nacoulma-Ouedraogo showed that this plant had spasmogenic effects [6] and according to the work of Koffi *et al*. [7], *Alternanthera pungens* induces parturition. In order to avoid or prevent possible toxicity risks, the general objective of this study was to evaluate the subacute toxicity of the aqueous extract of *Alternanthera pungens* in mammals to ensure its safe use in traditional medicine in various therapies.

2. Materials and Methods

2.1 Materials

2.1.1 Végétal material

The plant material consists of the whole plant of *Alternanthera pungens* (Amaranthaceae) collected during the month of June 2018, at the medicinal plant market of adjamé, a commune of the city of abidjan. This plant was authenticated at the National Floristic Center (CNF) of Abidjan of the Félix Houphouët Boigny University (UFHB).

2.1.2 Animal material

The animals used in our experiments were Wistar rats of the species *Rattus norvegicus* (Muridae). They weighed between 140 and 160 g were eight (8) weeks old. They were raised in the animal house of the Teaching and Research Unit in Animal Physiology Laboratory of Biology and Health in Biosciences Training and Research Unit of the Félix Houphouët Boigny University of Abidjan. The room had natural lighting and an average temperature of 25 °C. The animals were placed in cages with free access to water and food.

2.2 Methods

2.2.1 Preparation of the aqueous extract of *Alternanthera pungens*

The whole plant was dried in the shade at room temperature for one week. The whole plant was macerated as follows: one hundred (100) grams of crushed plant of *Alternanthera pungens* are macerated in two thousand (2.000) millilitres (ml) of distilled water for 24 hours using a RH-type magnetic stirrer (IKA, Labortechnik, Germany). The filtrate obtained was evaporated under vacuum at 70 °C using a "Bucchi" type Rotavapor evaporator. The paste obtained was frozen and then freeze-dried. A powder was obtained. This powder was dissolved in distilled water to prepare the different doses used of aqueous extract of *Alternanthera pungens* (AEAp)

2.2.2 Phytochemical study of aqueous extract of *Alternanthera pungens*

This study was carried out at the Pharmacognosy Department of the Formation and Research Unit of Pharmaceutical and Biological Sciences of the Felix Houphouët Boigny University in Abidjan. It has made it possible to highlight the major chemical groups of pharmacological interest, namely sterols, polyterpenes, flavonoids, tannins, quinone compounds, saponins and alkaloids. This was done by using a qualitative method described by ABO and Néné *Bi et al.* [8, 9].

2.2.3 Study of the subacute toxicity of an aqueous extract of *Alternanthera pungens* in Wistar rats

The subacute toxicity study was carried out according to the experimental protocol described by Ramirez-Farias *et al.* [10]. Twenty-four (24) Wistar rats were divided into two (2) groups of twelve (12) rats. Each batch contained an equal number of adult males and females with an average weight of between 140 and 160 g. The twenty-four rats were divided into two groups of four (4) batches of six (6) rats, each comprising three (3) males and three females. The rats in batch I, serving as controls, were given distilled water at a rate of 10 ml/kg. Rats in batches II, III and IV received doses of 500, 1000 and 2000 mg/kg bw of EAAp at a rate of two (2) ml of solution per animal. These different doses of extracts were administered daily and at the same time. The animals were observed daily to detect any physiological and/or behavioural changes. After twenty-eight (28) days, the rats were fasted and anaesthetised with diethyl ether. Their blood was then collected from the orbital sinus, without damaging the eye, using a pasteur pipette and collected in EDTA (Ethylene Diamine Tetra Acetic) tubes for haematological tests. These tests were carried out using a Beckman Coulter (FRANCE) automated system to determine the haematocrit percentage, haemoglobin concentration, erythrocyte and reticulocyte count, leucocyte formula and platelet count in the samples taken. The blood collected in dry tubes was used to measure biochemical parameters.

2.2.4 Statistical analysis

Statistical processing of the data and graphical representation of the values were carried out using Graph Pad Prism 8 (San Diego, California, USA) and Graph Pad Prism 4 (San Diego, California, USA) software respectively. Statistical differences between the means were determined by analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance level of $p<0.05$. All values are presented as mean±SEM (Standard Error on the Mean).

The following notation is used for any significant difference:

- Not significant (ns): $p>0.05$;
- Significant (*): $p<0.05$;
- Moderately significant (**): $p<0.01$;
- Very significant (***): $p<0.001$;
- Highly significant (****): $p<0.0001$.

3. Results

3.1 Phytochemical composition of the aqueous extract of *Alternanthera pungens*

The results of the phytochemical study showed that AEAp contains sterols and polyterpenes, saponosides, alkaloids, catechic tannins, anthocyanins. We notice the absence of polyphenols, flavonoids, quinonic compounds, gallic tannins and coumarins are absent (Table 1).

Table 1: Chem +/ical composition of an aqueous extract of *Alternanthera pungens*

Compounds	Reactions/Reagents	AEAp
Polyphenols	Reaction to ferric chloride	-
Sterols and Polyterpenes	Liebermann-Burchard reaction	+
Flavonoids	Reaction to cyanidin	-
Saponosides	Vigorous agitation	+
Quinonic compounds	Borntraeger's reagent	-
Alkaloids	Dragendorff and Bouchardât reagents	+
Catechic tannins	Stasny's reagent	+
Gallic tannins	Hydrochloric acid reactive	-
Anthocyanins	Phosphate buffer reagent	+
Coumarins	Lactonic cycle	-

AEAp: *Aqueous extract of Alternanthera pungens*
(+): Presence of the compound
(-): Absence of the compound

3.2 Effects of an aqueous extract of *Alternanthera pungens* on the behaviour of Wistar rats

Administration by gavage of doses of 500, 1000 and 2000 mg/kg bw of AEAp to the animals did not result in any significant change in their behaviour after twenty-height days of observation. Nevertheless, a few clinical signs such as tremors and polypnoea were observed during the first few days of administration, without leading to the death of the animals. Similarly, no cases of death were recorded in rats treated with different doses throughout the test.

3.3 Effects of an aqueous extract on haematological parameters and serum parameters in wistar rats after twenty-height (28) days of administration

3.3.1 Effects of an aqueous extract of *Alternanthera pungens* on haematological parameters in Wistar rats.

Table 2 shows the variations of haematological parameters of rats after 28 days of a continuous administration of increasing doses of AEAp. Our results show that this extracts cause no change in haematological parameters compared with those of control rats.

Continuous administration of increasing doses of AEAp at 500, 1000 and 2000 mg/kg bw for 28 days of treatment did not result in significant changes in red blood cell, haemoglobin and haematocrit levels in rats given these doses compared with control rats. However, at the same doses, highly significant decreases ($p<0.0001$) in white blood cell and platelet levels were observed in male rats and a highly significant increase ($p<0.0001$) in blood platelet levels was observed in female rats compared with control rats.

Table 2: Variation in haematological parameters in rats after administration of increasing doses of aqueous extract of *Alternanthera pungens* after 28 days of treatment.

Haematological paramètre	Gender	Control group	500 mg/kg bw	1000 mg/kg bw	2000 mg/kg bw
Red Blood Cells (× 10^6/µL)	Males	7,743±0,14	7,547±0,15	7,027±0,06 *	7,54±0,19
	Females	7,28±0	7,38±0,11	6,75±0,35	6,41±0,12
White Blood Cells (× 10^3/µL)	Males	19,27±0,3383	12,03±0,46****	12,13±0,29****	6,2±0,45****
	Females	8,45±0,15	8±0,4	8,6±0	6,55±0,25*
Haemoglobin (g/ dL)	Males	14,67±0,4978	13,73±0,37	13,43±0,318	14,5±0,057
	Females	14,9±0,7	14,8±0,8	14±1,2	13,2±0,6
Haematocrit (%)	Males	42±0,4583	45,07±0,35*	40,57±0,669	42,57±0,6888
	Females	45,3±0,1	43,55±0,85	40,2±0,6**	43,15±0,35
Blood Platelets (× 10^3/mm^3)	Males	1149±0,577	867±0,577****	779,7±0,33****	944,7±0,333****
	Females	440,5±1,5	736±1****	808±1****	864,5±0,5****

AEAp induced highly significant decreases in white blood cell and platelet levels in male rats and a highly significant increase in blood platelets in female rats compared to control rats (Mean±SEM; $p<0.05$, $p<0.0001$; n= 6). SEM: Standard Error of the Mean, Control group: Rats given 2ml of distilled water, 500, 1000 and 2000 mg/kg bw: Doses of AEAp f

3.3.2 Effects of aqueous extract of *Alternanthera pungens* on renal and liver biomarkers in Wistar rats

Variations in the levels of biochemical parameters in the kidneys and liver are summarised in Table 3. There was a highly significant increase ($p<0.0001$) in the urea level in males and a highly significant decrease ($p<0.0001$) in this level and a significant decrease ($p<0.01$) in the creatinine level in females at doses of 1000 and 2000 mg/kg bw compared to control animals. These results indicate a highly significant increase ($p<0.0001$) in uric acid levels in all animals at 2000 mg/kg bw of AEAp compared to those in controls. ALT and AST levels show a highly significant decrease ($p<0.0001$) at all doses of AEAp in both sexes compared to those in controls.

Table 3: Variation in liver and kidney biomarker levels in rats after administration of increasing doses of aqueous extract of *Alternanthera pungens* after 28 days of treatment.

Biochemical parameters	Gender	Control group	500 mg/kg bw	1000 mg/kg bw	2000 mg/kg bw
Urea (g/L)	Males	0,25±0	0,2333±0,003**	0,3433±0,003****	0,427±0,0033****
	Females	0,3967±0,003	0,33±0,00577 **	0,26±0,0116 ****	0,3267±0,0145 **
CREA (mg/L)	Males	4,333±0,3333	3,333±0,333	3,333±0,3333	3,667±0,333
	Females	5,333±0,3333	4,333±0,3333	3,333±0,3333 **	3,333±0,3333 **
Uric acid (mg/L)	Males	13,67±0,3333	12,33±0,333	19,33±0,6667***	27,33±0,667****
	Females	11,67±0,3333	20,33±0,33 ****	28±0,5774 ****	27±0,5774 ****
AST (UI/L)	Males	318±0,58	317,7±0,33 ****	290,3±0,88 ****	247±0,58 ****
	Females	235±1,155	206±0,58 ****	206±0,58 ****	201,7±0,88 ****
ALT (UI/L)	Males	50±0,57	69,33±0,67****	69,67±0,33****	62,33±1,20 ****
	Females	69±0,58	45±1,155****	43,67±0,88****	43,67±1,453****

AEAp causes a highly significant decrease in ALAT and ASAT at all doses in both sexes of animals compared to control groups, (Mean±SEM; $p<0.01$, $p<0.001$ $p<0.0001$; n= 6); SEM: Standard Error of the Mean; CREA: Créatinine; AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase, SEM: Standard Error of the Mean, Control group: Rats given 2ml of distilled water, 500, 1000 and 2000 mg/kg bw: Doses of AEAp

4. Discussion

The phytochemical study of AEAp revealed the presence of sterols and polyterpenes, saponosides, alkaloids, catechic tannins and anthocyanins. The presence of saponosides and tannins was confirmed by studies by Adéoti *et al*. [10], who noted the absence of alkaloids and anthocyanins, which would be due to the part of the plant used.

Blood cells are major targets of toxicity, thus the analysis of blood parameters in toxicological studies is relevant to assess the safety of a substance [11]. The results of the hematological parameter assay show highly significant decreases ($p<0.0001$) in white blood cell and platelet counts in male rats and a highly significant increase ($p<0.0001$) in blood platelets in female rats compared to rats in control groups. The decrease in white blood cell levels observed could involve a reduction in the immune system's ability to respond to an immune requirement. Our results are similar to those of Djoupo [12] who evaluated the antihypertensive, antidiabetic, cardioprotective, immunomodulating and diuretic activities of aqueous and ethanolic extracts of trunk bark of *Trichilia emetica* vahl (Meliaceae). The hyperplaquettosis observed in females may be associated with martial deficiency and myeloproliferative syndromes. These results are consistent with the work of Bain and Gupta [13] and Djetouan [14] with the aqueous extract of seeds of *Garcinia kola* Heckel (Clusiaceae) at a dose of 1000 mg/kg bw in rats.

Analysis of liver and kidney function is very important when assessing the toxicity of extracts, as they are necessary for the survival of an organism [15]. ALT is a cytosolic enzyme secreted into liver cells from where it is released into the blood in the event of hepatic cell necrosis [16]. C'est une enzyme spécifique au foie, ce qui en fait un important indicateur très sensible de l'hépatotoxicité. AST is an indicator of the destruction of hepatocytes, although in addition to the liver it is also found in the heart, skeletal muscles, lungs and kidneys. ALT and AST levels rise rapidly when the liver is damaged for various reasons including hepatic cell necrosis, hepatitis, cirrhosis, and hepatotoxicity of certain drugs [17]. In our study, the concentrations of these two enzymes were significantly decreased in all animals. As ALT is the specific marker of the liver, it can be said that our extract does not damage liver cells, a fact that is due to the presence in our extract of alkaloids and saponosides which, according to Derouiche and Kechrid [18], have hepatoprotective properties. Our results are contrary to those of Gbagbo *et al*. [19], who showed a gradual increase in liver markers in rats with prolonged administration of the total aqueous extract of the stem bark of *Spondias mombin* L. (Anacardiaceae). Creatinine and urea are excellent markers of renal function. Their increase indicates renal dysfunction [20]. In the present study, changes in plasma urea levels in rats treated with EAqAP at doses of 1000 and 2000 mg/kg bw in males alone cannot indicate impaired renal function since there was a significant decrease in creatinine levels in treated rats compared with control rats. These results are in agreement with those of Ouahchia *et al*. [21] who showed that elevation of urea alone did not necessarily justify kidney damage. The decreases in ALT, ASAT and creatinine levels in rats after daily administration of increasing doses of AEAp could indicate that this extract has no effect on the kidneys and liver. This reinforces the non-toxicity of this plant when administered orally at the doses studied.

5. Conclusion

Continuous administration of the aqueous extract of *Alternanthera pungens* shows no signs of toxicity and would have no notable effects on the kidneys and liver. However, its use should be moderated in view of the hyperplaquettosis observed in females.

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