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ACUTE AND SUBACUTE TOXICITY OF THE AQUEOUS EXTRACT OF THE STEM BARKS OF *PAUSINYSTALIA YOHIMBE* (K.SCHUM.) PIERRE EX BEILLE, IN LABORATORY RODENTS

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ABSTRACT

The acute toxicity study was performed in accordance with guideline 407. Mice were given the aqueous extract of *Pausinystalia yohimbe* (1000, 2500 and 5000 mg/kg) orally for 14 days. The subacute toxicity study was also performed according to the guideline 407. To assess subacute toxicity, rats were gavaged for 28 days at the dose of 250 mg/kg aqueous extract of *Pausinystalia yohimbe*. The results obtained after the acute toxicity study show that oral administration of *Pausinystalia yohimbe* aqueous extract (1000, 2500 and 5000 mg/kg) does not alter the general behavior of mice and does not cause mortality in animals up to the dose of 5000 mg/kg. The effects of *Pausinystalia yohimbe* aqueous extract on weight growth for 14 days in mice showed significant increases ($p<0.05$) in body gain in mice treated with the extract compared to those given distilled water. Administration of aqueous extract of *Pausinystalia yohimbe* (250 mg/kg, po), for 28 days caused a significant increase ($p< 0.05$) in ponderal growth and did not affect ($P>0.05$) organ weights (liver, heart, kidney) in rats. On the other hand, the administration of aqueous extract of *Pausinystalia yohimbe* aqueous extract at the dose of 250 mg/kg caused a significant decrease ($p<0.05$) in the number of white blood cells and lymphocytes in rats on the one hand and a non-significant decrease in the hematocrit rate, the number of red blood cells as well as that of platelets on the other hand. These results suggest that the aqueous extract of the stem barks of *Pausinystalia yohimbe* is a material whose use is favorable in pharmacology and justifies its use in traditional medicine in the treatment of erectile dysfunction.

KEYWORDS: *Pausinystalia yohimbe*, acute toxicity, subacute toxicity.

INTRODUCTION

The interest of the populations for phytotherapy has not stopped growing over the years. This is understandable when one considers the many positive aspects of herbal medicine. These include: its diversity, its flexibility, its availability in many parts of the world, its low cost and level of technological involvement, and its economic importance. While plants are often considered healthy and natural compared to the growing conventional drugs,[1] some scientific investigations have shown that they may contain toxic substances leading to serious side effects such as kidney and liver damage, diarrhea, constipation, vomiting, rectal bleeding.[2,3] and mortalities.[4,8] The plants could be toxic by ingestion or contact. The treatments based on plant extracts can often be spread over long periods. Hence the importance of controlling the doses to be administered in order to avoid other more serious pathologies in the long term.

Pausinystalia yohimbe, a plant from the great forests of Central Africa, is used in the Congolese pharmacopoeia for the treatment of numerous pathologies, including sexual impotence. Thus, due to its frequent use, it becomes essential to analyze it further in order to prove not only its therapeutic usefulness, but especially to rule out possible risks of toxicity. Previous work.[9,11] on two species of the genus *Pausinystalia* highlights the effect of the aqueous extract of the bark of *Pausinystalia macroceras* on testicular functions, the effect of the ethanolic extract of *Pausinystalia yohimbe* on the quality of spermatozoa in rats, the aphrodisiac activity and the study of the mechanism of action of the aqueous extract of the stem barks of *Pausinystalia yohimbe* in the wistar

rat,[12] the androgenic activity of the aqueous extract of the bark of the stem of *Pausinystalia yohimbe*,[13] and the effects of the aqueous extract of the bark of the stem of *Pausinystalia yohimbe* on the quality of the seed and the performances of reproduction in the male wistar rat.[14] However, no study on the acute and subacute toxicity of the aqueous extract of *Pausinystalia yohimbe* has been conducted. Therefore, this study was initiated to evaluate the acute and subacute toxicity of this extract in rodents.

MATERIALS

Materials and Methods

Plant material

Pausinystalia yohimbe stem barks from Mayombe in the Niari department (Republic of the Congo) were provided by vendors at the Total market in Brazzaville in November 2016. Identification was made at the Institut National de Recherche en Sciences Exactes et Naturelles (I.R.S.E.N.) by comparison with the reference sample of the National Herbarium registered under number 15694-2009.

Animal material

To study the acute and sub-acute toxicity, three (3) months old male albino mice with weight between 25 and 30 g and three (3) months old male Wistar rats with weight between 100 and 150 g were used. These rodents were raised at the used. These rodents were raised at the Faculty of Health Sciences animal facility used. These rodents were raised in the animal facility of the Faculty of Health Sciences and fed in a standard manner with free access to water and a night/day (12/12) lighting schedule.

Methods

Preparation of the aqueous extract of *Pausinystalia yohimbe*

The preparation of the bark of stem of *Pausinystalia yohimbe* was made by maceration. To do this, the bark of *Pausinystalia yohimbe* was cut into small pieces and then dried in the laboratory in the open air at room temperature (28-30 °C) for 15 days. They were then crushed and ground in a mortar to obtain a homogeneous powder. Fifty (50) grams of powder were mixed in 500 mL of distilled water. The obtained mixture was then placed under magnetic stirrer (model L-73) for 48 hours. The obtained macerate was filtered through a filter (model L-73) for 48 hours. The obtained macerate was filtered through a filter paper (Whatman paper n°3) and absorbent cotton. The filtrate obtained was concentrated on a water bath thermostated at 55°C for 4 days, which allowed to obtain 3.5 g of dry extract of brown color which was kept at + 4°C in a refrigerator of Appolo Brant brand for pharmacological tests.

Study of the acute toxicity in mice

Effects of the aqueous extract of *Pausinystalia yohimbe* on general behavior and mortality

The acute toxicity study was carried out in accordance with guideline 407,[16 - 17] in order to determine the lethal dose 50 (LD50) and the doses of extracts to be used during the pharmacological tests. For this purpose, twelve (12) albino mice, used during the pharmacological tests. For this purpose, twelve (12) albino mice, fasted 24 hours before the experiment, were divided into four lots of 3 mice each and then treated orally as follows:

- Lot 1: 0.5 mL distilled water/100g;
- Lot 2: 1000 mg/kg of the aqueous extract of *Pausinystalia yohimbe;*
- Lot 3: 2500 mg/kg of the aqueous extract of *Pausinystalia yohimbe;*
- Lot 4: 5000 mg/kg of the aqueous extract *Pausinystalia yohimbe*.

Effects on general behavior (ptosis, stool condition, aggression, response to external stimuli, vocalization, vomiting) of mice were observed every 30 min for the first 4 h. Mortality and LD50 were assessed after 48 hours.

Effect of the aqueous extract of *Pausinystalia yohimbe* on the weight evolution

The weight of each animal was measured every two days for fourteen (14) days using a **QUIGG®** scale (range 5000 g and accuracy 1g).

Study of the subacute toxicity in the rat

Effects of the aqueous extract of *Pausinystalia yohimbe*

The subacute toxicity study was performed (carried out) according to the guideline 407 (OCED, 2009). The aim was to demonstrate the functional and/or anatomo-pathological alterations following the repeated administration of the aqueous extract of *Pausinystalia yohimbe* at the supposedly therapeutic dose of 250 mg/kg.[18]

Effect of the aqueous extract of *Pausinystalia yohimbe* on the weight evolution

During the 28 days of treatment at the dose of 250 mg/kg, the body weight of the rats was measured every 2 days using a **QUIGG®** scale (range 5000 g and sensitivity 1g).

Effect of the aqueous extract of *Pausinystalia yohimbe* on organ weight

At the end of 28 days of treatment with aqueous extract of *Pausinystalia yohimbe* (250 mg/kg) and after dissection of rats per lot sacrificed by ethyl ether overdose, kidneys, the liver and the heart were carefully removed and their weights measured with a precision balance of 120 g capacity and 0.001 g sensitivity. In addition, a macroscopic observation of the color and size of these organs was made.

Effects of the aqueous extract of *Pausinystalia yohimbe* on hematological and biochemical parameters

At the end of the 28 days of treatment with the extract, at a dose of 250 mg/kg, the animals were anesthetized with ethyl ether. For each animal, approximately 2 mL of blood was collected from the ophthalmic vein in two types of tubes: EDTA tubes for analysis of hematological parameters and dry "Vacutest" tubes for analysis of biochemical parameters. The latter were centrifuged at 2500 rpm for thirty minutes using a centrifuge of Hospitex Diagnostics SRL Centrifuga C-60 type. The serum obtained was collected and stored in eppendorff tubes before analysis of biochemical parameters. Hematological and biochemical parameters were determined according to the classical methods.[19,20]

Statistical analysis

Statistical analysis of the collected data was performed using analysis of variance (ANOVA), the Student's and Mann-Whhitney «t" test to compare the "test" and "control" groups. Results are expressed as mean ± standard error with $p < 0.05$ as the significance level.

RESULTS AND DISCUSSION

Acute toxicity study

Effect of aqueous extract of P. *yohimbe* stem barks on the general behavior of mice Administration of the aqueous extract of *Pausinystalia yohimbe* at doses of 1000, 2500 and 5000mg/kg did not alter the general behavior of mice compared to the control lot (Table I). This extract did not cause any mortality in mice at these doses 48 hours after administration and up to the 14th day of observation; the mortality rate was 0%. The lethal dose (LD50) is therefore greater than 5000 mg/kg.

Table I: Effects of the aqueous extract of *Pausinystalia yohimbe* on general behavior and mortality.

Parameters	Treatments			
	Distilled water (0.5 Ml/100g)	*Pausinystalia yohimbe* (1000 mg/kg)	*Pausinystalia yohimbe* (2500 mg/kg)	*Pausinystalia yohimbe* (2500 mg/kg)
Mobility	N	N	N	N
Agression	N	N	N	N
Stool condition	N	N	N	N
Pain sensitivity	N	N	N	N
Vomiting	A	A	A	A
Vocalization	A	A	A	A
Erection pilot	A	A	A	A
State of the tail	N	N	N	N
Ptosis	A	A	A	A
Wariness	+	+	+	+
Number of dead	0	0	0	0

A: Absent; N: Norma;l +: yes

Effect of the aqueous extract of *Pausinystalia yohimbe* stem barks on the weight evolution in mice during 14 days

The evolution of the weight of the mice during the 14 days of the study of acute toxicity is presented in Figure 1. These results show that, the mice treated with *Pausinystalia yohimbe* at the doses of 1000, 2500 and 5000 mg/kg present an increase in the weight gain from D1 to D14 compared to those which received distilled water. These values evolved from 100 to 105.5 ± 2.85g, 100 to 112.8 ± 5.99g and from 100 to 107.5 ± 2.12g respectively for the doses 1000, 2500 and 5000 mg/kg of aqueous extract of *Pausinystalia yohimbe* for 100 to 100.6 ± 2.29g for the control lot, that is to say increases of 5.21, 11.34, 6.97 and 0.59.

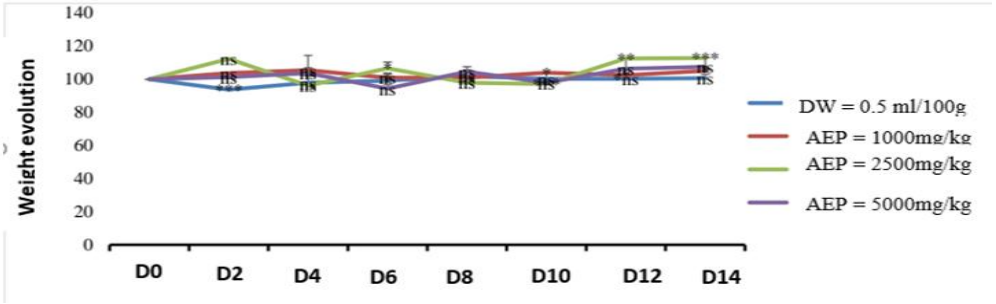

Figure 1: Effect of aqueous extract of Pausinystalia yohimbe on the weight development of mice. Values are means ± SEM with n = 3; ns = non-significant difference *= significative** and ***= highly significant, DW= distilled water ;AEP= aqueous extract of Pausinystalia yohimbe.

Subacute toxicity of the aqueous extract of *Pausinystalia yohimbe*

Effects of aqueous extract of *Pausinystalia yohimbe* on general condition, behavior and mortality rate in rats

The general condition and behavior of the rats were not altered compared to the control rats. The daily administration of the aqueous extract of *Pausinystalia yohimbe* at 250 mg/kg, during the 28 days of the test did not cause the death of the rats (the mortality rate was 0%).

Effect of the aqueous extract of *Pausinystalia yohimbe* on the weight evolution in rats during 28 days

The weight development of rats during the 28-day subacute study is presented in Figure 2. This figure shows that rats treated with *Pausinystalia yohimbe* at 250 mg/kg show an increase in weight gain from D1 to D21 compared to the control animals treated with distilled water. These values increase from 100 to 151.33 ± 3.98g for 100 to 145.97± 3.12g for the extract and distilled water i.e. 3.98g against 100 to 145.97 3.12g for the extract and distilled water i.e. respective increases of 33.91% and 31.49%.

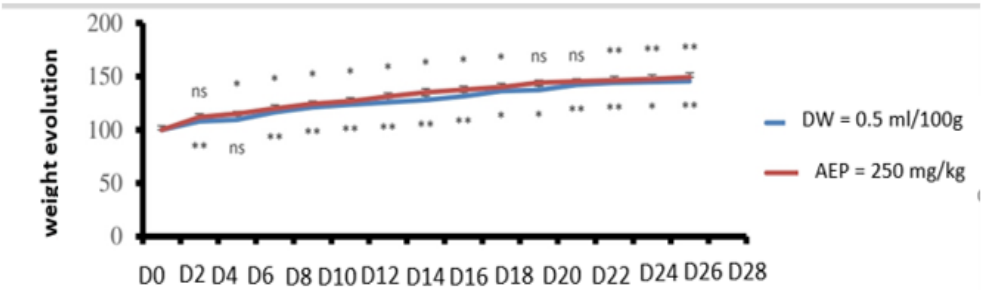

Figure 2: Effect of aqueous extract of Pausinystalia yohimbe administered for 28 days on weight gain in rats. values are means ± ESM with n = 5, *p < 0.05 ; **p < 0.01= significant difference from the first day ; DW= Distilled water ; AEP =Aqueous extract of Pausinystalia yohimbe.

Effect of aqueous extract of *Pausinystalia yohimbe* on organ weight

Table II presents the effect of aqueous extract of *Pausinystalia yohimbe* (250mg/kg) administered for 28 days on organ weight. It shows that, the weight of organs (Heart, Liver and kidneys) did not increase significantly in rats treated with aqueous extract of *Pausinystalia yohimbe* compared to those of the control lot that received distilled water.

Table II: Effect of aqueous extract of *Pausinystalia yohimbe* on organ weight.

Organ weights (g)	Treatements	
	distilled water (0.5 mL /100 g)	*Pausinystalia yohimbe*(250 mg/kg)
heart	0.87± 0.03	0.79 ± 0.03^{ns}
Liver	8.31 ± 0.17	8.29 ± 0.18 ^{ns}
Kidney	1.58 ± 0.03	1.51 ± 0.03 ^{ns}

*Values are means ± MSE, with n = 5. *: p< 0.05 significant difference from control (distilled water). ns: P >0.05 nonsignificant difference from control.*

Effect of aqueous extract of *Pausinystalia yohimbe* on biochemical parameters

The administration of the aqueous extract of *Pausinystalia yohimbe* (250 mg/kg) for 28 days caused a significant decrease of the ALT level compared to the controls, but without significant variations on the other biochemical parameters (Table III).

Table III: Effect of aqueous extract of *Pausinystalia yohimbe* on biochemical parameters.

Biochemical parameters	Treatments	
	Distilled water (0.5mL/100g)	*Pausinystalia yohimbe* (250mg/kg)
AST (UI/l)	161.33 ± 26.97	165.33 ± 10.60^{ns}
ALT (UI/l)	84.67± 20. 29	60 ± 2.85*
Creat (mg/l)	5.83 ± 0.28	7.30 ±0.18 ^{ns}
CT (g/l)	0.96 ± 0.02	1.03 ±0.02 ^{ns}
TG (g/l)	0.46 ± 0.09	0.36 ±0.02 ^{ns}
LDL (g/l)	0.26 ± 0.02	0.30 ± 0.04 ^{ns}
HDL (g/l)	0.1 ± 0.00	0.1±0.00^{ns}

*values are means ± SEM, with n = 5. *: p <0.05 significant difference from control (distilled water). ns: non-significant difference from control. AST : aspartate transaminase ; ALT : aminotransaminase ; TG: triglycerides ; TC: total cholesterol Creat: creatinine (LDL): Low-density lipoprotein (HDL): High- density lipoprotein..*

Effect of aqueous extract of *Pausinystalia yohimbe* on hematological parameters

The effects of the aqueous extract of *Pausinystalia yohimbe* on the hematological parameters are listed in Table I. It appears that the administration of the aqueous extract of *Pausinystalia yohimbe* (250 mg/kg) for 28 days, decreases significantly ($p < 0.05$) the rates of white blood cells and lymphocytes compared to the controls, but does not cause significant variations on the other hematological parameters.

Table IV: Effect of aqueous extract of *Pausinystalia yohimbe* on hematological parameters.

	Treatements	
Hematological parameters	**Distilled water (0.5mL/100g)**	***Pausinystalia yohimbe* (250mg/kg)**
WBCs (10^3/mm^3)	9.93 ± 0.53	7.90 ± 0.12*
LYM (%)	8.13 ± 0.46	6.43 ± 0.14*
MON (%)	0.86 ± 0.06	0.66 ± 0.08^{ns}
GRA (%)	0.93 ± 0.04	0.80 ± 0.04^{ns}
RBCs (10^3/mm^3)	7.03 ± 0.08	6.22 ± 0.47^{ns}
Hb (g/dl)	13.13 ± 0.54	12.20 ± 0.37^{ns}
HCT (%)	35.50 ± 0.54	32.9 ± 1.66^{ns}
MCV (µm^3)	50.66 ± 0.23	50.66 ± 0.47^{ns}
BPL (10^3/mm^3)	898.66 ± 55.58	774.00 ± 45.77^{ns}

Values are means ± SEM, with n= 5, p < 0.05 significant difference from control (distilled water) ns: non-significant difference from control. WBCs: white blood cells LYM: lymphocytes MON: monocytes, GRA: granulocytes, RBCs: red blood cells; Hb: hemoglobin MCV: mean corpuscular volume; BPL: blood platelets MPV: mean platelet volume; HCT: hematocrit.*

Acute toxicity of the aqueous extract of *Pausinystalia yohimbe* in mice

The results obtained after the acute toxicity study showed that the oral administration of the aqueous extract of *Pausinystalia yohimbe* stem barks (1000, 2500 and 5000 mg/kg) did not alter the general behavior of mice and did not cause mortality in animals up to the dose 5000 mg/kg. Our results are in disagreement with those obtained,[11] on the acute toxicity of the ethanolic extract of the stem barks of *Pausinystalia yohimbe*, which would be lethal at doses of 500, 1000, 2000 and 3000 mg/kg. Similar results were obtained in mice treated with chloroform extract of *Mansonia altissima* bark at the maximum dose of 137.5 mg/kg [21]. This difference could be attributed either to the concentration of the toxic substances contained in the bark of the plant, the animal species, the type of solvent, or the route of administration used. It has been scientifically proven that the toxicity of a substance varies according to the animal species, the dose, the concentration, the extraction technique and the route of administration of the substance [11 15]. These results allow to classify the toxicity of this extract in category 5 of the Harmonized System of Classification of Chemical Substances, with an LD50> 5000 mg/kg, category characterizing weakly toxic substances [15]. The effects of the aqueous extract of *Pausinystalia yohimbe* (1000, 2500 and 5000 mg/ kg) on weight gain over 14 days in mice showed significant increases in body gain in mice treated with the extract compared to those given distilled water. This increase in body weight could be related to an important food and water consumption suggesting an appetite stimulating effect of the extract in mice. Our results are similar to those obtained [18] which observed an increase in body gain in mice treated with the aqueous extract of Strychnos camptoneura stem bark. These results suggest that the aqueous extract of *Pausinystalia yohimbe* stem barks is a material with favorable use in pharmacology.

Subacute toxicity of the aqueous extract of *Pausinystalia yohimbe* in rats

The dose of 250 mg/kg is retained as the maximum therapeutic dose used for the pharmacological tests because up to 2500 mg/kg there were no dead mice and no side effects. Administration of the aqueous extract of *Pausinystalia yohimbe* (250 mg/kg, po), for 28 days did not modify the general state, the behavior of the animals and did not cause any mortality, which corroborates the results of the study of acute toxicity in the mouse. These results are similar to those obtained [10] which reported no mortality after administration of the ethanolic extract of the stem barks of *Pausinystalia yohimbe* (burantashi) in albino rats. The results in figure 2 show an increase in body weight growth of *Pausinystalia yohimbe* extract treated rats (250 mg/kg) compared to control rats confirming the acute toxicity results.

The increase in body weight is an indicator of the anabolic effects of the chemicals consumed by the animal. *Pausinystalia yohimbe* is thought to contain androgenic substances,[13] which are responsible for the anabolic effects observed in our work. This study showed no variation in organ weights (liver, heart, kidneys) and corroborates the results of the subacute toxicity conducted.[10] This study also showed that the aqueous extract of *Pausinystalia yohimbe* does not induce any variation on the hepatoprotective system. ALT is a cytosolic enzyme secreted in liver cells from which it is released into the bloodstream in case of hepatic cell necrosis.[22,23] It is a liver- specific enzyme, making it an important and highly sensitive indicator of hepatotoxicity.[24,25] AST is also an indicator of hepatocyte destruction although in addition to the liver it is found in the heart, skeletal muscle, lung and kidney.[22] ALT and AST levels rise rapidly when the liver is damaged for a variety of reasons including hepatic cell necrosis, hepatitis, cirrhosis as well as hepatotoxicity of certain drugs.[23,24] In our study, the concentration of these two enzymes (ALT and AST) did not change in animals treated with 250 mg/kg. Significant reductions in AST levels have already been obtained.[26,27] with the aqueous extracts of *Eleophorbia drupifera* and *Artemisia afra* leaves in rats, respectively. The chemical composition of *Pausinystalia Yohimbe* could provide reliable clues to identify the chemical compounds responsible for its effect on liver enzymes. Indeed, the chemical profile of this plant revealed the presence of flavonoids,[12] molecules known to be hepatoprotective [28] The hematopoietic system is one of the most sensitive targets of toxic compounds and an important index of the physiological and pathological status of humans and animals.[26] Changes in the hematopoietic system have a greater predictive value for human toxicity, when data are deduced from animal studies.[29] This hemolytic effect expressed in rats, could be attributed to the presence of saponosides.[3] Administration of aqueous extract of *Pausinystalia yohimbe* at the dose of 250 mg/kg caused a significant ($p< 0.05$) decrease in the number of white blood cells and lymphocytes in rats on the one hand and a non-significant decrease in the hematocrit rate, the number of red blood cells as well as that of platelets on the other hand. Similar results were observed in rats treated with extracts of *Mitragyna inermis* stem and root bark, which have physical properties characterized by an aphrogenic or foaming power and physiological properties expressed by a hemolytic index.[2] This reduction could also be explained by the presence of tannins,[12] in the extract; the latter having inhibiting effects on the synthesis of blood cells or hematopoiesis.

CONCLUSION

The present study shows that the aqueous extract of *Pausinystalia yohimbe* does not alter the general behaviour and causes no mortality of mice and rats no matter the doses used. This study reveals that the aqueous extract of *Pausinystalia yohimbe* induces no variation on biochemical parameters. However, the oral administration of the aqueous extract of *Pausinystalia yohimbe* at the dose of 250mg/kg causes a significant decrease in white blood cells and lymphocytes in rats on the one hand, and a decrease in hematocrit level, the number of red blood cells and the platelets. These results suggest that the aqueous extract of the stem barks of *Pausinystalia yohimbe* is a material whose use is favorable in pharmacology and justifies its use in traditional medicine in the treatment of erectile dysfunction.

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