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Safety of Extracts from Different Accessions of *Uvaria chamae*, an Anti-Sickle Cell Medicinal Plant

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Abstract

Uvaria chamae is a medicinal plant traditionally used in Benin to manage sickle cell disease, but the safety of its root extracts across its genetic diversity had not been systematically evaluated. This study evaluated the acute and 28-day repeated-dose oral toxicity of aqueous root extracts from five genetically distinct accessions (UV2, UV7, UV9, UV13, UV19). Female Wistar rats (n = 3 per group) received 2000 mg/kg once (acute oral toxicity, AOT) or 200 mg/kg/day for 28 days (subchronic oral toxicity, SCT) by gavage, following adapted OECD Guidelines 425 and 407. Body weight, AST, ALT, urea, creatinine, blood counts and liver and kidney histology were assessed. Changes from baseline were analyzed with paired t-tests and compared with controls by ANOVA and Dunnett's test. No deaths or overt clinical signs of toxicity occurred. Body weight decreased by 14.1% with UV7 in the AOT and by 8.1% ($P < 0.05$) and 7.9% with UV7 and UV19 in the SCT, whereas control rats gained weight. In the SCT, AST increased with UV2, UV7 and UV19 and ALT

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with UV2 and UV7 relative to baseline; compared with controls, only the ALT increase with UV7 (SCT) and the AST decrease with UV13 (AOT) were significant. Hemoglobin increased with UV9 and UV13 (AOT), as in controls, and platelets with UV2 (SCT). Histology showed no liver or kidney lesions. These preliminary findings suggest tolerability at the doses tested, but the small groups, single repeated dose and transaminase increases, especially with UV7, warrant liver monitoring in prolonged use.

Keywords

Biological Tolerance, *Uvaria chamae* Accessions, Acute Oral Toxicity, Subchronic Oral Toxicity, Sickle Cell Disease

1. Introduction

Sickle cell disease is the most widespread monogenic genetic disease in the world [1]. It disproportionately affects populations in sub-Saharan Africa and their diaspora. According to the latest analysis of the Global Burden of Disease, more than 7.7 million people were living with sickle cell disease worldwide in 2021, compared to 5.46 million in 2000, and more than 515,000 children are born with the disease each year [2]. The African region bears the brunt of this burden, with an estimated average prevalence of 2% and nearly 400,000 affected children born annually, 50% to 80% of whom die before the age of five in the absence of widespread newborn screening and structured care [1]. Benin is among the most affected countries in the West African sub-region, with a birth incidence exceeding 2% [2]. Beninese health authorities estimate that more than 4% of the population carries the disease, and approximately 5.1% of children under five years of age admitted to the pediatric emergency department of the National University Hospital Center-Hubert Koutoukou Maga in Cotonou were affected in 2019 [3]. Beyond the numbers, the disease imposes a considerable burden on patients, their families and the health system through unpredictable vaso-occlusive crises, chronic hemolytic anemia, increased susceptibility to infections and the high cost of optimal care, largely out of reach for a large part of the population of sub-Saharan Africa. The inaccessibility of conventional treatments naturally leads populations to turn to traditional medicine.

A recent systematic review encompassing all the literature published between 2000 and 2022 documented 117 plant species with proven anti-sickling activity, with Africa alone accounting for nearly 87% of the publications on the subject [4]. Among these plants, *Uvaria chamae* P. Beauv. (Annonaceae) occupies a special place in traditional West African medicine, where its roots are used in the management of sickle cell disease. This anti-sickling activity has been experimentally confirmed. The methanolic extract of the roots inhibits and reverses erythrocyte sickling *in vitro* [5], and more recent work conducted in Benin specifically validated the anti-sickling activity of the aqueous extract of the plant's roots [6], thus

confirming the local traditional use. Different parts of the plant are traditionally used to treat various health problems such as digestive disorders, fever, dysmenorrhea, and cancer, and to promote wound healing, among many other applications [7] [8]. However, the question of its safety remains a concern. Several toxicological studies conducted on rats have indeed qualified the image of a harmless plant. A subchronic toxicity study of the ethanolic extract of the roots showed a significant increase in serum aspartate aminotransferase at the highest doses, associated with significant mortality in the treated animals, with only 43% surviving to the end of the protocol [9]. Furthermore, an evaluation of the cytotoxicity and genotoxicity of the methanolic extract of the leaves of this plant in mice revealed a dose-dependent reduction in the mitotic index of bone marrow cells as well as a significant increase in chromosomal aberrations compared to controls, concluding that the plant has both cytotoxic and genotoxic potential [10]. These results contrast with those of acute toxicity trials conducted orally, which generally place the median lethal dose above 5000 mg/kg, a threshold classically considered reassuring but which does not, on its own, allow us to rule out a cumulative or specific tissue risk. Similarly, phytochemical screenings carried out on different parts of the plant have highlighted, in addition to flavonoids sought for their therapeutic properties, the recurring presence of tannins, alkaloids, cardiotonic glycosides and cyanogenic glycosides [5] [11], all families of compounds whose potential toxicity, at high doses or in prolonged use, is well documented in the pharmacological literature.

Larval toxicity tests provide additional but equally contrasting insights. Lethality tests on *Artemia salina* larvae (Brine Shrimp Lethality Test), frequently used as a preliminary cytotoxicity screening, generally classify *Uvaria chamae* extracts as non-toxic, with high median lethal concentrations, on the order of 27.8 mg/mL [12]; a more recent evaluation confirms this conclusion of non-toxicity on the same model [13]. This result, obtained in a simple aquatic organism, cannot, however, be directly extrapolated to systemic toxicity in mammals. Therefore, there is currently an unresolved discrepancy between the different models for assessing the plant's biological tolerance.

In addition to this toxicological uncertainty, there is a major methodological gap, rarely taken into account in published studies: the intraspecific variability of *Uvaria chamae*. Studies conducted in Benin have indeed revealed substantial genetic and morphological diversity among the plant populations distributed throughout the national territory, with a structuring into distinct genetic groups according to geographical areas [14] [15]. However, almost all toxicity studies available to date have been conducted on single accessions, without taking into account the genetic and environmental variability that can modulate the phytochemical composition and therefore the biological tolerance profile of the extracts. A recent ethnobotanical study conducted in Benin highlights the importance of examining the biochemical and toxicological properties of the plant extracts to confirm or refute the virtues traditionally attributed to it [16]. This gap is all the more worrying as the roots of *Uvaria chamae*, the most toxic part according to several phytochemical

screenings, are precisely those favored by traditional medicine in the treatment of sickle cell disease, potentially exposing patients already weakened by their chronic pathology to extracts whose biological tolerance has never been established on the scale of the different Beninese accessions.

It is in this context that the present study is justified. Faced with the widespread traditional use of *Uvaria chamae* in the management of sickle cell disease, but in the absence of solid toxicological data taking into account the intraspecific diversity of the plant in Benin, it appears essential to evaluate, in a systematic and comparative way, the biological safety of aqueous extracts of roots from several accessions representative of the national territory. This approach aims to bridge the gap between the pharmacological validation of the plant's anti-sickle cell activity and its safety, an essential prerequisite for any prospect of therapeutic development.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of aqueous extracts of *Uvaria chamae* roots collected in nine departments of Benin (Atacora, Donga, Collines, Zou, Couffo, Mono, Atlantique, Ouémé and Plateau) between March and May 2024 (Table 1).

Table 1. List of the *Uvaria chamae* accessions collected according to the departments of Benin.

Department	Municipality	Accession code
Atacora	Natitingou	UV18
	Toucountouna	UV19
Donga	Bassila	UV16
	Djougou	UV17
Collines	Dassa-Zoumè	UV13
	Savalou	UV14
	Savè	UV15
Zou	Zangnanado	UV1
	Zogbodomey	UV6
	Abomey	UV12
Couffo	Aplahoué	UV11
Mono	Comè	UV9
	Lokossa	UV10
Atlantique	Allada	UV7
	Ouidah	UV8
Ouémé	Bonou	UV5
	Kétou	UV2
Plateau	Pobè	UV3
	Sakété	UV4

In previous work on the genetic diversity of *Uvaria chamae*, we classified these 19 accessions from SSR microsatellite markers into two major genetic groups subdivided into five subgroups, with a similarity rate of 78% [14]. In the present study, we selected a representative from each of the five genetic subgroups for the safety study. These are accessions UV2 (Kétou), UV7 (Allada), UV9 (Comè), UV13 (Dassa-Zoumè) and UV19 (Toucountouna).

The plant material of *Uvaria chamae* collected in different communes of Benin was identified at the National Herbarium of the University of Abomey-Calavi under the number YH 268/HNB.

2.2. Extraction and Preparation of the Dosing Solutions

The collected root samples of each accession were dried in the laboratory at room temperature (20˚C - 25˚C), protected from sunlight and humidity, for 14 days. They were then ground into a fine powder and stored in airtight containers until extraction. Aqueous extracts were prepared by maceration as previously described [17] [18], following the chronological sequence below. For each accession, 10 g of root powder was macerated in 200 mL of distilled water heated to 77˚C for 30 min under intermittent stirring. The mixture was allowed to cool to room temperature and was filtered through Whatman filter paper. The residue was re-extracted twice under the same conditions, giving three successive extractions (3 × 200 mL). The three filtrates were pooled and then evaporated to dryness at 60˚C under reduced pressure using a Heidolph rotary evaporator. The dry extracts were weighed and stored in airtight containers until use. This extraction was repeated as many times as necessary to obtain the quantity of dry extract required for the tests; the yields given below refer to one 10 g batch.

The extraction yield was calculated using Equation (1):

$$\text{Yield}(\%) = \frac{m_{\text{extract}}}{m_{\text{powder}}} \times 100 \quad (1)$$

where m_{extract} is the mass of dry extract (g) and m_{powder} is the mass of root powder extracted (g). From 10 g of powder, the following masses of dry extract were obtained: UV2, 0.8 g (8%); UV7, 0.8 g (8%); UV9, 0.7 g (7%); UV13, 0.6 g (6%); and UV19, 1.2 g (12%).

On each day of administration, the dry extract was freshly dissolved in distilled water and the dose (2000 mg/kg for the acute test; 200 mg/kg/day for the repeated-dose test) was administered by oral gavage in a volume of 1 mL per rat, the amount of extract dissolved being calculated from the body weight of each animal measured on D0. Given the body weights of the animals, this corresponded to concentrations of about 200 to 340 mg/mL in the acute test and about 20 to 30 mg/mL in the repeated-dose test. Control animals received 1 mL of distilled water by the same route.

2.3. Animal Material

Female Wistar rats aged 8 to 10 weeks, nulliparous and non-pregnant, were used

for the toxicity tests; group mean body weights at the start of the studies ranged from 112 ± 8 g to 149 ± 10 g. These rats were acclimated to the ambient rearing conditions of the animal facility at the Laboratory of Experimental and Clinical Biology at the National Higher School of Biosciences and Applied Biotechnology in Benin. The rats were kept in wire cages equipped with feeders and waterers, with free access to food and water, under a 12-hour light/dark cycle. The breeding enclosure was regularly cleaned to ensure optimal development of the animals and to protect them from any infection.

2.4. Assessment of Acute and Subchronic Oral Toxicity

Both studies were designed with reference to the OECD Test Guidelines, with the deviations described below; they should therefore be regarded as adaptations of these guidelines rather than fully compliant studies.

2.4.1. Assessment of Acute Oral Toxicity (AOT)

The acute study was based on the limit test at 2000 mg/kg of OECD Test Guideline 425 (Up-and-Down Procedure) [19]. However, the sequential dosing and stopping rules of this guideline were not applied: three animals per group were dosed at 2000 mg/kg, together with a concurrent vehicle control group, instead of single animals dosed sequentially. This design therefore corresponds to a limit-dose acute study adapted from Test Guideline 425 and does not allow the median lethal dose to be estimated. The trial was conducted on 18 female Wistar rats. After a 15-hour fast, the rats were randomly divided into a control group of 3 rats receiving distilled water and five experimental groups of 3 rats each receiving the different extracts (UV2, UV7, UV9, UV13, UV19) at a single dose of 2000 mg/kg by gavage. Behavioral observation was conducted for 4 hours after administration. The rats were then given water and food daily for 14 days. During this period, signs of toxicity, including coat changes, motility, tremors, weight, grooming, respiration, noise sensitivity after metallic shock, fecal appearance, mobility, and death, were monitored. The rats were weighed and blood was collected by orbital puncture at the beginning of the experiment (D0) and at the end, after 14 days (D14).

2.4.2. Assessment of Subchronic Oral Toxicity (SCT)

The repeated-dose study was an adaptation of OECD Test Guideline 407 [20] [21]. It departs from the standard design of this guideline, which requires at least 10 animals per group (five of each sex) and at least three dose levels plus a control, in that it used three females per group, a single dose level (200 mg/kg/day) and no recovery group. It should therefore be regarded as an exploratory 28-day repeated-dose study. Six groups of three female rats each were used, randomly allocated to treatment groups. Group 1 received distilled water, while groups 2 through 6 were gavaged with the different plant extracts (UV2, UV7, UV9, UV13, UV19) at 200 mg/kg body weight daily for 28 consecutive days. The rats were observed daily throughout the study. They were weighed, and blood was collected

by orbital puncture at the beginning of the experiment (D0) and at the end, after 28 days (D28) [22] [23].

2.5. Blood Tests (AOT and SCT)

We measured serum urea and creatinine for the exploration of renal function, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) for liver function, and the complete blood count for the hematological assessment [22] [23]. Biochemical parameters were measured using a semi-automatic Biolabo spectrophotometer and Biolabo reagents. The complete blood count was performed on EDTA tubes using a Sysmex XN-550 hematology analyzer.

2.6. Histological Examination of the Removed Organs

The liver and kidneys were removed after dissection of the rats, fixed in 10% buffered formalin, and embedded in paraffin. Sections of 5 µm prepared with a microtome were mounted and stained with hematoxylin and eosin, according to a standard protocol [24]. Photographs were taken at 400× magnification. Histological slides were evaluated by an examiner blinded to treatment group allocation.

2.7. Statistical Analyses

Statistical analyses were performed on the individual data of each animal using the SciPy library (version 1.18) in Python. The results were expressed as mean ± standard error of the mean (SEM), with n = 3 rats per group; the individual rat was the experimental unit. The results of each parameter on D14 for the acute oral toxicity test and on D28 for the subchronic oral toxicity test were compared to their values on D0 using the paired Student's t-test, which takes into account that each rat was sampled at both time points, within each group, including the concurrent control group, whose results are reported alongside those of the treated groups. In addition, to compare each treated group with the concurrent control group, the change from baseline (value at D14 or D28 minus value at D0) was calculated for each rat, and the changes of the five treated groups were compared with those of the control group by one-way analysis of variance (ANOVA) followed by Dunnett's test, which adjusts for the five comparisons with the control. The significance level was set at 5%. Given the small group size (n = 3), the statistical power was low: non-significant results cannot be interpreted as evidence of the absence of an effect, and the P values of the paired tests, which were not adjusted for multiple comparisons, are presented as exploratory. The interpretation of the results therefore also relies on the direction and magnitude of the changes relative to the concurrent control group and on histological findings.

2.8. Ethical Considerations

The experimental protocol was conducted in accordance with the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines and international guidelines for the protection and use of laboratory animals. The animals were

housed in an animal facility at room temperature (25˚C ± 2˚C), under a 12-hour light/dark cycle, with free access to water and standard feed, and were acclimated for two weeks prior to the start of the trials. Every precaution was taken to minimize the number of animals used and to limit their suffering. At the end of the trials (D14 or D28), the animals were euthanized under anesthesia, and the liver and kidneys were collected for histology.

3. Results

3.1. Acute Oral Toxicity (AOT)

Oral administration of a single 2000 mg/kg dose of the aqueous extracts of the various *Uvaria chamae* accessions resulted in no deaths during the 14-day observation period. No clinical signs of toxicity, including agitation, aggression, convulsions, diarrhea, asthenia, drowsiness, anorexia or decreased mobility, were observed; body weight changes are described below.

Table 2 shows the variation in body weight, liver and kidney function in rats on D0 and D14 of the acute oral toxicity test.

Table 2. Variation in body weight, liver and kidney function in rats in the acute oral toxicity test of *Uvaria chamae* accessions.

Group	Day	Weight (g)	Creatinine (mg/L)	Urea (g/L)	AST (U/L)	ALT (U/L)
Control	D0	142 ± 9	6.2 ± 0.6	0.56 ± 0.03	7.89 ± 0.95	2.61 ± 1.06
	D14	147 ± 8	10.1 ± 1.6	0.56 ± 0.04	6.07 ± 2.05	1.29 ± 0.50
	P	0.37	0.18	0.94	0.35	0.18
	P†	—	—	—	—	—
UV2	D0	121 ± 5	4.5 ± 1.1	0.41 ± 0.11	2.65 ± 1.81	2.79 ± 0.09
	D14	115 ± 3	8.2 ± 0.8	0.47 ± 0.08	5.81 ± 3.32	9.38 ± 7.22
	P	0.47	0.12	0.23	0.56	0.46
	P†	0.83	1.00	0.99	0.73	0.31
UV7	D0	149 ± 10	5.5 ± 0.5	0.62 ± 0.04	4.60 ± 1.58	4.11 ± 1.42
	D14	128 ± 11	8.1 ± 0.5	0.47 ± 0.06	1.65 ± 0.24	1.33 ± 0.31
	P	0.38	0.08	0.28	0.24	0.17
	P†	0.18	0.92	0.59	1.00	1.00
UV9	D0	136 ± 5	6.6 ± 0.5	0.61 ± 0.10	13.50 ± 1.30	1.56 ± 1.02
	D14	135 ± 3	7.6 ± 0.6	0.59 ± 0.06	1.17 ± 0.05	1.18 ± 0.30
	P	0.79	0.22	0.75	**0.01**	0.66
	P†	0.98	0.41	1.00	0.15	1.00
UV13	D0	126 ± 6	4.6 ± 1.5	0.48 ± 0.13	18.40 ± 5.25	4.60 ± 1.61
	D14	130 ± 5	8.6 ± 0.8	0.39 ± 0.03	2.08 ± 0.07	3.03 ± 0.58

Continued

	P	**0.02**	0.10	0.50	0.09	0.45
	P†	1.00	1.00	0.90	**0.03**	1.00
UV19	D0	114 ± 6	4.8 ± 0.8	0.57 ± 0.09	5.59 ± 2.62	1.30 ± 0.39
	D14	118 ± 3	7.9 ± 0.5	0.46 ± 0.11	1.90 ± 0.31	1.60 ± 0.26
	P	0.34	0.13	0.46	0.33	0.33
	P†	1.00	0.99	0.83	0.99	0.99

Values are mean ± SEM (n = 3). P: paired Student's t-test (D0 versus end of study); P†: change from D0 compared with the control group (one-way ANOVA followed by Dunnett's test). D0, D14: days 0 and 14; AST: aspartate aminotransferase; ALT: alanine aminotransferase. Significant P values are in bold.

The mean weight of the different groups of rats on D0 ranged from 114 ± 6 g to 149 ± 10 g. On D14, the mean weight of the different groups ranged from 115 ± 3 g to 135 ± 3 g. The only statistically significant change in mean weight between D0 and D14 was a slight increase in the UV13 group (P = 0.02). However, mean body weight decreased by 14.1% in the UV7 group (from 149 ± 10 g to 128 ± 11 g) and by 5.0% in the UV2 group (from 121 ± 5 g to 115 ± 3 g), whereas it remained stable or increased slightly in the UV9 (−0.7%), UV13 (+3.2%) and UV19 (+3.5%) groups. In the control group, mean body weight increased by 3.4% (from 142 ± 9 g to 147 ± 8 g).

Serum creatinine levels ranged from 4.5 ± 1.1 mg/L to 6.6 ± 0.5 mg/L on D0 and from 7.6 ± 0.6 mg/L to 8.6 ± 0.8 mg/L on D14 in the treated groups. Serum creatinine increased between D0 and D14 in all groups, including the control group (from 6.2 ± 0.6 mg/L to 10.1 ± 1.6 mg/L), but none of these increases was statistically significant (P > 0.05; UV7, P = 0.08).

Blood urea levels ranged from 0.41 ± 0.11 g/L to 0.62 ± 0.04 g/L on D0 and from 0.39 ± 0.03 g/L to 0.59 ± 0.06 g/L on D14 in the treated groups. These levels did not vary significantly (P > 0.05).

AST levels ranged from 2.65 ± 1.81 U/L to 18.40 ± 5.25 U/L on D0 and from 1.17 ± 0.05 U/L to 5.81 ± 3.32 U/L on D14 in the treated groups. AST was significantly lower on D14 in the UV9 group (P = 0.01).

ALT levels ranged from 1.30 ± 0.39 U/L to 4.60 ± 1.61 U/L on D0 and from 1.18 ± 0.30 U/L to 9.38 ± 7.22 U/L on D14 in the treated groups. These levels did not vary significantly (P > 0.05). The absolute AST and ALT activities measured in this study were markedly lower than those usually reported for young Wistar rats [25] [26]; their interpretation is discussed in Section 4.

When the changes from D0 were compared with those of the control group (P† in **Table 2**), only the decrease in AST in the UV13 group differed significantly (ANOVA, P = 0.01; Dunnett's test, P = 0.03); this group had the highest AST value on D0 (18.40 ± 5.25 U/L). No other biochemical parameter or body weight change differed significantly from the control group.

Table 3 presents the hematological variations in rats in the acute oral toxicity test of *Uvaria chamae* accessions.

Table 3. Hematological variations in rats in the acute oral toxicity test of *Uvaria chamae* accessions.

Group	Day	White blood cells (G/L)	Hemoglobin (g/dL)	Platelets (G/L)
Control	D0	8.63 ± 0.34	13.2 ± 0.3	607 ± 63
	D14	6.46 ± 1.13	14.8 ± 0.3	530 ± 155
	P	0.18	0.07	0.53
	P†	—	—	—
UV2	D0	10.63 ± 1.40	12.5 ± 0.2	640 ± 101
	D14	9.98 ± 0.50	13.3 ± 1.4	598 ± 76
	P	0.67	0.57	0.83
	P†	0.92	0.85	1.00
UV7	D0	9.47 ± 1.11	13.8 ± 0.4	607 ± 101
	D14	8.11 ± 0.70	14.5 ± 0.3	624 ± 50
	P	0.52	0.26	0.92
	P†	0.99	0.80	0.97
UV9	D0	10.47 ± 0.56	13.6 ± 0.2	592 ± 77
	D14	8.88 ± 1.23	15.2 ± 0.3	586 ± 38
	P	0.16	**0.02**	0.96
	P†	1.00	1.00	0.99
UV13	D0	9.53 ± 1.21	13.6 ± 0.2	630 ± 41
	D14	7.29 ± 0.70	15.6 ± 0.3	464 ± 46
	P	0.33	**0.04**	0.20
	P†	1.00	1.00	0.98
UV19	D0	6.20 ± 0.50	14.5 ± 0.5	453 ± 22
	D14	6.95 ± 1.53	15.1 ± 0.8	419 ± 68
	P	0.74	0.64	0.54
	P†	0.53	0.74	1.00

Values are mean ± SEM (n = 3). P: paired Student's t-test (D0 versus end of study); P†: change from D0 compared with the control group (one-way ANOVA followed by Dunnett's test). D0, D14: days 0 and 14. Significant P values are in bold.

The mean leukocyte count ranged from 6.20 ± 0.50 G/L to 10.63 ± 1.40 G/L on D0 and from 6.95 ± 1.53 G/L to 9.98 ± 0.50 G/L on D14 in the treated groups. The mean leukocyte count did not vary significantly ($P > 0.05$).

In the groups of tested rats, the mean hemoglobin level varied from 12.5 ± 0.2

g/dL to 14.5 ± 0.5 g/dL on D0 and from 13.3 ± 1.4 g/dL to 15.6 ± 0.3 g/dL on D14. The mean hemoglobin level increased significantly in the UV9 (P = 0.02) and UV13 (P = 0.04) groups; it also increased in the control group, without reaching significance (from 13.2 ± 0.3 g/dL to 14.8 ± 0.3 g/dL, P = 0.07).

The mean platelet count ranged from 453 ± 22 G/L to 640 ± 101 G/L on D0 and from 419 ± 68 G/L to 624 ± 50 G/L on D14 in the treated groups. The mean platelet count did not vary significantly (P > 0.05).

None of the hematological changes differed significantly from those of the control group ($P^{\dagger} > 0.05$; Table 3), including the hemoglobin increases with UV9 and UV13, which were of similar magnitude to that of the control group.

3.2. Subchronic Oral Toxicity (SCT)

Daily oral administration of 200 mg/kg of the aqueous extracts of the various accessions of *Uvaria chamae* for 28 days resulted in no deaths during the experiment. No clinical signs of toxicity, including agitation, aggression, convulsions, diarrhea, asthenia, drowsiness, anorexia or decreased mobility, were observed; body weight changes are described below.

Table 4 shows the body weight, liver and kidney function of the rats in the subchronic oral toxicity test of *Uvaria chamae* accessions.

Table 4. Variation in body weight, liver and kidney function in rats in the subchronic oral toxicity test of *Uvaria chamae* accessions.

Group	Day	Weight (g)	Creatinine (mg/L)	Urea (g/L)	AST (U/L)	ALT (U/L)
Control	D0	131 ± 1	4.1 ± 1.6	0.45 ± 0.12	10.91 ± 5.75	4.08 ± 1.18
	D28	140 ± 5	4.8 ± 0.7	0.54 ± 0.04	12.48 ± 1.91	5.76 ± 1.68
	P	0.11	0.78	0.56	0.86	0.08
	$P^{\dagger}$	—	—	—	—	—
UV2	D0	112 ± 8	6.3 ± 0.5	0.76 ± 0.09	5.63 ± 1.74	1.33 ± 0.19
	D28	115 ± 3	5.1 ± 1.5	0.57 ± 0.03	14.86 ± 1.56	7.88 ± 1.33
	P	0.78	0.39	0.18	**0.03**	**0.03**
	$P^{\dagger}$	0.96	0.85	0.24	0.64	0.13
UV7	D0	119 ± 12	5.7 ± 0.9	0.62 ± 0.01	5.65 ± 0.91	1.63 ± 0.01
	D28	110 ± 13	6.9 ± 0.6	0.52 ± 0.03	18.24 ± 0.66	11.81 ± 0.37
	P	**0.03**	0.26	0.12	**0.006**	**0.001**
	$P^{\dagger}$	0.31	1.00	0.54	0.34	**0.006**
UV9	D0	128 ± 10	4.1 ± 1.4	0.61 ± 0.04	13.17 ± 8.59	4.32 ± 2.84
	D28	131 ± 5	7.5 ± 1.0	0.41 ± 0.01	15.33 ± 1.32	3.82 ± 0.66
	P	0.86	0.29	**0.03**	0.80	0.85
	$P^{\dagger}$	0.95	0.59	0.22	1.00	0.74

Continued

UV13	D0	134 ± 6	4.8 ± 1.2	0.38 ± 0.05	10.58 ± 3.29	2.35 ± 0.74
	D28	129 ± 5	4.8 ± 0.8	0.47 ± 0.00	9.80 ± 4.43	6.88 ± 0.38
	P	0.17	0.99	0.21	0.68	0.051
	P†	0.51	1.00	1.00	0.99	0.54
UV19	D0	143 ± 3	4.9 ± 1.2	0.60 ± 0.19	5.34 ± 1.73	1.18 ± 0.69
	D28	132 ± 8	5.0 ± 0.1	0.52 ± 0.03	15.36 ± 1.74	7.13 ± 1.50
	P	0.26	0.93	0.70	**0.02**	0.11
	P†	0.24	1.00	0.67	0.56	0.21

Values are mean ± SEM (n = 3). P: paired Student's t-test (D0 versus end of study); P†: change from D0 compared with the control group (one-way ANOVA followed by Dunnett's test). D0, D28: days 0 and 28; AST: aspartate aminotransferase; ALT: alanine aminotransferase. Significant P values are in bold.

The mean weight of the rats varied from 112 ± 8 g to 143 ± 3 g on D0 and from 110 ± 13 g to 132 ± 8 g on D28. Mean body weight decreased significantly by 8.1% in the UV7 group (P = 0.03; from 119 ± 12 g to 110 ± 13 g) and, without reaching significance, by 7.9% in the UV19 group (from 143 ± 3 g to 132 ± 8 g) and by 4.2% in the UV13 group, whereas it increased slightly in the UV2 (+2.7%) and UV9 (+2.1%) groups. In the control group, mean body weight increased by 7.1% (from 131 ± 1 g to 140 ± 5 g).

Serum creatinine levels ranged from 4.1 ± 1.4 mg/L to 6.3 ± 0.5 mg/L on D0 and from 4.8 ± 0.8 mg/L to 7.5 ± 1.0 mg/L on D28. Serum creatinine levels did not change significantly on D28 in the treated groups. Uremia varied from 0.38 ± 0.05 g/L to 0.76 ± 0.09 g/L on D0 and from 0.41 ± 0.01 g/L to 0.57 ± 0.03 g/L on D28. Uremia significantly decreased on D28 in the UV9 group (P = 0.03) and did not significantly vary in the other groups (P > 0.05). No significant increase in urea or creatinine was detected on D28 in any group.

AST levels ranged from 5.34 ± 1.73 U/L to 13.17 ± 8.59 U/L on D0 and from 9.80 ± 4.43 U/L to 18.24 ± 0.66 U/L on D28 in the treated groups. AST significantly increased on D28 in the UV2 (P = 0.03), UV7 (P = 0.006) and UV19 (P = 0.02) groups, corresponding to 2.6- to 3.2-fold increases in mean values. ALT ranged from 1.18 ± 0.69 U/L to 4.32 ± 2.84 U/L on D0 and from 3.82 ± 0.66 U/L to 11.81 ± 0.37 U/L on D28 in the treated groups. ALT significantly increased on D28 in the UV2 (P = 0.03) and UV7 (P = 0.001) groups, corresponding to 5.9- and 7.3-fold increases in mean values; ALT also increased in the UV13 (2.9-fold, P = 0.051) and UV19 (6.0-fold, P = 0.11) groups, without reaching significance. When the changes were compared with those of the control group (P† in Table 4), the increase in ALT was significant in the UV7 group (ANOVA, P = 0.004; Dunnett's test, P = 0.006), whereas the changes in AST, body weight, urea and creatinine did not differ significantly from the control group. The biological relevance of these increases is discussed in Section 4.

Table 5 presents the hematological profile of rats in the subchronic oral toxicity test of *Uvaria chamae* accessions.

Table 5. Hematological assessment of rats in the subchronic oral toxicity test of *Uvaria chamae* accessions.

Group	Day	White blood cells (G/L)	Hemoglobin (g/dL)	Platelets (G/L)
Control	D0	5.77 ± 1.66	12.7 ± 1.7	483 ± 41
	D28	5.00 ± 0.51	13.5 ± 1.2	431 ± 43
	P	0.59	0.26	0.28
	P†	—	—	—
UV2	D0	4.67 ± 0.12	14.0 ± 0.7	402 ± 6
	D28	4.30 ± 0.15	15.0 ± 0.2	501 ± 15
	P	0.053	0.15	**0.04**
	P†	0.99	1.00	0.24
UV7	D0	8.13 ± 1.20	15.4 ± 0.6	422 ± 33
	D28	6.73 ± 0.26	15.1 ± 0.1	484 ± 3
	P	0.31	0.58	0.22
	P†	0.94	0.58	0.47
UV9	D0	6.17 ± 0.22	14.5 ± 1.2	455 ± 86
	D28	5.63 ± 0.09	14.0 ± 0.7	450 ± 21
	P	0.06	0.57	0.97
	P†	1.00	0.42	0.95
UV13	D0	4.27 ± 0.41	13.7 ± 1.7	397 ± 85
	D28	4.85 ± 0.14	13.8 ± 1.2	437 ± 40
	P	0.16	0.90	0.59
	P†	0.50	0.90	0.65
UV19	D0	4.80 ± 0.29	15.4 ± 0.1	460 ± 14
	D28	4.83 ± 0.20	15.8 ± 0.2	507 ± 7
	P	0.90	0.07	0.13
	P†	0.86	0.98	0.59

Values are mean ± SEM (n = 3). P: paired Student's t-test (D0 versus end of study); P†: change from D0 compared with the control group (one-way ANOVA followed by Dunnett's test). D0, D28: days 0 and 28. Significant P values are in bold.

The mean leukocyte count ranged from 4.27 ± 0.41 G/L to 8.13 ± 1.20 G/L on D0 and from 4.30 ± 0.15 G/L to 6.73 ± 0.26 G/L on D28. It did not change significantly on D28 ($P > 0.05$).

Hemoglobin levels ranged from 13.7 ± 1.7 g/dL to 15.4 ± 0.1 g/dL on D0 and

from 13.8 ± 1.2 g/dL to 15.8 ± 0.2 g/dL on D28 in the treated groups. There was no significant change on D28 ($P > 0.05$).

The mean blood platelet count varied from 397 ± 85 G/L to 460 ± 14 G/L on D0 and from 437 ± 40 G/L to 507 ± 7 G/L on D28. It increased significantly on D28 in the UV2 group ($P = 0.04$).

Apart from this platelet increase, no significant change in hematological parameters was observed in the subchronic oral toxicity test, and none of the hematological changes differed significantly from those of the control group ($P^{\dagger} > 0.05$; Table 5).

3.3. Histological Study of the Liver and Kidneys

3.3.1. Liver Histology: AOT

Figure 1 shows the liver histology in the acute oral toxicity test.

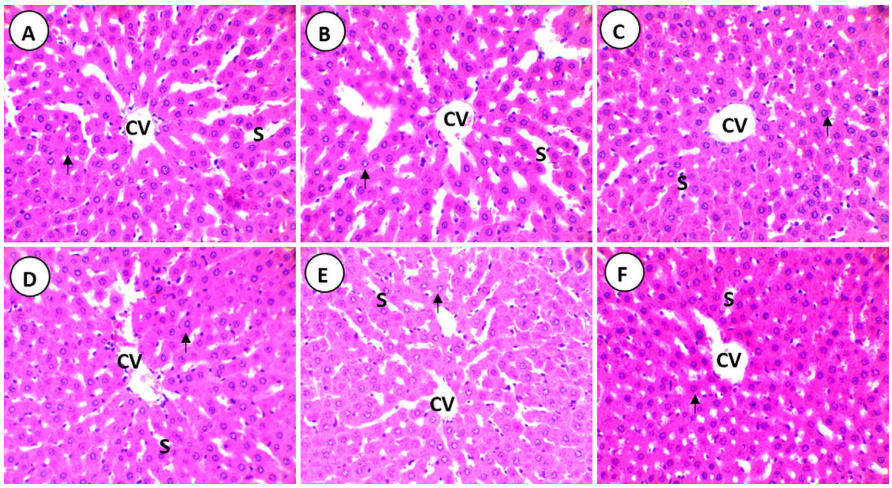


Figure 1. Liver histology in the acute oral toxicity test (H&E, 400×). (A) Control; (B) UV2; (C) UV7; (D) UV9; (E) UV13; (F) UV19. CV: centrilobular vein; S: sinusoids; arrow: hepatocyte cords.

In the acute oral toxicity test, the liver parenchyma of rats treated with root extracts from the different accessions of *Uvaria chamae*, UV2 (Figure 1(B)), UV7 (Figure 1(C)), UV9 (Figure 1(D)), UV13 (Figure 1(E)) and UV19 (Figure 1(F)), did not show any visible atypia. The centrilobular veins (CV), sinusoids (S) and hepatocyte cords (arrow) retained the characteristic appearance observed in control rats (Figure 1(A)).

3.3.2. Kidney Histology: AOT

Figure 2 shows the renal histology in the acute oral toxicity test.

In the acute oral toxicity test, the renal parenchyma of rats treated with root extracts from the different accessions of *Uvaria chamae*, UV2 (Figure 2(B)), UV7 (Figure 2(C)), UV9 (Figure 2(D)), UV13 (Figure 2(E)) and UV19 (Figure 2(F)), showed no visible atypia. The renal glomeruli (G) and renal tubules (T) retained their characteristic appearance, as observed in control rats (Figure 2(A)).

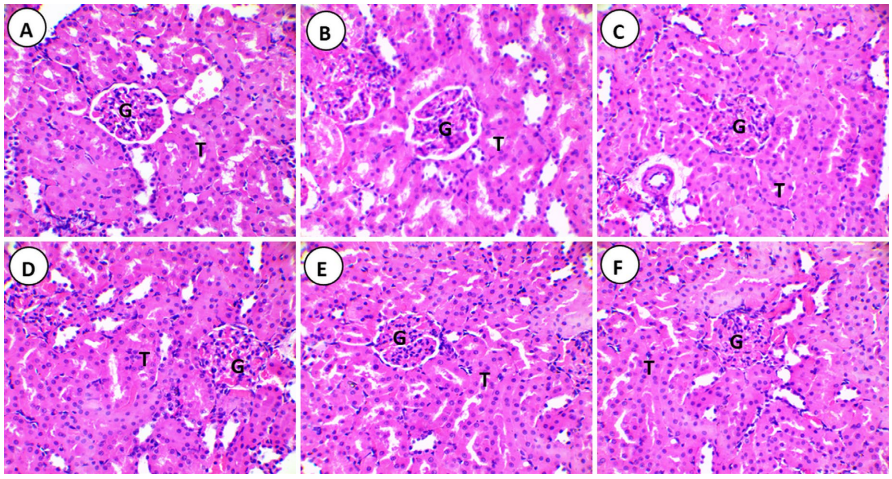


Figure 2. Renal histology in the acute oral toxicity test (H&E, 400×). (A) Control; (B) UV2; (C) UV7; (D) UV9; (E) UV13; (F) UV19. G: glomerulus; T: renal tubules.

3.3.3. Liver Histology: SCT

Figure 3 shows the liver histology in the subchronic oral toxicity test.

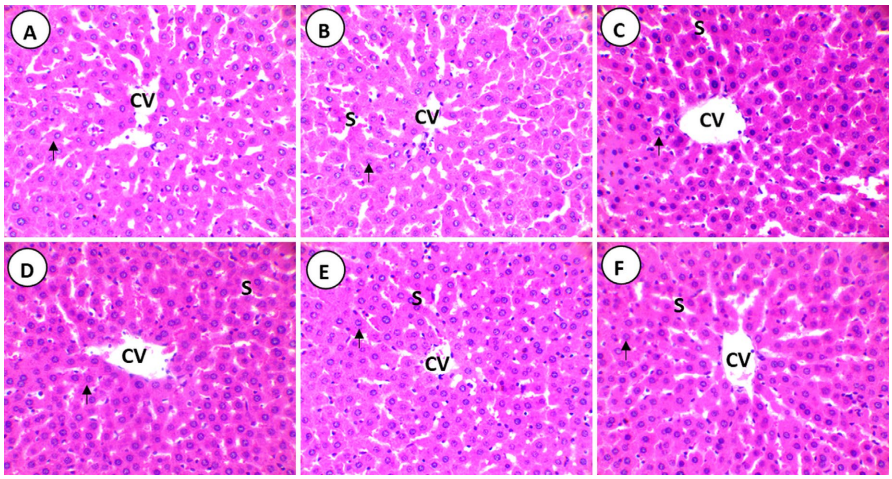


Figure 3. Liver histology in the subchronic oral toxicity test (H&E, 400×). (A) Control; (B) UV2; (C) UV7; (D) UV9; (E) UV13; (F) UV19. CV: centrilobular vein; S: sinusoids; arrow: hepatocyte cords.

In the subchronic oral toxicity test, the liver parenchyma of rats treated with root extracts from the different accessions of *Uvaria chamae*, UV2 (**Figure 3(B)**), UV7 (**Figure 3(C)**), UV9 (**Figure 3(D)**), UV13 (**Figure 3(E)**) and UV19 (**Figure 3(F)**), showed no visible atypia. The centrilobular veins (CV), sinusoids (S) and hepatocyte cords (arrow) retained the characteristic appearance observed in control rats (**Figure 3(A)**).

3.3.4. Kidney Histology: SCT

Figure 4 shows the kidney histology in the subchronic oral toxicity test.

In the subchronic oral toxicity test, the renal parenchyma of rats treated with root extracts from the different accessions of *Uvaria chamae*, UV2 (**Figure 4(B)**),

UV7 (Figure 4(C)), UV9 (Figure 4(D)), UV13 (Figure 4(E)) and UV19 (Figure 4(F)), showed no visible atypia. The renal glomeruli (G) and renal tubules (T) retained their characteristic appearance, as observed in control rats (Figure 4(A)).

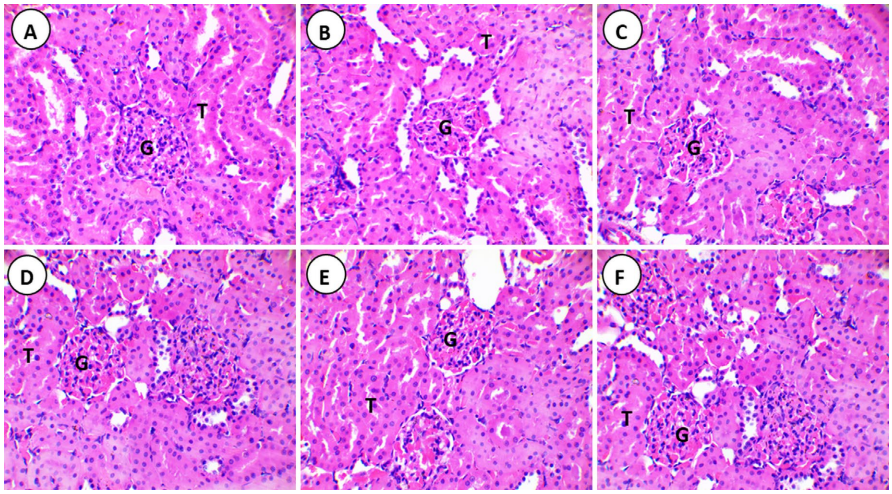


Figure 4. Renal histology in the subchronic oral toxicity test (H&E, 400×). (A) Control; (B) UV2; (C) UV7; (D) UV9; (E) UV13; (F) UV19. G: glomerulus; T: renal tubules.

4. Discussion

This study aimed to evaluate the biological safety of aqueous extracts of five accessions of *Uvaria chamae* (Annonaceae), a medicinal plant whose roots are traditionally used in Benin for the management of sickle cell disease.

In the acute oral toxicity study, oral administration of the aqueous extract of each accession of *Uvaria chamae* at a single dose of 2000 mg/kg did not cause death in rats. During the experimental period, there were no signs of toxicity such as agitation, aggression, body twisting, convulsions, diarrhea, asthenia, drowsiness, anorexia or decreased mobility. We also noted no significant difference in urea levels, ALT and platelet count between D0 and D14 ($P > 0.05$). The absence of mortality at 2000 mg/kg is consistent with acute oral toxicity studies conducted in Nigeria on aqueous root [27] and leaf [28] extracts of *Uvaria chamae*. Serum creatinine increased in all five treated groups between D0 and D14, although not significantly. Because this rise was observed with all accessions, factors unrelated to treatment, such as growth or analytical variation between runs, are likely to contribute. The control group showed a rise of similar magnitude (from 6.2 ± 0.6 mg/L to 10.1 ± 1.6 mg/L), which suggests that this change is not attributable to the extracts. Renal histology showed no visible lesions and blood urea did not increase; together, these findings do not indicate overt acute renal injury, although a subtle functional effect cannot be ruled out. The AST level significantly decreased on D14 in the group of rats treated with the UV9 extract ($P = 0.01$). Compared with the control group, however, this decrease did not differ significantly (Dunnett's test, $P = 0.15$), and the decrease in AST was significant only with UV13 ($P = 0.03$), a group with a high baseline value. A similar, though not statistically

significant, decrease in AST was observed with UV7 and UV19, and ALT decreased in most groups, including the control group. The decrease in serum levels of these two markers, combined with normal liver parenchyma on histology, does not suggest hepatic cytolysis. However, it cannot be interpreted as a hepatoprotective effect, the demonstration of which would require an induced hepatotoxicity model such as carbon tetrachloride intoxication, used by Omoruyi *et al.* [29] to demonstrate the hepatoprotective effect of the aqueous extract of *Uvaria afzelii* root in the Wistar rat. In contrast, Legba *et al.* [30], in Benin, reported significant increases in AST and ALT after acute oral administration of *Uvaria chamae* root extracts, without histological abnormalities, and recommended using doses below 2000 mg/kg. Olumese *et al.* [27] reported no major atypia in the histological examinations of rat livers, apart from slight vascular congestion.

The mean hemoglobin level increased significantly in rats treated with extracts from accessions UV9 and UV13 ($P = 0.02$ and $P = 0.04$). Furthermore, a non-significant increase was observed in the remaining groups (UV2, UV7 and UV19). This observation could be compatible with stimulation of erythropoiesis by these accessions, but other explanations remain plausible, including transient hemoconcentration after administration of a single high dose or a physiological increase related to growth; differentiating between these would require reticulocyte counts and erythrocyte indices, which were not measured. The increase in hemoglobin also observed in the control group ($P = 0.07$) favors a physiological increase related to growth rather than an effect of the extracts. Increases in hemoglobin have also been reported in two studies conducted in Nigeria with root and leaf extracts of the plant [27] [28].

The body weight decreases observed with UV7 in the acute test (−14.1%) and with UV7 and UV19 in the subchronic test (−8.1% and −7.9%) deserve attention; the decrease with UV7 in the subchronic test was statistically significant ($P = 0.03$). The rats used were young and still growing, so that a stable or decreasing body weight over 14 to 28 days represents a deviation from the expected growth trajectory; by comparison, mean body weight of control rats increased by 3.4% over 14 days and by 7.1% over 28 days. A decrease of this magnitude may reflect reduced food intake, reduced palatability of the extract, gastrointestinal discomfort or a systemic effect; food consumption was not measured, which prevents these possibilities from being distinguished. With UV7, the weight decrease coincided with the largest transaminase increases in the subchronic test, including the only ALT increase that differed significantly from the control group, so a treatment-related effect of this accession cannot be excluded. These observations temper the conclusion of good tolerability for UV7 and, to a lesser extent, for UV19, and justify including food intake and body-weight gain relative to controls as endpoints in future studies.

For the study of subchronic oral toxicity, oral administration of the aqueous extract of each of the five *Uvaria chamae* accessions at a dose of 200 mg/kg per day for 28 consecutive days induced a significant decrease in body weight only with UV7, and a non-significant decrease with UV19, as discussed above. For the

evaluation of renal toxicity, serum creatinine did not significantly increase between D0 and D28 ($P > 0.05$). Blood urea levels did not increase on D28 in the treated rat groups and, conversely, decreased significantly in the group receiving the UV9 extract ($P = 0.03$). These blood urea and creatinine results do not suggest renal toxicity, and the normal histology of the renal parenchyma observed in the subchronic oral toxicity test is consistent with this interpretation. Comparable observations have been reported with other aqueous extracts of medicinal plants administered orally for 28 days in rats. Tcheutchoua *et al.* [31] noted no significant effect on serum creatinine or uremia at doses of 200 to 800 mg/kg, with serum creatinine even decreasing significantly in males at doses of 200 and 400 mg/kg, without any link to hepatic, renal, pulmonary or splenic damage on histology. In contrast, Dossou-Agoin *et al.* [32], applying OECD Test Guideline 407 in male rats to aqueous extracts of *Pedalium murex* at the same dose levels in Benin, observed proteinuria and renal damage indicating potential nephrotoxicity, which shows that the absence of renal effects cannot be generalized from one plant extract to another. An isolated decrease in blood urea or creatinine levels, not accompanied by renal histological lesions, is generally not considered harmful.

Addressing the liver toxicity of *Uvaria chamae* extracts, the groups treated with extracts from accessions UV2 and UV7 showed a significant increase in AST and ALT ($P < 0.05$), and the UV19 group a significant increase in AST ($P = 0.02$); ALT also increased with UV13 and UV19, without reaching significance. In interpreting these changes, it should be noted that the absolute AST and ALT activities measured in this study (group means of about 1 to 18 U/L) were markedly lower than those generally reported for young Wistar rats with standard methods [25] [26], for example 61 to 153 U/L for AST and 23 to 67 U/L for ALT in female Wistar rats [26]. Such a systematic difference suggests an analytical offset related to the assay method, calibration or sample handling. Consequently, these values cannot be meaningfully compared with published physiological limits. The control rats also showed low activities (**Table 2** and **Table 4**), which is consistent with an analytical offset affecting all samples rather than an effect of treatment. The changes must therefore be assessed against the concurrent control group. On this basis, the AST increases observed after 28 days largely reflect low baseline values in the treated groups: on D28, AST in the treated groups (9.80 to 18.24 U/L) was close to that of the control group (12.48 U/L), and none of the AST changes differed significantly from the control (Dunnett's test, $P \geq 0.34$). On D28, ALT was 0.66 to 1.37 times the control value in the UV2, UV9, UV13 and UV19 groups, but 2.05 times the control value in the UV7 group. Because an analytical offset may be additive, fold changes calculated from very low baseline values should not be overinterpreted, and the ALT increase with UV7, ALT being the more liver-specific enzyme in the rat [33], is the most robust signal of possible hepatocellular stress. The adversity of such changes should be judged by integrating clinical pathology with histopathology [34]-[36]. In the subchronic test, only the ALT increase with UV7 differed significantly from the control group (Dunnett's test, $P = 0.006$), which

identifies UV7 as the accession of greatest concern; given the low power of a design with three animals per group, the absence of significance for the other accessions does not exclude an effect. These results are consistent with those of Olumese *et al.* [9], who observed a decrease in urea levels and a significant increase in AST and ALT. The same team had previously reported, with the aqueous root extract, a significant increase in AST at 5000 mg/kg and in ALT at 2000 mg/kg [27]. In contrast, Tcheutchoua *et al.* [31] observed no significant variation in mean AST and ALT levels after 28 days of oral administration of an aqueous plant extract in rats, illustrating the variability of enzymatic responses depending on the accession and plant species considered.

In histology, the liver parenchyma of rats treated with extracts from the five accessions of *Uvaria chamae* did not show any visible atypia in the subchronic oral toxicity test. This biochemical-histological discrepancy is a well-documented phenomenon in experimental toxicology and underscores the need never to interpret an enzymatic parameter in isolation, but always in light of the overall functional and morphological profile [33] [35] [36]. The heterogeneity observed between accessions may reflect differences in phytochemical composition; however, because the extracts were not chemically characterized in this study, this explanation remains a hypothesis to be tested. Moreover, the toxicological profile of *Uvaria chamae* also depends on the organ sampled and the extraction solvent, as illustrated by the work carried out separately on the aqueous root extract [27], the ethanolic root extract [9] and the n-hexane fraction of leaves [37]. Comparisons between studies must therefore take these parameters into account before any generalizations are made. These results also call for caution in interpreting the data. Indeed, while the traditional use of *Uvaria chamae* to relieve sickle cell crises appears to present a low risk in view of the results of the acute toxicity test, repeated or prolonged administration, as might be envisaged in a long-term prevention strategy for sickle cell disease, would require monitoring of liver function. This precaution appears particularly relevant for UV7 and, to a lesser extent, for UV2 and UV19, which showed increases in liver enzymes relative to baseline.

Regarding hematological parameters, the number of leukocytes did not significantly vary on D28 in the different groups of rats compared to D0. However, total leukocyte counts alone do not assess immune function, and no conclusion on immunotoxicity can be drawn from this result. This result corroborates those obtained by Tcheutchoua *et al.* [31], who noted no significant variation in leukocyte count after 28 days of oral administration of an aqueous extract in rats.

Hemoglobin levels did not significantly change in the treated groups on D28, and no evidence of an effect on the erythrocyte lineage was detected. In the thrombocyte lineage, only the UV2 extract significantly increased the platelet count on D28 ($P = 0.04$). A significant increase in platelet count has also been reported in rats treated with *Uvaria chamae* root extract at a dose of 500 mg/kg [9]. This concordance could point to an effect of the species on platelet counts rather than a peculiarity of the UV2 accession; this remains speculative, since megakaryopoiesis

and bone marrow were not examined.

In summary, no deaths, overt clinical signs or histological lesions of the liver and kidneys were observed. However, body weight decreased in some groups, notably with UV7, and, relative to baseline, repeated administration increased AST with UV2, UV7 and UV19 and ALT with UV2 and UV7, although only the ALT increase with UV7 differed significantly from the control group. Lokonon *et al.* [38], who studied aqueous root extracts from a single accession of *Uvaria chamae* at the same doses in Wistar rats, also observed no deaths or histological lesions, but found no change in AST or ALT; the transaminase increases observed here with some accessions illustrate the value of testing several accessions. After 28 days, analysis of hematological parameters showed no variation in leukocyte or hemoglobin levels and an isolated increase in platelets in the group treated with the UV2 accession, a finding also reported in Nigeria by Olumese *et al.* [9]. Overall, the data do not show overt subchronic toxicity at 200 mg/kg/day, but they are not sufficient to establish the safety of prolonged use.

Limitations of the Study

This work has several limitations that should be kept in mind when interpreting the results.

First, the small number of animals per group (n = 3) results in low statistical power: non-significant differences cannot be interpreted as evidence of the absence of an effect, and isolated significant results should be interpreted with caution. In addition, the paired comparisons with baseline were not adjusted for multiple comparisons, and Dunnett's test adjusts for the comparisons with the control within each parameter but not across the many parameters examined.

Second, the subchronic toxicity trial was conducted at only one dose (200 mg/kg/day), which does not allow for establishing a dose-response relationship or determining a no-observed-adverse-effect level (NOAEL). Both studies were adaptations of OECD Test Guidelines 425 and 407 rather than fully compliant studies, and only female rats were used.

Third, food and water consumption, organ weights, reticulocyte counts and erythrocyte indices were not measured, and the extracts were not chemically characterized, so that differences between accessions cannot be linked to their composition. In addition, the low absolute transaminase activities limit comparison with published reference values, and the calibration records of the transaminase assays were not available for this revision.

Finally, the 28-day exposure period does not guarantee the safety of prolonged chronic use, and the results obtained in healthy Wistar rats are not directly applicable to sickle cell patients, whose liver and kidneys are already under stress from the disease. Chronic toxicity studies, at multiple doses and combined with phytochemical analysis of the extracts, are needed to complete this work.

5. Conclusions

The main objective of this study was to systematically and comparatively evaluate

the safety of aqueous extracts of *Uvaria chamae* roots from several representative accessions in Benin. This approach aims to bridge the gap between the pharmacological validation of the plant's anti-sickle cell activity and its safety, an essential prerequisite for any potential therapeutic use. Acute and subchronic oral toxicity tests revealed no deaths or overt clinical signs of toxicity in Wistar rats, and histological examinations showed no visible atypia in the hepatic and renal parenchyma of rats treated with root extracts from the five accessions. However, several biochemical changes were observed, in particular increases in AST (UV2, UV7 and UV19) and ALT (UV2 and UV7) relative to baseline after 28 days, of which only the ALT increase with UV7 differed significantly from the control group, together with body weight decreases with UV7 and UV19. UV7 was therefore the accession of greatest concern, as it also caused a significant decrease in body weight after 28 days.

Overall, these results provide preliminary evidence that aqueous extracts of *Uvaria chamae* roots are tolerated at the doses tested, namely 2000 mg/kg as a single dose and 200 mg/kg/day for 28 days. Given the small sample size of three animals per group, the single subchronic dose assessed, and the absence of an established dose-response relationship, these findings remain exploratory. Liver function monitoring is recommended in cases of repeated or prolonged administration, particularly for UV7.

Chronic toxicity studies, at multiple doses and coupled with phytochemical characterization of accessions, are necessary before any prospect of therapeutic development.

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Author Contributions

HWH, FA and JEL participated in the experimental work and in drafting and revising the manuscript. TDMA, RCH, GA, CN, EM, GD, PAT, AM, CA and AAEA participated in reviewing and validating the manuscript. MS supervised the entire study, controlled and revised the statistical analysis, and reviewed, corrected, validated, and translated the manuscript. All authors read and approved the final version of the manuscript.

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Data Availability

The data generated and analyzed during this study are available from the corresponding author upon reasonable request.

Use of Generative Artificial Intelligence

During the preparation of this manuscript, the authors used the Claude assistant (Anthropic) for editorial and technical assistance, including: language editing and proofreading of the English text; consolidation and formatting of the reference list; verification and correction of citations and bibliographic references; verification of internal numerical and terminological consistency throughout the manuscript; reformatting of the manuscript to comply with the submission requirements of the journal; and, during the revision, execution of the statistical calculations (descriptive statistics, paired Student's t-tests, one-way ANOVA and Dunnett's tests) on the individual raw data provided by the authors, using the SciPy library in Python, according to the analysis plan defined in response to the reviewer. The authors reviewed, verified, and approved all AI-assisted edits and calculations and take full responsibility for the accuracy and content of the publication. No artificial intelligence tool was used to generate experimental data, and no such tool is listed among the authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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