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RESEARCH ARTICLE

SAFETY OF UVARIA CHAMAE P. BEAUV ROOTS AQUEOUS EXTRACTS IN WISTAR RATS

Lokonon Jacques Ezéchiel[1], Agbogba Félicienne[1,2], Medoatinsa Espérance[1], Abissi Gloria[1], Tchogou Pascal[1], Koudoro Yaya[3], Agbangnan Pascal[3], Anago Eugénie[2], Akpovi D. Casimir[2] and Senou Maximin[1]

1. Laboratoire de Biologie Expérimentale et Clinique (LaBEC), Ecole Nationale Supérieure des Biosciences et Biotechnologie Appliquées de Dassa-Zoumé (ENSBBA), Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques d'Abomey (UNSTIM).
2. Laboratoire de Biologie Appliquée (LARBA), Ecole Polytechnique d'Abomey-Calavi (EPAC), Université d'Abomey-Calavi (UAC), R Bénin.
3. Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA); Ecole Polytechnique d'Abomey-Calavi / Université d'Abomey-Calavi (UAC), R. Bénin.

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Abstract

Uvaria chamae is used in the treatment of sickle cell crisis in Benin. The aim of this work is to determine the safety of Uvaria chamae roots aqueous extract in wistar rats. The aqueous extract was obtained by maceration. The tests for Acute Oral Toxicity (AOT) and of Sub-Chronic Oral Toxicity (SCT) respectively by forced gavage in a single dose of 2000 mg / Kg and 200 mg / Kg of body weight for 28 days were performed on female Wistar rats. The weight of the animals, serum creatinine, transaminases and the number of white blood cells were determined on day 0 and then on day 14 and day 28 respectively for AOT and SCT. Histologic analysis of the liver, kidneys and spleen was performed for both tests. There were no deaths in toxicity testing and organ histology did not show atypia. Weight, serum AST and ALT transaminases, and mean white blood cell count did not change significantly between the start and end of the experiment for both AOT and SCT. Only creatinine decreased significantly for SCT, indicating more protection of kidney function. Uvaria chamae roots did not show hepatic, renal and immune toxicity in the acute or sub-chronic state.

Introduction:-

Medicinal plants were and remain the most widely the way used, especially in rural areas, to solve human and animal health problems. There were an estimated 400,000 to 500,000 plant species on earth and more than 200,000 medicinal species were found in tropical countries of Africa. In Benin, out of the 5,000 plant species inventoried in forest ecosystems, 172 were consumed by local populations as food plants and 814 as medicinal plants. Indeed, the use of traditional medicine was widespread and was of growing health and economic importance in the country (Koudoro et al., 2018). Indeed, it was estimated that traditional medicine accounts for 80% to 90% of health care in Africa and the WHO was increasingly encouraging herbal research used in herbal medicine (WHO, 2002; Pingali et al., 2015; Agbogba et al., 2019). But there was a problem that of the safety of these different plants used in traditional medicine. Several phytochemical screenings have revealed the presence of highly toxic substances in plant extracts which could prove fatal in the short or long term (Oduola et al., 2007).

Corresponding Author:- Senou Maximin
Address:- Laboratoire de Biologie Expérimentale et Clinique (LaBEC), Ecole Nationale Supérieure des Biosciences et Biotechnologie Appliquées de Dassa-Zoumé (ENSBBA), Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques d'Abomey (UNSTIM).

In addition, studies have found that plant extracts used to cure certain ailments could be responsible for several adverse effects and even cause damage to various organs including the liver, kidneys and spleen (Mapanga and Musabayane, 2010). It was therefore urgent to verify the toxicity of plant extracts for safe use for the treatment of diseases (Haq, 2004; Philomena, 2011; Nasri, 2013). Uveriachamae was one of the herbs from the Beninese pharmacopoeia used in the treatment of sickle cell anemia. The aim of this work was to evaluate the toxicity of aqueous extracts of the roots of Uvaria chamae P. Beauv on rats of wistar strains.

Materials and methods:

Plant material and aqueous extraction:

The roots of Uvaria chamae were collected in Hêvié in the commune of Abomey-Calavi in Benin. The identification and certification of the plant were made at the National Herbarium of the University of Abomey-Calavi on number YH268 / HNB. The plant was dried at laboratory temperature (20 °C - 25 °C) out of direct sunlight and moisture for three weeks. They were then powdered and stored in black sachets (Koudoro et al., 2018; Tchogou et al., 2021). The technique used to prepare the extracts was that of maceration. After filtration, the extracts were evaporated to dryness at 60 °C using a Heidolph type rotary evaporator (Koudoro et al., 2018 ;Tchogou et al., 2021).

Animal material:

The animal material was composed of nine (9) strain female Wistar albino rats of approximately three months old from the animal house of Institute of Applied Biomedical Sciences whose mean weight is from 125 to 155 g. These rats were acclimatized to ambient conditions in the animal house of the laboratory of the National School of Applied Biosciences and Biotechnologies in Benin. They had access to water and food. They were lit for 12 hours a day and have been put in spacious cages. The cage was cleaned regularly and the water was renewed very often. The behavior of the animals was observed during the two weeks of acclimatization (Tchogou et al., 2021).

Acute oral toxicity:

An acute toxicity test (AOT) was performed as recommended by the Organization for Economic Co-operation and Development guideline 423 for the testing of chemicals (OECD, 2002). Two groups of rats were formed, namely the control group and the test group. Each group consists of three female wistar rats. Each animal in the control group received by force gavage and in a single dose of distilled water and the animals in the test group received by force gavage and in a single dose 2000 mg / kg body weight of the aqueous extract of Uvaria chamae. Animals were observed carefully for four hours and then daily for 14 days. They were weighed and the blood was collected by orbital puncture at the start of the experiment and then after 14 days (Agbogba et al., 2019 ;Tchogou et al., 2021).

Sub-chronic oral toxicity:

The test group for sub-chronic oral toxicity (TSC) consisted of three Wistar rats which received by force gavage the aqueous extract of Uvaria chamae at 200 mg / kg body weight, daily for 28 consecutive days (Biswas et al., 2010). They were weighed and blood was collected by orbital puncture at the start of the experiment and then after 28 days (Agbogba et al., 2019; Tchogou et al., 2021).

Blood tests:

The following blood tests were performed. Serum creatinine for the exploration of kidney function. AST and ALT transaminases were assayed for hepatic function. The leukocyte count in the blood aimed to analyze immune function (Agbogba et al., 2019; Tchogou et al., 2021).

Histopathological analysis:

At the end of the experiment, the animals were dissected. The liver, the kidney and the spleen were removed, fixed in 10% buffered formalin, and embedded in paraffin. The specimen's sections (5 µm) were mounted on glass slides, deparaffinated, and hydrated. For histological analysis, sections were stained with hematoxylin and eosin (H&E), following a standard protocol (Sènou et al., 2010). The pictures were taken at 400X magnification.

Statistical analysis:

To assess the biological effect of the extract, Mann Whitney test was used. The significance level was set at 5%. The graphs were drawn using Graphpad software.

Results and discussion:-

In both acute and sub-chronic oral toxicity tests, no dead animals were recorded.

The aqueous root extracts of Uvaria chamae did not exhibit acute oral toxicity:

Table 1 compared the weight of the animals, the serum levels of creatinine, transaminases (ASAT and ALAT) and the number of leukocytes on the day 0 (D0) to the day 14 (D14) in rats during acute oral toxicity.

The mean weight of the rats treated with 2000 mg of extract / Kg was 136 ± 8.69 g on D0 and 140 ± 9.84 g on D14. The mean weight did not change significantly with treatment.

The mean serum creatinine levels of the rats treated with 2000 mg of extract / Kg was 13.6 ± 1.25 mg / mL on D0 and 14.0 ± 1.29 mg / mL on D14. The mean creatinine levels did not change significantly with treatment, indicating no deterioration in kidney function.

The mean AST levels of the rats treated with 2000 mg of extract / Kg was 160 ± 15.4 U / L on D0 and 182 ± 13.9 U / L on D14. The mean ASAT levels did not vary significantly with treatment, suggesting an absence of cytolysis.

Table 1:- Physical, biochemical and immune assessments in the Acute Oral Toxicity test.

Parameters	Means at J0	Means at J14	P-value	Difference
Body weight (g)	136 ± 8.69	140 ± 9.84	0.8	No significant
Creatinine (mg / L)	13.6 ± 1.25	14.0 ± 1.29	0.8	No significant
Transaminase AST (U/L)	160 ± 15.4	182 ± 13.9	0.3	No significant
Transaminase ALT (U/L)	72.3 ± 6.49	61.0 ± 7.00	0.3	No significant
White blood cells (G / L)	8.90 ± 1.05	7.22 ± 0.897	0.3	No significant

The aqueous root extracts of Uvaria chamae did not exhibit sub-chronic oral toxicity

Table 2 compared the weight of the animals, the serum levels of creatinine, transaminases (ASAT and ALAT) and the number of leukocytes on the day 0 (D0) to the day 14 (D14) in rats during sub-chronic toxicity test.

The mean weight of the rats treated with 200 mg of extract / Kg / day was 149 ± 6.81 g on D0 and 166 ± 6.64 g on D28. The mean weight did not change significantly with treatment.

The mean serum creatinine levels of the rats treated with 200 mg of extract / Kg / day was 12.2 ± 0.617 mg / mL and 9.80 ± 0.458 mg / mL on D28. The mean creatinine levels decreased significantly with treatment ($P < 0.05$), indicating improvement in renal function.

The mean serum AST levels of the rats treated with 200 mg of extract / Kg / day was 143 ± 13.0 U / L on D0 and 150 ± 11.3 U / L on D28. The mean ASAT levels did not vary significantly with treatment, suggesting an absence of cytolysis.

The mean ALT levels of the rats treated with 200 mg of extract / Kg / day was 106 ± 7.45 U / L on D0 and 100 ± 10.0 U / L on D28. The mean ALT levels did not vary significantly with treatment, suggesting an absence of hepatic cytolysis.

The mean number of white blood cells in rats treated with 200 mg of extract / Kg / day was 11.3 ± 1.08 G / L on D0 and 9.90 ± 1.21 G / L on D28. The mean white blood cell count did not change significantly with treatment, suggesting no immunity disturbance.

Table 2:- Physical, biochemical and immune assessments for the Sub Chronic Oral Toxicity test.

Parameters	Means at J0	Means at J28	P-value	Différence
Body weight (g)	149 ± 6.81	166 ± 6.64	0.15	No significant
Creatinine (mg / L)	12.2 ± 0.617	9.80 ± 0.458	**0.03**	**Significant**
Transaminase AST (U/L)	143 ± 13.0	150 ± 11.3	0.7	No significant
Transaminase ALT (U/L)	106± 7.45	100 ± 10.0	0.7	No significant
White blood cells (G / L)	11.3± 1.08	9.90 ± 1.21	0.4	No significant

The Aqueous Extract of Uvaria chamae did not Alter the Hepatic, Renal and Splenic Parenchyma in the Acute or Sub-chronic Tests.

In acute (Figure 1B) and sub-chronic (Figure 1C) oral toxicity tests, the liver of rats fed with aqueous extract of Uvaria chamae did not show any visible atypia. Hepatocytes normal appearance are neatly arranged in radial cords around the central vein. The venous sinusoids were clearly visible as observed in the control rats (Figure 1A).

In the acute oral toxicity tests (Figure 2B) and sub-chronic (Figure 2C), the renal parenchyma of the rats fed with the aqueous extract of Uvaria chamae kept its typical appearance as observed in the control rats (Figure 2A). The glomeruli, proximal and distal tubes as well as collecting ducts did not exhibit any visible atypia.

In the acute oral toxicity tests (figure 3B) and sub-chronic (figure 3C), the splenic architecture of the rats force-fed with the aqueous extract of Uvaria chamae was not modified and was normal as in the control rats (Figure 3A). The central arteries, the periarteriolar sleeves and the germinal centers of the white pulp appeared typical. It was the same for the venous sinusoids and the Billroth cords of the red pulp which have kept the typical architecture.

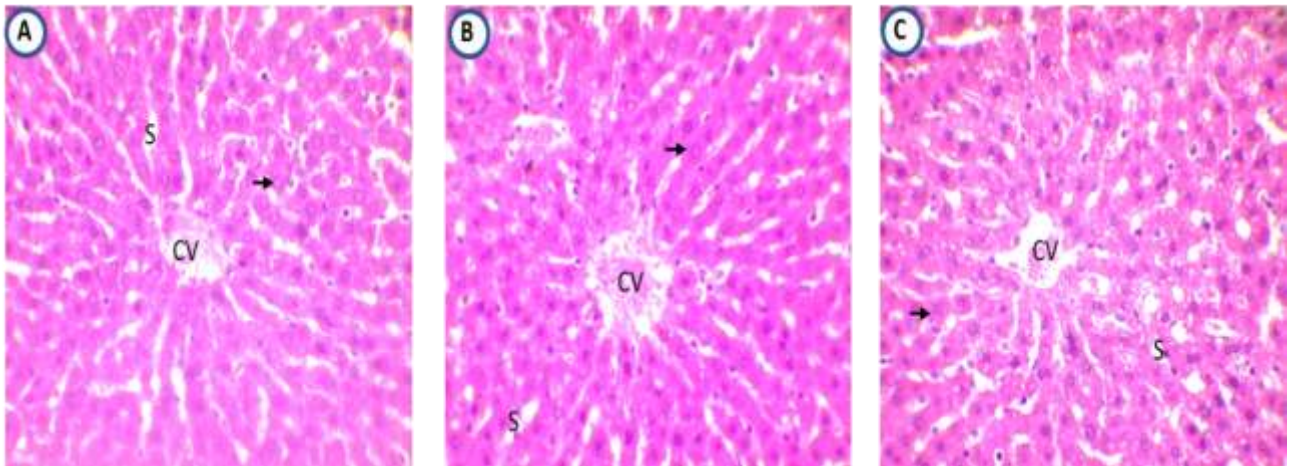

Figure 1:- Liver histology in acute and sub-chronic oral toxicity tests of the roots aqueous extract of Uvaria chamae (magnification 400X).
Figure 1A:- control rats; Figure 1B: acute toxicity test; Figure 1C: sub-chronic toxicity test S: venous sinusoids; CV: central vein; Arrows: Hepatocytes.

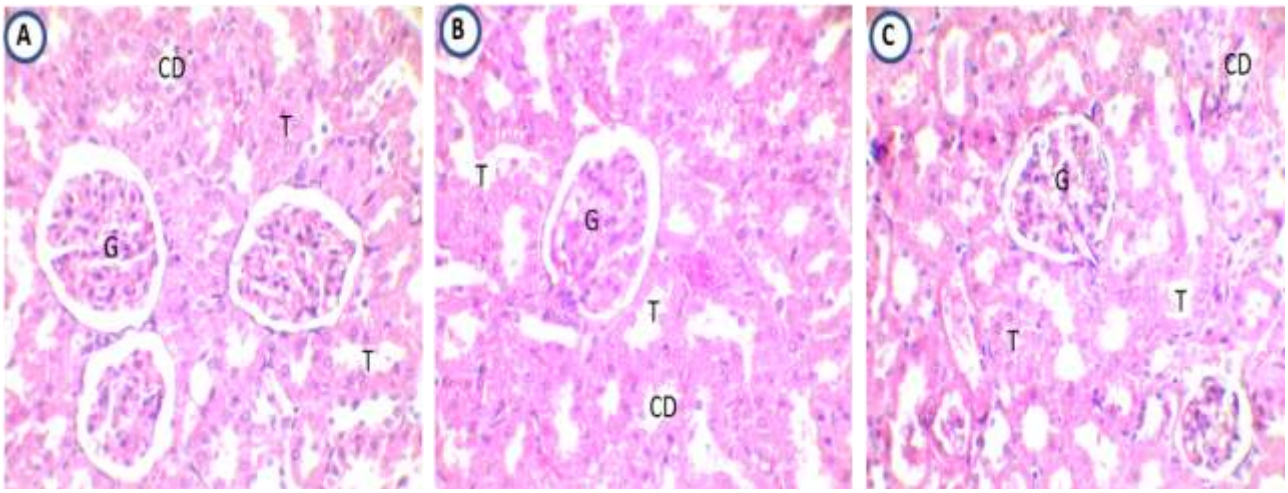

Figure 2:- Renal histology in acute and sub-chronic oral toxicity tests of the roots aqueous extract of Uvaria chamae (magnification 400X).
Figure 2A:- Control rats; Figure 2B: acute toxicity test; Figure 2C: sub-chronic toxicity test; G: Glomeruli; T: Proximal and distal tubes; CD: collecting ducts.

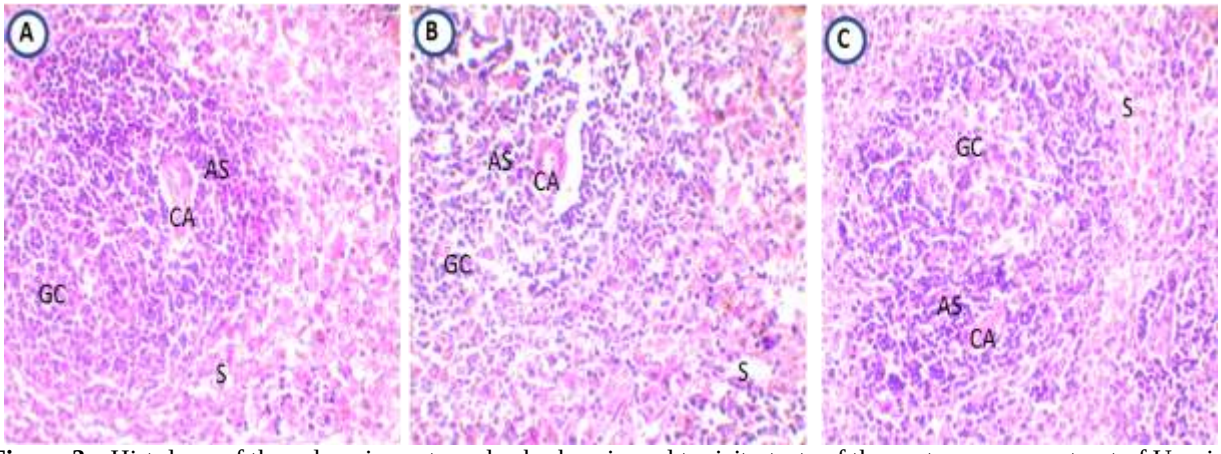

Figure 3:- Histology of the spleen in acute and sub-chronic oral toxicity tests of the roots aqueous extract of Uvaria chamae (magnification 400X).

Figure 3A:- Control rats; Figure 3B: acute toxicity test; Figure 3C: sub-chronic toxicity test; AS: the periarteriolar sleeves; CA: The central arteries; S: the venous sinusoids; BC: the Billroth cords.

Uvariachamae P. Beauv, which was the subject of this present work, was used in Benin to relieve crises in patients suffering from sickle cell disease in traditional Beninese medicine. We have sought to determine its safety.

The weight of the rats did not vary significantly and those treated with acute or sub-chronic oral toxicity from the aqueous extract of Uvaria chamae. These results were observed in the cases of toxicity studies on plants of Psorospermumfebrifugum and Cocos nucifera (Agbogba et al., 2019; Tchogou et al., 2021) which are anti-anemic plants used by Beninese traditional therapists. Similar results were also reported when studying aqueous extracts of Jatropha tanjorensis leaves and methanoic extracts of Sphenostylisstenocarpa which were anti-anemic plants (Idu, 2014; Okonkwo, 2013).

Relative to liver function, AST and ALT transaminases did not vary significantly with aqueous extract of Uvaria chamae either in the acute or sub-chronic oral toxicity test. The extract was therefore not toxic to the liver. These results were confirmed by the histological sections which did not show any visible atypia in the liver cells. These results were consistent with the toxicity work of Sènou et al. (2017b) on aqueous extracts Sorghum bicolor which did not exhibit hepatic toxicity. Similar results have also been reported on Psorospermumfebrifugum by Agbogba (2019).

Furthermore, serum creatinine did not vary significantly with respect to acute oral toxicity. But at sub-chronic oral toxicity, creatinine levels were significantly reduced in rats treated with Uvaria chamae root extract, indicating protection of renal function. The extract therefore had a beneficial effect on the kidneys. The absence of renal toxicity was reported for certain plants such as Psorospermumfebrifugum and Cocos nucifera whose root bark is traditionally used in the treatment of anemia (Gandhi, 2013; Sènou et al., 2017a; Tchogou et al., 2017). This lack of toxicity was confirmed by histological sections of the kidneys.

Finally, we thought of an absence of immune disturbance because the spleen histology, a peripheral immune organ was not modified by the treatment with the extract. In addition, the number of blood leukocytes did not significantly change with treatment with the aqueous extract of Uvaria chamae root, whether for acute or sub-chronic oral toxicity. Such preservation of immunity was noted in the aqueous extract of the leaf sheath of Sorghum bicolor in the treatment of anemia (Sènou et al. 2017b).

Conclusion:-

The aqueous extract of the roots of Uvaria chamae did not show acute or sub-chronic toxicity to the liver and kidneys. It also does not appear to interfere with the immunity or weight of the rats. These safety results open up great prospects for its safe use in relieving sickle cell crises.

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