ORIGINAL ARTICLE

SAFETY OF AQUEOUS EXTRACTS FROM THE BARK OF *Daniellia Oliveri* (ROLFE) HUTCH. AND *Dalziel* IN WISTAR RATS

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ABSTRACT

Introduction: Beninese traditional used of several plants in the treatment of sickle cell disease. The safety of many of these plants did not be established and *Daniellia Oliveri* is one of them. To counter this, the present work aimed to determine the safety of the aqueous extract of *Daniellia Oliveri* on female rats of wistar strains. **Methods**: The aqueous extract of the barks of *Daniellia Oliveri* was obtained by maceration. The Acute Oral Toxicity (AOT) by gavage in a single dose of 2000 mg/Kg of body weight and Sub-Chronic Oral Toxicity (COT) tests by forced gavage of 200 mg of extract/D/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 COT. Histological analysis of liver, kidney and spleen was performed for both tests. **Results**: There were no deaths during toxicity testing and organ histology showed no atypia. Serum creatinine, serum levels of AST and ALT transaminases and the mean number of white blood cells did not vary significantly between the beginning and the end of the experiment for both AOT and COT. Only weight increased significantly ($P < 0.05$) for COT. **Conclusion**: The aqueous extract of the bark of *Daniellia Oliveri* did not show hepatic, renal and immune toxicity in acute or sub-chronic state. Its use in traditional medicine can be recommended.

Keywords: *Sickle cell disease; Daniellia oliveri; acute toxicity; sub-chronic toxicity; Benin.*

1. INTRODUCTION

Traditional medicine was widespread in Africa and according to estimates by the World Health Organization; it provided 80% of health care in Africa. The OMS stipulated that traditional medicine is the body of knowledge and practices, explainable or not, used to diagnose, prevent or eliminate an imbalance, physical, mental, or social, based exclusively on knowledge acquired or transmitted from generation to generation, orally or in writing [1]. The use of medicinal plants was taking on increasing health and especially economic importance due to their accessibility and low cost [2]. In Benin, 814 plants were used by Beninese to treat illnesses [3,4]. These plants contained metabolites that have multiple interests used in several fields, including pharmacology, the food and cosmetics industry. It included, among others, phenolic compounds, coumarins, saponosides, mucilages, volatile compounds, sterols and terpenes which are of therapeutic interest [5,6].

Nevertheless, there was a problem with the biological tolerance of the different plant extracts used in traditional medicine. Several phytochemical screens revealed the presence of highly toxic substances in plant extracts that were lethal in the short or long term [7]. Several studies indicated that certain 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 [8]. It was therefore very important to check the toxicity of plant extracts for safe use in the treatment of diseases [9,10,11]. Traditional Beninese medicine used the bark of *Daniellia Oliveri* to relieve sickle cell crises. The aim of this work was to evaluate in vivo the acute and sub-chronic oral toxicity of aqueous extracts of *Daniellia Oliveri* in female Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant material and aqueous extraction:

The bark of *Daniellia Oliveri* were collected in Abomey in Benin. The identification and certification of the plant were made at the National Herbarium of the University of Abomey-Calavi on number YH267 / HNB. The plant was dried at laboratory temperature (20 ° - 25 °) out of direct sunlight and moisture for three weeks. They were then powdered and

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stored in black sachets [6-12]. 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 [6-12].

2.2 Ethics Statement:

The study was approved by the National Research Ethic review Boards of Benin. The Wistar rats used in this study were handled according to the institutional animal safety guidelines (Animal facility, National School of Applied Biosciences and Biotechnologies, National University of Sciences, Technologies, Engineering and Mathematics, Benin).

2.3 Animal material:

The animal material was composed of nine (9) strain albino Wistar female rats from the animal house of IBSA 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.

2.4 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 [13]. 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 Daniellia oliveri. 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 [14-12].

2.5 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 *Daniellia Oliveri* at 200 mg / kg body weight, daily for 28 consecutive days (Biswas et al.). They were weighed and blood was collected by orbital puncture at the start of the experiment and then after 28 days [14-12].

2.6 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 [14-12].

2.7 Histology

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 specimens 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 [15]. The pictures were taken at 400X magnification.

2.8 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.

3. RESULTS

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

The aqueous bark extracts of *Daniellia Oliveri* did not exhibit acute oral toxicity

Table 1 showed the weight of the animals, the serum levels of creatinine, transaminases (ASAT and ALAT) and the number of leukocytes on Day 0 (D0) and on Day 14 (D14) in rats during acute oral toxicity. The mean weight of the rats treated with 2000 mg of extract / Kg was 144 ± 5.93 g on D0 and 145 ± 5.49 g on D14. The mean weight did not change significantly with treatment. The mean serum creatinine level of the rats treated with 2000 mg of extract / Kg was 11.7 ± 1.80mg/mL on D0 and 13.0 ± 1.40 mg / mL on D14. The mean creatinine level did not change significantly with treatment, indicating no deterioration in kidney function.

The mean AST level of the rats treated with 2000 mg of extract / Kg was 221 ± 47.4 U/L on D0 and 237 ± 47.8 U / L on D14. The mean ASAT level did not vary significantly with treatment, suggesting an absence of cytolysis. The mean ALT levels for rats treated with 2000 mg of extract / kg was 63.3 ± 4.06 U/L at day 0 and 72.0 ± 6.56 U / L at day 14. The mean ALT level did not vary significantly with treatment, suggesting an absence of hepatic cytolysis. The mean number of white blood cells in rats treated with 2000 mg of extract / Kg was 11.9 ± 1.43G/L on D0 and 8.73 ± 1.29 G/L on D14. The mean white blood cell count did not change significantly with treatment, suggesting no disturbance in immune function.

Table 1: The table shows the physical, biochemical and immune assessments in the Acute Oral Toxicity test.

Parameters	Means at J0	Means at J14	P-value	Difference
Body weight (g)	144 ± 5.93	145 ± 5.49	0.85	No significant
Creatinine (mg/L)	11.7 ± 1.80	13.0 ± 1.40	0.59	No significant
Transaminase AST (U/L)	221 ± 47.4	237 ± 47.8	0.83	No significant
Transaminase ALT (U/L)	63.3 ± 4.06	72.0 ± 6.56	0.32	No significant
White blood cells (G/L)	11.9 ± 1.43	8.73 ± 1.29	0.17	No significant

The aqueous bark extracts of *Daniellia Oliveri* did not exhibit sub-chronic oral toxicity

Table 2 showed the weight, the serum levels of creatinine, transaminases (AST and ALT) and the number of leukocytes on Day 0 (D0) and on Day 28 (D28) in rats during sub-chronic toxicity test.

The mean weight of the rats treated with 200 mg of extract / Kg / day was 124 ± 7.51 g on D0 and 156 ± 6.69 g on D28. The mean weight increased significantly with treatment. The mean serum creatinine level of the rats treated with 200 mg of extract / Kg / day was 12.7 ± 1.29 mg / mL and 9.90 ± 1,42 mg / mL on D28. The mean creatinine level did not change significantly with treatment (P <0.05), indicating improvement in renal function.

The mean serum AST level of the rats treated with 200 mg of extract / Kg / day was 147 ± 18.7 U/L on D0 and 127 ± 21.5 U / L on D28. The mean ASAT level did not vary significantly with treatment, suggesting an absence of cytolysis.
The mean ALT level of the rats treated with 200 mg of extract / Kg / day was 69 ± 9.50 U / L on D0 and 86.3 ± 6.69 U / L on D28. The mean ALT level 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 11.2 ± 1.23 G / L on D0 and 9.47 ± 1.58 G / L on D28. The mean white blood cell count did not change significantly with treatment, suggesting no immunity disturbance.

Table 2: The table shows the physical, biochemical and immune assessments for the Sub Chronic Oral Toxicity test.

Parameters	Means at J0	Means at J14	P-value	Difference
Body weight (g)	124 ± 7,51	156 ± 6,69	**0.03**	**Significant**
Creatinine (mg / L)	12.7 ± 1.29	9.90 ± 1.42	0.2	No Significant
Transaminase AST (U/L)	147 ± 18.7	127 ± 21.5	0.5	No significant
Transaminase ALT (U/L)	69 ± 9.50	86.3 ± 6.69	0.21	No significant
White blood cells (G / L)	11.2 ± 1.23	9.47 ± 1.58	0.4	No significant

The Aqueous Extract of *Daniellia Oliveri* 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 *Daniellia Oliveri* did not show any visible atypia. Hepatocytes normal appearances 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 *Daniellia Oliveri* 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 *Daniellia Oliveri* 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.

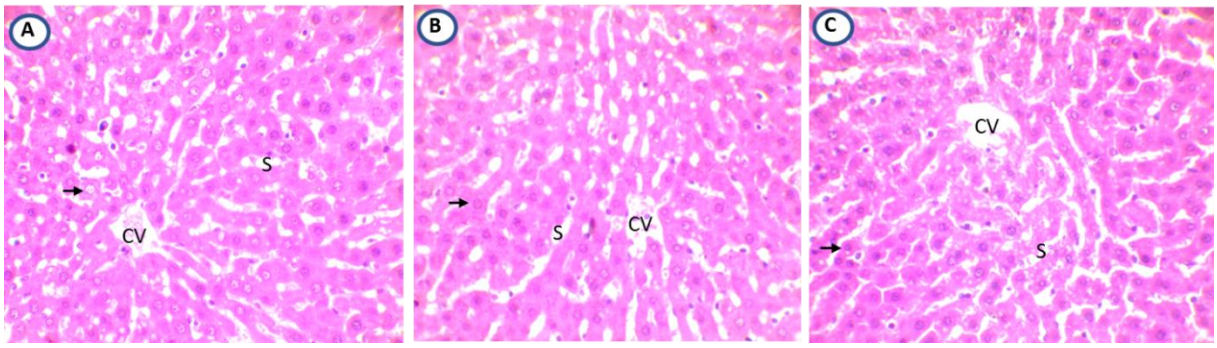

Figure 1: The figure show the liver histology in acute and sub-chronic oral toxicity tests of the roots aqueous extract of *Daniellia Oliveri* (magnification 400X).

Figure 1A: controls rats; Figure 1B: acute toxicity test; Figure 1C: sub-chronic toxicity test S: venous sinusoids; CV: central vein; Arrows: Hepatocytes.

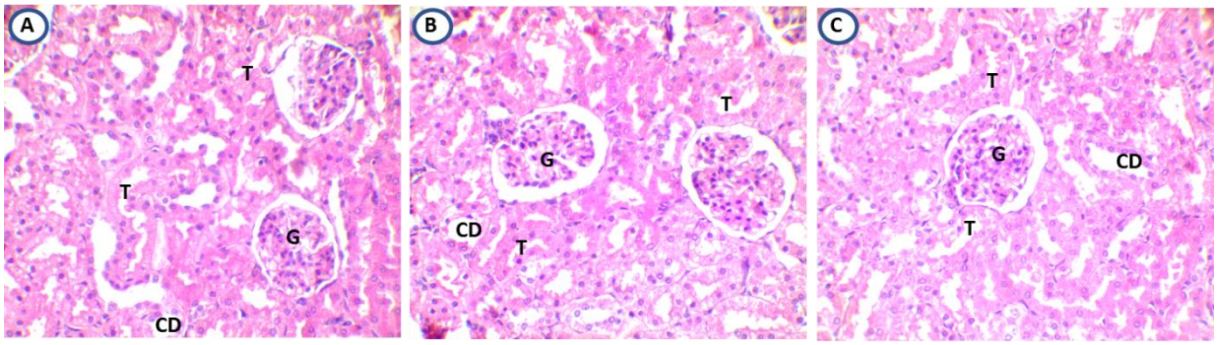

Figure 2: Renal histology in acute and sub-chronic oral toxicity tests of the roots aqueous extract of *Daniellia Oliveri* (magnification 400X).

Figure 2A: Controls rats; Figure 2B: acute toxicity test; Figure 2C: sub-chronic toxicity test; G: Glomeruli; T: Proximal and distal tubes; CD: collecting ducts.

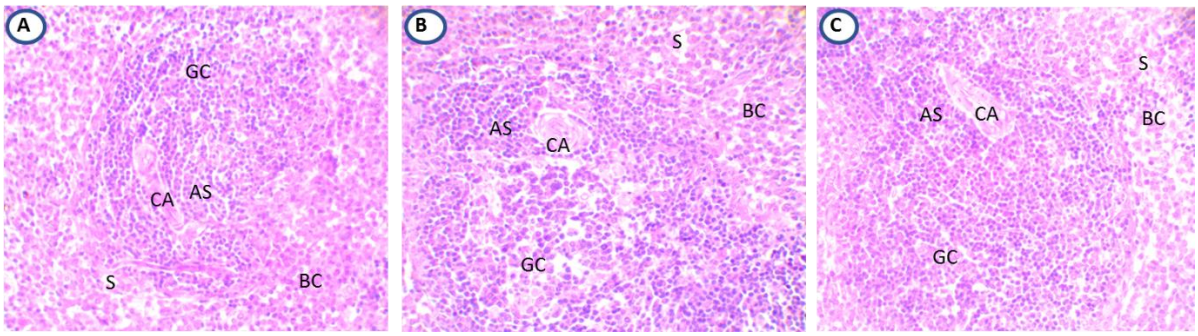

Figure 3: Histology of the spleen in acute and sub-chronic oral toxicity tests of the roots aqueous extract of *Daniellia Oliveri* (magnification 400X).

Figure 3A: Controls 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.

4. DISCUSSION

The population of poor countries in Africa made massive use of traditional medicine because of its affordability, but also its accessibility [14]. To relieve sickle cell crises, patients suffering from sickle cell anemia use *Daniellia Oliveri* prescribed by Beninese traditional healers. This study sought to determine its safety.

The weight of the rats did not statistically vary between the control rats and those treated with the aqueous extract of the barks of Daniellia oliveri with acute oral toxicity. But with regard to sub-chronic oral toxicity the weight of the rats increased significantly. Similar results were reported by Agbogba (2019) on Psorospermum febrifugum and by Tchogou (2021) on Cocos nucifera which did not vary the weight during the acute oral toxicity study [14-12]. The increase in the weight of the rats during the sub-chronic oral toxicity would be due to the duration of the experiment which favored weight gain.

Furthermore, serum creatinine did not show any significant variation with respect to acute and sub-chronic oral toxicity. The extract was therefore not toxic to the kidney. These results were confirmed by the histological sections performed on the kidneys where the renal parenchyma retained its typical appearance. The absence of renal toxicity was reported in several studies such as those on Psorospermum febrifugum and Cocos nucifera [16,14] which were plants used in Benin against anemia and which did not display any acute and sub-chronic kidney toxicity.

Regarding the exploration of liver function, AST and ALT transaminases did not vary significantly between control rats and those treated with the aqueous extract of Daniellia oliveri, whether for acute or sub-chronic oral toxicity. The extract was therefore not toxic to the liver. In addition, the histological section confirmed the absence of hepatic toxicity. Indeed, the liver of rats force-fed with the aqueous extract of *Daniellia Oliveri* did not show visible atypia. These results were comparable to those obtained by Sènou (2017b) on Sorghum bicolor which did not show liver toxicity [17].

Finally, it was noted an absence of immune toxicity. In fact, leukocytes did not vary significantly between control rats and those treated with the aqueous extract of Daniellia oliveri, whether for acute or sub-chronic oral toxicity. In addition, on histology, the splenic architecture was not modified, indicating an absence of toxicity in the spleen, which was an immune organ. These results were similar to those obtained by Agbogba (2019) on Psorospermum febrifugum, Sènou (2017a) on Cocos nucifera and Sènou (2017b) on Sorghum bicolor which did not show immune toxicity [14,16,17].

5. CONCLUSION

The aqueous extract of *Daniellia Oliveri* was devoid of toxicity on hepatic, renal and immune functions in the acute or sub-chronic state. It was the same for the morphology of these organs. Its use in traditional medicine can be recommended in the treatment of sickle cell crises. Insérez votre conclusion ici et indiquer clairement les idées majors émanant de votre recherche d'étude.

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