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# RESEARCH ARTICLE

## EVALUATION OF THE ACUTE AND SUBACUTE TOXICITY OF THE AQUEOUS EXTRACT OF THE RECIPE BASED ON THE STEAM BARK OF *DACRYODES BUTTNERIE* (ENGL.) H.J. LAM (BURCERACAE) AND *PSEUDOPSONDIAS MICROCARPA* (A. RICH.) ENGL.(ANACARDIACEA)

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**ABSTRACT**

*Dacryodes buttneri* (Burseraceae) and *Pseudospndias microcarpa* (Anacardiaceae) are two plants used in traditional Congolese medicine in the form of a recipe in the treatment of several diseases. This study aimed to estimate the acute and subacute toxicity of the recipe. The acute toxicity of the aqueous extract (2000 and 5000 mg/kg) of the recipe was evaluated according to the OECD guideline in single administration. It appears that the aqueous extract of the recipe does not modify the general behavior of the animals and does not cause any mortality. This result suggests that the toxic doses would be greater than 5000 mg/kg. Subacute toxicity was evaluated in rats for 28 days using the high dose of 800 mg/kg. It appears from this study that the aqueous extract (800 mg/ kg) of the recipe in regular administration for 28 days does not modify the general behavior of the animals, does not cause any mortality and does not modify significantly ($p>0.05$) the relative weight of organs, biochemical and hematological parameters compared to control animals. On the other hand, histological examinations revealed venous toxicity in the liver, intestine and kidneys. This recipe should be used with caution in patients with a known history of liver, kidney and bowel disorders.



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# INTRODUCTION

The use of medicinal plants is growing in most countries of the world. This use is mainly based on the idea that plants are a natural means of treatment devoid of any risk. Consumers often believe that natural is synonymous with harmless. However, a plant can be both useful and toxic (Zekkour, 2008). A plant is said to be toxic when it contains substances harmful to humans (or animals) and whose ingestion, or simple contact, causes various more or less serious or fatal disorders. These disorders can be morphological and functional lesions in a living organism, caused by a substance introduced in a relatively high single dose or in small doses repeated for a long time. It is estimated that 4% of plants worldwide are toxic to varying degrees (Rockett, 2015). As such, plant toxicity deserves greater attention. Therefore, the toxicological impact of medicinal plants in humans, especially in sensitive periods, is of great concern. *D.buttnerie* and *P.microcarpa* are two Congolese medicinal plants whose recipe is used in the treatment of several pathologies. Traditional healers usually use a combination of at least two plants to treat patients in order to increase therapeutic efficacy or to reduce certain toxic effects.

To date, no study on the toxicity of the *D.buttnerie* and *P.microcarpa* steam bark recipe has been carried out to assess its safety. It is therefore in this context that this present research work takes place, which focuses on the acute and subacute toxicity of the aqueous extract of the based on the steam bark *D.buttnerie* and *P.microcarpa* in vivo.

# MATERIALS AND METHODS

**Plant material:** The plant material consisted of steam bark from *Dacryodes buttneri* (Burseraceae) and *Pseudospndias microcarpa* (Anacardiaceae). The collections took place from July 2020 to February 2021 in the Mousendjo forest (Niari department, in the south of the country). Botanical identification of the plant material was done by Mousamboté, botanist systematistof Higher Normal School of Agronomy and Forestry (HNSAF) and confirmed at the Herbarium of the National Institute for Research in Exact and Natural Sciences (NIRENS) witch a collected sample was compared to a reference

sample (IEC025849 for *Dacryodes buttneri* and IEC001226 for *Pseudospndias microcarpa*). After harvesting, the samples were cleaned and then dried at the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE) of the Faculty of Science and Technology (F.S.T) for 14 days at a temperature of 26 ± 1°C. After drying, the samples were ground separately using a mortar and filtered through a sieve. The powder obtained from each sample was used to formulate the recipe. 10 g of powdered trunk bark of *D. buttneri* and 10 g of *P.microcarpa* were mixed and boiled in 200 ml of distilled water for 15 minutes. After cooling and then filtration, the decoction obtained was concentrated in a water bath (55° C). The solid residue obtained constituting the aqueous extract was kept to assess acute and subacute toxicity.

**Animal material:** Female albino Wistar rats (200 - 250 g) and female albino Swiss mices (20 - 30 g) of either sex obtained from the Faculty of Science and Technical of Marien NGOUABI-University were used. These animals were provided to us by the animal facility of the Faculty of Science and Technology (F.S.T). They were fed with a standard feed and water ad libitum. They were acclimatized during one week before experimentation and were housed under standard conditions (12 hours light and 12 hours dark) and at the temperature of 27°C ± 1°C. The rules of ethics published by the International Association for the Study of Pain (Zimmermann, 1983) have been considered.

**Acute toxicity:** Acute toxicity was estimated in accordance with OECD Guideline No. 425 (OECD, 2008a) (OCDE, 2008a). The mice were fasted for 24 hours before oral administration of the aqueous extract of the recipe. Three (03) groups of three (03) mice each were formed and treated with different doses of physiological saline (control group, 0.5 ml/100g) and aqueous extract at the doses of 2000 and 5000 mg/kg. After oral administration, macroscopic observations were made every thirty (30) minutes during four (OCDE, 2008a) hours to assess the general behavior of the animals. They focused on piloerection, aggression, mobility, alertness, stool status, vomiting and mortality. Mortality was evaluated for forty-eight (48) hours after oral administration of the products. The animals' body weight was measured every two day for fourteen (14) days.

**Subacute toxicity:** To evaluate the tolerance of the aqueous extract of the recipe intended for long-term use, we used OECD guideline no. 407 (OECD, 2008b) (OCDE, 2008b). The animals were fasted for 24 hours before oral administration of the aqueous extract of the recipe and divided into two (02) groups of five (05) rats each. They were treated for 28 days with different doses of water physiological (0.5 ml/100g) and the aqueous extract of the recipe at a single dose of 800 mg/kg. Macroscopic observations were made 2 hours after each administration of the test products. Rats were fed and hydrated daily, then weighed daily. Behavioral changes, clinical signs of toxicity and mortality were observed and recorded, and the weight of each rat was noted respectively on the 7th, 14th, 21st and 28th days. At the end of the treatment, the animals were anesthetized and the blood samples were collected by recto-orbital sampling in the dry tubes to dose biochemical parameters (creatinine, alanine aminotransferase, aspartate aminotransferase, amylase and triglycerides) and EDTA citrated tubes to dose hematological (blood count and sedimentation rate). After euthanasia, the target organs (liver, kidneys and small intestine) were removed for anatomic pathology analyses.

**Hemato-biochemical study:** Hematological and biochemical parameters were analyzed at the National Public Health Laboratory (L.N.S.P) in Brazzaville by an ABX Micros 60 automatic hematology analyzer (HORIBA ABX Diagnostics) and a Raytochemray 120 automatic biochemistry analyzer, including white blood cells (GB ); lymphocytes (Lym); monocytes (Mon); granulocytes (Gra); red blood cells (RB); sedimentation rate (ESR); hemoglobin concentration (HGB); hematocrit (HCT) and platelets (PLA); Creatinine (CRE); alanine aminotransferase (ALT); aspartateaminotransferase (AST); total cholesterol (TC); amylase (Amyl) and triglycerides (TG).

**Histopathological study:** The liver, kidneys and small intestine removed were fixed in 10% formalin, then the following steps: inclusion, sectioning and staining were carried out at the level of the anatomopathology laboratory of the hospital and university center of Brazzaville (CHU - B). The preserved organs underwent sections with a thickness of 5 µm using a microtome after their inclusion in paraffin. They were then stained with hematoxylin-eosin, fixed between slide and coverslip before being observed using a microscope fitted with a camera.

**Statistical analysis of results:** The Excel 2016 software was used to process the data. All values were expressed as mean ± standard error of mean (SEM). Analysis of variance followed by Student-Fischer t test "t" was performed. The significance level was set at $p < 0.05$.

# RESULTS

**Effect of the aqueous extract of the recipe on the general behavior of rats after acute administration:** Oral administration of the aqueous extract (2000 and 5000 mg/kg) of the recipe of steam bark of *D.buttnerie* and *P. microcarpa* did not modify the general behavior of the animals compared to control group (distilled water 0.5mL/100g). No mortality was observed up to the dose of 5000 mg/kg. However, there is a significant ($p < 0.05$, $**p < 0.01$) weight gain between day 4 and day 8 compared to the control group in the animals treated with the aqueous extract (2000 mg/kg) of recipe (Figure 1).

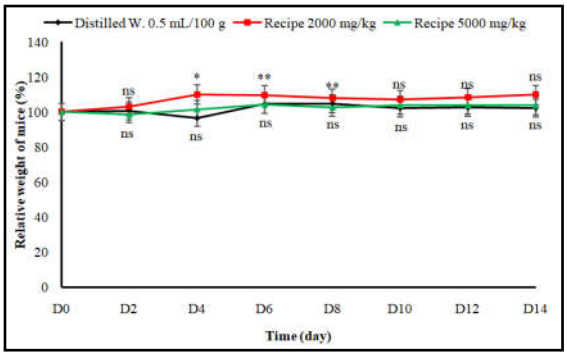



**Figure 1: Effect of aqueous extract (2000 and 5000 mg/kg) of recipe on body weight change in mice. Each value represents the mean ± ESM. *p < 0.05, ** p < 0.01Significantdifferent (Student t-test) versus D0 ; ns (p>0.05) = no signicant different (Student t-test) versus D0**

**Effect of the aqueous extract of the recipe on the general behavior of rats after 28 days of treatment:** The results of the effect of the aqueous extract (800 mg/kg) of the steam bark of recipe of on the general behavior and the weight evolution of the rats for 28 days are presented in Table 2 and Figure 2. Observation of the behavior of the rats throughout the study period revealed no behavioral changes, no mortality. However, after 28 days of treatment, there is a significant weight gain in the animals treated with the aqueous extract compared to the controlgroup (Figure2).

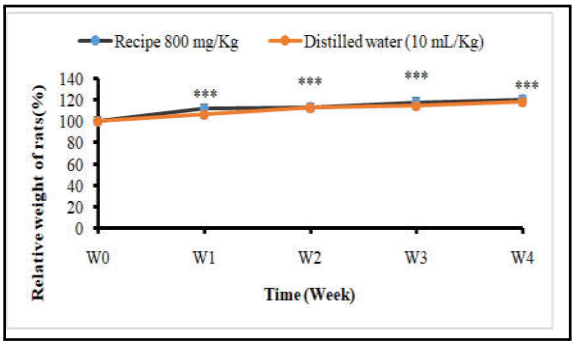



**Figure 2. Effect of aqueous extract on body weight changes in rats during 28 days of treatment. Results are expressed as mean ± standard error, n =5 rats; ***p < 0.001 Student's t test significant compared to day 1. ns= Student's t test not significant compared to day 1.**

**Effect of the aqueous extract of the recipe on the relative weight of the organs of rats during 28 days of treatment:** After 28 days of treatment with aqueous extract of recipe macroscopic observation of the organs (kidneys, spleen, intestine, heart and liver) showed no color variation of the organs of the treated rats (800 mg/kg) compared to the control rats (10 mL/kg). Moreover the treatment of rat withthe aqueous extract of the recipe at a dose of 800 mg/kg on the relative weights does not significantly increase (table 3) the weights of the organs (kidneys, spleen, small intestine, heart and liver) ($p > 0.05$) compared to the control group (10 mL/kg).

**Table 3. Under the effect of aqueous extract of recipe in relative organ weights of rat 28 days after treatment**

| Organs | Treatment | |
|---|---|---|
| | Control group (10mL/Kg) | Aqueous extract (800 mg/kg) |
| Left kidney | 0.50 ±0.03 | 0.56 ±0.04ns |
| Right kidney | 0.54 ±0.02 | 0.54 ±0.04ns |
| Liver | 4.59 ±0.13 | 5.6 ±0.47ns |
| Small intestine | 4.93 ±0.11 | 4.5 ±0.26ns |
| Spleen | 0.39 ±0.03 | 0.44 ±0.09ns |
| Heart | 0.76 ±0.02 | 0.75 ±0.05ns |

**Effect of the aqueous extract of the recipe on the haematological and biochemical parameters of rats during 28 days of treatment:** The results of the hematological and biochemical parameters of the rats during 28 days of treatment are presented in Tables 4 and 5. With regard to the hematological parameters, the results show non-significant variations ($p > 0.05$) after 28 days of treatment compared to the control animals (Table 4). With regard to the biochemical parameters, no significant variation ($P > 0.05$) is observed in the animals treated with the aqueous extract (800 mg/Kg) compared to the control animals (Tables 5).

| Treatment (28 days) | | |
|---|---|---|
| Parameters haematological | Distilled water (10 mL/Kg) | Aqueous extract of recipe (800 mg/kg) |
| SR (mm) | 5±0.94 | 2.8±0.58ns |
| WBC ($10^3$/mm$^3$) | 7 ± 0.31 | 6.94 ± 0.48ns |
| Lym (%) | 96.1 ±0.16 | 95.02 ±1.06ns |
| Mon (%) | 3.1 ±0.1 | 4.05 ±0.89ns |
| Gra (%) | 0.8 ± 0.14 | 0.92 ±0.38ns |
| RBC ($10^6$/mm$^3$) | 7.61 ±0.01 | 5.48 ± 1.18ns |
| HGB (g/dl) | 17.7 ± 0.24 | 16.46 ± 1.17ns |
| HCT (%) | 49.3 ± 0.1 | 33.38 ±7.09ns |
| PLA ($10^3$/mm$^3$) | 828 ±2.70 | 1255.6 ±197.64ns |

**Histopathological study of rats for 28 days of treatment:** Figure 3 presents the photomicrographs of the liver, kidneys and small intestine of rats treated with the aqueous extract of the recipe (800 mg/kg) and the control group (10 mL/Kg).

**Table 5. Effetof aqueous extract of recipe in biochemical parameters of rat 28 days after treatment**

| Treatment (28 days) | | |
|---|---|---|
| Parameters biochemical | Distilled water (10mL/kg) | Aqueous extract of the recipe (800 mg/Kg) |
| ASAT (UI/L) | 146 ± 11.25 | 150 ± 10.71ns |
| ALAT (UI/L) | 52.2 ± 5.57 | 46 ± 3.12ns |
| Créat (mg/L) | 9.18 ±0.18 | 8.46 ±0.57ns |
| TC (g/L) | 1.74 ± 0.02 | 0.76 ±0.05ns |
| TG (g/L) | 1.3 ± 0.09 | 1.36 ±0.05ns |
| Amyl | 236 ± 17.33 | 250 ± 36.79ns |

Each value represents the mean ± ESM. ns ($p>0.05$) = no signicant different (Student t-test) versus control group. ASAT: aspartic acid aminotransaminase; ALAT: alanine aminotransaminase; TG: triglycerids; TC: total cholesterol; Creat: creatinine level; Amyl: amylase.

The results showed that after the administration of the aqueous extract of the recipe to rats for 28 consecutive days, histopathological changes are observed during histological examination of organ sections in the tissue of the rats (Figures 3 A,B,C,E,F,H) compared to the control group (distilled water 10 mL/kg) (Figure 3 D,G,I) in particular a frankly congestive venule at the level of the kidney (renal cortex, renal medulla and renal papilla), liver (hepatic parenchyma) and a dilated venule in the intestine (serosa of the jejunal wall) while for the control group (distilled water 10 mL/Kg) the organs appear morphologically integrated.

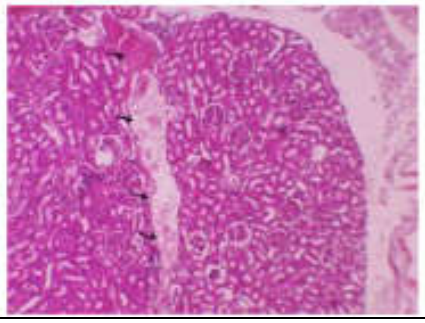
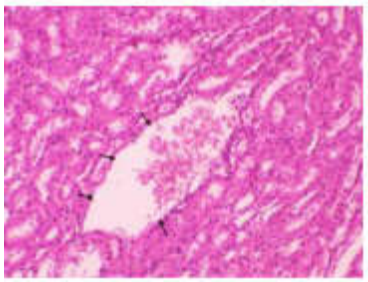
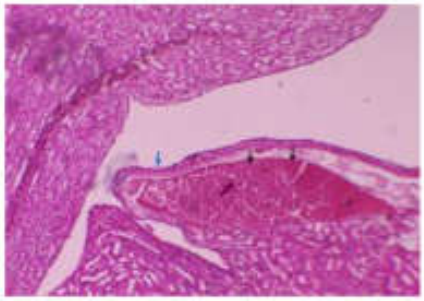
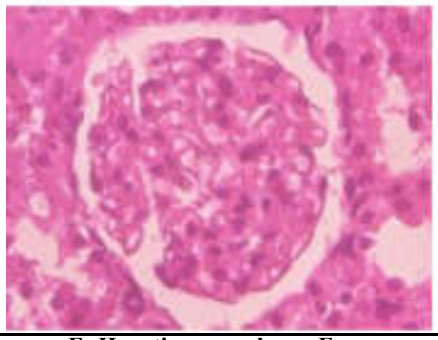
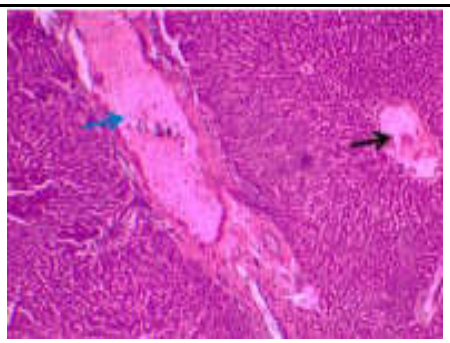
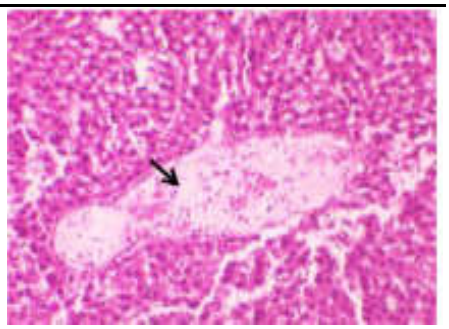
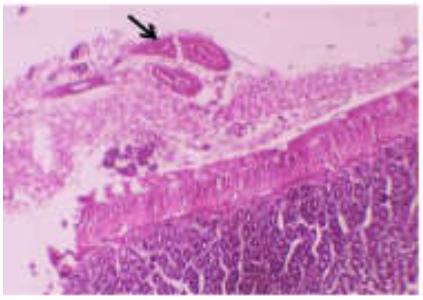
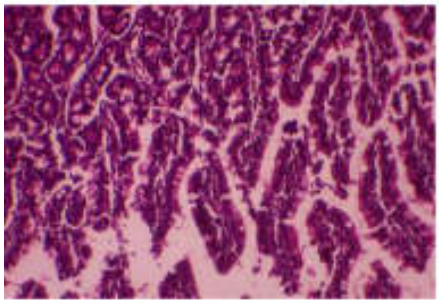
# DISCUSSION

The present study was initiated to estimate the acute and sub-acute toxicity of the recipe for stem bark from *D.buttneri* (Burceracae) and *P.microcarpa* (Anacardiacea). Before estimating sub-acute toxicity, this study involved first estimating acute toxicity in mice in order to determine the LD50 and the therapeutic doses. It emerges from this study that the aqueous extract of the recipe at a dose of 2000 and 5000 mg/kg does not modify the general behavior of the animals and no mortality was observed, just as in the control batch treated with distilled water (0.5ml/100g). This result suggests that the aqueous extract of the recipe would not be toxic and that the toxic doses would be higher than the dose of 5000 mg/kg. According to the globally harmonized classification system, the recipe for the stem barks of *Dacryodesbuttneri* (Burceracae) and *Pseudospondiasmicrocarpa* (Anacardiacea) would be classified in category 5 of plants not presenting a danger to the organism (OCDE, 2001).

The aqueous extract of the recipe not having caused the death of the mice up to the dose of 5000 mg/kg, the dose of 800 mg/kg (which is approximately one fifth of the therapeutic dose) was retained for assess sub-acute toxicity in rats. It appears from this study that the aqueous extract (800 mg/kg) of the recipe in regular administration for 28 days does not modify the general behavior of the animals, does not cause any mortality and does not modify significantly ($P>0.05$) the relative weight of organs (liver, kidneys, intestine, spleen and heart), hematological and biochemical parameters compared to control animals (distilled water 10 mL/Kg). According to Lu (1992) (Lu Franck, 1992), variation in the color and appearance of organs often indicates the nature of toxicity. Indeed the liver and the kidneys are two organs which regulate metabolism, excretion, and which are particularly sensitive to potential toxic agents, their functions must be monitored in toxicological studies (OMS, 2000).

On the other hand, anatomopathological examinations revealed the presence of localized lesions in the veins, more specifically congestion with inflammatory infiltrate in the liver, intestine and kidneys, while the arteries presented a morphologically integrated appearance compared to control rats (distilled water 10 mg/Kg) where very slight inflammation was observed. These results obtained with distilled water would suggest that its lesions could be linked to the aqueous extract (800 mg/Kg) of the recipe. Similarly, the presence of localized lesions in the veins unlike the arteries which are morphologically integrated could be due to the fact that the aqueous extract (800 mg/Kg) of the recipe has more affinity for oxygen in the absence of which it becomes toxic.

It has been reported by several authors that venous toxicity would be the precursor sign by which many toxic agents manifest themselves (Koudou, 2019). This result suggests that the dose of 800 mg/Kg in repeated administration would be close to the toxic dose and the result of a toxic process due to cell necrosis. The toxic effect observed could be due to one of its constituents, in particular *Pseudopsondias microcarpa* (Anacardiacea). Indeed, this plant is part of the medicinal and toxic plants of the Congolese pharmacopoeia (Bouquet, 1964) which could explain or justify its use in recipe by traditional health practitioners.

| | | |
|---|---|---|
|  |  |  |
| **A: Renal cortex Exp. Microphotograph shows a frankly congestive venule (→) x4** | **B: Renal medulla Exp. Microphotograph shows a congestive venule (→) x10** | **C: Renal papilla Exp. Microphotograph shows a congestive venule (→) in the papilla (→) that protrudes into a calyx. X4** |
|  |  |  |
| **E: Hepatic parenchyma Exp. Microphotograph shows a congestive portal vein branch (→) and centro- lobular vein (→). x4** | **F: Hepatic parenchyma Exp. Microphotograph shows a congestive centrilobular (→) vein. The surrounding hepatocytes do not have an altered appearance. X10** | **G: Hepatic parenchyma ED Microphotograph shows microvesicles appearing integral. X40** |
|  |  | |
| **H: Jejunal wall Exp. Microphotograph shows a dilated venule (→) in the serosa. X4** | **I: Jejunal wall ED Microphotograph shows intestinal villi appearing intact.X4** | |

# CONCLUSION

*D.buttnerie* (Burceracae) and *P.microcarpa* (Anacardiacea) are two Congolese medicinal plants whose recipe is used in the treatment of several pathologies. It appears from this study that the aqueous extract of the recipe based on the bark of the trunk *of D.buttnerie* (Burceracae) and *P.microcarpa* (Anacardiacea) is not toxic in single administration (acute toxicity) up to a dose of 5000 mg/Kg or repeated administration at a dose of 800 mg/Kg (subacute toxicity). However, histological examinations revealed venous toxicity of the liver, intestine and kidneys after repeated administration at a dose of 800 mg/Kg. This result suggests that the dose of 800 mg/Kg would be close to the toxic dose. It should therefore be used with caution in patients with a known history of liver, kidney and bowel disorders. Therefore, additional sub-chronic and chronic toxicity research should be carried out to identify the mechanisms of this venous toxicity.

**Conflict of interest**: The authors declare that they have no conflict of interest

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