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(RESEARCH ARTICLE)

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# Toxicity analysis of the methanolic extract of *Gaertnera phanerophlebia* Baker (RUBIACEAE): Aerial parts

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## Abstract

Recently, herbal medicines have become increasingly popular, with high usage rates in Africa (90 %) and in India (70 %). In Madagascar, they are considered a good, natural alternative to allopathic medicines. However, as more and more herbal drugs are recommended, concerns about their safety have emerged. Consumers and some practitioners have limited knowledge of the potential risks of herbal remedies. As part of the promoting and innovating of the practice of Malagasy traditional medicine, acute and subacute toxicological studies were carried out on *Gaertnera phanerophlebia* Baker, an endemic species of the RUBIACEAE family. It has been used as a febrifuge and wound healing but there is very little information about its direct toxic effects on organisms. This study aimed to characterize the toxicological profile of the methanolic extract from the aerial parts in male and female mice via oral administration. *In vivo* experiment indicates that, while the methanolic extract (ST380RF) of the aerial parts has a high acute safety profile (non-toxic up to 5,000 mg/kg of b.w), repeated 28-day exposure at lower doses (5 mg/kg of b.w) caused significant hepatotoxicity and nephrotoxicity, as evidenced by biochemical, hematological and anatomopathological studies of vital organs. These results demonstrate that even if a substance is harmless, its gradual accumulation in the body following repeated exposure over a long period can lead to serious adverse effects that are often impossible to detect until a critical threshold is reached. They also confirm that pharmacological benefits cannot replace comprehensive subacute safety evaluations before human application.

**Keywords.** *Gaertnera phanerophlebia*; Aerial Parts; Methanolic Extract; Toxicity; Subacute

## 1. Introduction

Herbs and natural products are essential components of healthcare around the world. In fact, 25 % to 40 % of modern pharmaceutical products are derived from plants. Many essential medications, including Aspirin (derived from willow bark), Artemisinin (derived from sweet wormwood), and various cancer treatments stem from traditional botanical knowledge [1]. Plants offer a vast, largely untapped source of structural diversity for new drugs, often providing safer alternatives to synthetic compounds. The World Health Organization (WHO) actively supports the integration of traditional, complementary, and integrative medicine (TCIM) into national health systems to ensure safe, effective,

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and evidence-based care. The WHO recognizes that up to 80 % of the world's population uses TCIM [2]. However, a major challenge is the lack of strict, standardized regulation for herbal products that would ensure their quality, efficacy, and safety.

Studies have revealed that the aerial parts of *Gaertnera phanerophlebia* Baker, also known as "Tsitsirontafika", and belonging to the RUBIACEAE, have long been used in Traditional Malagasy Medicine to treat wounds, pain and fever [3]. Its biological and pharmacological properties have already been studied and demonstrate its therapeutic potential in both conventional and alternative medicine [4,5]. The present study aims to determine this plant's safety margin, considering its numerous applications, with a view to its potential development into drugs.

## 2. Materials and Methods

### 2.1. Raw material

The fresh aerial parts of *Gaertnera phanerophlebia* Baker were collected in December 2019 from the Alaotra Mangoro region of Madagascar, specifically from the commune of Ambohibary and the Fokontany of Ampitambe Ambatomainty (Fig.1). Botanists at the National Center for Pharmaceutical Research Applications (CNARP) identified and authenticated the plant. A voucher specimen was archived in the Department of Botany herbarium under the registration number ST380RF (Photo collection: Rakotoarisoa Mbolatiana Abigaila, Andasibe, 2019; Rakotoarisoa Falitiana Marrino).


**Figure 1** Collection of the aerial parts of *Gaertnera phanerophlebia* (RUBIACEAE)

### 2.2. Experimental animals

Healthy male and female mice (*Mus musculus*) weighing between 20 and 30 g, were used as the animal model. They were sourced from the animal house of the Department of IMVAVET (Malgache Institute of Veterinary Vaccines) and acclimatized for seven days with standard, feed and water unrestricted. The mice were housed in same sex groups of three. The toxicological study was conducted in alignment with the OECD guidelines and the 3Rs principles. It was carried out according to the ethnical protocol to ensure humane treatment and minimal usage of animal. The study was also approved by the Pasteur Institute of Madagascar (IPM) Ethics Committee [6,7].

### 2.3. Extract preparation

The aerial parts were air-dried and subsequently milled into a fine powder using mechanical grinder. Then, based on the procedure, 350 g of the powdered plant underwent methanolic extraction via cold maceration at room temperature using 1,800 ml of solvent for 24 hours. The mixture was filtered with filter paper, and the filtrate was concentrated in a rotary evaporated with a water bath at 40 °C (Fig.2). The resulting crude extract (ST380RF) was weighted and stored 4 °C until needed [8]. The extraction yield was calculated using the following formula:

$$Yield\ of\ extract(\%) = \frac{weight\ of\ extract\ x100}{weight\ of\ plant\ material}$$


**Figure 2** Preparation of the extract (ST380RF)

### 2.4. Oral acute toxicological study: single administration

The oral acute toxicological study identifies all adverse effects that occur immediately or shortly after short-term exposure to a toxic substance administered in a single dose over a period not exceeding 24 hours [9]. The goal of this procedure is to evaluate and classify potential hazards by determining the dose thar causes 50 % mortality. Based on Organization for Economic Co-operation and Development (OECD) test guidelines 423/425 and Lorke's method, this study will assesses the acute oral toxicity of *G. phanerophlebia* (aerial parts) within 24 hours, determining the $LD_{50}$ (median lethal dose) in mice [5,10,11,12].

#### *2.4.1. Substance administration*

The animals were deprived of food for 18 hours prior to dosing. They were weighed, and randomly divided into groups of three per sex (n = 3/group/sex). The solution was prepared by diluting the crude extract in 5 % DMSO. Each animal received 1 ml per 100 g of body weight by gavage. The control group received physiological water (9 ‰), while the treated groups were given with the prepared ST380RF solution at different gradual dosed (10 to 5, 000 mg/kg of b. w).

#### *2.4.2. Study monitoring*

The study was carried out in two phases according to the Lorke method (1983) with some modifications [10].

In the first phase, after regrouping the animals by sex, graded doses of the plant extract (10, 100, 1000 mg/kg of b.w) were administered orally to the various groups. Daily observations of the animals were performed immediately following administration. Since there were no deaths during 24 hours of this preliminary study, the second phase was performed with other groups that were administered graded doses of 2,000 3,000, 4,000, and 5,000 mg/kg of b.w. The control group was treated with the vehicle alone. Then, for 24 -72 hours, the mice in both phases, were observed for changes in behavior, signs of toxicity, and mortality [13]. For each dose of the substance, the number of animals that died was recorded, along with the time of death and the signs shown by the animals before death [14]. Surviving animals were observed for behavioral changes, including variations in skin, membrane, and pupils color, changes in body posture; changes in body weight; movements; rearing; and tremors [15,16]. Finally, euthanized animals underwent necropsy (macroscopic autopsy) to examine vital organs, including, the liver, heart, kidneys, stomach, lungs, and brain, for damage. This procedure identifies pathological changes, such as hemorrhages, congestion, necrosis, and organ discoloration caused by toxic substances [17].

#### *2.4.3. Determination of $LD_{50}$*

The median lethal dose ($LD_{50}$) represents the statistically derived single dose of a substance that can be expected to cause death in 50 % of the animals tested [18]. It was calculated using the following formula, and were interpreted according to the classification rate of HODGE et STERNER (1949), as shows in table 1 [19]. Lower numbers indicate higher toxicity (below 5 mg/kg), while higher numbers indicate lower toxicity (above 15,000 mg/kg) [20,21].

*Oral Median Lethal Dose ($LD_{50}$) =[M0+M1] /2*

**M0**: the highest dose of the test substance that produces no mortality; **M1**: the lowest dose that produces mortality; **$LD_{50}$**: expressed in mg/kg body weight

**Table 1** Classification of $LD_{50}$

| **$LD_{50}$ value** | **Classification** |
|---|---|
| <5 mg/kg | Extremely toxic |
| 5-50 mg/kg | Highly toxic |
| 50-500 mg/kg | Moderately toxic |
| 500-5,000 mg/kg | Slightly toxic |
| 5, 000- 15, 000 mg/kg | Practically non-toxic |
| >15, 000 mg/kg | Relatively harmless |

$LD_{50}$: Lethal dose 50 %

### 2.5. Oral subacute toxicological study: repeated administration

As described in OECD guidelines, the subacute toxicity study involves a 28-day repeated-dose administration to evaluate toxic effects, and histopathological changes (OECD, 1979). This study investigates adverse effects associated with repeated dosing that do not occur immediately, due to product accumulation in tissues or other mechanisms. To identify intersexual variability, the experiment was conducted on both female and male mice [22].

#### *2.5.1. Clinical observation and dosage titration*

The methanolic extract (ST380RF) was administered orally via gavage once daily in the morning at a dose of 5 mg/kg of b.w, for 28 days. The control groups (male and female) were administered only physiological water (9 ‰). All animals had free and unlimited access to food and water. However, to maintain consistent dosages relative to growth, body weights were measured weekly to allow for adjustments in the quantity of extract administered based on any weight changes acquired by the mice [23]. Additionally, the animals were monitored daily for mortality and toxicity. Clinical signs were recorded twice daily (before and after administration) throughout the dosing period. Gross necropsies were performed on all surviving animals at the end of the trial and on those that died during the study.

#### *2.5.2. Organs harvesting*

After the 28-day cycle is complete, the following are recorded: clinical signs, final body weight, and pathological examination. At the end of the drug administration, all surviving mice in each group are humanely sacrificed using chloroform anesthetic. Thereafter, the vital organs (heart, lungs, spleen, kidney and liver) were surgically excised and prepared for morphological and weight-based assessments [24]. This test included determining the relative organ weights and performing a histopathological examination of the organs. Organ weights were expressed in absolute and relative terms (**Pr**) (g and g/100 g of body weight, respectively), calculated using the following formula :

$$Pr(g)=Po/Pa \times 100$$

Where, $\mathbf{P_o}$ : the weight of the organ ; $\mathbf{P_a}$ : the animal's body weight, both in grams

#### *2.5.3. Biochemical and hematological parameters analysis*

At the end of the trial, prior to sacrifice, the surviving animals were fasted for 12 hours. Immediately after euthanized by inhalation of chloroform vapor, 1 ml of blood was collected via cardiac puncture into sterilized dry tubes [25].

Samples stored in EDTA (Ethylenediaminetetraacetate) tubes were used for hematological analysis to determine complete blood count. Hematological evaluation includes red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count, and hematocrit (HTC).

Samples collected in anticoagulant-free tubes were centrifuged at 2580 rpm for 10 minutes and stored at -20 °C. The collected sera were used to determination the effect of biochemical markers of toxicity. This included measuring Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total and Conjugated bilirubin, Total cholesterol, Triglycerides, and Creatinine. Theses parameters were determined at the Medical Biology Analysis Laboratory of the

Central Directorate of the Military Health Service (DCSSM) using automated hematological systems and biochemical analyzers.

*2.5.4. Histopathological studies of vital organs*

The histopathological study was performed according to the method described by Lamb (1981). Vital organs (liver, lungs, spleen, kidneys) of each animal were removed, weighted and preserved in 10 % formalin for 48 hours. Then, the organs were sectioned to a thickness of 4 µm using a microtome after paraffin embedding. The organs were stained with hematoxylin and eosin, fixed between a slide and a coverslip, and examined under a microscope [26]. Tissue processing was conducted by an anatomist certified from the Department of Anatomical Pathology Laboratory of the Joseph Ravoahangy Andrianavalona University Hospital Center (CHU- J.R.A).

### 2.6. Statistical analysis

All experimental measurements were carried out in triplicates and all measured variables were expressed as the mean ± standard error of the mean (SEM). The statistical significance of each test was estimated by Student's t test. Significance of the differences was established at the 5 % level ($p<0.05$).

## 3. Results

### 3.1. Extraction yield

Extraction by methanolic maceration at room temperature using powdered aerial part of *G. phanerophlebia* yielded 27.78 g (7.36 %) of crude extract (ST380RF).

**Table 2** Crude extraction yield on aerial parts of *G. phanerophlebia*

| Plant material | Crud Extract | Mass (g) | Yield (%) | Appearance |
|---|---|---|---|---|
| *Gaertnera phanerophlebia* (aerial parts) 350 g | ST380RF | 27.78 | 7.36 | Green paste |

### 3.2. Oral Acute toxicity

Overall, the study of the oral acute toxicity revealed no adverse behavioral changes in female and male mice at doses ranging from 10 to 5,000 mg/kg of b.w, compared to the control. Additionally, no animals died within 24-72 hours of observation. Conversely, there was no significant change in body weight (Table 3), which is an indicator of toxicity. Macroscopic anatomopathological studies did not show any alteration in the analyzed organs (Fig.3).

Since no mortality was observed at this dose rate, the median lethal dose of the crude extract (ST380RF) via oral route is therefore greater than 5,000mg/kg of b.w. According to the Hodge et Sterner classification (1943) and the OECD guidance (2008), the methanolic extract of the aerial parts of *G. phanerophlebia* is classified as having the lowest toxicity (class 5) [19,27]. These results show that orally administering the aerial parts of *G. phanerophlebia* is not toxic to mice, suggesting a safe human use.

**Table 3** Body weight observation during oral acute toxicity study of the crude extract of *G. phanerophlebia* aerial parts in mice

| Group n° | Dose received (mg/kg b.w) | Average weight of animals (g) | | p-value | Signs of toxicity | Duration of study |
|---|---|---|---|---|---|---|
| | | Before test | After test | | | |
| 1 | 10 | 24.66 ± 1.15 | 24.76 ± 1.02 | 0.915 | | |
| 2 | 100 | 22.66 ± 0.57 | 22.10 ± 2.50 | 0.792 | | |
| 3 | 1,000 | 22.33 ± 1.52 | 22.44 ± 1.36 | 0.930 | | |
| 4 | 2,000 | 28.33 ± 0.57 | 28.20 ± 1.01 | 0.880 | No signs of toxicity | 72 hours |
| 5 | 3,000 | 25.83 ± 0.76 | 25.85 ± 1.84 | 0.447 | | |
| 6 | 4,000 | 26.66 ± 0.57 | 26.71 ± 0.53 | 0.570 | | |
| 7 | 5,000 | 29.50 ± 0.50 | 29.40 ± 0.66 | 0.904 | | |

Values are expressed as mean ± SD; *significantly different from the control at $p<0.05$

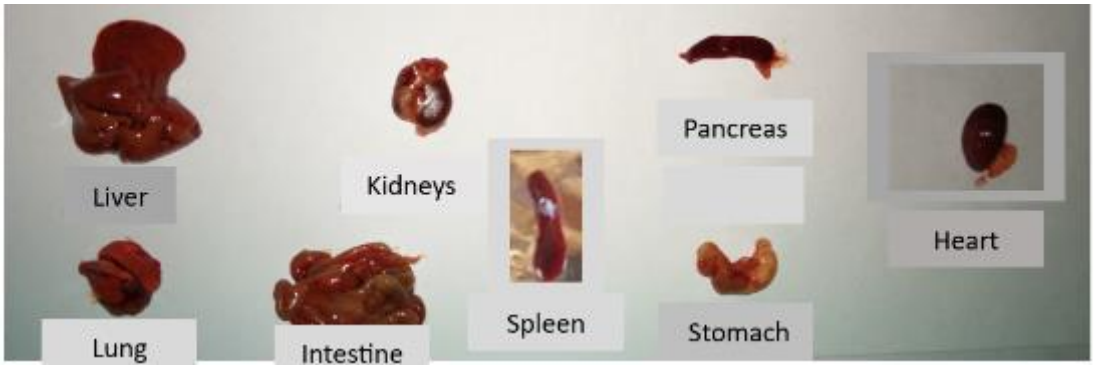


**Figure 3** Macroscopic appearance of vital organs treated with aerial part extract of *G. phanerophlebia* (ST380RF)

### 3.3. Oral subacute toxicity

Observations of subacute toxicity focused on the animal behavior and included monitoring changes in body weight, analyzing hematological and blood biochemical data, and performing an anatomopathological examination of internal vital organs. Administering a daily dose of 5 mg/kg b.w of crude extract (ST380RF) to mice orally for 28 days did not caused mortality. All animals survived to the end of the study.

#### *3.3.1. Animal behavior*

Male and female mice treated with the ST380RF extract showed no changes in general physical appearance or somatomotor function. There were no manifestations of tremors, seizures, salivation, diarrhea, coma, or abnormal behaviors such as self-mutilation or backward walking were observed in either sex. All treated animals were clinically normal during the experimental period.

#### *3.3.2. General growth*

Figure 4 illustrates the results relating to the weight change of male and female mice that survived the 28-days subacute toxicity study. The figure shows how the treatments affected the growth of the mice compared to their body mass on the first day of the experiment. For male mice, there was no significant difference in mean body mass variation between those treated with ST380RF extract (5 mg/kg b.w) and the control group ($p = 0.2$). The weight of the male mice remained constant throughout the treatment. In contrast, the average mass of the treated female mice increased highly significantly ($p = 0.000$).

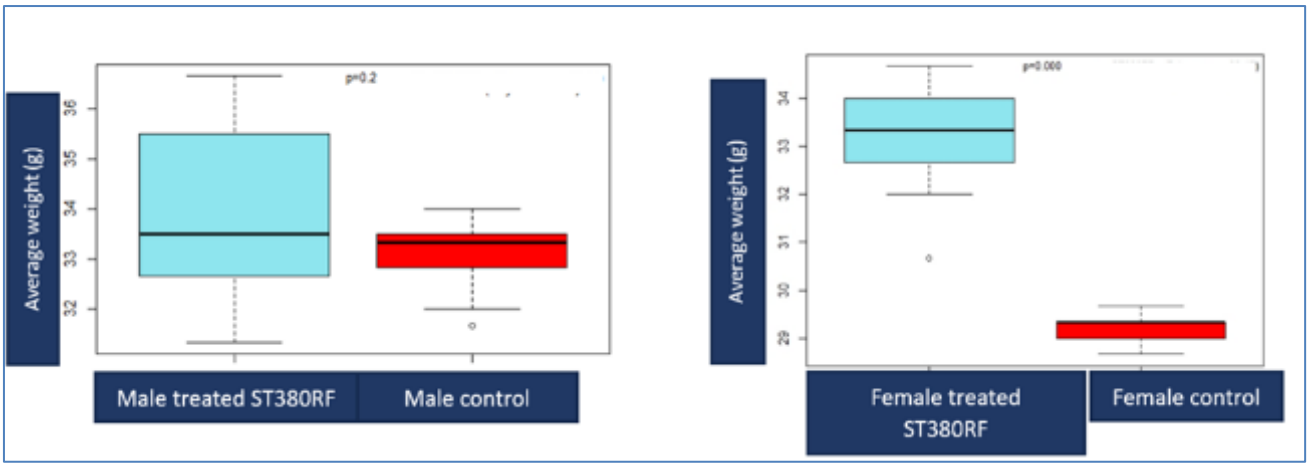


**Figure 4** Comparison of mean body masses of male and female mice treated with ST380RF extract in oral subacute toxicity relative to the control

### 3.4. Biochemical and hematological parameters

At week four, all mice in each group were sacrificed, and the biochemical and hematological balances of each group were examined in relation to the control using Principal Component Analysis (PCA). The studied parameters were : Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Triglycerides, Cholesterol, Alkaline phosphatases (ALP),

Hight Density Lipoprotein (HDL), Total protein, Low Density Lipoprotein (LDL), Albumin and Gamma GT, Creatinine, Urea, Uric acid, Calcium, Cholesterol, Blood glucose and Complete Blood Count (red blood cells, white blood cells, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Content (MCHC), Mean Corpuscular Hemoglobin Concentration (MCHC), and platelets.

**Effects on hematological parameters:** Regarding hematological parameters, variable effects were observed in the tested animals. Animals treated with the crude extract of *G. phanerophlebia* (ST380RF) experienced increase MCV (Mean Corpuscular Volume), hemoglobin, WBC (white blood cells), and platelet count after repeated oral administration of 5 mg/kg of b.w for 28 days (Table 4).

**Table 4** Effects of subacute oral administration of the crude extract (ST380RF) of the aerial part of *G. phanerophlebia* on hematologic parameters in mice

| **Parameters** | **Control group** | | **Treated group (ST380RF: 5 mg/kg b.w)** | |
|---|---|---|---|---|
| Sexes | Males | Females | Males | Females |
| WBC (T/l) | 4.4± 0.42 | 2.066 ± 0.23 | 4.133 ± 0.40 | 6.7 ± 0.30* |
| RBC (g/l) | 9.2333 ± 0.464 | 8.873 ± 0.45 | 8.893 ± 0.63 | 8.97 ± 0.29 |
| Hematocrit (%) | 35.666 ± 0.33 | 42.333 ± 0.62 | 40.5 ± 0.04* | 40.1 ± 0.81 |
| Platelets (g/l) | 618.333 ± 0.69 | 440.001 ± 0.93 | 587.5 ± 0.95* | 567.2 ± 0.61* |
| Hemoglobin (g/dl) | 14.133 ± 0.83 | 14.663 ± 0.71 | 13.766 ± 0.83 | 13.8 ± 0.48 |
| MCV (fl) | 45.666 ± 0.73 | 49.221 ± 0.94 | 46.666 ± 0.24 | 47.12 ± 0.81* |
| MCHC (pg) | 15.466 ± 0.69 | 17.07 ± 0.32 | 15.15 ± 0.36 | 15.17 ± 0.12 |
| MCHC (g/dl) | 32.366 ± 0.16 | 33.65 ± 0.04 | 32.9 ± 0.16 | 31.995 ± 0.04 |

*Values significantly different at ($p<0.05$) from those of the control group. Each value represents the mean ± s.e.m of the values for 3 animals; WBC: white blood cells; RBC: red blood cells; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Haemoglobin Content; MCHC: Corpuscular haemoglobin Concentration

**Effects on biochemical parameters:** Subacute treatment with the crude extract of *G. phanerophlebia* (ST380RF) at a dose of 5 mg/kg b.w caused significant changes in liver and renal markers, as well as other general parameters. Male mice that received increased levels of cholesterol, total protein, and LDL. In contrast, females exhibed increased AST, ALT, ALP, albumin, HDL, and triglyceride levels. In both sexes, increases in uremia, in blood glucose, cholesterol, and calcium were observed compared to the control group.

**Table 5** Effects of subacute oral administration of the crude extract (ST380RF) of the aerial parts of *G. phanerophlebia* on biochemical parameters in mice

| **Parameters** | **Control group** | | **Treated group (ST380RF: 5 mg/kg b.w)** | |
|---|---|---|---|---|
| Sexes | Males | Females | Males | Females |
| Urea (mmol/l) | 9.793 ± 0.70 | 9.563 ± 0.38 | 12.44 ± 0.90* | 12.745 ± 0.60* |
| Uric acid (µmol/l) | 557.12 ± 0.94 | 690.08 ± 0.61 | 530.17 ± 0.09 | 538.22 ± 0.36 |
| Creatinine (µmol/l) | 52.333 ± 0.23 | 39.664 ± 0.94 | 54.12 ± 0.48 | 34.5 ± 0.67 |
| Blood glucose (mmol/l) | 5.853 ± 0.96 | 5.64 ± 0.56 | 8.323 ± 0.35* | 6.44 ± 0.73 |
| Total protein (g/l) | 72.3 ± 0.30 | 64.533 ± 0.81 | 82.333 ± 0.46 | 93.666 ± 0.34 |
| Albumin (g/l) | 39.333 ± 0.94 | 38.122 ± 0.41 | 36.667 ± 0.85* | 46.333 ±0.54* |
| AST (UI/l) | 132.666 ± 0.88 | 240.15 ± 0.43 | 169.5 ± 0.28* | 503.14 ± 0.83* |
| ALT (UI/l) | 78.333 ± 0.08 | 167.333 ± 0.97 | 93.12 ± 0.82* | 66.666 ± 0.58* |

| | | | | |
|---|---|---|---|---|
| ALP (UI/l) | 103.85 ± 0.33 | 99.666 ± 0.32 | 90.523 ± 0.04* | 73.966 ± 0.36* |
| ALT/ALP (UI/l) | 0.754 | 1.678 | 1.028 | 0.9.1 |
| Total calcium (mmol/l) | 1.066 ± 0.14 | 1.71 ± 0.04 | 2.63 ± 0.39 | 1.70 ± 0.49 |
| Total cholesterol (mmol/l) | 2.86 ± 0.33 | 2.61 ± 0.74 | 3.06 ± 0.12 | 2.72 ± 0.25 |
| HDL (mmol/l) | 1.761 ± 0.29 | 1.233 ± 0.34 | 1.16 ± 0.12 | 1.99 ± 0.48 |
| LDL calculated (mmol/l) | 1.22 ± 0.56 | 0.88 ± 0.89 | 1.315 ± 0.30 | 1.07 ± 0.10 |
| Triglyceride (mmol/l) | 1.506 ± 0.34 | 1.29 ± 0.21 | 1.29 ± 0.13 | 3.78 ± 0.72* |

*Values significantly different at ($p<0.05$) from those of the control group. Each value represents the mean ± s.e.m of the values for 3 animals.

*3.4.1. Organ weight effects*

The effects of repeatedly administering the crude extract ST380RF orally were studied in both sexes and compared to the control group. The study examined the effects on the weight of vital organs (liver, spleen and kidneys). The results are presented in the following text and figure 5.

- **Liver:** No significant difference was observed in the treated group for either male ($p = 0.724$) or female ($p = 0.0983$) mice.
- **Spleen:** An increase in organ weight was observed in the treated females, and the difference was highly significant ($p = 0.000$).
- **Renal:** Compared to the control group, variations in organ weight were observed according to sex. Treated males showed a significant increase in kidney weight ($p = 0.0316$), while treated in females, a non-significant decrease ($p = 0.155$).

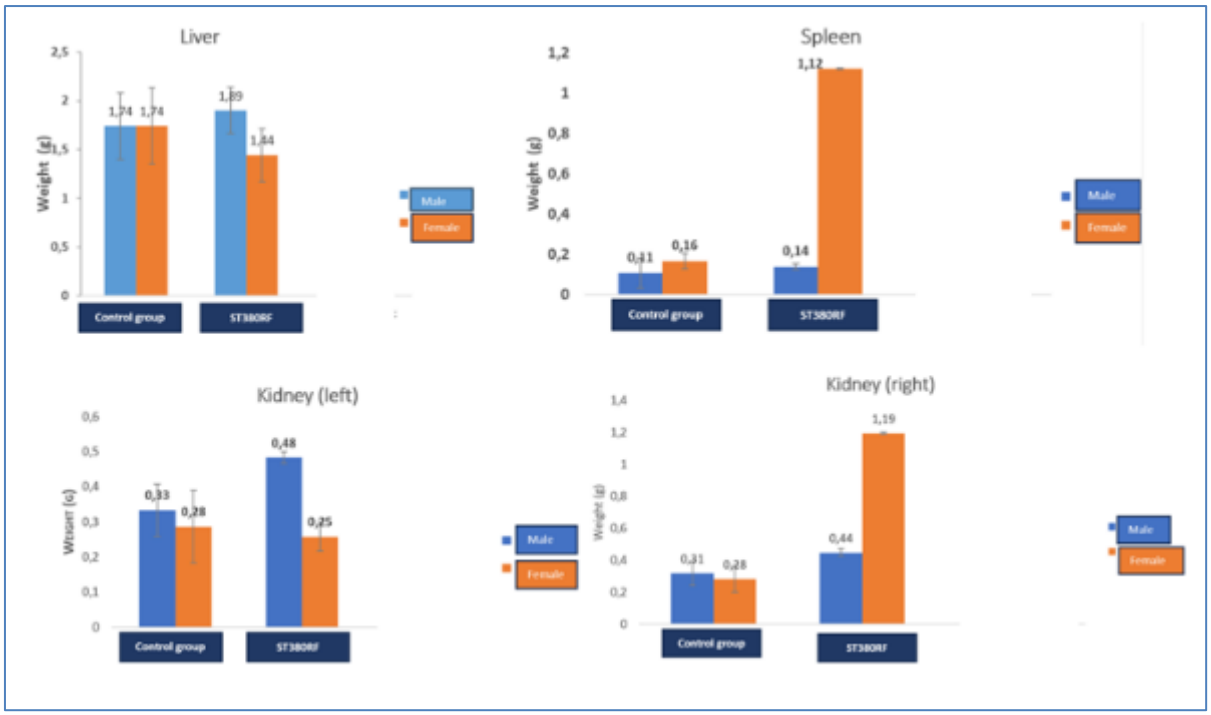


**Figure 5** Effects of subacute oral administration of the aerial parts extract (ST380RF) of *G. phanerophlebia* on relative organ weights in mice

### 3.5. Anatomopathological aspects of vital organs

The results of the histological aspects of the organs are presented in the Table 6. Gross and histopathological examination of vital organs in the treated group showed no serious alterations or remarkable histological changes in the kidneys and spleen, compared to the controls. However, the livers of male mice that received the ST380RF extract contained discrete focal microvacuoles.

**Table 6** Results of histological studies of organs treated with crude extracts of aerial parts of *G. phanerophlebia* (ST380RF) after oral subacute toxicity in mice

| Sexe | **Untreated group** | | |
|---|---|---|---|
| | Kidneys | Liver | Spleen |
| Male | No tissue damage | No tissue damage | No tissue damage |
| Female | | | |
| | Treated group with ST380RF | | |
| Male | Mild lymphocytic inflammation | Focal and discrete microvacuole | No tissue damage |
| Female | No tissue damage | Vacuolar hepatocyte (macro and micro, scattered), Scattered nodular lymphocytic inflammation | No tissue damage |


**Figure 6** Histological observation of vital organs

## 4. Discussion

This study aimed to evaluate the acute and subacute oral toxicity of the methanolic extract of the aerial parts of *G. phanerophlebia*. The RUBIACEAE family is a promising source of herbal medicines. Many species are endemic and are widely used to treat various diseases in traditional medicine. Likewise, the aerial parts of *G. phanerophbleia* have many medicinal properties described in the Malagasy pharmacopoeia [3]. While this beneficial plant is interesting, it should be evaluated for broader applications considering its health benefits and safety. Since the pharmacological efficacies of traditional medicines alone do not validate human use, toxicity studies like this one determine potential, safety thresholds, and ensure that there are no significant adverse histopathological changes in vital organs [5].

Based on the toxicity results, the methanolic extract of *G. phanerophlebia* (ST380RF) is considered safe and non-toxic. There were no harmful effects or mortality in mice at doses up to 5,000 mg/kg of b.w via the oral route after a single dose was administered to both sexes. Acute toxicity studies are usually carried out on laboratory animals using a sufficient high dose to produce morbidity or mortality from the substance in question, based on previous report of its toxicity or the toxicity of structurally related compounds [28]. However, the $LD_{50}$ has limitations : as it is a preliminary assessment, it can be influenced by factors such as animal species, sex, age, and time of day. It only concerns mortality and provides no information on the reaction mechanisms involved or the nature of the damage caused. This is why a toxicity study by determining the toxic effects of repeated administration over 28 days was necessary. These acute toxicity results provide guidelines for selecting doses for the subacute studies, which may be more clinically more relevant [29].

The 28-day subacute study of the aerial parts extract was initiated due to the absence of observed acute mortality, which suggests an $LD_{50}$ greater than 5,000 mg/kg of b.w. The study revealed cumulative toxic effects at a low dose of 5 mg/kg of b.w. Histopathology demonstrated that chronic and repeated exposure causes renal and hepatic damage. The liver is

the metabolic site, and the kidneys are the primary route of elimination. This can lead to structural or functional changes [30]. In fact, the repeated dosing showed that lower, non-lethal doses can cause significant accumulation-based toxicity over time. These results demonstrate the importance of repeated toxicity study because extracts showing no acute mortality (class 5 or better) cannot be considered safe for chronic consumption without subacute testing [31].

The type of organs damage was determined by examining the biochemical parameters. The ALT/ALP ratio is important for valuating liver damage caused by hepatotoxins. In this study, an ALT/ALP ratio of less than 2 in both sexes indicates that the liver damage induced by *G. phanerophlebia* (ST380RF) extract, is cholestatic rather hepatocellular [32,33]. A low ratio indicates mild histological changes, such as microvacuoles and nodular lymphocytic inflammation, which are not strongly associated with acute liver dysfunction because biochemical markers are more definitively indicate the type of injury.

Creatinine, urea and electrolytes are common parameters measured to assess kidney's function [34]. Based on the provided results, administering ST380RF at a dosage of 5 mg/kg of b.w does not cause nephrotoxicity or impair kidney function. This is indicated by stable creatinine levels, which are a key indicator of glomerular filtration rate (GFR), as well as the absence of negative histological or morphological effects. The observed increase in urinary urea (12.44 ± 0.90 mmol/l vs control 9.793 ± 0.70 mmol/l) suggests improved kidney filtration rather than damage. The absence of morphological or histological changes in the kidneys confirms that ST380RF is not harmful but may even improve their function.

Measuring hematological parameters is an essential method for assessing the toxic potential of plant extracts or foreign substances. Variations in blood constituents, for example, can have high predictive value for toxicity in humans [35]. In this study, the absence of significant variations in red blood cell count, hemoglobin, hematocrit, and MCV in mice indicates that the ST380RF extract of *G. phanerophlebia* does not alter the production, destruction, morphology, or osmotic fragility of red blood cells. Furthermore, the stability of the white blood cell count suggests that the extract has a minimal impact on the immune system. These tests are relevant assessing the toxicity of a substance since changes in an animal's hematological system have greater predictive value for human toxicity [36].

During subacute toxicity evaluation, changes in body weight serve as a sensitive indication of an animal's general health status [37]. According to standard preclinical toxicology guidelines, the absence of significant body weight changes in animals over a 28-day study suggests that the ST380RF extract is unlikely to cause adverse metabolic, nutritional, or toxicological effects at the tested dosage. Stability of body weight compared to the control group suggests a favorable safety profile regarding systemic toxicity.

The outcomes of this experiment and the present study suggested that, while the methanolic aerial parts extract of *G. phanerophlebia* is safe, it may be toxic at a repetitive dosage. This hypothesis has been verified and is valid for any use of medicinal plants for therapeutic purposes.

## 5. Conclusion

These results represent the first toxicological investigations of the methanolic extract from the aerial parts of *G. phanerophlebia*. This scientific data could help determine the conditions for the oral administration of this plant to avoid or limit adverse effects. The results also allow for consideration of its potential use in herbal medicine and justify its use in Traditional Malagasy Medicine. Consequently, further research is needed to better understand its mechanism of action. Herbal medicine requires more thorough evaluation of its efficacy and safety using toxicological techniques, given the need to validate medical use before clinical application.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

All authors declare that they have no conflicts of interest.

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