

Ethanollic leaf extracts of *Uvariondendron Calophyllum*: investigations on acute and subacute oral toxicity

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Abstract

Background: *Uvariodendron calophyllum* is used in traditional medicine for the treatment of infectious diseases and has been shown to possess some biological properties. This study aimed to assess the acute and subacute toxicological profile of the ethanolic leaf extract of *Uvariodendron calophyllum* (UCL EtOH).

Methods: The acute and subacute toxicity experiments on mice were done following the OECD 425 and OECD 407 standards, respectively. In the investigation on acute toxicity, mice received an oral dose of 2000 mg/kg and were monitored for the first four hours, then throughout the course of 24 hours, and once per day for 14 days. UCL EtOH was administered orally to male and female mice for 28 days at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, respectively, in the subacute toxicity experiments. The body weight, organ weight, hematological, biochemical markers, and histological alterations were assessed.

Results: The acute toxicity of UCL EtOH revealed LD₅₀ values >2000 mg/kg with no symptoms of mortality. The subacute toxicity of UCL EtOH at different dosages demonstrated no significant adverse responses to the organs or hematological parameters.

Conclusion: The oral lethal dose was greater than 2000 mg/kg. 400 mg/kg of the extract daily for 28 days had no observable adverse effects (NOAEL) in both male and female mice.

Keywords: Acute toxicity study; biochemical parameters; hematological parameters; subacute toxicity study; *Uvariodendron calophyllum*.

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Citation on this article: Menkem Zeuko'o E, Toghueo Kuipou RM, Ngouana V, Majoumou MS, Keumoe R, Siwe Tchokomeni G, Fekam Boyom F. Ethanolic leaf extracts of *Uvariondendron Calophyllum*: investigations on acute and subacute oral toxicity. *Investigational Medicinal Chemistry and Pharmacology* (2025) 8(3):123; Doi: <https://dx.doi.org/10.31183/imcp.2025.00123>



Background

Herbal remedies made from medicinal plants have gained notoriety in developing nations' healthcare systems for being misrepresented as safe and coming from natural sources [1–3]. These bioactive substances from conventionally used medicinal plants are nevertheless frequently utilized as self-medication because it has been determined that they are safe without comprehending the potential health impacts [4,5]. Information on the toxicological profile and negative effects of these products is lacking. Numerous national and international regulatory bodies have been prompted by the toxicity of herbal products to create and put into practice guidelines for evaluating, observing, and avoiding toxicity. The WHO's Uppsala Monitoring Committee (UMC) gathers and disseminates data on the harmful effects of herbal drugs, while the Organization for economic cooperation and Development (OECD) establishes standards for carrying out different toxicity studies. Most frequently, toxicity tests are employed to investigate side effects or endpoints like cancer, cardiotoxicity, and skin/eye irritation. Toxicology testing is useful for further clinical studies and for calculating the No Observed Adverse Effect Level (NOAEL). To determine the additional range of doses in animal experiments and to explain the likely clinical symptoms elicited by the test chemicals under investigation, acute and subacute toxicity studies are necessary. It also plays a crucial role in determining the therapeutic index of chemicals and medications [6]. If it is determined that medicinal plants have the necessary potential to evolve into pharmaceutical substances, the results of animal toxicity studies will be crucial for making an informed decision about their safety [8]. The screening of possible toxicity of natural products is attention-seeking, even if many therapeutic plants may have pharmacological activities that are advantageous to human health if consumed [2,7,8]. Long-term usage of natural treatments without any indication of a health risk may show that the medication is safe [2,5,9]. Despite the widespread use of plants in traditional medicine, no thorough study has been done to determine the toxicity of any plant. However, local Cameroonians use plants like *Uvariadendron calophyllum* to treat many different diseases, including malaria [10]. These assertions have been supported by interesting research on extracts and essential oils from various plant sections, as well as by scientific studies that have been published. The antiplasmodial activities of extracts and essential oils [10–12], the antimycobacterial activity of extracts and fractions [13,14], antifungal activities and the anti-yeast activities of extracts [15–17] are a few examples of these activities. It is crucial to check the toxicological profile of this plant to ensure the safety and efficacy, given the overall confirmed pharmacological activity of these plants and the rise in the usage of plant-based goods. Consequently, this study sought to assess the ethanolic leaf's safety *Uvariadendron calophyllum* extract, both acute and subacute.

Methods

Plant material and preparation

On September 11, 2011, *U. calophyllum* leaves were gathered at Mount Kalla near Yaoundé, Cameroon. A voucher specimen with the identification code 28734/SFR/CAM was deposited at the National Herbarium of Cameroon. After being cleaned, the leaves were dried at 30°C in the shade. An electric grinder was used to compress the dry leaves. Then, 2 liters of 95% ethanol were used to extract 500 grams (500g) of the resulting powder over the

course of 72 hours at room temperature. The mixture was then filtered before being evaporated with a rotary evaporator (Rotavapor BÜCHI 011) under low pressure. The obtained crude extract was evaporated and stored at 4 degrees until use.

Preparation of test solutions

The crude extract obtained was dissolved in distilled water. Afterward, the mixture was stirred (3–5 min) using a magnetic stirrer. The solution obtained was kept in a refrigerator after each oral administration. The volume of solutions selected for administration was determined by the following mathematical formula.

$$V=D \times P / C$$

V=volume of solution selected to be administered (mL),

D=dose (mg/kg),

P=weight of animal (kg), and

C=concentration of the solution selected to be administered (mg/mL).

Animals

The animals used in our investigation were male and female BALB/c mice aged between 3 weeks to 2 months and weight 18 g and 30 g provided, respectively, by the animal house of the Faculty of Medicine of the University of Yaoundé I, where they were submitted to natural day/night cycles. They were fed a standard laboratory diet, with unrestricted access to tap water. Each experiment was approved by the Institutional Ethical Committee, which followed all guidelines set forth by the European Union for the protection of animals used in research (CEE Council 86/609; Ref N° FWA-IRD 0001954).

Ethical Approval

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Acute oral toxicity profile

The oral acute toxicity of - UCL EtOH was evaluated using BALB/C mice according to the procedure outlined by the OECD 425 guidelines (OECD, 2008), with some modifications. Following the fasting period, the mice were weighed and the volume of the extract was determined with respect to their body weight. The volume of the extract given to the mice was 2mL/100g body weight. The extract was suspended in distilled water. A limit dose of 2000mg/kg body weight of UCL EtOH was administered to 3 female mice (group 1), while the control group (group 0) received vehicle, 10% ethanol. Food was provided for the mice 1 to 2 hours after treatment. The animals were observed 30 minutes after, followed by hourly observations for 8 hours and once a day for the next 13 days. The food and water consumption were noted daily for 14 days. All observations were systematically recorded, with individual records being maintained for each animal. Surviving animals were weighed, and daily visual observations were

performed to assess mortality, behavioral patterns, changes in physical appearance, injuries, pain, and signs of illness. Animals were under observation for a period of 14 days [18,19].

Sub-acute oral toxicity

With some modifications to the procedure outlined by OECD 407 guidelines (OECD, 2008), the oral subacute toxicity of UCL EtOH extract was assessed in BALB/c mice. Six groups of five male and five female mice each were created from a total of sixty mice. The test groups got the test extract at doses of 100, 200, and 400 mg/kg every day for a total of 28 days, whereas the control group received 10% ethanol. The extract satellite group received 400 mg/kg of extract, while the control satellite group received 10% ethanol. For a total of 28 days, the animals were monitored for indicators of toxicity and mortality. Mice's individual body weights were measured at first and then every two days till the experiment was over. Individual animals' feed intake and weight gain were tracked weekly. On the 29th and 41st days, blood samples were taken in EDTA and dry tubes for hematological and biochemical analyses after all animals were sacrificed under anesthesia, diazepam (30 mg/kg) and ketamine (15 mg/kg). Animal deaths and unusual clinical symptoms were monitored daily throughout the experiment.

Hematological Parameters

The blood samples were analyzed for white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), erythrocyte sedimentation rate (ESR), platelets, clotting time, and packed cellular volume (PCV) using an automated machine « globular counter HYCEL Diagnostics (Celly, type CA 4001 series N°: CA40D 1975).

Organs Weight

The weights of the heart, lungs, liver, kidneys, ovary, and testes were among the vital organs whose quantitative data were evaluated. Organs from the sacrificed animals were meticulously removed and placed in a petri dish with 10% normal saline. The isolated organs were carefully cleaned, patted dry with cotton wool, and weighed using an exact balance. Each organ's weight was normalized to a mouse's 100 g body weight.

Histopathologic examination

The liver, kidneys, lungs, and spleen tissue samples were fixed in 10% formalin, overnight dehydrated using an improved ethanol series, and embedded in paraffin blocks. Hematoxylin and eosin (H&E) were used to stain ultrathin sections after they had been dewaxed by xylene and rehydrated using a series of ethanol that had been degraded. The histopathologic examination was carried out by a pathologist using an optical microscope while they were blinded to the treatments in order to observe the various abnormalities [20].

Data analysis:

The gathered information was entered into Excel, exported to SPSS 20.00 and GraphPad Prism 5.00, and the outcomes were presented as Mean SEM (standard error of mean). One-way analysis of variance (ANOVA), the significance by F-test at 5% probability, and contrast between the means by Tukey-Kramer test were employed to find differences between the treatments in trials

comparing more than two means. The GraphPad Prism 5.00® tool (GraphPad Software, Inc., San Diego, CA) was used for all statistical analyses.

Results

Acute Toxicity

In female mice given UCL EtOH at a dose of 2000 mg/kg body weight, there were no deaths noted. When compared to the control at the end of the 14 days of general observation, the test animals did not exhibit any appreciable behavioral changes such as shaking, diarrhea, salivation, breathing, impairment in food intake, water consumption, postural abnormalities, hair loss, sleep, lethargy, restlessness, or changes in physical appearance such as eye color, mucous membranes, salivation, skin/fur effects, body weight, or injury. The animals' body weights (Table 1) and the weights of their critical organs (Figure 1) were computed. Weight changes were not found to be substantial. Figure 1 depicts how the extract affected the relative weights of the major organs. No appreciable variations from the corresponding controls were found. The outcomes showed that vital organs such as the kidney, liver, heart, lungs, and spleen were not negatively impacted during treatment. In comparison to controls, a macroscopic examination of the treated animals' organs revealed no differences in color. The liver, kidney, lungs, heart, and spleen organs from both control and treatment mice that were autopsied at the conclusion of the trial showed no obvious abnormalities in the histopathological study.

Sub-acute oral toxicity

The animals studied did not die because of the ethanol extract of *U. calophyllum* at doses of 100, 200, and 400 mg/kg given orally every 24 hours for 28 days. During the experiment, no toxicity that could be seen was found. Figures 2 and 3 show how the body weight of the control and extract-treated mice changed. The weight gain did not differ noticeably from that of the control group. After receiving an ethanol extract of *U. calophyllum* for 28 days, mice's body weight gradually increased at doses of 100, 200, and 400 mg/kg, which may be a sign that the animal's nutritional status has improved. In most cases, reducing body weight gain and internal organ weight is a straightforward process after being exposed to harmful compounds, and a sensitive index of toxicity.

Effects of extracts on the body weight of mice

There was a constant increase in the weight of male and female mice in the different groups during the experimental period (Figures 2 and 3).

Effects of extracts on Relative Organ Weight

The macroscopic examinations did not show any change in organ color and weights of all animals (Figures 4 and 5). Macroscopic examination of the organs (liver, kidney, lungs, heart, and spleen) at the end of the experiment of males and females' mice treated with the extract at 100mg/kg, 400mg/kg, control 10%EtOH and satellite 400mg/kg showed no changes in color compared to control. Inflammation of the liver and spleen was observed in the groups treated with 200 mg/kg and influenced the relative organ weight. Figures 4 and 5 showed no variation in the relative organ weights of the kidney, lungs, heart, testes, ovaries, and stomach of

the treated groups, male and female mice with respect to the control.

Effects of extracts on hematological parameters

The effects of the plant extract on the red blood cell (RBC) components and white blood cell differentials are summarized in Tables 2 and 3. Normal values were obtained for lymphocytes, MID mean corpuscular hemoglobin concentration (MCHC), Hemoglobin (HGB), RBC, and WBC. A significant ($p < 0.05$) increase in hemoglobin, RBC and WBC levels was observed compared to the control. No significant ($p \geq 0.05$) changes were observed in the levels of procalcitonin (PCT), mean corpuscular hemoglobin (MCH), lymphocytes (Lym), and mean MCHC, respectively in all the treated animals compared to the control, while a significant decrease occurred in mean corpuscular volume (MCV). In the female, normal values were observed for lymphocytes (Lymphocytes), MID, MID%, RBC, Hematocrit (HCT), MCHC, HGB, and Mean platelet volume (MPV).

Effects of extracts on histopathological parameters of liver, lung, and kidney

When compared to the control, there were no visible signs of inflammation or color changes in the internal organs (Figures 6, 7, and 8).

Discussion

In this investigation, mice in the treatment and control groups received the crude extract (UCL, EtOH) and a vehicle, respectively. Up until day 14, the mice were observed every day for any hazardous indications and death. One of the most significant findings to show the toxic effects on organs in the treated groups is the clinical symptom [21]. Mice that were given a single dose of 2000 mg/kg of crude extract (UCL, EtOH) orally during the 14-day observation period displayed no overt signs of distress, and there were no discernible indicators of toxicity or death. All the mice became heavier, but their behavior remained largely unchanged. Skin, fur, and other physical characteristics, including eyes, were confirmed to be in normal condition. Although the mice's body weight increased (Table 1), this might mean that the extract's administration had only a minimally toxic effect on the mice's growth. Additionally, the measurement of food and water intake is crucial when examining the safety of a product intended for therapeutic use because it is necessary for the animal's physiological well-being and the achievement of the proper response to the tested fractions [22,23].

Mice's internal organ weight and body weight changes upon exposure to harmful chemicals typically reflect the toxicity [24]. Body weight fluctuations are markers of the harmful effects of medications and chemicals and are important if the body weight loss is greater than 10% of the starting weight [25,26]. However, the tested groups showed increased body weight. Animals' organ weight is a significant indicator of their physiological and pathological conditions. The relative organ weight is crucial for determining whether the organ was damaged. The main organs impacted by the metabolic disorder include the liver, heart, lungs, spleen, stomach, ovaries, and kidneys (both left and right), as well as toxicant-induced responses [27].

Both the control and treated groups both gained body weight, and the difference was not statistically different. The administration of the crude extract (UCL, EtOH) did not hurt any of

the critical organs' organ weights. This investigation concluded that *U. calophyllum* extracts had essentially no acute hazardous effects and have an LD₅₀ value greater than 2000 mg/kg. The limit test approach serves as a guideline for categorizing the fractions based on the anticipation at which dose level the animals are likely to survive [28]. It is not designed to determine a precise LD₅₀ value. The OECD's recommended chemical labeling and acute systemic toxicity classification categorizes the *U. calophyllum*. The lowest toxicity rating, class 5, or LD₅₀ > 2000 mg/kg, was given to the crude extract. Drugs with LD₅₀ values by oral route more than 2000 mg/kg are regarded as harmless or basically non-toxic. This is the first time the crude extract's acute oral toxicity has been brought to light [29,30].

The subacute dose 1/5, 1/10, and 1/20 of 2000 mg/kg was chosen based on the mice's LD₅₀ value which kept the mice alive. In the 28-day oral toxicity study with repeated doses, no evidence of treatment-related illness or death was seen in any of the animal groups. Changes in body weight increase and internal organ weight following exposure to a few potentially harmful compounds might indicate toxicity [25,26]. The loss of more than 10% of the initial body weight is regarded as statistically significant, and changes in body weight are indicators of the harmful effects of medications and chemicals [25,26]. An increase in animal body weight is rather than the negative side effects of medications or chemicals; this is more closely tied to the buildup of body fat. However, reductions in the body weight of animals used in toxicity studies may be related to their regular physiological adaptations to plant extracts or chemicals, which cause them to have a decreased appetite and, as a result, consume fewer calories. High concentrations of plant extracts may also cause the animals to get stressed, which may cause them to eat less and lose weight [29].

The hematopoietic system is one of the most sensitive targets for hazardous substances and a crucial indicator of the physiological and pathological status in both humans and animals [29,31]. Changes in the hematological system have a better predictive value for human toxicity. Hematological indices analysis revealed that *U. calophyllum* extracts (UCL, EtOH) had minimal to no impact on WBC, Lym, RBC, HGB, MCV, and MCHC, but dramatically enhanced HCT at 400 mg/kg body weight. This suggests that, given the substantial rise in HCT levels, *U. calophyllum* may only be having a moderate impact on erythropoiesis. Nevertheless, the normal MCV and MCHC levels suggested that the morphology and the red blood cells' osmotic fragility were unaffected [32,33].

All treatment groups experienced a significant rise in WBC, Lym, HCT, and MCHC levels following administration of the *U. calophyllum* ethanolic extract. The *U. calophyllum* extract's considerable rise in WBC and Lym suggests that it may include biologically active compounds that can strengthen the immune system. The aforementioned rise may potentially be the result of an imbalance in the rates of synthesis and catabolism of hematological parameters [34–37]. The presence of steroid saponins, which may be known to have hemolytic action, may be the cause of the large decrease in HCT shown in mice treated with *U. calophyllum* extract and is suggestive of anemia. In contrast, no discernible differences in MCHC and MCH levels were found in the treated animals; the *U. calophyllum* extract could not have produced regeneration anemia compared to the control group. The extract could be deemed non-toxic to the immune systems; nonetheless, because the results of the experiment were within the reference range for most parameters.

Additionally, after exposure to a toxic substance, a reduction in body and internal organ weights is thought to be a sensitive indicator of toxicity [38]. Because of their ability to

accurately predict toxicity and strong correlation with histological alterations, the liver and kidney weights were thought to be helpful in toxicity studies. It is typically a target organ of toxicity because there is little inter-animal heterogeneity. The liver is also recognized as the main detoxifying organ. In comparison to histology, weight alterations were observed to correlate with toxicity less frequently and with less sensitivity [39]. Consequently, it could be said that the kidney and liver can be the targets of subacute oral poisoning. The livers of the treated rats showed no histological alterations, which supported this. In the metabolism and excretion of medications or plant products, the liver and kidneys play crucial roles. These organs may become toxic or suffer cell damage as a result of exogenous chemicals and their metabolites [34,37,40].

The mouse's liver (Figure 6) has four main lobes with different lobation patterns. Hepatocellular fatty alteration is typical in several inbred mouse strains (BALB/c). The central vein, a tributary of the hepatic vein, is in the middle and at the corners of the portal triad, which is made up of branches of the hepatic artery, the portal vein, and the bile duct. The fibrous capsule of the liver projects connective tissue septa into the liver tissue, dividing the liver lobes into indistinct lobules. Hepatocytes, huge polygonal cells with large central nuclei that are organized in cords, make up the liver parenchyma. The sinusoids that are surrounded by fenestrated endothelium are between the hepatocyte cords. The Kupffer cells are found in the sinusoids and are connected to the

endothelium lining, and macrophages that live in the liver. The bile canaliculi are formed by the apical surfaces of nearby hepatocytes, and they come together to form bile ducts that are bordered by cuboidal epithelium [41].

Like the lungs of the rat, the mouse's lungs (Figure 8) are covered by visceral pleura and comprise an undivided left lung and a right lung divided into four lobes (Figure 6, control). The only bronchi in the mouse that has cartilage are the primary bronchi, which split off from the trachea. These bronchi are lined with respiratory epithelium, a ciliated pseudostratified columnar epithelium with goblet cells scattered throughout. The smaller intrapulmonary bronchi, the terminal bronchiole, and the respiratory bronchiole are the next branch in the bronchial tree. The smaller bronchi are lined by ciliated simple columnar epithelium, whereas the terminal and respiratory bronchioles are lined by ciliated simple columnar epithelium. As the airways get smaller, their epithelium gets simpler, and their walls become thinner with less connective tissue and smooth muscles by cuboidal epithelium [42–44]. The standard index for assessing treatment-related pathological alterations in tissues and organs is the histopathological examination. The results of the histopathological study often concur with those of the body weight and organ weight measurements [20,27,45]. The liver, kidney, and lung histopathology analyses did not reveal any morphological changes to the cellular structures.

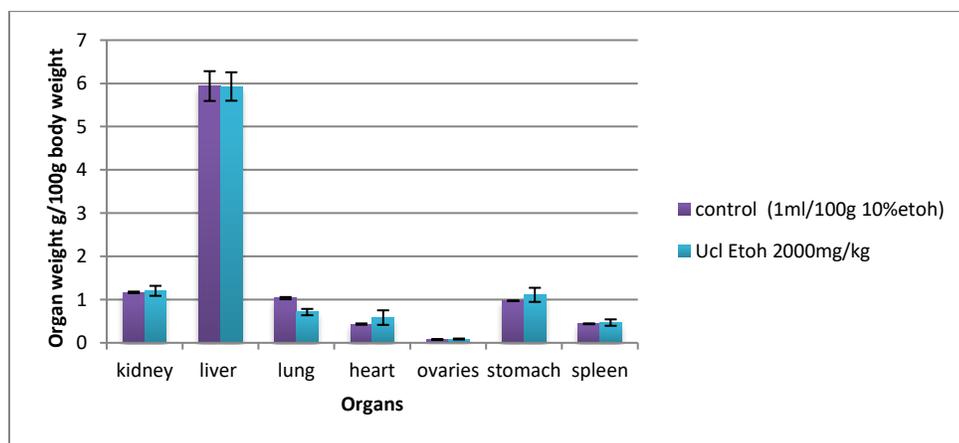


Figure 1. Relative Organ Weight of mice (ROW) per 100 g body weight recorded at the end of the acute toxicity study from experimental mice after 14 days

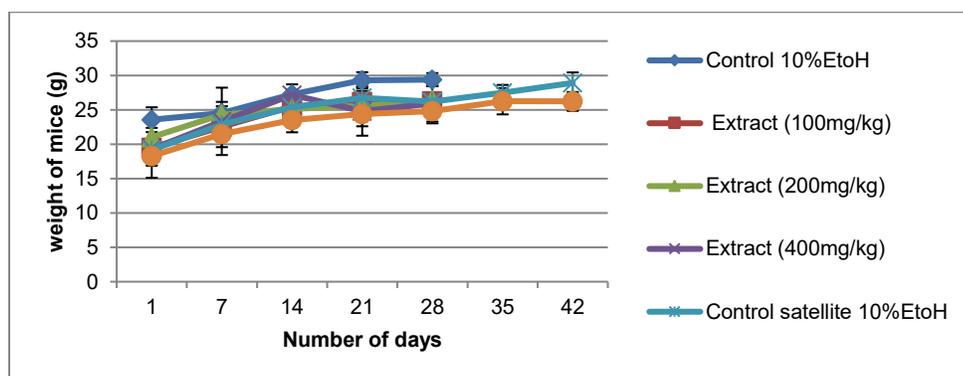


Figure 2. Variation of the weight of male mice during the experimental period

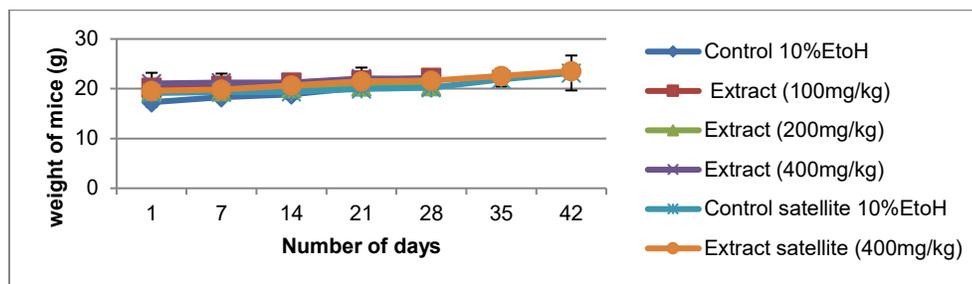


Figure 3. Variation of the weight of female mice during the experimental period

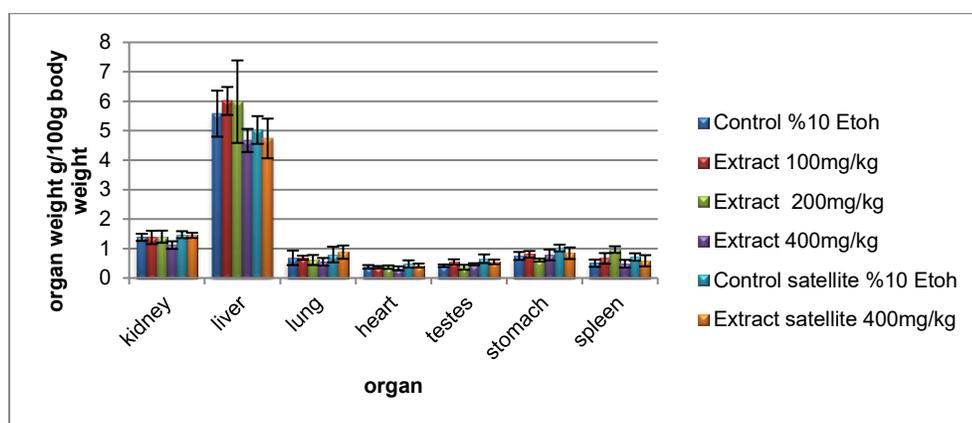


Figure 4. Relative Organ Weight of mice (ROW) per 100 g body weight recorded at the end of the study from experimental male mice after 28 and 42 days

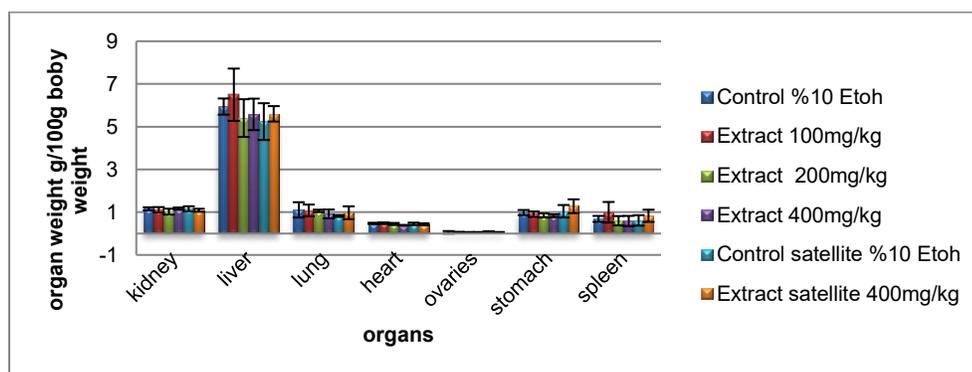


Figure 5. Relative Organ Weight of mice (ROW) per 100 g body weight recorded at the end of the study from experimental female mice after 28 and 42 days

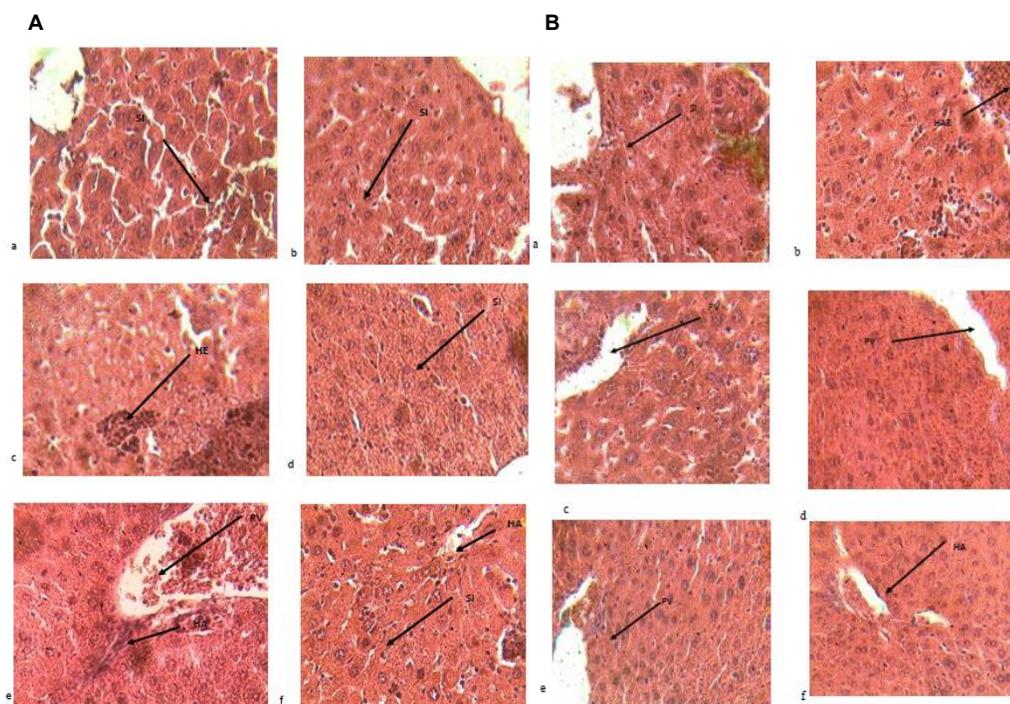


Figure 6. Histology of the liver of different groups A: Female and B: male mice at 400X following 28 and 42 days of extract administration.

a. control (10% EtOH), b. UCI EtOH Extract 100 mg/kg, c. UCI EtOH Extract 200 mg/kg, d. UCI EtOH Extract 400 mg/kg, e. Satellite control (10% EtOH), f. satellite UCI EtOH Extract 400 mg/kg, HE: Hepatocytes, PV: Portal vein, HA: Hepatic artery, HAE: Hemorrhage, SI: Sinusoids; The effect of the extract on the liver of female mice over a treatment period of 28 days showed degeneration (b, c) and disorganisation of hepatocytes (b, c), and inflammation (c and d). The control satellite and the extract 400 mg/kg (e and f) satellite showed normal tissues, indicating an over healing effect. Following treatment with extracts, the liver tissue showed some abnormalities in the male mice. These effects included vascular congestion, inflammatory nodules (extract with 100 mg/kg picture b), capillary dilatation of granulomas (extract 200 mg/kg picture c), vascular congestion, and leucocytary infiltration (extract 400 mg/kg Picture d). Normal cells were observed with the control satellite and extract 400 mg/kg satellite (Pictures e and f).

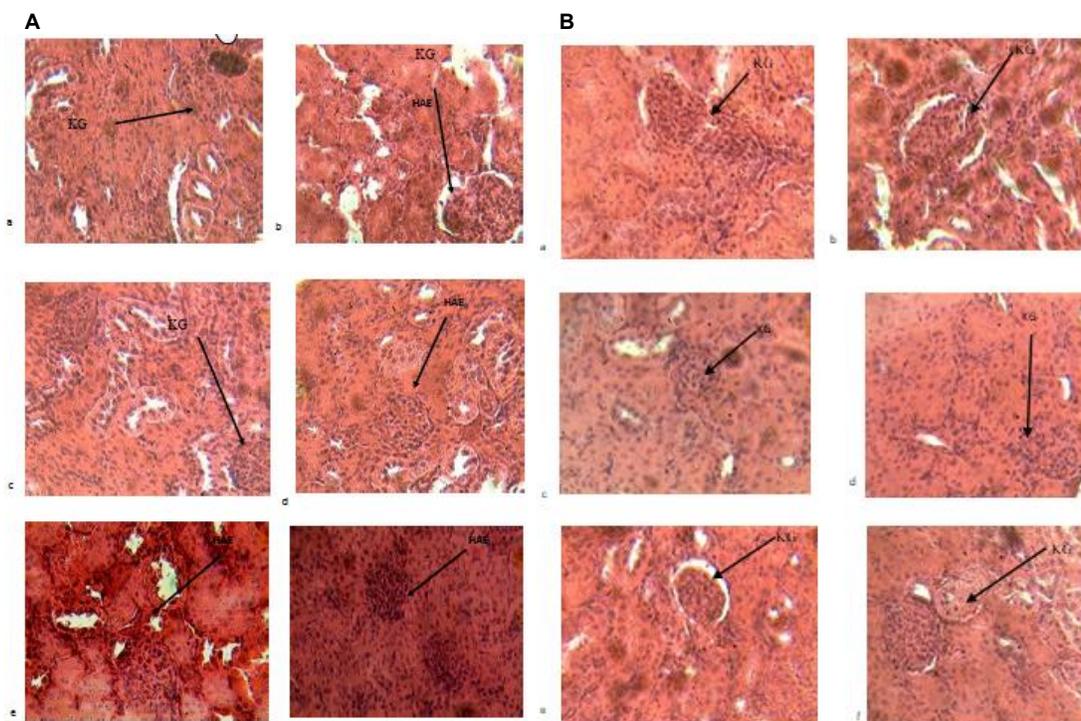


Figure 7. Histology of kidneys of A: Female and B: male mice in different groups following 28 and 42 days of extract administration.

a. control (10% EtOH), b. UCI EtOH Extract 100mg/kg, c. UCI EtOH Extract 200mg/kg, d. UCI EtOH Extract 400mg/kg, e. Satellite control (10% EtOH), f. satellite UCI EtOH Extract 400mg/kg, KG: kidney glomerulus, HAE: Hemorrhage; The tissues of the kidneys of female mice after 28 days of treatment showed some abnormalities at the different doses. Tubular clearance and mesangial expansion were observed in all the groups treated with the extract. Normal cells were observed with satellite control, and mesangial expansion was observed in the extract at 400mg/kg for the satellite group. The tissues of the kidneys of the male mice showed tubular clarification in the group treated with the extract 100 mg/kg (Picture b). Normal tissue was observed in the control treated with ethanol. Mesangial expansion was observed with the groups treated at 200 mg/kg and 400 mg/kg (Pictures c and e); the abnormality was not healed in the satellite 400 mg/kg (Picture f).

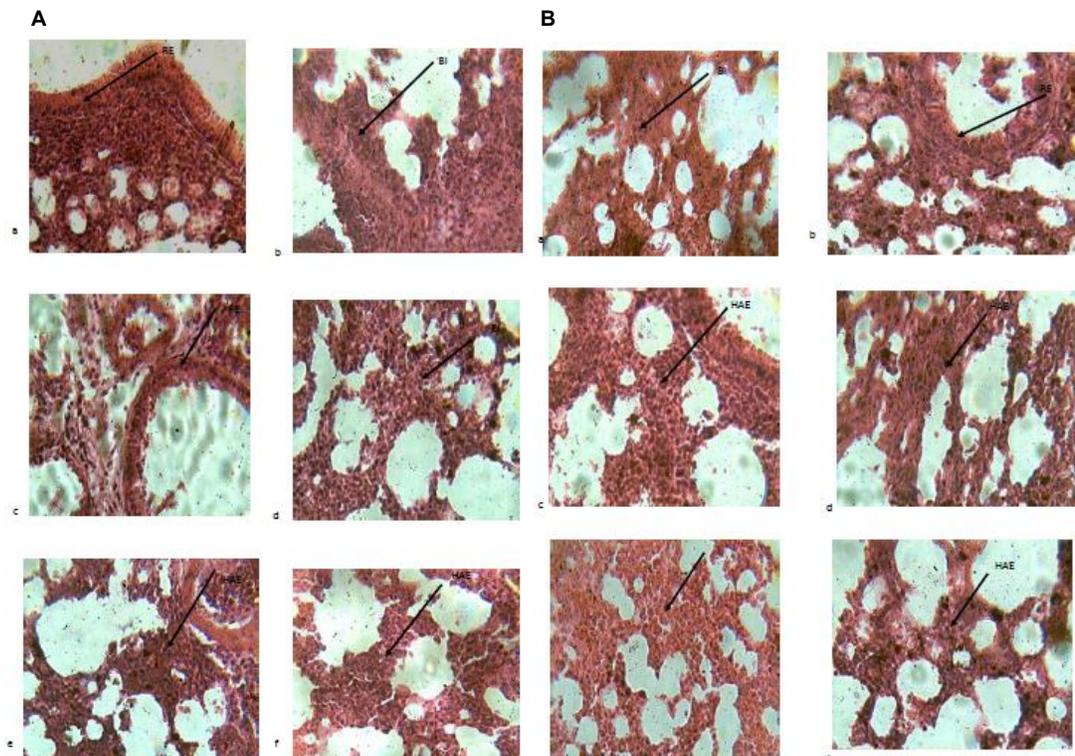


Figure 8. Histology of lungs of A: female and B: male mice of different groups at 400x following 28 and 42 days of extract administration. a. control (10% EtOH), b. UCL EtOH Extract 100 mg/kg, c. UCL EtOH Extract 200 mg/kg, d. UCL EtOH Extract 400mg/kg, e. Satellite control (10% EtOH), f. satellite UCL EtOH Extract 400mg/kg. HAE: Hemorrhage, RE: Respiratory Epithelium, BI: Bronchioles; The control and treated group at 100 mg/kg (picture a and b) showed normal cells, while inflammation and leukocyturia infiltration were noted in the group treated with 200 mg/kg and 400 mg/kg (picture c and d). The control and group treated at 400 mg/kg satellite groups revealed normal cells, indicating a post healing effect at the end of drug administration. The lungs of the male mice after 28 days of oral administration of the extract at different doses showed normal cells in all the treated groups (Pictures a, b, c, d, e, and f).

Table 1. Body weight of mice on day 1, day 7, and day 14

Groups/ Days	1	7	14
	Body weight ± S.D (g)		
Control 10% EtOH (1mL/100g)	18.55±0.54	19.17±0.53	22.34±0.055
UCL EtOH (2000mg/kg)	18.13±0.42	21.98±1.34	23.05±1.01

UCL EtOH, Ethanolic extract of *Uvariadendron Calophyllum*

Table 2. Hematological parameters of Male mice

Hematological Parameters	Control (10% EtOH)	Extract 100 mg/kg	Extract 200 mg/kg	Extract 400 mg/kg	Control satellite 10% EtOH	Extract Satellite 400 mg/kg
WBCx10 ³ /μL	3.07±0.95 ^a	1.88±0.38 ^a	4.03±1.76 ^a	2.1±0.64 ^a	2.95±1.34 ^a	2.02±0.82 ^a
LYM# x10 ³ / μL	2.44±0.66 ^a	1.59±0.35 ^a	3.02±1.09 ^a	1.77±0.55 ^a	2.13±0.93 ^a	1.53±0.65 ^a
MID# x10 ³ / μL	0.41±0.29 ^a	0.22±0.11 ^a	0.66±0.46 ^a	0.23±0.09 ^a	0.48±0.24 ^a	0.31±0.13 ^a
GRA# x10 ³ / μL	0.40±0.11 ^a	0.08±0.03 ^a	0.38±0.30 ^a	0.11±0.07 ^a	0.32±0.27 ^a	0.17±0.08 ^a
LYM%	81±9.95 ^a	84.28±7.63 ^a	76.94±6.30 ^a	83.70±5.01 ^a	74.10±7.84 ^a	76.10±5.33 ^a
MID%	12.90±5.98 ^a	11.50±5.68 ^a	14.68±3.79 ^a	11.26±3.53 ^a	15.97±2.85 ^a	15.66±2.68 ^a
GRA%	6.07±3.95 ^a	4.25±1.93 ^a	8.42±2.72 ^a	5.04±2.32 ^a	9.92±5.81 ^a	8.24±2.78 ^a
RBC x10 ⁶ / μL	6.23±0.02 ^a	9.65±5.64 ^a	5.83±2.85 ^a	6.78±2.02 ^a	5.78±2.63 ^a	4.89±1.29 ^a
HCT	43.90±5.20 ^a	61.93±36.14 ^a	35.56±19.63 ^a	43.70±15.33 ^a	40.47±18.44 ^a	34.86±9.16 ^a
MCV (fL)	67.33±4.62 ^a	64.50±3.11 ^a	59.60±4.51 ^a	64.00±4.18 ^a	70.00±3.35 ^a	71.40±3.58 ^a
RDW (%)	27.03±11.14 ^a	22.18±0.96 ^a	25.74±1.91 ^a	22.92±2.95 ^a	19.42±0.59 ^a	19.68±0.71 ^a
MCH (pg)	18.80±2.69 ^a	13.55±6.88 ^a	14.36±5.69 ^a	17.18±3.12 ^a	19.40±2.29 ^a	20.02±1.82 ^a
MCHC (g/dL)	19.33±15.57 ^a	21.05±10.57 ^a	24.28±9.58 ^a	27.06±5.97 ^a	27.80±3.53 ^a	28.00±2.03 ^a
HGB (g/dL)	10.17±2.87 ^a	10.23±2.71 ^a	7.56±4.01 ^a	11.26±1.81 ^a	10.77±3.89 ^a	9.66±2.15 ^a
RDW-SD (fL)	43.00±15.72 ^a	34.50±1.29 ^a	35.80±2.05 ^a	35.00±2.83 ^a	33.50±1.52 ^a	34.00±1.87 ^a
PLT (x10 ³ / μL)	111.5±12.02 ^a	115.67±20.59 ^a	168.80±89.42 ^a	140.20±32.90 ^a	153.17±95.09 ^a	141.20±23.54 ^a
MPV (fL)	7.40±0.36 ^a	7.10±0.22 ^a	7.28±0.48 ^a	7.54±0.62 ^a	7.87±0.73 ^a	8.22±0.59 ^a
PCT (%)	0.083±0.01 ^a	0.082±0.01 ^a	0.12±0.07 ^a	0.10±0.03 ^a	0.12±0.07 ^a	0.12±0.02 ^a
PDW (%)	45.77±5.61 ^a	44.53±2.57 ^a	45.94±4.46 ^a	45.36±4.68 ^a	45.05±22.29 ^a	50.70±10.19 ^a

MCHC- Mean Corpuscular Hemoglobin concentration, MCH- Mean Corpuscular Hemoglobin, HCT- Hematocrit MCV- Mean Corpuscular Volume, RBC-Red Blood Cell, PCT- Procalcitonin, PLT- Platelet, WBC- White Blood Cell; a, b indicate there is statistical difference at p≤0.05; The same letters on the same line indicate no statistical differences; Extract means Ethanolic extract of *Uvariadendron Calophyllum*

Table 3. Hematological parameters of female mice

Hematological Parameters	Control (10% EtOH)	Extract 100 mg/kg	Extract 200 mg/kg	Extract 400 mg/kg	Control satellite 10% EtOH	Extract Satellite 400 mg/kg
WBCx10 ³ /μL	1.50±0.53 ^a	1.82±0.75 ^a	3.60±1.10 ^a	6.47±3.60 ^a	4.15±1.99 ^a	4.03±2.85 ^a
LYM# x10 ³ /μL	1.25±0.44 ^a	1.52±0.59 ^a	2.85±0.96 ^a	5.55±3.16 ^a	3.00±1.24 ^a	2.82±2.29 ^a
MID# x10 ³ /μL	0.17±0.08 ^a	0.24±0.12 ^a	0.38±0.06 ^a	0.62±0.35 ^a	0.65±0.36 ^a	0.48±0.28 ^a
GRA# x10 ³ /μL	0.08±0.02 ^a	0.09±0.05 ^a	0.42±0.05 ^a	0.33±0.26 ^a	0.61±0.51 ^a	0.35±0.18 ^a
LYM%	83.20±0.44 ^a	85.08±3.13 ^a	83.95±8.13 ^a	85.50±4.76 ^a	74.43±8.48 ^a	76.72±8.17 ^a
MID%	11.17±1.33 ^a	9.82±2.28 ^a	8.00±3.25 ^a	9.37±1.32 ^a	15.50±2.57 ^a	14.22±3.21 ^a
GRA%	5.60±0.87 ^a	5.10±1.78 ^a	8.05±4.88 ^a	5.17±3.52 ^a	12.60±4.68 ^a	9.04±5.51 ^a
RBC x10 ⁶ /μL	6.49±0.62 ^a	5.22±2.16 ^b	4.24±3.64 ^b	6.33±1.69 ^a	6.07±2.45 ^a	5.70±0.69 ^a
HCT	42.47±5.81 ^a	33.03±14.46 ^b	47.00±11.50 ^a	40.13±13.76 ^a	43.35±18.35 ^a	40.52±6.17 ^a
MCV (fL)	65.33±4.04 ^a	62.83±3.43 ^a	69.00±0.00 ^a	62.33±5.85 ^a	71.00±2.94 ^b	70.80±3.49 ^b
RDW (%)	20.23±1.10 ^a	22.03±2.22 ^a	20.05±0.21 ^a	20.73±1.32 ^a	19.25±0.95 ^a	19.14±0.34 ^b
MCH (pg)	19.23±0.51 ^a	17.00±4.90 ^b	20.70±0.57 ^a	18.57±0.97 ^a	19.73±1.76 ^a	19.34±1.49 ^a
MCHC(g/dL)	29.5±1.14 ^a	27.28±8.29 ^a	29.95±0.78 ^a	29.83±2.17 ^a	27.78±2.46 ^a	27.36±2.34 ^a
HGB (g/dL)	12.47±1.33 ^a	8.07±1.95 ^a	8.65±7.28 ^a	11.80±3.35 ^a	11.70±3.89 ^a	11.02±1.63 ^a
RDW-SD (fL)	33.33±2.31 ^a	33.83±2.23 ^a	34.00±1.41 ^a	32.00±1.00 ^a	33.50±1.73 ^a	33.80±1.64 ^a
PLT (x10 ³ /μL)	92.33±10.26 ^a	128.50±69.54 ^a	139.00±27.00 ^a	139.33±25.32 ^a	129.75±72.54 ^a	156.75±59.56 ^a
MPV (fL)	7.23±0.58 ^a	7.05±0.24 ^a	7.40±0.99 ^a	7.33±0.58 ^a	7.80±1.08 ^a	8.10±1.12 ^a
PCT (%)	0.07±0.01 ^a	0.09±0.04 ^a	0.11±0.08 ^a	0.10±0.02 ^a	0.10±0.06 ^a	0.11±0.06 ^a
PDW (%)	45.53±2.14 ^a	43.25±3.50 ^a	49.4±0.00 ^a	44.53±2.35 ^a	51.95±6.15 ^a	52.04±8.48 ^a

MCHC- Mean Corpuscular Hemoglobin concentration, MCH- Mean Corpuscular Hemoglobin HCT- Hematocrit MCV- Mean Corpuscular Volume, RBC-Red Blood Cell, PCT- Procalcitonin, PLT- Platelet PCV-packed cell volume; a, b indicate there is statistical difference at p≤0.05; The same letters on the same line indicate no statistical differences; Extract means Ethanolic extract of *Uvariadendron calophyllum*

Conclusion

According to the current findings, there is no detectable in vivo toxicity brought on by the extracts (UCL, EtOH) in a mouse model. Both the control and treatment groups of mice had the same internal organ architecture, according to the macroscopic examination. The chronic toxicity of the crude ethanolic extract also showed no mortality and severe negative effects on the treated mice's organs, histopathology, and hematological parameters. *Uvariadendron calophyllum* could therefore be utilized as a medication in established dosages, particularly in remote areas where conventional medications are too expensive.

Abbreviations

HCT : Hematocrit
HGB: Hemoglobin
LD : Lethal Dose
MCH: Mean Corpuscular Hemoglobin
MCHC: Mean Corpuscular Hemoglobin Concentration
MCV : Mean Corpuscular Volume
OECD : Organization for Economic Co-operation and Development
PCV : Packed Cell Volume Platelets
PLT : Platelets
RBC : Red Blood Cells
ROW: Relative Organ Weight
MPV : Mean Platelet Volume
Ucl EtOH: Ethanolic extract of *U. calophyllum* leaves

Authors' Contribution

EMZ: Conceptualization, Investigation, Methodology, formal analysis, data curation, Software, Writing – original draft, Writing – review & editing; RMTK: Methodology Writing – review & editing, Validation, Supervision; VN: Investigation, Methodology; MSM: Investigation, Methodology; RK: Investigation, Methodology; GST: Investigation, Methodology; FFB: Writing – review & editing,

Supervision, Resources. All authors read and approved the manuscript.

Acknowledgments

The authors acknowledge the laboratory of anatomy and pathology of the Faculty of Medicine, University of Yaoundé 1.

Conflict of interest

The authors declare no conflict of interest

Article history:

Received: 14 November 2025

Received in revised form: 6 January 2026

Accepted: 23 January 2026

Available online: 23 January 2026

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