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Toxicity Assessment Of Terminalia Monoceros (Myrtales; Combretatceae), A Plant Used For Water Purification In The South Of Madagascar

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Abstract: Terminalia monoceros, a plant endemic to Madagascar, is traditionally used to purify water in the Beloha Androy district. This study evaluates its acute and sub-acute toxicity in mice through clinical, biochemical and hematological analyses. Oral administration of doses between 50 and 5000 mg/kg for acute toxicity revealed no signs of toxicity or mortality, indicating low toxicity and precluding determination of a median lethal dose (LD50). For subacute toxicity, daily administration of 10 mg/kg for 30 days produced no significant changes in body weight or organs (heart, liver, spleen, kidneys), suggesting no adverse effects on growth and physiology. Biochemical (liver enzymes, renal function) and hematological (leukocyte count, hemoglobin, platelets) analyses revealed no abnormalities, ruling out any hepatotoxicity, nephrotoxicity or hematological toxicity. Terminalia monoceros extracts are therefore well tolerated, even at high doses, and show no obvious toxicity. These results reinforce their potential as a natural water treatment agent. However, further studies are still needed to further investigate their safety and efficacy, thus contributing to the valorization of medicinal plants in environmental and therapeutic applications.

1. Introduction

Currently, as the world continues to urbanize, industrialize, and develop, surface waters are becoming increasingly vulnerable to pollution. This pollution not only poses direct risks when consumed by humans, but also poses significant risks [1]. Also, water pollution has a major impact on people's lives, particularly on health, education, the economy, and the environment. Consumption of unsafe water and poor hygiene and sanitation conditions are the cause of numerous diseases, including cholera, diarrhea, dysentery, hepatitis A, typhoid fever, and polio [2]. Unsafe drinking water and poor sanitation are the second leading cause of morbidity in Madagascar, causing the death of one in five children each year [2].

Southern Madagascar being a region facing water problems and ecological water treatment by *Terminalia monoceros* plant is the water treatment method and could be a key element in finding solutions to these problems. The population of Beloha Androy

uses it to treat unsanitary water because the plant acts as a coagulant to ensure effective removal of pollutants during the water treatment process and also adsorbs water contamination by chemicals [4]. Reference [5] indicates that coagulation-flocculation is increasingly appearing as not only a clarification process but also a specific removal treatment capable, to some extent, of competing with more expensive treatments such as activated carbon adsorption.

On the other hand, inhaling, touching, or even ingesting chemical substances does not necessarily lead to toxic effects. For example, carbon dioxide (CO_2) is a metabolite of the human body exhaled by the lungs and is also present in the environment. It can cause asphyxiation if present in sufficient quantities in a confined or poorly ventilated space. Paradoxically, the absorption of a substance in small amounts can be highly toxic and cause severe damage, while the absorption of a less toxic substance in large quantities may produce a benign effect. The toxic effect is thus linked to the concept of toxicity; and this toxicity of a substance can be defined as its ability to produce harmful effects on a living organism [5]. It varies depending on the dose, frequency, duration of exposure, and the time of onset of clinical signs. Clinically, there are three essential forms of toxicity: acute toxicity, short-term or subacute (subchronic) toxicity, and long-term or chronic toxicity. When an individual absorbs chemical products, various biological effects can occur, which may be beneficial (e.g., improved health after administering a drug) or harmful (e.g., lung damage following the inhalation of a corrosive gas). The concept of a toxic effect implies harmful consequences for the organism [6] and this effect will be an acute effect is felt within a relatively short time (minutes, hours, days), or a chronic effect manifest only after a relatively long exposure time and persists (weeks, months, years) [6]. Exposure to certain toxic substances can affect various systems and organs of the human body, leading to a wide range of clinical effects. These effects vary depending on the nature, concentration, and duration of exposure to toxic agents. Among the most common manifestations are irritations, corrosive injuries, neurological disorders, and alterations in cardiovascular and hematological functions [6].

The gGeneral Aim is to evaluate the acute and sub-acute toxicity of *Terminalia monoceros* extracts in mice to assess their safety for potential use in water purification.

So the specifics aims are :

1. To determine the acute toxicity of *Terminalia monoceros* extracts by administering doses and observing clinical signs, mortality, and LD50.

2. To assess its extracts sub-acute toxicity through daily administration

3. To evaluate the biochemical effects of *Terminalia monoceros* extracts on liver enzymes and renal function

4. To analyze the hematological profile and to identify any hematotoxic effects.

2- Materials and Methods

In this study, albino mice (*Mus musculus albinos*) of the "Swiss" strain were used as experimental models. The subjects were exclusively male, aged 16 to 20 weeks, with an average weight of 30 grams.

The plant was collected on Androy region Between Beloha and Tsihombe and the tested products are lyophilized extracts of the aerial parts of the plant, as well as a mixture of equal parts (0.5 + 0.5) of the plant. These extracts are solubilized in a solution of wood ash diluted in distilled water, with a concentration of 4 mg/ml. This concentration corresponds to a preparation of 60 g of ash in a 15-liter bucket of water.

2.1- Determination of LD50

Each test dose is administered to a group of five mice, while a control group receives only the vehicle used (1 ml per 100 g of body weight). The day before the experiment, the animals are fasted for 24 hours, with access to water but no food. After administration of the product, they are kept without food or water for the first six hours.

The doses administered orally are 50, 100, 1000, 2500, and 5000 mg/kg of body weight. Administration is performed via gavage in mice fasted for 24 hours, with a volume of 1 ml of the product containing the corresponding dose per 100 g of body weight. Observations are made during the first six hours after administration, then at 24 hours, and finally at 72 hours.

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If a lethal dose is observed, the LD50 is determined by testing intermediate doses in an arithmetic sequence. The pH of the product corresponding to the highest test dose is measured using a pH meter.

The LD50 (median lethal dose) is determined by identifying the dose of a substance that kills half of the population of mice to which it is administered [7]. The LD50 is a quantitative indicator of a substance's toxicity. Mortality is observed over a 72-hour follow-up period. Determining the LD50 requires testing at least three doses in duplicate (repeated twice). Based on the results of preliminary tests, several doses are selected to achieve mortality rates between 10% and 90%. Observations are conducted over 72 hours [7].

2.2- Subacute Toxicity

Subacute toxicity involves the repeated administration of a product over a period not exceeding three months. It helps identify the organ or system preferentially affected by the toxic substance. So, for the preparation of the solution, 60 g of wood ash are added to 15 liters of water and left to decant for 30 minutes, resulting in a solution containing 4 g of ash per liter. This solution is then used to prepare the product concentrations, administering a dose of 10 mg of product per kg of mouse in a volume of 0.5 ml per mouse. In practical terms, 750 mg of the product are dissolved in 15 liters of the ash solution, left to decant, and then 1.5 liters per day of this solution is used as drinking water for a 70 kg human.

A dose of 10 mg/kg/day was administered orally to the mice, equivalent to 1.11 mg/kg/day for a 70 kg human. Each group, composed of males and females, received this daily dose for 30 days. The control group received 0.5 ml of distilled water per mouse, while another control group received 0.5 ml of the ash solution.

The evaluation of visceral organ weights (heart, liver, spleen, kidneys, etc.) in treated and untreated mice after 30 days of treatment with aqueous extracts of Terminalia monoceros allows for the examination of the plant's potential effects on these organs. Observed variations may reflect potential toxicity, inflammation, or metabolic stress. This analysis aims to determine whether the administration of Terminalia monoceros extract induces significant changes in organ structure or function, assessing its safety and identifying potential adverse effects. Such an approach is essential to ensure the safe use of this plant in therapeutic or environmental applications.

2.3- Analysis of Biochemical Parameters in Treated and Untreated Mice

The evaluation of biochemical parameters in mice treated and untreated with aqueous extracts of *Terminalia monoceros* helps determine the plant's impact on physiological and metabolic functions. These parameters, such as liver enzymes (AST, ALT), renal markers (urea, creatinine), lipids (cholesterol, triglycerides), and proteins, provide valuable insights into the potential toxicity or safety of the extract. By comparing results between treated and untreated groups, this study aims to verify whether the administration of *Terminalia monoceros* affects hepatic, renal, or metabolic functions and to confirm its safe use in water treatment or other therapeutic applications.

2.4- Analysis of Hematological Parameters in Treated and Untreated Mice

The analysis of hematological parameters in mice treated and untreated with aqueous extracts of *Terminalia monoceros* evaluates the plant's impact on the blood and immune systems. These parameters, such as white blood cell count (WBC), hemoglobin levels (HGB), red blood cells (RBC), hematocrit (HCT), and platelets (PLT), provide essential information on overall health, immune response, and hematopoietic function. By comparing results between treated and untreated groups, this study aims to determine whether *Terminalia monoceros* extract causes significant changes in blood composition, confirming its safety and absence of adverse effects on the hematological system

3- Results

3.1- Acute Toxicity

No mortality (0% MRT) was observed on all tested doses (from 50 mg/kg to 5000 mg/kg). If 5000 mg/kg is the highest tested dose, the substance appears to have a wide safety margin, meaning it does not cause lethal effects even at high concentrations.

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So, the lethal dose 50% (LD50) could be much higher than 5000 mg/kg, or the substance may not be lethal at practical doses (Table 1).

Table 1. summarizes the mortality rate observed following the oral administration of different doses of *Terminalia monoceros* extracts in mice.

Doses Administered	Mortality Rate (% MRT)
50 mg/kg	0%
100 mg/kg	0%
1000 mg/kg	0%
2500 mg/kg	0%
5000 mg/kg	0%

3.2- Comparison of Visceral Organ Weights

After 30 days of treatment, the *Terminalia monoceros* extract had no significant impact on the weight of organs such as the heart, spleen, liver, gallbladder, and kidneys. The observed differences compared to the control groups were not statistically significant (Table 2).

It is worth noting that the ash solution, used as a vehicle, also did not affect the weight of the organs (heart, spleen, liver, gallbladder, kidneys, and adrenal glands). The differences compared to the control groups were not significant (Table 2).

The results for *terminalia monoceros* on male and female mice show that the t-value for the liver and gallbladder (respectively -2.445; -2.41) is close to the critical value but remains slightly below -2.776. This suggests a trend toward a significant difference but not enough to conclude a statistically significant change. For the other organs, they have t-values well below the critical value, indicating no significant difference between treated and untreated groups (Table 2).

About ash, all t-values are below the critical value, indicating no significant differences in organ weights between treated and untreated groups. None of the t-values reach or exceed the critical value, meaning no significant differences are observed between groups for the ash with male and female mice. The analysis of t-values shows that none reach or exceed the critical value of 2.776 at a significance level of 0.05 with 4 degrees of freedom. Thus, the results do not support a significant effect of the treatment on the weight of the studied organs (Table 2).

Table 2: Comparison of Visceral Organ Weights After 30 Days of Treatment (Student's t-test)

Group	t (Heart)	t (Spleen)	t (Liver & Gallbladder)	t (Kidneys & Adrenals)	t (Table)	Degrees of Freedom	Significance Level (α)
Plant + Male Mice	-0.627	-0.854	-2.445	-1.128	2.776	4	0.05
Plant + Female Mice	-1.023	-0.332	-2.41	1.81			
Ash + Male Mice	-0.016	-1.578	-1.265	-0.259			
Ash + Female Mice	-1.697	-1.98	-0.982	-1.204			

3.3- Biochemical and hematological parameters

The following results show the biochemical and hematological analyses in mice and show that for the administration of the lyophilized extract of *Terminalia monoceros* at a dose of 10 mg/kg/day no hepatotoxic or nephrotoxic effects were found and it seems to be well tolerated (Table 3) and did not cause significant hematological changes, indicating the absence of myelotoxic effects, anemia, or immune system disruption (Table 4).

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Table 3: Biochemical Parameters of Mice Treated and Untreated with Aqueous Extracts of Terminalia monoceros

Biochemical Parameters	Control	Lyophilized Extracts (10 mg/kg/day)
Gly Glycemia (g/l)	1.07 ± 0.06	1.13 ± 0.05
AST Aspartate Aminotransferase (UI/l)	130.13 ± 3.12	131.83 ± 2.97
ALT Alanine Aminotransferase (UI/l)	52.81 ± 2.51	52.5 ± 2.13
AP Alkaline Phosphatase (UI/l)	118.9 ± 4.09	118.67 ± 2.72
GT Gamma-glutamyl Transferase (UI/l)	1.83 ± 0.46	2.33 ± 0.42
BT Total Bilirubin (mg/l)	1.54 ± 0.32	1.68 ± 0.24
TG Triglycerides (g/l)	0.63 ± 0.05	0.57 ± 0.05
Chol Cholesterol(g/l)	0.68 ± 0.06	0.49 ± 0.03
Urea (mg/l)	0.34 ± 0.02	0.36 ± 0.03
Creat Creatinin (mg/l)	5.80 ± 0.30	4.83 ± 0.32
AU Uric Acid (mg/l)	20.70 ± 0.73	21.03 ± 0.49
Prot Proteins (g/l)	74.34 ± 1.69	74 ± 1.46
Albu Albulin (g/l)	31.79 ± 1.06	32.67 ± 2.29

Table 4: Hematological Parameters of Mice Treated and Untreated with Aqueous Extracts of *Terminalia monoceros*

Hematological Parameters	Control	Lyophilized Extracts (10 mg/kg/day)
WBC White Blood Cells ($\times 10^3/\mu l$)	7.78 ± 0.55	7.7 ± 0.48
HGB Hemoglobin (g/dL)	13.35 ± 0.35	13.2 ± 0.48
RBC Red Blood Cells ($\times 10^6/\mu l$)	7.45 ± 0.2	7.28 ± 0.18
HCT Hematocrit (%)	48.93 ± 2.68	50.92 ± 1.02
PLT Platelets ($\times 10^3/\mu l$)	713.33 ± 67.18	681.66 ± 39.62
MCV Mean Corpuscular Volume (fL)	58.78 ± 1.87	58.71 ± 0.71
MCH Mean Corpuscular Hemoglobin (pg)	16.63 ± 0.47	16.63 ± 0.24
MCHC Mean Corpuscular Hemoglobin Concentration (g/dL)	28.21 ± 0.54	28.13 ± 0.43
LYM Lymphocytes Percentage (%)	81.71 ± 4.08	81.36 ± 4.73

4- Discussion

The toxicity studies conducted on mice via oral administration revealed that *Terminalia monoceros* extracts do not exhibit acute toxicity, even at high doses. Indeed, the maximum tolerated dose (MTD), the minimum lethal dose, and the LD50 (median lethal dose) are all greater than 5000 mg/kg. For comparison, vitamin C, with an LD50 of 11,900 mg/kg, is considered practically non-toxic, while table salt (LD50 = 3000 mg/kg) is slightly toxic, caffeine (LD50 = 192 mg/kg) is moderately toxic, and strychnine (LD50 = 1 mg/kg) is classified as extremely toxic. These results indicate that *Terminalia monoceros* extracts are well-tolerated and have a favorable safety profile.

Although medicinal plants are recognized for their numerous biological activities, the toxic potential of their bioactive substances remains understudied [8]. *Terminalia monoceros*, an endemic plant of Madagascar, is traditionally used by local populations for the treatment of unsafe water. In this study, the oral administration of *Terminalia monoceros* extracts at doses ranging from 50 mg/kg to 5000 mg/kg did not result in mortality or behavioral changes in mice. These observations confirm the short-term safety of the extracts.

To evaluate longer-term effects, a subchronic toxicity study was conducted over a period of 30 days. Changes in body weight were used as an indicator of potential adverse effects of bioactive substances [9]. No significant changes in body weight were observed

in treated mice compared to the control group, suggesting that prolonged administration of the extracts does not affect normal animal growth. Weight loss could have indicated metabolic disturbances, such as alterations in carbohydrate, protein, or lipid metabolism [10], [11], [12].

Similarly, no significant changes were observed in the weight of organs (heart, liver, spleen, kidneys, and lungs), indicating that the extracts have no harmful effects on the growth or function of these organs. The relative weight of organs is a sensitive indicator in toxicity studies [9], and the absence of significant variation reinforces the safety of the extracts.

The liver and kidneys play a crucial role in detoxification and the elimination of toxic substances [13]. Analysis of liver enzymes (AST and ALT) and renal markers (urea, creatinine, and uric acid) was performed to assess potential toxic effects. No significant increase in AST and ALT levels was observed, suggesting that the extracts do not damage liver cells [14]. Similarly, levels of urea, creatinine, and uric acid remained stable, indicating that renal function was not impaired [15], [16], [17]. Analysis of blood parameters revealed no significant differences between treated and control mice. This confirms that the extracts do not affect hematopoietic function, cause allergies, or induce intravascular effects such as hemolysis [18] [19].

5- Conclusion

The results of this study demonstrate that *Terminalia monoceros* extracts do not exhibit acute or subchronic toxicity in mice, even at high doses. No adverse effects were observed on body weight, organs, hepatic and renal functions, or hematological parameters. These findings confirm the safety of *Terminalia monoceros* extracts and support their potential use as a natural agent for water treatment and other therapeutic applications.

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