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Original Research Article

Evaluation of the acute and subacute toxicity of the hydroalcoholic extract of *Leucas martinicensis* in rats

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Abstract

Introduction: The leaves of *Leucas martinicensis* are used in Burkina Faso to treat many diseases such as fever, bronchitis, insomnia and malaria. But there is a lack of toxicity documentation to inform the users. Therefore, the absence these crucial information's can cause serious public health problems.

Materials and Methods: To fill this gap, we investigated on acute and subacute toxicity study of the hydroalcoholic extract of *L. martinicensis* following the OECD method. For acute toxicity, the limit dose of 5000 mg/kg body weight was administered. In the subacute toxicity study, four groups of rats were formed, including a control group that received distilled water for 28 days and three other groups that received doses of the hydroalcoholic extract of *L. martinicensis* of 100, 400, and 700 mg/kg, respectively, for the same period.

Results: The LD_{50} was determined and hematological and biochemical parameters were analyzed. The LD_{50} was greater than 5000 mg/kg, and there were no significant changes in the rats body weight or the relative weight of their organs (heart, lungs, liver, kidneys, spleen). Urea was the only biochemical parameter that showed a significant increase.

Conclusion: The hematological analysis did not show any significant variation. The hydroalcoholic extract of *L. martinicensis* is practically non-toxic. However, at high doses, the extract has a moderate toxic effect characterized by an increase in uremia.

Keywords: *Leucas martinicensis*, Acute toxicity, Subacute toxicity, Rat, LD_{50}

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1. Introduction

Plants are a valuable source of natural products. In developing countries, nearly 80% of the population uses them for medicinal purposes.[1]

L. martinicensis is a grass from Lamiaceae family, often found in tropical and subtropical regions. Leaves are opposite, ovate to lanceolate, with a slightly serrated margin. Its inflorescences consist of small tubular flowers grouped in spikes or terminal clusters. They are often white or cream-colored, sometimes with shades of pink or purple. The species occurs in tropical regions, and are mainly found in wetland and dry areas.[2] *L. martinicensis* is used in traditional medicine in several regions of Africa.[3,4]

In Burkina Faso, *L. martinicensis* is used by traditional practitioners to cure many diseases including fever, bronchitis, insomnia, nervousness, nervous disorders, anxiety, heart palpitations, and malaria.[2,5] Many studies on *L. martinicensis* have shown that extracts contain several pharmacological substances such as, larvicidal, antimicrobial and antioxidant properties.[6-8]

However, few studies have been conducted on the toxicity of these extracts, particularly hydroalcoholic extracts. The objective of this study is to determine the acute toxicity and subacute toxicity (of the hydroalcoholic extract of *L. martinicensis*.

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2. Materials and Methods

2.1 Plant material

Leaves of *L. martinicensis* were collected in Ouagadougou. After identification, herbarium specimens were deposited at the biodiversity information center of the University Joseph Ki-Zerbo. Leaves were dried in the laboratory between 25 and 30°C, then they were crushed and sieved. The powder was macerated to obtain a hydroalcoholic extract.

2.2. Animal material

Male and female Wistar rats were used in the present study. Rats were gathered from animal laboratory center of University of Joseph KI-ZERBO. They were raised 24 ± 2 °C in the laboratory, fed and cared. All studies were performed according to the Guiding Principles for the Care and Use of Laboratory Animals. All studies were performed according to the Guiding Principles for the Care and Use of Laboratory Animals of the Ethics Committee of Joseph KIZERBO University (CE-UJKZ/2025–06).

2.3. Acute toxicity assessment

For acute toxicity, a single dose of 5000 mg/kg body weight was administered. Prior to administration, fasting rats (4 hours) were first weighed, and then they were administered by extracts of *L. martinicensis*. After administration of the extract, the rats were kept for additiona l 2 hours following Organization for Economic Cooperation and Development (OECD) guidelines 423.[9] According to this protocol, three animals are required. If the first treated animal dies, treatment continues with 2000 mg/kg. If the first animal treated survives, two other animals are treated. If only one of the three animals dies, the LD50 value is estimated to be greater than 5000 mg/kg. If both animals die, then dosing proceeds at 2000mg/kg. Fourteen (14) days of observation period was necessary to record toxicity effects in this study.[10]

2.4. Subacute toxicity assessment

The subacute toxicity study was conducted following Organization for Economic Cooperation and Development (OECD) Protocol 407.[11] Male and female rats were divided into four groups of six. Three (3) groups received daily doses of extracts during 28 days. These three doses, consisting of minimum of (100 mg/kg), intermediate of (400 mg/kg), and maximum of (700 mg/kg) were used based on the LD_{50}. A control group received distilled water. During the experiment each specimen was weighted. At the end of the 28 days, each animal was sacrificed using ketamine plus xylazine at a ratio of 2:1 (v/v) an estimated dose of 1 mL/kg. Blood samples were collected through the cardiac using tubes: one containing an anticoagulant (EDTA) and the another without anticoagulant for the respective measurement of hematological and biochemical parameters.[1,10] Organs of each specimen kidneys, liver, heart, lungs and spleen were removed, and weighed.

2.5. Hematological parameters

The hematological examination was performed using a hematology analyzer based on the principle of variation in the electrical impedance emitted by the cells being studied. This allowed the following parameters to be obtained: white blood cells count (WBC), red blood cells count (RBC), hemoglobin level (Hb), mean corpuscular volume (MCV), hematocrit (Ht), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT).[12]

2.6. Biochemical parameters

Biochemical parameters were determined using a spectrophotometer with the kinetic and endpoint methods.[13,14] The following parameters: Glucose (mmol/L); Creatinine (µmol/L); Urea (mmol/L); Alanine and Aspartate aminotransferases (ALT and AST) (UI/L) were measured.

2.7. Statistical analyses

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software Inc., California). Multiple *t tests* – One per row was used to compare treated groups and control groups at threshold of $p < 0.05$.

3. Results

3.1. Acute toxicity

The results on acute toxicity did not show a sign of toxicity to rats after oral administration of 5000 mg/kg of the hydroalcoholic extract of *L. martinicensis*. All rats survived after the 14-day experiment, reflecting the absence of toxidrome in extracts.

3.2. Subacute toxicity

The results of **Figure 1** reveal that the body weight of treated animals increased during the 28 days of treatment, but those did not differ significantly compared to the controls ones.

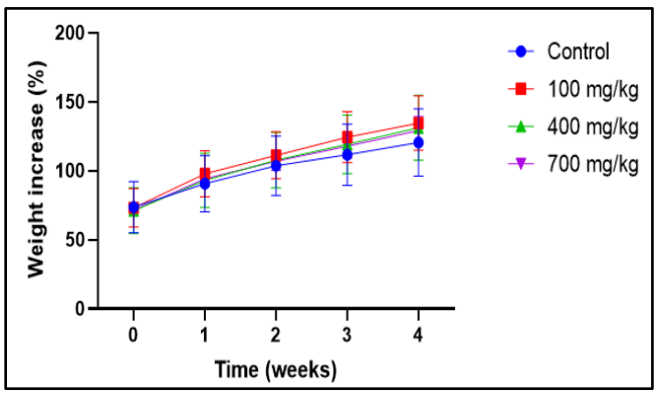

Figure 1: Effect of the hydroalcoholic extract of *L. martinicensis* on weight gain in rats during 28 days of treatment. Data are expressed as mean ± SD, n = 6. No

statistically significant difference was observed between the test and control groups.

There were no significant differences between organs of treated animals and controls groups (**Table 1**)

Table 1: Effects of hydroalcoholic extract of *L. martinicensis* on the relative weight of rat organs after 28 days of treatment.

Doses	Spleen	Kidney	Liver	Heart	Lungs
Control	0,47 ± 0,04	0,59 ± 0,08	3,59 ± 0,54	0,38 ± 0,05	0,60 ± 0,06
100 mg/kg	0,47 ± 0,05	0,48 ± 0,04	3,26 ± 0,66	0,32 ± 0,04	0,56 ± 0,06
400 mg/kg	0,45 ± 0,03	0,63 ± 0,03	3,50 ± 0,24	0,38 ± 0,03	0,61 ± 0,04
700 mg/kg	0,48 ± 0,03	0,59 ± 0,03	3,63 ± 0,36	0,35 ± 0,02	0,55 ± 0,04

Data are expressed as mean ± SD, n = 6. No statistically significant difference was observed between the test and control groups.

The same trends were observed with hematological variables, where no significant difference was found between treated groups and those of control group (**Table 2**).

Table 2: Effects of the hydroalcoholic extract of *L. martinicensis* on blood cells of rats after 28 days of treatment.

Doses	0 mg/kg	100 mg/kg	400 mg/kg	700 mg/kg
WBC (10 ^{9}/L)	3,46 ± 0,80	2,78 ± 1,05	2,78 ± 0,73	5,62 ± 1,91
RBC (10^{12}/L)	6,20 ± 0,99	6,53 ± 0,40	6,24 ± 0,35	6,874 ± 0,20
HGB (g/dl)	11,08 ± 1,76	11,58 ± 0,69	11,14 ± 0,71	11,58 ± 0,54
HCT (%)	37,64 ± 5,94	40,2 ± 2,38	37,82 ± 2,48	40,84 ± 1,29
MCV (Fl)	60,42± 0,96	61,58± 0,38	60,46± 0,54	59,42± 0,78
MCH (pg)	17,78± 0,24	17,72 ± 0,11	17,82± 0,27	16,84± 0,39
MCHC (g/Dl)	29,42± 0,39	28,8± 0,30	29,5± 0,39	28,5± 0,84
PLT (10^{9}/L)	655,6 ± 35,55	674,2± 71,32	619± 50,80	729,4± 74,66

WBC: White Blood Cell Count, RBC: Red Blood Cell Count, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Cell Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PLT: Platelet Count. Data are expressed as mean ± SD, n = 5. No statistically significant difference was observed between the test and control groups.

The serum molecular parameters such as glucose, creatinine, and transaminases do not show a significant difference, excepted, urea value ($p < 0.05$) (**Table 3**).

Table 3: Effects of *L. martinicensis* hydroalcoholic extract on biochemical parameters in rats after 28 days of treatment.

Doses	0 mg/kg	100 mg/kg	400 mg/kg	700 mg/kg
Blood glucose (mmol/L)	4,56 ± 0,26	5,02± 0,23	4,73 ± 0,24	5,33 ± 0,25
Creatinine (µmol/L)	70,50 ± 3,31	76,24± 2,65	67,42 ± 1,69	71,80 ± 2,16
Uremia (mmol/L)	8,22 ± 0,49	8,87± 0,27	7,93 ± 0,11	10,13* ± 0,11
ALAT (UI/L)	89,59 ± 5,65	81,27± 2,62	95,78 ± 6,32	91,37 ± 3,46
ASAT (UI/L)	214,98 ± 9,60	197,85± 7,78	225,65 ± 8,51	195,14 ± 4,22

Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT). Data are expressed as mean ± SD, n = 5. *Indicate a significant difference at $p < 0.05$ compared to the control group.

4. Discussion

The hydroalcoholic extract of L. martinicensis administered orally at a dose of 5000 mg/kg did not cause any deaths during the 14 days of the acute toxicity. The hydroalcoholic extract of L. martinicensis is therefore classified at category 5 according to the OECD chemical toxicity scale. This class includes substances with low toxicity.

Eze et al.[15] conducted an acute toxicity study of the aqueous extract of L. martinicensis leaves using a single dose of 3000 mg/kg, and did not observe any rats' deaths during the experiment. They concluded that the LD50 was greater than 3000 mg/kg. Our results corroborate those of Eze et al.[15] The hydroalcoholic extract of *L. martinicensis* can be considered practically non-toxic.

The study of the subacute toxicity study of the hydroalcoholic extract of *L. martinicensis* did not induce any observable showed no behavioral changes in rats. A steady weight gain was observed across all groups, including the controls, indicating that the extract did not adversely affect the general health or growth of the animals.

There was no significant difference in body weight or relative organ weight between the treated groups and the control group. Although, the biochemical parameters showed a significant increase in urea levels, but Eze et al.[15] showed a non-significant increase in urea. The increase in uremia was not accompanied by a concurrent rise an increase in creatinine levels. The other biochemical parameters remained unchanged.

Uremia is one of the biochemical parameters used to assess renal function like creatinemia.[16] Urea is a nitrogenous end product derived from the catabolism of proteins and amino acids, while creatinine is a degradation product of creatine phosphate in muscles. Both molecules are excreted by the kidneys.[17-19] An isolated elevation in urea (azotemia) without a corresponding increase in creatinine typically suggests prerenal causes rather than intrinsic renal damage. Common prerenal causes include dehydration or a reduced renal perfusion rate, while metabolic factors such as a high-

protein diet or a hypercatabolic state can also lead to increased urea production.[20]

Hematological analyses showed that hydroalcoholic extract of L. martinicensis did not cause significant changes in the number of erythrocytes leucocytes and thrombocytes. The results show that the extract has no significant effect on these parameters.

5. Conclusion

The results of the hydroalcoholic extract of Leucas martinicensis showed that the LD50 was greater than 5000 mg/kg body weight, suggesting that the extract is practically non-toxic. However, prolonged oral administration of the hydroalcoholic extract of *L. martinicensis* may cause slight variations in blood biochemical parameters. Further study of the effect of the extract on water consumption in rats is needed to explain the uremia.

6. Source of Funding

None.

7. Conflict of Interest

None.

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