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EVALUATION OF THE ACUTE TOXICITY OF LIANA BARK OF *LANDOLPHIA OWARIENSIS* P. BEAUV. (APOCYNACEAE)

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Abstract

The objective of this study was to assess the acute toxicity of the decocted bark of the liana bark of *L. owriensis P. Beauv. (Apocynaceae)* in the Wistar strain rat. The study of acute toxicity was carried out according to the OECD 423 method. This study was carried out up to a limit dose of 5000 mg / kg of body weight. After administration of different oral doses, observations on behavior, respiration, skin effects, response of the sensory nervous system or the gastrointestinal effects of animals were made on the 1st, 2nd, 3rd and 14 days (OECD 423). During 14 days of observation, a decoction of the bark of liana from *L. owriensis* did not show any mortality or lead to obvious toxicity on the mice at the respective doses of 300, 2000 and 5000 mg / kg. The decoction is not toxic to rats, even in large doses (5000 mg / kg of body weight). Therefore, the vine barks of this plant could be used for effective therapeutic purposes.

Keywords: Apocynaceae; Landolphia Owariensis; Acute Toxicity; LD_{50}.

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1. Introduction

Plants are used in medicine, providing many drugs from the earliest times to the present, and as the feedstock for many industrial products as well as a wide range of chemicals [1]. Plant species are important for drug development and pharmacological research, not only when plant constituents are used directly as therapeutic agents [2, 3].

In recent years, however, research on medicinal plants has been carried out using a combination of pharmacological studies [4]. These studies are conducted on plants to determine their toxicity and the activities possessed by the species under study. Acute toxicity

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2. Materials and Methods

2.1. Materiel

2.1.1. Plant Material

Landolphia owariensis liana bark was collected in November 2017 in Séguéla (Djibrosso-Kani, northern Côte d'Ivoire). They were identified by Professor Tra Bi Fezan Honora of Nangui Abrogoua University (Côte d'Ivoire).

2.1.2. Animal Material and Method of Administration

Eighteen rats (Wistar) weighing between 160 and 202 g were used in this study. They were purchased and maintained at Nangui Abrogoua University pet store for experimental purposes. The animals were kept under controlled conditions of temperature (23 ± 2) °C, humidity (50 ± 5) % and 12-hour light-dark cycles. All animals were acclimatized for seven days prior to the study. Animals were randomized into groups (experimental and control) and individually housed in sanitized polypropylene cages containing a sterile paddy wrap as bedding. They had free access to standard pellets as a staple diet and water at will. The animals were accustomed to the laboratory conditions 48 hours prior to the experimental protocol in order to minimize any non-specific stress.

2.1.3. Apparatus and Other Equipment

- Retsch GM 300 shredder
- Rotary Evaporator RC600-KnF
- 1 mL syringes with a 50-12 metal mouse feeding tube for intraesophageal administration.

2.2. Extraction

2.2.1. Procedure

The preparation of the decocted of *L. owariensis* was carried out as follows: the powder of the dried liana bark (20 g) was introduced into a glass jar with a capacity of 350 mL. Then 200 mL of distilled water was added and hermetically sealed. The closed container containing the mixture was placed in a boiling bath (100 °C) for 30 minutes. The mixture (powder and distilled water) after heating was filtered through Whatmann filter paper No 3 and then evaporated under reduced pressure by a rotary evaporator to yield 485.7 mg of crude aqueous extracts of *Landolphia owariensis*.

2.3. Acute Toxicity Assessment Method

The acute toxicity of *L. owariensis* decocted was determined in rats using OECD Method 423 [4].

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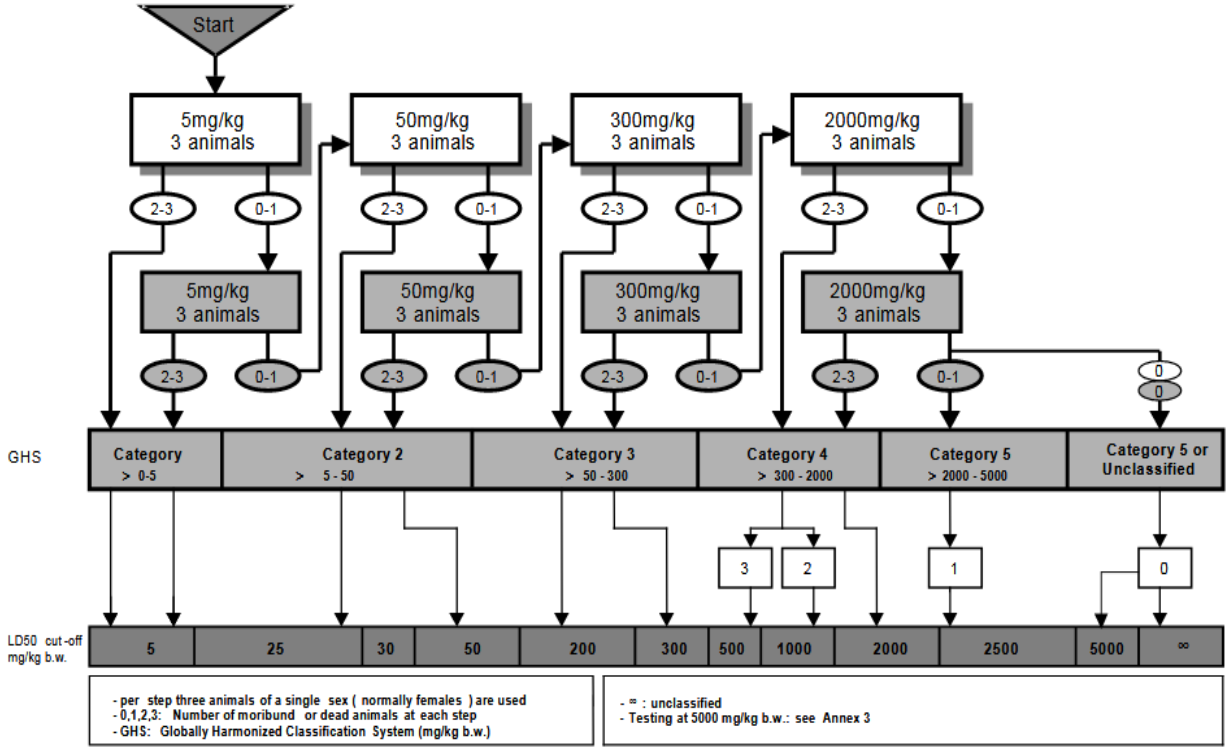

Figure 1: Acute toxicity assessment method for our extract (OECD 423)

Rats fasted for 16 h were randomly assigned to groups of three rats per group. Graduated doses of the extract (5, 50, 300, 2000 and 5000 mg/kg bw) were administered separately to the rats in each group using a curved steel needle. All rats were then allowed free access to food and water. They were observed for 24 hours to look for signs of acute toxicity. The number of deaths during this period was recorded.

- 0 mg/kg (0 mg/mL) < category 1 ≤ 5mg/kg (0.25 mg/mL)
- 5 mg/kg (0.25 mg/mL) < category 2 ≤ 50mg/kg (2.5 mg/mL)
- 50 mg/kg (2.5 mg/mL) < category 3 ≤ 300mg/kg (15 mg/mL)
- 300 mg/kg (15 mg/mL) < category 4 ≤2000 mg/kg (100 mg/mL)
- -2000 mg/kg (100 mg/mL) < category 5 ≤5000 mg/kg (250 mg/mL)

3. Results and discussion

3.1. Results

3.1.1. Acute Toxicity Test

For the acute toxicity study, the oral administration to rats of graduated doses (5, 50, 300, 2000 and 5000 mg / kg bw) of decoction of *L. owariensis* did not show any significant change in behavior, respiration, skin effects, the sensory or gastrointestinal nervous system responses during the observation period. No mortality or toxic reaction was recorded in the different groups, 24 hours after administration. A decoction of *L. owariensis* is safe up to a dose of 5000 mg / kg of body weight.

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3.1.2. Weight Development

All treated animals showed a positive weight change. The evolution of the weight of the animals during the 14 days of the experiment is recorded in **Table 1**.

Table 1: Change in average animal weight

Doses administered (mg / Kg)	**Average body weight of animals (g)**		**Variation**	**Test t of Student (5 %)**
	J0	**J14**		
5	160.60 ± 28	170.20 ± 25	9.60	NS
50	170.60 ± 28	180.20 ± 25	9.60	NS
300	192.15 ± 25	197.30 ± 17	5.20	NS
2000	201.60 ± 30	206.75 ± 15	5.15	NS
5000	196.20 ± 13	208.70 ± 11	12.90	NS

What does NS mean: Not significant ($p > 0.05$); Jo: 1st day of administration of the decoction; D14: 14 days after administration of the decoction.

3.2. Observations for 24 hours and 14 days

Table 2: Different observations made in 24 hours and 14 days during the test on the control and the experimental batch

Observations	**Control lot**		**Experimental Lot**	
	24 hours	14 days	24 hours	14 days
Skin and Fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membranes	Normal	Normal	Normal	Normal
Diarrhea	Absence	Absence	Absence	Absence
Salivation	Absence	Absence	Absence	Absence
Lethargy	Absence	Absence	Absence	Absence
Heartbeats	Normal	Normal	Normal	Normal
Aggressiveness	Absence	Absence	Absence	Absence
Sleepiness	No	No	yes	No
Power supply	yes	yes	yes	yes
Mobility	yes	yes	yes	yes
Mortality	No	No	No	No

During the 14 days following treatment no mortality was observed.

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4. Discussion

Table 3: Organ Mass of Treated Rats and Rats Receiving 5000 mg / kg body weight

Group of animals	Treatment and doses	Organ weights			
		Liver	Heart	Kidney 1	Kidney 2
Control Rats	Weight in (g) of untreated control animals	3.79 ± 0.30 3.81 ± 0.32 3.83 ± 0.29	0.42 ± 0.04 0.41 ± 0.02 0.43 ± 0.04	0.33 ± 0.023 0.32 ± 0.025 0.34 ± 0.024	0.34 ± 0.023 0.33 ± 0.026 0.33 ± 0.023
Experimental Rats	Weight in (g) of animals treated at 5000 mg / kg body weight dose	3.84 ± 0.28 3.85 ± 0.27 3.84 ± 0.29	0.45 ± 0.04 0.44 ± 0.03 0.42 ± 0.02	0.36 ± 0.021 0.39 ± 0.020 0.38 ± 0.021	0.35 ± 0.022 0.38 ± 0.019 0.39 ± 0.022

Landolphia owariensis is an Apocynaceae used in several regions of Africa for its various therapeutic virtues. In this study, we are carrying out in upstream stages of the design of an Improved Traditional Medicine (ATM) for the treatment of haemorrhoidal attacks.

The evaluation of acute toxicity consists in measuring and recording the various adverse effects that appeared after the administration of the tested substance. Indeed, in this work, the animals which received the doses higher than 2000 mg / Kg did not show changes in behaviour and neither more or less serious signs of intoxication (apathy, drowsiness, difficult movement of the animals during the experiment). No cases of mortality were recorded. At the end of the acute toxicity evaluation test, the LD_{50} is higher than 5000 mg / Kg orally. According to the Globally Harmonized System of Classification (GHS), the decoction can be classified as non-toxic [8]. The maximum tolerated dose is 5000 mg / kg bw / vo. This confirms the idea that the MTD is higher than the doses necessary to have pharmacological effects. Thus, thanks to its DMT of 5000 mg/kg bw/vol, the decoction offers an appreciable safety margin [9, 10]. This could reassure us about its possible use as a phytomedicinal agent.

The toxicity study is much more necessary, it will not only identify the range and concentration of the dose that could be used subsequently, but also reveals the possible clinical signs caused by the substances under investigation. In addition, it is also a useful parameter for investigating the therapeutic index of drugs.

5. Conclusions and Recommendations

Our work aimed to encourage the use of *Landolphia owariensis* as a medicinal plant in Côte d'Ivoire in general, and in Seguela in particular. We carried out pre-formulation tests on the decoction of *L. owariensis*. The results of the acute and subacute toxicity studies showed that a dose of 5000 mg / kg of body weight of bark of deciduous liana administered by the oral route appeared to be non-toxic. This study confirms that this plant is safe for people who use it in the north of Ivory Coast as a medicine. To our knowledge, this is the first time that this activity has been carried out on the vine bark of this plant. We wish to continue this study, by carrying out the evaluation of antihemorrhoidal activity of the decoction of this plant.

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