

Original Research Article

EVALUATION OF THE ACUTE TOXICITY OF AQUEOUS EXTRACTS FROM TWO SPECIES OF *CORCHORUS* GENUS: *CORCHORUS AESTUANS* L. AND *CORCHORUS OLITORIUS* L. (MALVACEAE)

ABSTRACT

Aims: *Corchorusaestuans* and *Corchorusolitorius* are two species of the genus commonly mentioned in Côte d'Ivoire as medicinal plants whose leaves decoctions are used to treat various diseases. To avoid cases of intoxication in traditional treatments, this study evaluates the acute toxicity of these plant species by having mice gorge on increasing doses of crude aqueous extract.

Place and Duration of Study: This study was carried out in the Pharmacognosy Laboratory of Félix HOUPHOUËT-BOIGNY University (Côte d'Ivoire), between march 2016 and september 2016.

Methodology: To assess the acute toxicity of the two extracts, we used 110 mice of SWISS breed. These animals aged four to six weeks were of both sexes (55 mice for each sex) and weighed between 13 and 26 g. Before the manipulations, the animals were fasted 12 hours before being divided into 11 batches of 10 mice receiving extract 1 (*Corchorusaestuans*) and extract 2 (*Corchorusolitorius*). We observed the effects of each phytomedicine on mice in different batches.

Results: Aqueous extracts of *Corchorusaestuans* and *Corchorusolitorius* (Malvaceae), administered orally, exert a dose-response effect. Concerning toxicity activities, *Corchorusolitorius* is not toxic. However, *Corchorusaestuans* is slightly toxic in the conditions of this study. The dose which kills half of the animals (LD_{50}) is 3999.93 mg/kg by oral voice (vo). This dose is the threshold of toxicity of the plant in the conditions of this study. Fortunately, this toxicity threshold is not accessible in therapy. The phytomedicine, in the conditions of use in traditional medicine in Côte d'Ivoire, is not toxic.

Conclusion: The two plant species thus offer an interesting margin of safety, which is reassuring as to the use of the decoction, for the treatment of various pathologies.

Keywords: *Corchorusolitorius*, *Corchorusaestuans*, *Toxicity*, *Phytomedicine*, Côte d'Ivoire

1. INTRODUCTION

Since antiquity, humans have used plants for their various needs [1]. The main areas of use of plants are, among others, construction, housing, health and food. Some of these plants are exploited for multiple uses. Among these plants with many uses, the *Corchorus* species genus are well known throughout the world. In India, China, Nepal and Bangladesh, these plants are cultivated for their undeniable economic interest [2]. In West Africa, species of the *Corchorus* genus are commonly found in open places such as fields where they are tropical weeds of crops. They are found in crops of millet, rice, sugar cane, cotton, peanuts and beans [3]. In Côte d'Ivoire, species of the *Corchorus* genus are also found. They have always aroused research interest and have been the subject of several studies [4, 5]. This work was devoted to the census of plants in Côte d'Ivoire and evoked the use of plants of the *Corchorus* genus in the diet and in the therapy of Ivorian populations. They reported that four (4) species representing this genus are eaten in Côte d'Ivoire [6, 7]. *Corchorusaestuans* and *C. olitorius* are two species of the genus commonly evoked in the country as medicinal plants whose leaf decoctions are used to treat various diseases. However, studies on the toxicity of these plants are little known in Côte d'Ivoire. This study responds to this concern. It is dedicated to the evaluation of the acute toxicity of the aqueous extracts of these two plants in accordance with the practices of traditional healers.

2. MATERIAL AND METHODS

2.1. MATERIAL

Biological material

The plant material used for the toxicological study concern extracts of dried leaves from *Corchorusaestuans* and *Corchorusolitorius*. The plant species come from District of Abidjan precisely from the market gardening sites of Bingerville (Côte d'Ivoire).

Animals used

The assessment of the acute toxicity of the two extracts was carried out on white mice (*Mus musculus*) of the SWISS race whose age varies between four and six weeks. The weight of these mice was between 13 and 26 grams. They were fed with pellets from the company FACI (Food Manufacturing of Côte d'Ivoire) and tap water.

Technical equipment for the preparation of the extract

An oven of 40°C was used to evaporate the decoction filtrate and obtain drug powders. The powders were weighed using a SHIMADZU AUX 320 Uni Bloc electronic scale. The mice were weighed on a TESTUT electric scale. In addition to this material, we also needed cotton wool and Wattman paper used as a filter, a porcelain mortar and pestle to finely grind the crystals obtained, sterile glass jars and a refrigerator for the conservation of extract, metal cages lined with wood chip bedding, spatulas and a cannula for incubating the animals. All the technical material cited comes from the Pharmacognosy laboratory of Pharmaceutical and Biological Sciences belonging to Félix HOUPHOUËT-BOIGNY University (Côte d'Ivoire).

2.2. METHOD

Plant identification

Plant samples came from Bingerville in Côte d'Ivoire. The identification of species was done by the National Floristic Center within Félix Houphouët-Boigny University. Scientific names have been assigned according to APG IV [8].

Preparation of concentrated extracts in the laboratory

Three (3) liters of decoction from *Corchorusaestuans* and *Corchorusolitorius* leaves were first wrung out in a square of clean cloth, successively filtered twice on absorbent cotton then on Wattman paper. The volume of the filtrate (approximately 3 liters) was evaporated in a rotavapor then in an oven at 60°C. After 2 days, the crystals obtained were pulverized using a porcelain mortar and pestle. The fine powders collected (32.45; 32.59 g), respectively for *Corchorusestuans* and *Corchorusolitorius*, constituted the total dry extracts. We kept them in the refrigerator, in a sterile glass jar, hermetically closed. The maximum concentration which corresponds to a concentration at the limit of the solubility of each extract was sought. For extract 1 (ECA) obtained from *Corchorusaestuans* leaves, we used 5 g of total extract in 20 ml of distilled water. Thus, we have developed a phytomedicine 1, with a maximum concentration of 250 mg/ml. The same operation made it possible to obtain extract 2 (ECO), from the leaves of *Corchorusolitorius* and prepared phytomedicine 2, also by dissolving 4.92 g of total extract in 20 ml of distilled water. We obtained a maximum concentration of 246 mg/ml. These concentrations of 246 and 250 mg/ml, obtained with the phytomedicines prepared respectively from the leaves of *Corchorusestuans* and *Corchorusolitorius*, made it possible to conduct the acute toxicity study. From each limiting solution, successive dilutions were prepared at 1/2, 1/3, 1/4 and at 1/5. This allowed us to have the following concentrations:

250; 125; 83.33; 62.5 and 50 mg/ml for *Corchorusaestuans*

245; 122.5; 81.66; 61.25 and 49 mg/ml for *Corchorusolitorius*.

Conditioning and composition of batches of mice for the evaluation of acute toxicity

To assess the acute toxicity of the two extracts, we used 110 mice of SWISS breed. These animals aged four to six weeks were of both sexes (55 mice for each sex) and weighed between 13 and 26 g. They were kept in metal cages of the Pharmacognosy Laboratory of Félix HOUPOUËT-BOIGNY University (Côte d'Ivoire) which served as a framework for these experiments. The mice were fed with pellets from FACI (Food manufacturing of Côte d'Ivoire) and tap water. Before the manipulations, the animals were fasted 12 hours before being divided into 11 batches of 10 mice receiving extract 1 (*Corchorusaestuans*) and extract 2 (*Corchorusolitorius*) as follows:

- batch 01: control mice receiving distilled water
- batch 02: mice treated with extract 1 at 250 mg/ml
- batch 03: mice treated with extract 1 at 125 mg/ml
- batch 04: mice treated with extract 1 at 83.33 mg/ml
- batch 05: mice treated with extract 1 at 62.5 mg/ml
- batch 06: mice treated with extract 1 at 50 mg/ml
- batch 07: mice treated with extract 2 at 245 mg/ml
- batch 08: mice treated with extract 2 at 122.5 mg/ml
- batch 09: mice treated with extract 2 at 81.66 mg/ml
- batch 10: mice treated with extract 2 at 61.25 mg/ml
- batch 11: mice treated with extract 2 at 49 mg/ml.

Gavage of mice

After subjecting the mice to 12-hour fast, solutions with concentrations ranging from 49 to 250 mg/ml were administered by gavage, to the different batches formed, according to the method already used in previous works [9]. Force-feeding was performed through a slightly curved intubation cannula. It was made respecting the proportion of 0.6 ml per 20 grams of body weight according to pharmacological standards [10].

Doses of phytomedicines administered

The doses of extract administered were expressed in mg/kg/vo of body weight. The different concentrations calculated above correspond to the following doses: 7500; 3750; 2500; 1875; 1500 mg/kg/vo for *Corchorusaestuans* extract (ECA) and 7350; 3675; 2450; 1837.5; 1470 mg/kg/vo for *Corchorusolitorius* extract (ECO). Tables 1 and 2 provide informations about concentrations and doses of ECA and ECO administered to the different batches made up. After the administration of the extracts, the mice are placed back in the respective metal cages and they again have access to the granules. As soon as the animals were placed in the cage, an initial observation was made, then every 30 minutes on the first day and once on the second day, for 48 hours. These observations were aimed at looking for symptomatic disorders (agitation, stretching, tremors, motor difficulties and dyspnoea) and mortalities.

Table 1. Administered concentrations and doses of ECA (*Corchorusaestuans* extract)

Batches formed	1	2	3	4	5	6
Substance administered by gavage	Distilled water	Extract	Extract	Extract	Extract	Extract
Concentration in mg/ml	0.6ml/20g	250	125	83.33	62.5	50
Corresponding dose (mg /kg/vo of body weight)	30ml/kg	7500	3750	2500	1875	1500
Number of mice per batch	10	10	10	10	10	10

Table 2. Administered concentrations and doses of ECO (*Corchorusolitorius* extract)

Batches formed	1	7	8	9	10	11
Substance administered by gavage	Distilled water	Extract	Extract	Extract	Extract	Extract
Concentration in mg/ml	0.6ml/20g	245	122.5	81.66	61.25	49
Corresponding dose (mg/kg/vo of body weight)	30ml/kg	7350	3675	2450	1837.5	1470
Number of mice per group	10	10	10	10	10	10

Toxicological parameters evaluated**Determination of the Maximum Tolerated Dose (MTD) and the Lethal Dose 100% (LD₁₀₀)**

The determination of toxicological parameters consists of establishing a relationship between a dose and one or more effects considered to be toxic. Here, the only effect of acute toxicity sought is death. Thus, the maximum tolerated dose (MTD) is the dose that does not kill any animal and the lethal dose 100% (LD₁₀₀) is the dose that kills all animals, when the extract is administered [11, 12].

Determination of the Lethal Dose 50% (LD₅₀)

The acute toxicity of a substance is observed after administration of a single dose of product or repeated doses not exceeding 24 hours. It is assessed by determining the LD₅₀. LD₅₀ is the dose leading to death in half of the subjects experimented after administration of a product. The calculation of the LD₅₀ is made from the following formula:

$$LD_{50} = LD_{100} - \frac{\sum(axb)}{n} (\text{mg/kg/vo}) \quad [11]$$

LD₅₀: Lethal dose 50%; LD₁₀₀: Lethal dose 100%; a: mean sum of deaths between two successive doses; b: difference between two successive doses; n: average number of animals used per group.

The toxicity classes recorded in Table 3 made it possible to assess the values obtained.

Table 3. Classes of toxicity[13]

Index or class	Commonly term used	Toxicological parameter (LD ₅₀) of toxicity
1	Extremely toxic	LD ₅₀ ≤1 mg/kg
2	Highly toxic	1 mg/kg ≤LD ₅₀ ≤50 mg/kg
3	Moderately toxic	50 mg/kg ≤LD ₅₀ ≤500 mg/kg
4	Slightly toxic	500 mg/kg ≤LD ₅₀ ≤5 g/kg
5	Almost toxic	5 g/kg ≤LD ₅₀ ≤15 g/kg
6	Relatively harmless	LD ₅₀ ≥50 mg/kg

3. RESULTS

3.1. Symptomatic disorders observed after gavage with phytomedicines

The doses of phytomedicines administered to mice by gavage range from 1500 to 7500 mg/kg/vo, for ECA (extract of *Corchorusaestuans*) and from 1470 to 7350 mg/kg/vo, for ECO (extract of *Corchorusolitorius*). A few moments after gavage of the two extracts of ECA and ECO at these different doses, the behavior of the mice was not the same for the two extracts. For ECO, motor difficulties and dyspnea were observed when doses of 7350 mg/kg/vo were administered. A short period of restlessness followed by drowsiness and stretching were also noted. Twenty minutes later, the animals resumed their usual behavior. For ECA, the mice having received the doses of 7500 mg/kg/vo and of 3750 mg/kg/vo experienced, about fifteen minutes after gavage of the product, a slight acceleration of respiration. After thirty minutes, the animals became listless, sleepy and motionless. Changes relating to the general appearance of the mice (hairiness, skin, condition of the ears, eyes and mouth) were not observed during the 48 hours of observation.

3.2. Laboratory concentrated doses

The different concentrations of the extracts as well as the doses corresponding to each extract are recorded in tables 4 and 5. For the *Corchorusaestuans* extract, the concentrations are 50 mg/ml, 62.5mg/ml, 83.33mg/ml, 125mg/ml and 250mg/ml. The 250 mg/ml correspond to the maximum concentration (saturation concentration) of ECA (extract of *Corchorusaestuans*). The doses corresponding to these different concentrations are expressed in mg/kg according to oral voice (vo). They are: 1500, 1875, 2500, 3750 and 7500 mg/kg/vo. Related to *Corchorusolitorius* extract, the concentrations are : 49; 61.25; 81.66; 122.5 and 245mg/ml. These concentrations correspond to doses of 1470; 1837.5; 2450; 3675 and 7350 mg/kg /vo.

3.3. Effect of gavage of phytomedicines on the mortality of mice

In batches that received *Corchorusolitorius* extract, there were no mouse deaths during the two days of observation. On the other hand, in batches that received *Corchorusaestuans* extract, mouse deaths were noted. The results are recorded in Tables 4 and 5. After three hours, the first death was recorded among the mice that received the doses of 7500 mg/kg/vo. In the batch, four mice died five hours later. A day after the experiment, all the other mice of the 7500 mg/kg/vo batch died. Concerning batches that received 3750 mg/kg/vo, four mice died. For batches that received doses ranging from 1500 mg/kg/vo to 2500 mg/kg/vo, there was no death of mice during the two days of observation. The number of dead mice increases when the dose of ECA is increased. There is a dose-response effect with *Corchorusaestuans* extract.

3.4. Evaluation of toxicological parameters

The highest dose is 7500 mg/kg/vo for ECA (extract of *Corchorusaestuans*) and 7350 mg/kg/vo for ECO (extract of *Corchorusolitorius*). These two doses correspond respectively to the maximum doses (doses at saturation or doses at the solubility limit) for the two plant extracts. For the *Corchorusaestuans*, this dose (7500 mg/kg/vo) is the 100% lethal dose (DL_{100}) because it kills all animals. The maximum tolerated dose (MTD), dose which does not kill any animal in the event of administration of the extract is 2500 mg/kg/vo. The dose which kills half of the animals (LD_{50}) is 3999.93 mg/kg/vo. This LD_{50} (3999.93 mg/kg/vo) shows that the aqueous extract of *Corchorusaestuans* is slightly toxic in the conditions of this study, according to the toxicity scale. For *Corchorusolitorius*, the LD_{50} is zero over two days of observation because the mean of the sum of dead mice between two successive doses is zero and the lethal dose does not exist. The aqueous extract of *Corchorusolitorius* is therefore harmless according to said scale. *Corchorusolitorius* extract (ECO) is devoid of acute toxicity, the maximum tolerated dose (MTD) was evaluated, in these conditions, at 7350 mg/kg/vo.

Table 4. Mortality of mice after gavage of *Corchorusaestuans* extracts

Batches formed	1	2	3	4	5	6
Substance administered by force- feeding	Distilled water	Extract	Extract	Extract	Extract	Extract
Concentration (mg/ml)	0.6ml/20g	250	125	83.33	62.5	50
Corresponding dose in mg/kg/vo	30ml/kg	7500	3750	2500	1875	1500
Number of mice per batch	10	10	10	10	10	10
Number of dead mice	0	10	4	0	0	0
Mortality (%)	0	100	40	0	0	0

Table 5. Mortality rate of mice after gavage of *Corchorusolitorius* extracts

Batches formed	1	7	8	9	10	11
Substance administered by force- feeding	Distilled water	Extract	Extract	Extract	Extract	Extract
Concentration (mg/ml)	0.6ml/20g	245	122.5	81.66	61.25	49
Corresponding dose in mg/kg /vo	30 ml/kg	7350	3675	2450	1837.5	1470
Number of mice per batch	10	10	10	10	10	10
Number of dead mice	0	0	0	0	0	0
Mortality (%)	0	0	0	0	0	0

4. DISCUSSION

Symptomatic disorders observed after force-feeding of phytomedicines

Administration of the aqueous extract of *Corchorusolitorius* caused dyspnea in mice. The dyspnea is thought to be due to therapeutic and above all hypotensive effect of the plant [15]. This hypotensive effect would be due to flavonoids and comparable to that of Acetylcholine [16]. Indeed, in the ears, stimulation of muscarinic receptors activates a G protein comprising 3 subunits: α , β and γ . The subunit stimulates K^+ . The outflow of K^+ causes a deficit of positive charges inside the cell. This leads to hyperpolarization and causes the entry of Ca^{2+} which results in a sharp decrease in the force of contraction of the heart and subsequently hypotension. Thus, the inhibition of voltage-gated calcium channels, by flavonoids, would be the cause of the hypotension observed with this plant in animals [17]. These observations confirm the therapeutic properties of the plant.

Effect of extracts on mouse mortality

The aqueous extract of *Corchorusolitorius* is harmless in the conditions of this study. This is a reassuring result for a widely consumed plant. On the other hand, *Corchorusaestuans* administered at doses between 7500 mg/kg/vo and 3750 mg/kg/vo, caused the death of the mice, depending on the batches formed. The maximum tolerated dose (MTD) is the dose necessary to have pharmacological effects [18]. This dose was 2500 mg/kg/vo for *Corchorusestuans*. There is therefore a dose-response effect in this species. The dose which kills half of the animals (LD_{50}) is 3999.93 mg/kg/vo. The plant is therefore slightly toxic. This result is not alarming because this toxicity threshold is difficult to reach through consumption. A 70 kg person will need 279995.09 mg of dry extract. However, according to the yield of 3.24%, this goes back to 8628.35 g of dry leaves consumed. This is an impossible threshold for the daily meal. However, the death of mice could be explained by the abundance of alkaloids in the chemical composition of the plant. Indeed, in large quantities, alkaloids become powerful poisons [19]. In all these cases, it is not an absolute toxicity but a relative toxicity linked to the quantity of matter ingested.

4. CONCLUSION

Aqueous extracts of *Corchorusaestuans* and *C. olitorius* (Malvaceae), administered orally, exert a dose-response effect. Concerning toxicity activities, *Corchorusolitorius* is a non-toxic plant. However, *Corchorusaestuans* is slightly toxic under the conditions of this study. This toxicity is at the LD_{50} of 3999.93 mg/kg/vo. This toxicity threshold is not accessible in therapy. Phytomedicines, in the conditions of use in traditional medicine in Côte d'Ivoire, is not toxic. The two plants thus offer an interesting margin of safety, which is reassuring as to the use of the decoction, for the treatment of various pathologies.

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