

Sub-acute Oral Toxicity of *Pancratium trianthum* Herb. (Amaryllidaceae) Bulb's Aqueous and Ethanolic Extracts in Wistar Rat

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Abstract: *Pancratium trianthum* Herb. is a herb whose leaves and seeds are reportedly highly toxic. However, the bulb is used in the form of aqueous decoctions, orally, in the treatment of cough and asthma in traditional medicine in southern Benin. The interest in the use of *Pancratium trianthum* Herb. bulb requires that an approach of its toxicity be undertaken in order to establish the safety of this treatment. Larval and acute toxicity tests were conducted. The larval toxicity test showed that the mortality of the larvae followed a dose-response relationship in that the number of dead larvae increased with increasing concentration using the logarithmic adjustment performed. For the acute toxicity test on rats, due to the doses administered and the treatments carried out, we obtained for the bulb of *Pancratium trianthum* Herb. an MTD of about 290 mg/kg.bw, an LD50 of 275 mg/kg.bw for the ethanolic extract and an LD50 of 365 mg/kg.bw for the aqueous extract. We thus retain that the bulb of *Pancratium trianthum* Herb. is not devoid of toxicity. It is slightly toxic and can be classified, especially its aqueous extract, as a product of category 4 of plant extracts according to the OECD guidelines (2008). However, these results reassure us about the safety of the plant when used at a suitable dose and the analysis of biochemical parameters further confirms the relative safety of the plant. Phytochemical studies revealed that the plant has a heterogeneity of chemical groups and a significant antioxidant activity, which could be responsible for its anti-asthmatic properties.

Keywords: *Pancratium trianthum* Herb, Traditional Medicine, Safety, MTD and LD50

1. Introduction

Since the 1980s, there has been an unprecedented surge of interest in the use of herbal medicine worldwide. The World Health Organization estimates that traditional medicine, including the treatment of ailments with plants, covers the health care needs of 80% of the population in developing countries [11].

At present, medicinal plants can provide interesting solutions to health-related constraints, especially in developing countries [2]. In Benin, for rural populations,

especially those far from health centers, the demand for treatment by plants is quite high [3]; also, this use of plants remains partly based on the idea that plants are a natural means of treatment without any risk. However, the use of medicinal plants in our countries is essentially based on oral knowledge transmitted from generation to generation, which is mostly unaware of the data relating to their phytochemistry and the "pharmaco-toxicological" properties of these plants; this increases the risks for the users of these plants [4]. A plant can be both useful and toxic. In fact, the use of plants for therapeutic purposes in traditional rural areas reveals high risks of intoxication, linked to the lack of control over the

doses used in the preparation and administration of medicinal recipes. This inaccuracy constitutes a real problem because the lack of knowledge of the doses of traditionally administered extracts exposes the populations that use them to real risks of therapeutic accidents that can sometimes prove tragic [5]. Therefore, the objective of this work is to evaluate the acute toxicity of the bulb of *Pancratium trianthum* Herb. Which is a herb used in the treatment of cough and asthma in southern Benin and to determine the toxicological parameters that are necessary to better rationalize its use. There is still no phytochemical investigation carried out on *Pancratium trianthum* Herb, [6]. So we undertook to verify the safety of the bulb of this plant by studying the toxicity of its extracts on shrimp larvae and its acute toxicity on wistar rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

The plant material is the bulb of *Pancratium trianthum* Herb. a herb with a subspherical bulb of 5-6 cm in diameter, often compound, with a collar of old leaf bases about 6 cm long. Collections were made in Vakon, Danto and Katagon, Akpro-Misséréte commune, Ouémé department, Benin. The identification and authentication of the plant was done at Benin's National Herbarium of Abomey-Calavi's University, where the specimen is kept under the number AAC 890/HNB.

2.1.2. Animal Material

The animal material consisted of *Artemia salina* shrimp larvae and Wistar rats. These rats were kept and acclimatized under the conditions of the animal house to the Laboratory of Effort Physiology, National Institute of Youth, Physical Education and Sport, University of Abomey-Calavi (12 h light, 12 h darkness and 28°C temperature). Their average weight was 169 ± 4.57 g for the acute *in vivo* toxicity study. *Artemia salina* shrimp larvae were used for the larval toxicity study (*in vitro* toxicity).

2.2. Methods

2.2.1. Preparation of Extracts

Preparation of the ethanolic extract: A test sample of 15 bulbs of *Pancratium trianthum* was cut, crushed, macerated in 500 mL of 96° ethanol for 48 h under magnetic stirring and filtered. The filtrate was placed in an oven and scraped.

Preparation of the aqueous extract: A test sample of 20 bulbs of different sizes of *Pancratium trianthum* Herb. were cut, crushed and put in decoction for 30 min. The decoction, after being allowed to cool, was filtered and the filtrate was placed in the oven and scraped.

The extracts (*ethanolic and aqueous*) obtained are kept in a cool place throughout our work, to be used for the various analyses.

2.2.2. Phytochemical Screening

The method used for the phytochemical screening is that

of Houghton P. J. and Raman A. [7] and repeated by Assogba and al [8]. It is based on differential precipitation and colouration reactions of the main groups of chemical compounds contained. This analysis includes the search for alkaloids, polyphenolic compounds, quinone derivatives, saponosides, triterpenoids, steroids, cardenolides, cyanogenic derivatives, mucilages, coumarins, reducing compounds, anthracene derivatives and others.

2.2.3. Larval Toxicity Test

The larval toxicity test is performed on *Artemia* shrimp larvae according to the method proposed by Michael and al [9] and repeated by Ahoton and al [10]. The data (dose-response) are transformed by logarithm and the LC50 is determined from the graph showing the number of dead larvae as a function of the different concentrations. This value is obtained from the best fit line of half the number of deaths on the curve and then on the concentration axis [11].

2.2.4. Sub-acute Oral Toxicity

We conducted our toxicity study on Wistar strain rats according to the recommendations of the OECD (Organisation for Economic Co-operation and Development) guidelines for chemical testing (section 4: health effects; test #423: acute oral toxicity; toxicity class method adopted in 2008). [12]. For our study, nulliparous and non-pregnant female Wistar rats are used for this study. They are divided into six (06) batches of ten (10) rats per dose of extract administered and one (01) control batch, that is to say thirteen (13) batches of ten (10) rats for the two extracts including one control batch. The following steps were rigorously followed:

a) Observation of symptomatic disorders

After gavage of the aqueous and ethanolic extracts, the animals were returned to their metal cages where they could access the pellets again. They were observed immediately and then every 30 minutes, for 08 hours on the first day and then every 06 hours during the entire experimental period. During this period, symptomatic disorders (*agitation, mobility, lack of appetite, sensitivity to noise and pinching, breathing, appearance of faeces, motor difficulties and dyspnoea*) were noted in the animals of the batches.

b) Evaluation of toxicological parameters: MTD, LD50 and LD100

After administration of the extracts, at decreasing doses of 1000, 800, 600, 500, 300 and 200 mg/kg body weight, dead rats were counted in each batch for 48 hours.

This acute toxicity experiment was conducted to determine the toxicological parameters of the Lethal Dose 50% (LD50), the dose that kills 50% of the animals, the Lethal Dose 100% (LD100), the dose that kills all the animals, and the Maximum Tolerated Dose (MTD), which represents the maximum dose that does not kill any animal when the extract is administered. These doses are obtained from the TREVAN curve given by the percentage of mortality of rats as a function of the decimal logarithm of the doses administered.

The Lethal Dose 50% (LD50) was determined from the formula of Behrens and Kärber [13]. It is calculated as follows:

$$LD_{50} = LD_{100} - \frac{\Sigma(axb)}{n}$$

LD50: 50% lethal dose; LD100: 100% lethal dose; a: average of the sum of deaths between two successive doses; b: difference between two successive doses; n: average number of rats used per batch.

A follow-up on 14 days after administration allowed to look for possible clinical signs of morbidity or mortality and to assess the evolution of the body weight of the tested rats. On day 14, biochemical assays were performed on blood samples from the rats.

2.3. Statistical Analysis

The recorded data were processed using Statistical software Version 7.1 from STAT Soft Inc. For each variable, the mean (m) and standard error of the mean (SEM) were calculated. The results of the different groups were compared using the Kruskal Wallis analysis of variance (ANOVA) and the Mann Whitney U test. The Wilcoxon rank test was used for intra-group comparisons. The level of statistical significance of the results was set at $p < 0.05$.

3. Results

3.1. Phytochemical Screening

The extract from aqueous maceration of *Pancratium trianthum* Herb. is viscous and yellowish in color while the ethanolic extract is orange in color. The extraction yield is 6.2% for the aqueous extract and 4.9% for the ethanolic extract.

The phytochemical screening led to the characterization of several chemical groups (Table 1).

These phytochemical tests revealed the presence in the

plant of several chemical groups such as: reducing compounds, alkaloids, mucilages, saponosides, polyphenolic compounds such as tannins and flavonoids; and anthracenics such as free anthracenics. The results of the tests for cyanogenic derivatives were negative.

Table 1. Results of phytochemical analysis of extracts.

CHEMICAL GROUPS	IDENTIFICATION	
	Aqueous extract of Pt	Ethanolic extract of Pt
Reducing compounds	+	+
Alkaloids	+	+
Coumarins	+	-
Mucilage	+	+
Cyanogenic derivatives	-	-
Flavonoids	+	+
Gallic tannins	+	+
Catechic tannins	+	+
Anthocyanins	-	+
Leucoanthocyane	-	-
Quinone derivatives	-	-
Triterpenoids	-	-
Cardenolides	-	-
Steroids	-	-
Saponosides	+	-
Free Anthracene	-	+
O-heterosides	-	-
C-heterosides	-	-

-: absence; +: presence; Pt: *Pancratium trianthum* Herb.

3.2. Larval Toxicity of Extracts

Figure 1 shows the variation of larval mortality as a function of the concentrations of *Pancratium trianthum* Herb. extracts.

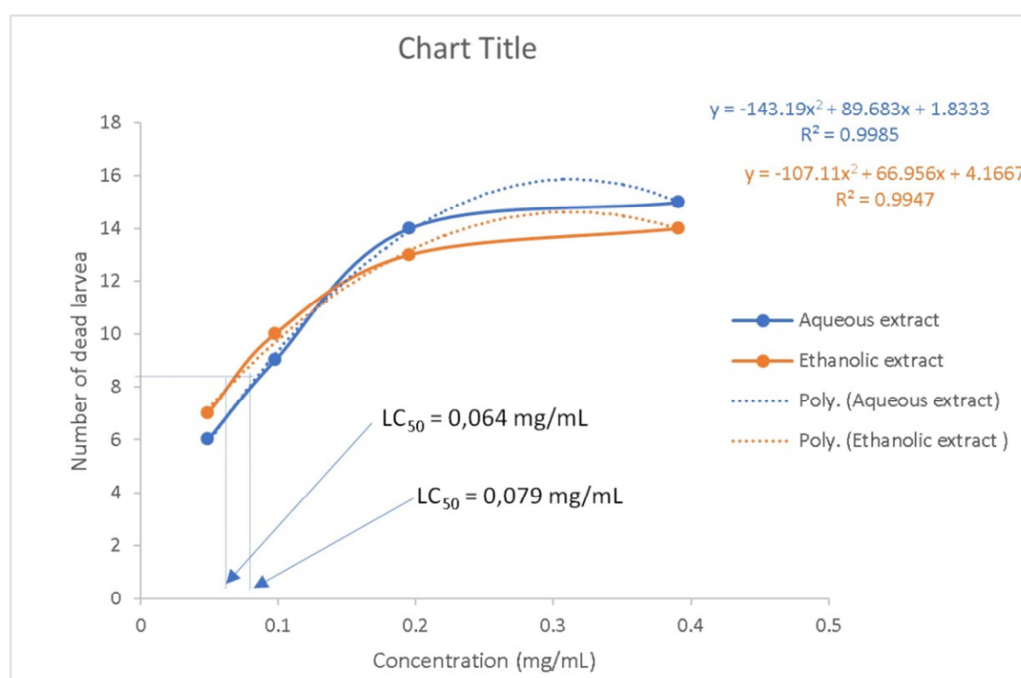


Figure 1. Variation curve of larval mortality as a function of the concentration of aqueous and ethanolic extracts of *Pancratium trianthum* Herb.

The reading of the two curves allows to deduce that the mortality of the larvae respects a dose-response relation because the number of dead larvae increases when the concentration increases by means of the polynomial adjustment presented on Figure 1; the concentration which causes the death of half of the 16 introduced larvae (LC_{50}) gives respectively for the two extracts:

$$(LC_{50})_{Pt_{aqueous}} = 0.079 \text{ mg/mL and } (LC_{50})_{Pt_{ethanolic}} = 0.064 \text{ mg/mL}$$

Let us note that the LC_{50} values of the two extracts are between 0.050 mg/mL and 0.1 mg/mL while the bulb of *Pancretrium trianthum Herb.* reveals a low toxicity on the

larvae of *Artemia salina* [14], under the conditions of our study. These two values, apparently close, are very significantly different with $P = 0.0106$ ($P < 0.05$).

3.3. Sub-acute Oral Toxicity

An increasing mortality rate in rats was observed, which is proportional to the increase in the concentration of *Pancretrium trianthum Herb.* extracts. From the doses administered and the treatments carried out, we can conclude that *Pancretrium trianthum Herb.* Administered orally, therefore, exerts a dose-response effect [15].

Table 2. Toxicological parameters (LD100 and LD50) of *Pancretrium trianthum* bulb's aqueous extract determination.

Lot number	Mortality rate of rats after gavage of <i>Pancretrium trianthum</i> bulb's aqueous extract						
	1*	2	3	4	5	6	7
Number of rats per batch (n)	10	10	10	10	10	10	10
Administered dose (mg/kg bw/v)	ED	1000	800	600	500	300	200
Number of deaths after 48 hours of force-feeding	00	10	10	07	02	01	00
Mortality (%)	0	100	100	70	20	10	0
a	-	-	10	08,5	04,5	01,5	0,5
B	-	-	200	200	100	100	100
LD ₁₀₀	800 mg/kg bw/v						
$LD_{50} = LD_{100} - \frac{\Sigma(ax \cdot b)}{n}$	365 mg/kg bw/v						

1*: control batch; ED: distilled water; LD50: Lethal dose 50%; LD100: Lethal dose 100%; a: average of the sum of deaths between two successive doses; b: difference between two successive doses; n: average number of rats used per batch.

Table 3. Toxicological parameters (LD100 and LD50) of *Pancretrium trianthum* bulb's ethanolic extract determination.

Lot number	Mortality rate of rats after gavage of <i>Pancretrium trianthum</i> bulb's ethanolic extract						
	1*	2	3	4	5	6	7
Number of rats per batch (n)	10	10	10	10	10	10	10
Administered dose (mg/kg bw/v)	ED	1000	800	600	500	300	200
Number of deaths after 48 hours of force-feeding	00	10	10	09	06	03	00
Mortality (%)	0	100	100	90	60	30	0
a	-	-	10	09,5	07,5	04,5	1,5
b	-	-	200	200	100	100	100
LD ₁₀₀	800 mg/kg bw/v						
$LD_{50} = LD_{100} - \frac{\Sigma(ax \cdot b)}{n}$	275 mg/kg bw/v						

1*: control batch; ED: distilled water; LD50: Lethal dose 50%; LD100: Lethal dose 100%; a: average of the sum of the deaths between two successive doses; b: difference between two successive doses; n: average number of rats used per batch.

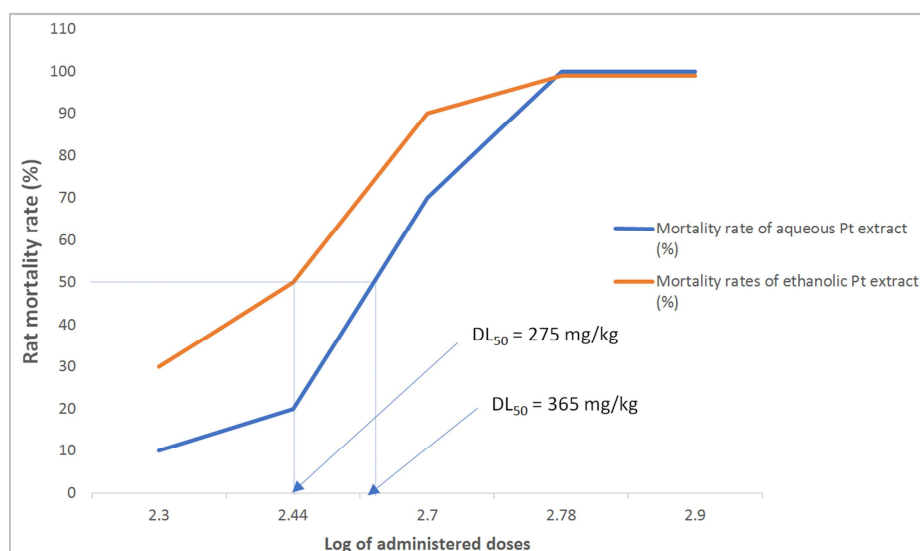


Figure 2. Mortality curves for rats as a function of aqueous extract doses and ethanolic of *Pancretrium trianthum* Herb.

The projections made on the TREVAN curves (Figure 2) confirmed the acute toxicity parameters obtained from the formula of Behrens and Kärber [13]. Thus, we obtained the significant values of acute toxicity in mice treated with the aqueous and ethanolic extracts of *Pancreatium trianthum* Herb.

The toxicological characteristics obtained under the experimental conditions are:

- 1) The maximum tolerated dose or MTD is approximately 290 mg/kg body weight.
- 2) The 50% lethal dose or LD50 of the ethanolic extract is 275 mg/kg body weight.
- 3) The 50% lethal dose or LD50 of the aqueous extract is

365 mg/kg body weight.

- 4) The 100% lethal dose or LD100 of both extracts is 800 mg/kg body weight.

We therefore retain that the plant is not devoid of toxicity. The bulb of *Pancreatium trianthum* Herb. can be classified, especially the aqueous extract, as a moderately toxic product according to the classification of Hodge and Sterner [16], under the conditions of this study. All animals that survived the experiment had a slight weight gain, throughout the study.

The results of the biochemical parameters measured are presented in the table (Table 4) opposite.

Table 4. Result of biochemical parameters in rats after the toxicity test.

Determined parameters	Aqueous extract of <i>Pancreatium trianthum</i> Herb.			Ethanolic extract of <i>Pancreatium trianthum</i> Herb.		
	Control lot	Batch 300 mg/kg	Batch 500 mg/kg	Lot Witness	Batch 200 mg/kg	Batch 300 mg/kg
Blood glucose (g/L)	0.77 ± 0.13	0.69 ± 0.05	0.89 ± 0.02	0.90 ± 0.10	1.09 ± 0.28	0.90 ± 0.08
Blood calcium (mg/L)	95 ± 5	91 ± 5	97 ± 1	93 ± 8	97 ± 7	90 ± 8
Magnesium (mg/L)	30 ± 2	25 ± 4	28 ± 1	29 ± 2	29 ± 1	30 ± 1
Creatinemia (mg/L)	7.5 ± 0.58	6.5 ± 0.58	7.0 ± 1.15	7.0 ± 0.81	7.5 ± 0.58	5.5 ± 0.58*
Transaminases TGO (IU/L)	219 ± 23	224 ± 29	179 ± 33	262 ± 21	253 ± 38	268 ± 2
Transaminases TGP (IU/L)	96 ± 9	84 ± 4	110 ± 1	91 ± 3	90 ± 0.58	85 ± 2
Total Cholesterol (g/L)	0.77 ± 0.08	0.75 ± 0.10	0.76 ± 0.07	0.82 ± 0.08	0.77 ± 0.10	0.80 ± 0.01
HDL Cholesterol (g/L)	0.42 ± 0.06	0.38 ± 0.04	0.45 ± 0.06	0.46 ± 0.04	0.44 ± 0.02	0.46 ± 0.03
Triglycerides (g/L)	1.4 ± 0.92	1.63 ± 1.22	0.84 ± 0.05	1.34 ± 0.35	0.93 ± 0.13	1.39 ± 0.13
Natraemia (mEq/L)	144 ± 3	145 ± 2	148 ± 1	143 ± 5	149 ± 1	143 ± 3
Kalemia (mEq/L)	5.2 ± 0.5	5.5 ± 1.03	5.1 ± 0.0	5.2 ± 0.5	5.0 ± 0.17	5.5 ± 0.6
Blood chloride (mEq/L)	102 ± 3	102 ± 1	102 ± 2	102 ± 2	103 ± 2	99 ± 1

The biochemical parameters obtained show no significant difference ($p > 0.05$) between the Control lot and the lots having received 300 mg/kg and 500 mg/kg of the aqueous extract on the one hand and between the Control lot and the lots having received 200 mg/kg and 500 mg/kg of the ethanolic extract of *Pancreatium trianthum* Herb. on the other hand, whatever the biochemical parameter considered.

However, a decrease in mobility and transient anorexia were observed during the first three days after administration of the extracts. At identical doses, the effects were more noticeable in the ethanolic extract than in the aqueous extract.

4. Discussion

The classical phytochemical screening performed on the bulb of *Pancreatium trianthum* Herb. showed the presence of reducing compounds, alkaloids, polyphenols, flavonoids, tannins and saponosides. Also abundant mucilages were observed [6]. The presence of these chemical compounds could explain the use of this herb for the treatment of cough and asthma. The antiasthmatic effect would be due to saponosides as well as alkaloids, responsible for vasodilation; alkaloids would slow down alveolar secretions and calm asthma attacks [17]. Indeed, Amaryllidaceae alkaloids are a group of alkaloids with many documented biological activities [18]. They are endowed with vasodilator, anti-tumor, analgesic, spasmolytic, antitussive properties [19]. Flavonoids and tannins have antioxidant [20], anti-inflammatory [21] properties; they relieve hay fever,

sinusitis, asthma [22].

The larval toxicity results for both extracts indicate low larval toxicity. The acute toxicity test on Wistar rats confirmed the observations made on the larvae. In fact, many studies have shown that the larval toxicity test is an excellent method for preliminary toxicity investigations [23]. It has been proved that there is a positive correlation between the larval toxicity test and the oral lethal dose of phytomedicines in rats [24]. The different toxicity tests of *Pancreatium trianthum* Herb. The maximum tolerated dose or MTD was 290 mg/kg body weight for the ethanolic extract and 365 mg/kg body weight for the aqueous extract. The ethanolic extract seems to reveal more toxicity of the plant than the aqueous extract. This relative toxicity of the bulb of this herb could be due to the toxicity of some alkaloids that the bulb of *Pancreatium trianthum* Herb. would contain. [25]. However, the analysis of acute toxicity parameters thus obtained show that at doses between 0 and 300 mg/kg body weight, there is an absence of mortality in rats. Thus, a dose-response effect of *Pancreatium trianthum* Herb. bulb extracts, on Wistar rats is evident [26].

The values of LD50; LD100; and MTD obtained for the extracts of *Pancreatium trianthum* Herb. The values of LD50, LD100 and MTD obtained for the extracts of *Pancreatium trianthum* Herb. in rats allow the extracts studied to be classified as moderately toxic substances on the Hodge and Sterner classification scale, developed by Cotonat [27].

Almost all the mean values of biochemical parameters in agreement with the reference values [28]. The lack of significant difference ($p > 0.05$) between the Control batches

and the different batches that received the extracts is probably due to the fact that the 200 mg/kg and 300 mg/kg body weight; 300 and 500 mg/kg body weight doses administered to the rats are the doses framing the lethal doses 50 of the ethanolic extract and the aqueous extract respectively.

On the other hand, the value of 365 mg/kg of body weight for the LD₅₀ of the aqueous extract *Pancratium trianthum* Herb. in reference to the method of administration by traditional therapists, is equivalent for a person of 70 kg to 365 x 70 which gives 25550 mg or 25.55g of product in a single dose to run the same risks. This dose of 25.55 g of aqueous extract of *Pancratium trianthum* Herb. on the classification scale of Gosselin Smith and Hodge [29], is classified as a slightly toxic substance for humans. However, in practice, referring to the average yield of the extraction of the bulb of *Pancratium trianthum* Herb which is about 5.55%, the doses administered for the treatment of cough and asthma by traditional therapists in South Benin hardly exceed 250 mg/kg of body weight. Thus, according to the classification scale of Gosselin Smith and Hodge [29], the therapeutic doses applied are safe in vivo, which validates the safety of the treatment of these diseases by the bulb of *Pancratium trianthum* Herb.

5. Conclusion

The larval and acute oral toxicity tests of the aqueous and ethanolic extracts of *Pancratium trianthum* Herb. showed that the oral administration of increasing doses of both extracts, has a dose-response effect on *Artemia salina* larvae and Wistar rats. The LC₅₀, LD₅₀ and MTD values obtained show in comparison to the standards, that the plant bulb could be classified as a mildly toxic substance for humans. However, considering the toxicological parameters found and the doses administered for the treatment of respiratory ailments such as cough and asthma, it can be retained that the use of the bulb of *Pancratium trianthum* Herb. at low doses (doses lower than 300 mg/kg body weight) is practically safe for humans. The acute oral toxicity test of the aqueous and ethanolic extracts of *Pancratium trianthum* Herb. reassures us that the bulb of this plant is safe when used at a suitable dose. However, tests of subacute or chronic toxicity by the oral route deserve to be carried out in order to confirm the non-toxic character of the bulb of *Pancratium trianthum* Herb.

Conflicts of Interest

The authors declare they have no conflict of interest in this work.

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