

THE ACUTE TOXICITY OF THE AQUEOUS EXTRACT OF *ARISTOLOCHIA LONGA* L, « MOROCCO » PREPARED BY THE TRADITIONAL METHOD

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ABSTRACT

Medicinal plants are one of the main resources of therapeutic agents. Among Moroccan medicinal plants, *Aristolochia longa*, it is a species commonly used in Moroccan traditional medicine. The present study was conducted to investigate the effect of the aqueous extract of *aristolochia longa* in albinos mice. Acute toxicity test was performed to determine lethal 50, lethal dose 100 and minimum lethal dose. The administration of the aqueous extract induced the appearance of several symptoms of toxicity like Weakness, convulsion, diarrhé, hypoactivity. Our results show that the highest dose killing all animals or lethal dose 100 is 12 g / kg, maximum tolerated dose is 5 g / kg and the lethal dose 50 is 7.6 ± 1.33 g/kg. Our study suggest non toxicity of the *aristolochia longa* aqueous extract.

KEYWORDS: *Aristolochia longa*, medicinal plant, toxicity, lethal dose 50.

INTRODUCTION

Aristolochia longa locally called berztame is among the most toxic Moroccan plants. It belongs to the family Aristolochiaceae which has up to 500 species, (Neinhuis C et al, 2005), we cite : *Aristolochia indica*, *Aristolochia baetica*, *Aristolochia albida* (APG, 2009), *Aristolochia mollissima* (Yu JQ et al, 2007), *Aristolochia acuminata* (Graham JG et al 2000), *Aristolochia constricta* (De Tommasi N et al 1998), *Aristolochia rotunda* (Pistelli L et al 1995), *Aristolochia Clematitis* (Kostalova D et al, 1991) and *Aristolochia longa*. The species of this family are recognized by their anti-cancer activity (Benzakour G et al, 2011) (Cherif HS et al 2009) (Saidi F et al, 2009).

This plant is a perennial plant glabrescent (high: 20-50 cm), with slender stems, spreading and branching. Its leaves are triangular oval (wide: 3-5 cm), at the base corded, with entire margins. It has solitary flowers, brownish green, glabrescent perianth, lanceolate tongue is characteristic by the presence of oval or pyriform Capsules. (A.majdouline 2017).

Aristolochia longa is used as sources of abortifacient, emmenagogue, sedative, analgesic, anti-inflammatory, anti-feedant, muscle relaxant, antihistaminic, and anti-allergic drugs (Pacheco AG et al 2009). it is very rich in chemical compound we find polyphenols flavonoide saponin tannins carbohydrate and c-heteroside (Bachir Benarba et al 2014), but its toxicity is due to the presence of a very powerful acid called aristolochic acid

(C17H11NO7), it is present in the plant in two forms aristolochic acid I and aristolochic acid II. They are recognized as the active ingredients of *aristolochia longa* (Stiborova M 2001).

Several studies have shown that AAs is responsible for developing of nephropathy syndrome in the persons treated by *aristolochia longa*. It is characterized by chronic renal failure, tubule-interstitial fibrosis and urothelial cancer. (Wu TS et al 2004) (Shaw D et al, 2012)

MATERIALS AND METHODS

The tubers of *aristolochia longa* L were cleaned of impurities, washed with tap water and dried out of the light for four weeks, then crushed using a medium fine powdered mortar. Dried tubers of *aristolochia longa* L were collected in june and jully and authenticated by Dr Abdelilah RAHOU, botanist in Moulay Ismail Faculty.

The decoction is made with 60 g of the seed powder in 1000 ml of warm distilled water for a few hours at a moderately high temperature. Then a Filtration is carried out on hydrophilic cotton, then on Wattman paper No 3 was carried out, from the filtrate obtained we prepared the different doses used during the experiment.

Experimental animals

The male Albinos mice, which are derived from the Swiss strain, are animals that all come from the Pasteur

Institute. The mice used in acute toxicity have an average weight of 30 ± 5 g (males). The breeding of these animals was carried out in faculty pet shop. The animals had free access to tap water and standard pellet diet. These mice have been deprived of food for 12 h before the beginning of the experiment. The animals were acclimatized in cages under standard environmental conditions of light/dark cycles, temperature (25°C) and air changes.

Administration of the aqueous extract

Divide the animals into 11 lots, each batch contains 7 mice. Consider a lot as a control. The aqueous extract

was administered by intraperitoneal injection to 10 groups of mice ($n = 7$). The doses injected are (4g/kg; 5g/kg; 6g/kg; 7g/kg; 8g/kg; 9g/kg; 10g/kg; 11g/kg; 11.5g/kg; 12g/kg). The control group received distilled water only. After the injection of the extract, the animals are replaced in their metal cages where they could have access to water and standard pellet diet.

Table 1: Doses injected into mice during the experiment.

Lot	Control	1	2	3	4	5	6	7	8	9	10
Dose g/kg	distilled water	4	5	6	7	8	9	10	11	11.5	12

After the administration of the extract, the mice are continuously monitored in the first, 4th, 6th, 8th and 24th hour after treatment, to notice any expected mortality or change of behavior. These signs of toxicity were monitored daily for 15 days.

RESULT

After administration of the aqueous extract we observed the mice during the first hours to record the behavioral

changes caused by *aristolochia longa L*. The observation lasts 15 days (the duration of the experiment) to detect the delayed effects of the aqueous extract. Control mice showed no signs of toxicity or mortality. The injection of the aqueous extract has led to behavioral changes in mice, the following table presents the different symptoms of toxicity observed in the mice treated with *aristolochia longa L*

Table 2: Toxicity symptom of *aristolochia longa L*.

Dose g/kg	Toxicity symptom
Distilled water	Nothing
4	Nothing
5	Weakness
6	Weakness, convulsion, death
7	Weakness, convulsion, diarrhé, hypoactivity, death
8	Weakness, convulsion, diarrhé, hypoactivity, death
9	Weakness, convulsion, diarrhé, hypoactivity anorexia, death
10	Weakness, convulsion, diarrhé, hypoactivity, anorexia, death
11	Weakness, convulsion, diarrhé, hypoactivity, anorexia, death
11.5	Weakness, convulsion, diarrhé, hypoactivity, anorexia, excitaion and death
12	Weakness, convulsion, diarrhé, hypoactivity, anorexia, excitaion and death

Mortality according to dose

The injection of *aristolochia longa* induced the death of certain mice. The number of dead animals increases with

the increase of the injected dose. Table 3 shows the number of dead mice according to the different doses.

Table 3: Number of dead mice.

Dose g/kg	4	5	6	7	8	9	10	11	11.5	12
Number of mortality	0	0	1	2	3	4	5	6	7	7

Determination of the LD50

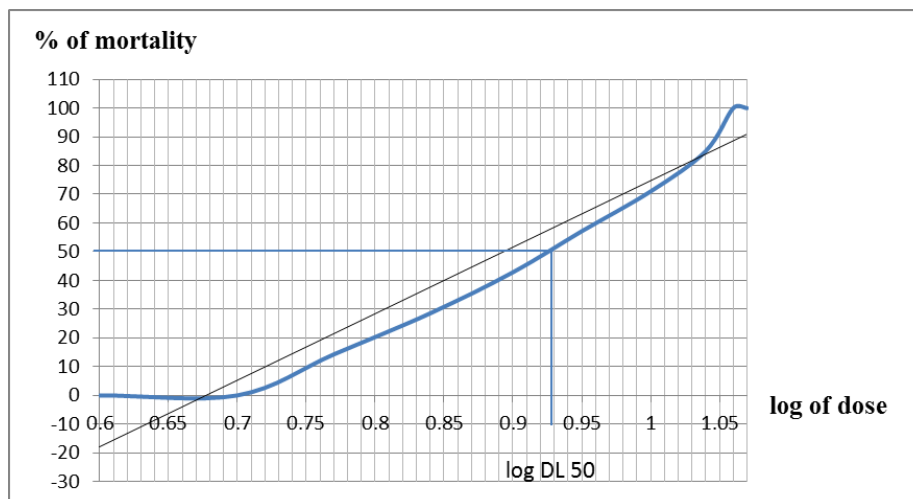
The lethal dose 50 is a very important toxicology parameter because it makes possible to classify the plants according to their degree of toxicity. To determine the lethal dose 50 of the aqueous extract of *aristolochia longa L* we adopted two methods: Trevane 1927 method, Bliss (1938) method.

Trevane (1927) method

The method of trevane is based on the presentation of death rates according to the decimal logarithm of the dose.

Table 4: Dose transformation in log dose and mortality in percentage of mortality.

Dose g/kg	4	5	6	7	8	9	10	11	11.5	12
Mortality	0	0	1	2	3	4	5	6	7	7
Log dose	0.6	0.7	0.77	0.84	0.9	0.95	1	1.04	1.06	1.07
%of mortality	0	0	14	28	42	57	71	85	100	100

**Figure 2: The log dose according to the percentage of dead mice.**

The curve obtained will be used to determine the lethal dose 50, then it is sufficient to project the 50 percent mortality value on the graph to determine the log of DL50. The LD50 corresponds to the inverse of log LD 50. The value of LD 50 is 7.9 g / kg.

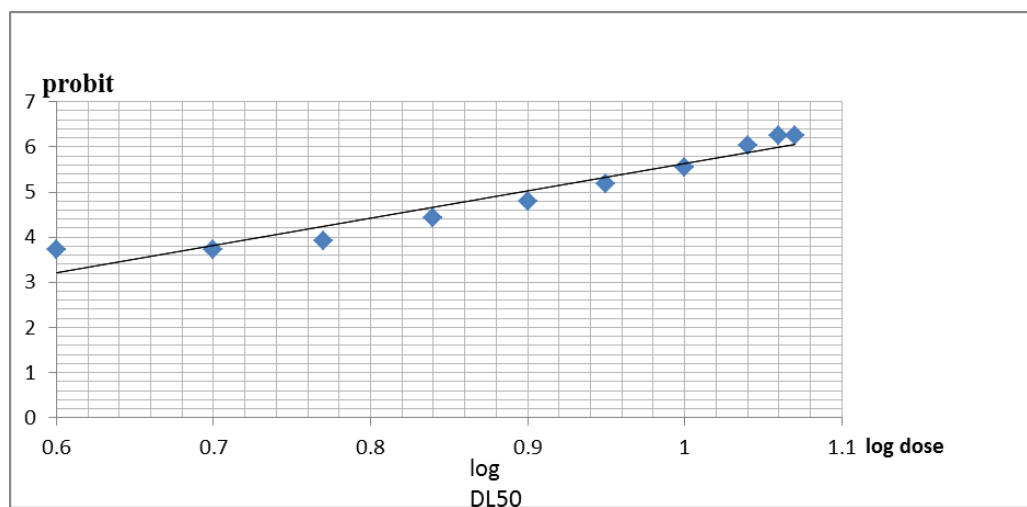
transformation it is necessary to express the percentage of mortality in unit probite and the dose in logarithmic decimal, from this line the value of the lethal dose 50 can be determined 7.6 ± 1.33 g/kg.

Bliss method (1938)

The method of Bliss is a transformation of the curve of trevane to a linear straight line, to carry out this

Table 5: Dose transformation in log dose and % of mortality in probite unit.

Dose g/kg	4	5	6	7	8	9	10	11	11.5	12
Mortality	0	0	1	2	3	4	5	6	7	7
Log dose	0.6	0.7	0.77	0.84	0.9	0.95	1	1.04	1.06	1.07
%of mortality	0	0	14	28	42	57	71	85	100	100
Probite	3.72	3.72	3.92	4.42	4.8	5.18	5.55	6.04	6.26	6.26

**Figure 2: The log dose according to the unit of probity.**

The determination of the LD50 is carried out by graphic projection. We target the probit of 5 on the Y axis, then we extrapolate it on the X axis to find the log of the lethal dose 50 that corresponds to it. 7.6 ± 1.33 g/kg.

DISCUSSION

Aristolochia longa L is a well-known plant in the Maghreb region, especially in Morocco, Tunisia and Algeria. its chemical composition (polyphenols, flavonoids, tannins, c-heterosides, carbohydrates, and saponins) make it a plant very used in the traditional medicine, but the presence of aristolochic acid I and II classifies this plant in the box of poisonous plants.

In the present study we noticed that injection of the 4g / kg dose showed no change in the behavior level of the rats, however from the 5g / kg dose we recorded some signs of toxicity such as Weakness, convulsion, diarrhea. With the increase of the dose of the aqueous extract injected the symptoms of toxicity become very remarkable this is why after the administration of the dose 12g/kg we noticed the following symptoms: Weakness, convulsion, diarrhea, hypoactivity, anorexia, excitaion and death. The same symptoms of toxicity were reported by Ghita BENZAKOUR and all 2011 who

studied Immunostimulatory potential of *Aristolochia longa L*. According to our experience, the lethal dose 100 is equal to 12g / kg while the minimum lethal dose is equal to 6g / kg. Our result is very close to that proven by Chrif HS and all 2014, they evaluated the toxicity of *aristolochia longa L* extract by administering different doses of this extract to mice, after a certain time they observed mortalities in batches receiving a dose of 4g / kg and 5g / kg.

The determination of the lethal dose 50 was carried out by two methods : Trevane (1927) and Bliss (1938). We found 7.9g/kg for Trevane method (1927) and 7.6g/kg for that of Bliss (1938). The calculation of the lethal dose 50 is an important step in the toxicological study because it allows us to indicate the degree of toxicity of the plant, to evaluate the danger in case of overdose, to program experiments of subacute and chronic toxicity in rats and determine the therapeutic index. In order to specify the degree of toxicity of our plant we used the table of Viau (2003) , this table classifies the substances according to their LD50 in five categories Ultra toxic, Extremely toxic, Very toxic, Moderately toxic, Slightly toxic, Non toxic. From this reference we can consider *aristolochia longa* as a Slightly toxic plant.

Table 6: Degree of toxicity according to LD50 Viau (2003).

Category	LD50
Ultra toxic	Less than or equal to 5mg/kg
Extremely toxic	5-50mg /kg
Very toxic	50-500mg/kg
Moderately toxic	0.5-5g/kg
Slightly toxic	5-15g/kg
Non toxic	Superior to 15g/kg

The same observation was also mentioned by Bachir Benarba and his collaborators 2016, they showed that LD 50 *aristolochia longa* is greater than 5g / kg. In another study Cherif et all 2014 classified this plant as non toxic based on the scale of Viala (1998).

The acute toxicity of certain species of the aristolochiaceae family has been the goal of several scientific studies, in order to determine their degrees of toxicity, the following table presents the lethal dose 50 of three species of aristolochiaceae.

Table 7: Lethal dose 50 of three species of aristolochiaceae.

Species	<i>Aristolochia indica</i>	<i>Aristolochia baetica</i>	<i>Aristolochia albida</i>	<i>Aristolochia longa L</i>
Lethal dose 50	7,5g/kg (mall mridula, 2011)	4g/kg (Mohammed Bourhia et all 2019)	2g/kg (Felix F. D. Guinnin et all, 2017)	7.9g/kg

Based on this table we conclude that the lethal dose 50 of *aristolochia longa* is the lowest, so it can be considered as the least toxic compared to other species.

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