

## THE ACUTE TOXICITY OF THE AQUEOUS EXTRACT OF PEGANUM HARMALA L, « MOROCCO » PREPARED BY THE TRADITIONAL METHOD

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### ABSTRACT

Peganum Harmel is a medicinal plant widely used in traditional medicine for its richness in alkaloid (harmalol, and harman and quinazoline harmine, harmaline). Unfortunately the bad use, the non respect of the doses during the treatments transforms this plant with a very powerful poison. The present study was initiated to study the acute toxicity of the aqueous extract of peganum harmala L. Administration of the aqueous extract at different doses taken intraperitoneal injection on male mice to severe symptoms of toxicity. These symptoms are presented by Drowsiness, hypoactivity, anorexia, isolation, bradycardia, difficulty breathing, excitaion, death the results obtained show that, the highest dose killing all animals or 100% lethal dose (LD 100) is 10 g / kg for peganum harmala L. while for the maximum tolerated dose it is 1g / kg. The LD50 was determined by three methods Kraber and Behrens, trevane (1927) and Bliss (1938). we obtained very close values, 1.8 g/kg for the Kraber and Behrens method, 2.4g/kg for trevane (1927) and 2.5±0.75g/kg for that of Bliss (1938). this result indicates that the aqueous extract of the *peganum harmala* L plant administered by intraperitoneal injection is moderately toxic.

**KEYWORDS:** *Peganum Harmala*, Acute Toxicity, Lethal Dose 50, Phytotherapeutics.

### INTRODUCTION

The use of plants for medicinal purposes has been practiced for many centuries by a substantial proportion of the Moroccan population. Due to economic conditions and availability, plants are the main medicinal source to treat infectious diseases in some developing countries (Sofowora, 1996). Phytotherapeutics are extracted from medicinal plants and their active ingredients may relieve symptoms and even cure diseases though they occasionally present adverse effects.

*Peganum harmala*, also called in Morocco harmal, is a species of medicinal plant of the family Zygophyllaceae, perennial, with yellowish-white flowers and small dark brown seeds (S. Achour and al., 2012). It is a perennial herbaceous glabrous plant that grows in semi-arid range lands and sandy soils, especially along the Mediterranean region in North Africa and the Middle East (El-Bahri and all., 1991). In Morocco peganum harmala intoxication, is responsible for 4.6% of all plant poisonings received at the Poison Control Center of Morocco (CAPM) in 2010.

*Peganum harmala* is among the richest plants in alkaloid (harmalol, and harman and quinazoline harmine, harmaline) according to the bibliographical study harmaline is the most abundant alkaloid in this plant it

presents 3% of the seed (Ben Salah and al., 1986; Frison and al., 2008 ; Yuruktumen and al., 2008 ; Herraiz and al., 2010 ; Marwa and al., 2011). Harmaline toxicity is more dangerous than the toxicity of other alkaloids. It causes several toxicological symptom such as respiratory paralysis and hypothermia, central nervous system depression, tremor and convulsion (Jahaniani et al., 2005).

To confirm the toxic potency of this plant, we studied The acute toxicity of the aqueous extract of *peganum harmala*, « Morocco » prepared by the traditional method on male Albinos mice.

### MATERIALS AND METHODS

The seeds of *Peganum harmala* were cleaned of impurities, washed with tap water and dried out of the light for two weeks, then crushed using a medium fine powdered mortar. Dried seeds of *P. harmala* were collected in May and June and authenticated by Dr Abdelilah RAHOU, botanist in Moulay Ismail Faculty.

The decoction is made with 40 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 Albino 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  $30 \pm 5\text{g}$  (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 ( $25^\circ\text{C}$ ) and air changes.

### Administration of the aqueous extract

Divide the animals into 11 lots, each batch contains 5 mice. Consider a lot as a control. The aqueous extract was administered by intraperitoneal injection to 10 groups of mice ( $n = 5$ ). The doses injected are (0.6g/kg; 1g/kg; 1.6g/kg; 2g/kg; 3g/kg; 4g/kg; 5g/kg; 6g/kg; 8g/kg; 10g/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	0.6	1	1.6	2	3	4	5	6	8	10

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.

### RESULTS

After the administration of the extract, the behavioral changes of the mice during the first hours were followed

to record the symptoms of toxicity caused by *peganum harmala*. The observation was extended along the duration of the acute toxicity study (15 days) to detect the delayed effects of the crude extract. The control group, containing the mice fed with distilled water, did not show any sign of immediate toxicity or mortality.

The following tables summarize all the results obtained during the experiment.

**Table 2: Toxicity symptom of peganum harmala.**

Doses g/kg	Toxicity symptom
0.6	Nothing
1	Nothing
1.6	Vibration, hypoactivity, isolation, anorexia and death
2	Vibration, hypoactivity, isolation, anorexia and death
3	Vibration, hypoactivity, isolation, anorexia and death
4	Vibration, hypoactivity, isolation, anorexia, difficulty breathing, and death
5	Vibration Hypoactivity, isolation, anorexia, difficulty breathing and death
6	Vibration, hypoactivity, isolation, anorexia, difficulty breathing, and death
8	Vibration, hypoactivity, isolation, difficulty breathing, excitation and death
10	Vibration, hypoactivity, isolation, bradycardia, difficulty breathing, excitation and death

### Mortality according to dose

The following table shows the number of dead mice according to the different doses.

**Table 3: Number of dead mice.**

Dose g /kg	0.6	1	1.6	2	3	4	5	6	8	10
Number of dead	0	0	1	2	3	4	4	4	4	5

### Determination of the LD50

There are several methods for determining LD50. In our work we use three methods: Kraber and Behrens method, Trevane 1927 method, and Bliss (1938) method.

### Karber and Behrens method

It is a close approximation method,  $\text{LD50} = (\text{LD100-AB}) / N$

A = the difference between 2 successive doses.

B = mean of death between two successive doses.

N = average number of animals per batch.

According to the relation, our LD50 is **1.8g /kg**

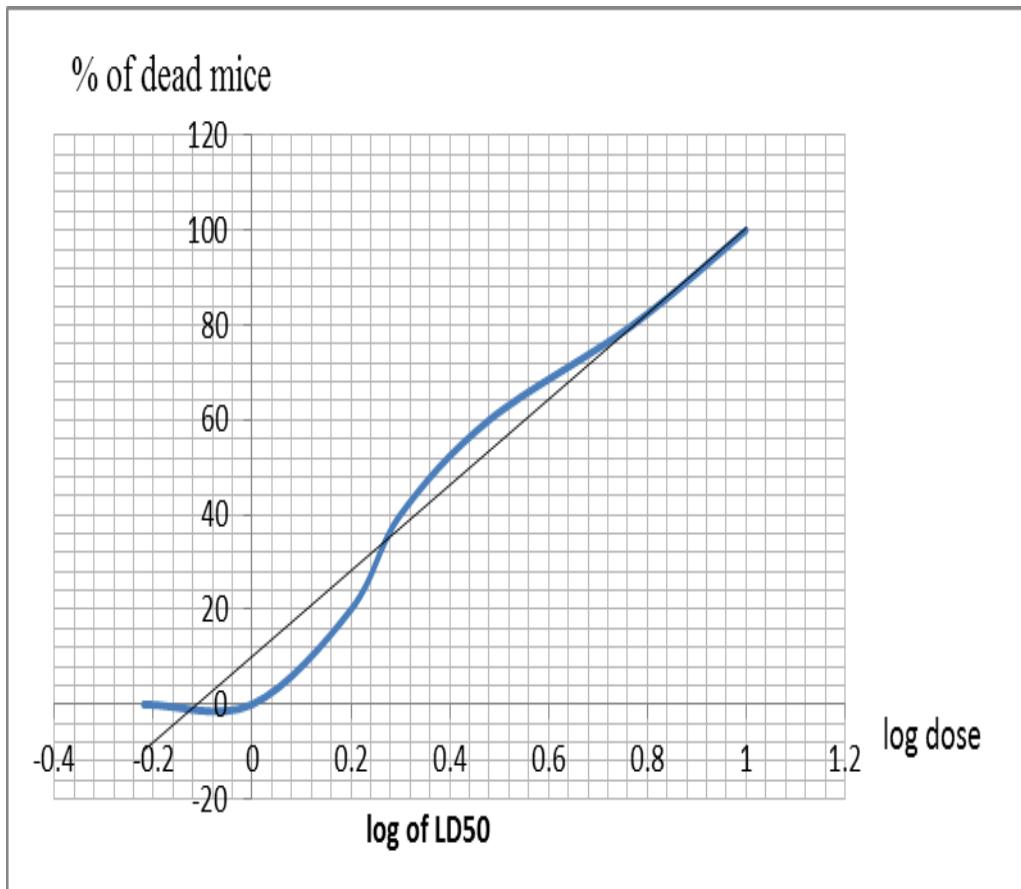
**Trevane 1927**

It is a graphical method. It is based on the truncation of the trevane curve. This curve represents the percentage

of dead mice according to the logarithm of injected doses.

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

Dose g/kg	0.6	1	1.6	2	3	4	5	6	8	10
Mortality	0	0	1	2	3	4	4	4	4	5
% of mortality	0	0	20	40	60	80	80	80	80	100
Log dose	-0.22	0	0.20	0.30	0.48	0.60	0.70	0.77	0.90	1

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

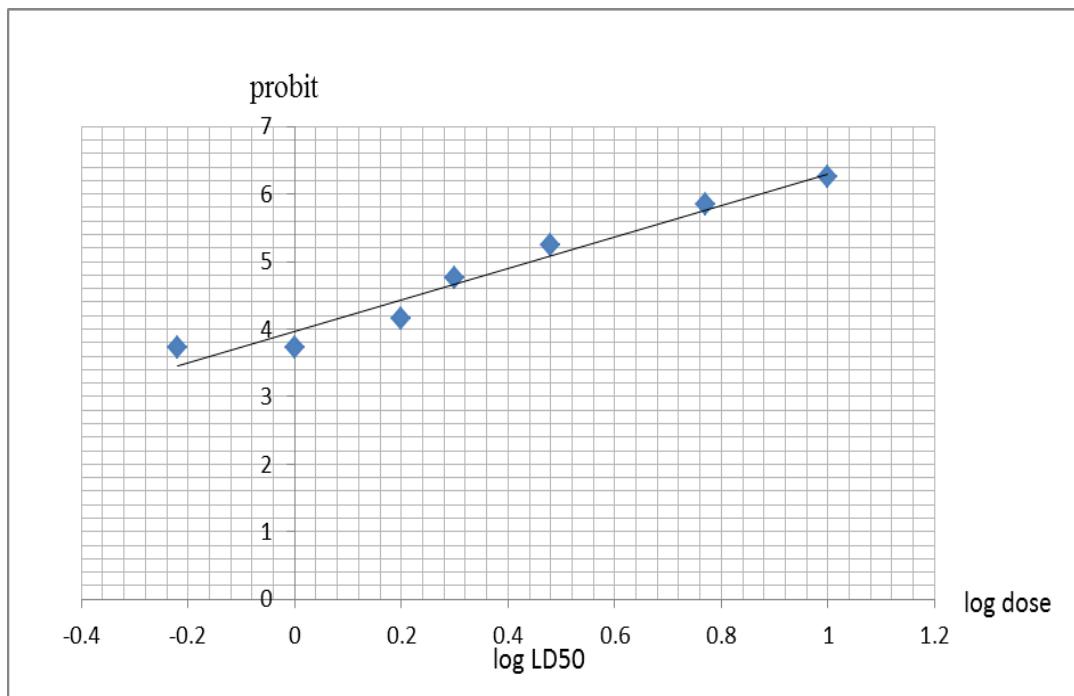
The determination of the lethal dose 50 is made graphically by extrapolation on the graph. The LD50 corresponds to the inverse of log LD 50. The value of LD 50 is 2.4 g / kg.

**Bliss method (1938)**

This method is based on the transformation of mortality percentage into probit unit, the dose in logarithmic value to obtain a regression line. from this line the value of the lethal dose 50 can be determined. In order to obtain a linear curve we took into consideration the most significant values.

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

Dose g/kg	0.6	1	1.6	2	3	4	5	6	8	10
Mortality	0	0	1	2	3	4	4	4	4	5
% of mortality	0	0	20	40	60	80	80	80	80	100
Log dose	-0.22	0	0.20	0.30	0.48	0.60	0.70	0.77	0.90	1
Probit	3.72	3.72	4.16	4.75	5.25	5.84	5.84	5.84	5.84	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. The LD50 corresponds to the inverse of log LD50, our LD50 is  $2.5 \pm 0.75$  g/kg.

## DISCUSSION

*Peganum harmala* is a plant rich in alkaloids namely harmine, harmaline, harmol and harmalol (Duke, 1985; Bruneton 1987). This richness in alkaloids gives our plant a very important toxic power (bellakhdar, 1997). In our study it was noted that the injection of doses 0.6 and 1 g / kg showed no change in behavior against the dose 1.6 g / kg caused the, vibration, hypoactivity, isolation, anorexia. Fure is as the injected dose increases the symptom become very remarkable, vibration, hypoactivity, isolation, bradycardia, difficulty breathing, excitaion, death. Frison and al., 2008 and Mahmoudian and al., 2002 described the same symptoms of toxicity observed in our work. We noted the 10g / kg dose as an LD100.

The determination of the lethal dose 50 was carried out by three methods Kraber and Behrens and trevane (1927) and Bliss (1938), we obtained as a result three values close to 1.8 g/kg for the Kraber and Behrens method, 2.4g/kg for trevane (1927) and  $2.5 \pm 0.75$ g/kg for that of Bliss (1938) then we conclude that the three values are very close. Horn (1956) revealed that a substance can be considered toxic only if it has an LD50 of less than 3g /kg, based on this result we can confirm that *peganum harmala* morocco is a toxic plant. To clarify the degree of toxicity of *peganum harmala* we have taken as a reference the table of Viau (2003). From this table we

have classified *peganum harmala* as a moderately toxic plant LD50 is between 0.5-5g / kg.

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

Catégories	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

Our LD50 is close to that of REZZAGUI Abire (2012), she found a value of 2.8585g / kg in the mice administered by the aqueous extract of *peganum harmala*, such a result was also reported by Lamchouri and al., 2002, that they studied the acute and chronic toxicity of *Peganum harmala* seeds by oral rou2te, they recorded an LD50 equal to 2.70g / kg, of a Marua H. AL-Hammoshi (2010) rated 1.07g / kg as LD 50.

In another study by Muhi-eldeen and al., 2008, that they administered well-determined doses of the aqueous extract of *peganum harmala* namely, 200, 300, 350, 400, 450, 500 and 550 mg / kg to albino mice, the LD50 was 420 mg / kg. So this value is much lower than that found in our experience.

Ahmed Melhaoui (2006) explained that the variation of the LD50 is according to the stages of development of this plant, so the toxicity of *peganum harmala* is related to the total alkaloid levels recorded during its growth. It also varies with species, sex, and age of the animal,

temperature, diet, rearing conditions, and timing of administration.

## CONCLUSION

*Peganum harmala* is a plant widely used in traditional medicine. In Morocco, it is considered an abortifacient, emmenagogue, oxytocic, sedative, anthelmintic, antimalarial, purgative, emetic, antiseptic, cicatrizant, sudorific and diuretic agent. It is still a subject of complicated research because it presents a remarkable risk of toxicity. Its toxicity is due to the presence of a high rate of alkaloids (harmine, harmaline, harmalol and harman), especially in the seed. The study of the acute toxicity of the peganum harma aqueous extract allowed us to consider this moderately toxic plant, so the scientific work and the sensitization will be a very important step to reduce the risk of toxicity by this plant.

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