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ACUTE TOXICITY STUDY IN MICE OF RUTA CHALEPENSIS L AQUEOUS AERIAL PART EXTRACT AN ANCIENT MOROCCAN TRADITIONAL MEDICINAL PLANT

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ABSTRACT

Ruta chalepensis L (Rutaceae) is an aromatic medicinal plant. It is still used in traditional medicine in many countries as laxative, anti-inflammatory, analgesic, antispasmodic, abortifacient, antiepileptic, emmenagogue and for the treatment of cutaneous pathologies. Many of *Ruta chalepensis L* therapeutic properties have been studied for decades in many countries around the world so as to understand its mechanism of action. The purpose of this study is to determine the acute toxicity parameters of the aqueous extract of *Ruta chalepensis L* on white males mice divided into several batches treated with different doses of the extract. The results obtained at the end of the experiment give a Lethal Dose 100 (LD100), 30 g / kg / vi body weight, a lethal dose 50 (LD50) of 19.6 g / kg /vi body weight and a Maximum Tolerated Dosage (DMT) of 4.5g / Kg / vi of body weight. The value of the Lethal Dose 50 obtained makes it possible to deduce that the total aqueous extract of *Ruta chalepensis L* is relatively harmless in Swiss albino white mice.

KEYWORDS: *Ruta chalepensis L,* Acute toxicity, LD50, LD100, DMT.

INTRODUCTION

The treatment of diseases with herbal remedies is still popular (WHO Regional Office for the Western Pacific, 1977; Qureshi et al., 1989). Currently, according to WHO (World Health Organization) , more than 80% of the world's population, especially in underdeveloped countries, use traditional treatments to meet their health and primary care needs (Eddouks et al.,2007).

Previous studies of the Poison Control Center and Pharmacovigilance of Morocco (CAPM) showed that plants were involved in 3 to 5% of all intoxications, but resulted in fairly high mortality (17%) (Ouamni L et al., 2009., Khattabi et al., 2000).

The choice of the plant for the evaluation of the acute toxicity was realized in view of the multitude of therapeutic uses that presents *Ruta chalepensis L* and also at different ages.

Ruta chalepensis L is a medicinal plant still used in traditional medicine in many countries as laxative, analgesic, antispasmodic, abortive, antiepileptic, emmenagogue and for the treatment of cutaneous pathologies. Also *Ruta chalepensis L* was said, in addition to its medicinal properties, to be used as antifertility plant in Turkish and Chinese civilizations for hundreds of years.

Phytochemical screening has highlighted the presence of coumarins (chalepensin, chalpin, rutamarin ... etc.) and alkaloids (kokusaginine, skimmianine, arborinin, etc.) (S.Mansour et al., 1990).

Ruta chalepensis L is an aromatic plant. It belongs to the family Rutaceae, commonly called by the local population "Fidgel". It is spontaneous, largely in North Africa, particularly in Morocco. It is found frequently in rock gardens, lawns and dry hillsides (Nt Beniston, 1984). Pharmacological investigations have shown that the ethanolic extract of the plant aerial parts of *Ruta chalepensis L* has anti-inflammatory activity and antipyretic activity (T.Johnson, 1999).

MATERIALS AND METHODS

Preparation of aqueous extract

The plant was harvested, dried and stored in the sun at room temperature for about 6 weeks it has been identified by RAHOU Abdelilah Botanist of the department of Biology of the Faculty of Sciences of Meknès.

In order to be in accordance with the traditional form of use of the *Ruta chalepensis L* as recommended by traditional therapists, we proceeded to an aqueous decoction to obtain study extracts. 80 g of dry matter (areal parts plant blossoms and leaves) were decocted in a flask containing 2000ml of distilled water, the decoction was kept under continuous reflux for two hours, at the end of this operation, the decoction obtained after cooling was filtered through a funnel containing a cotton wool and centrifuged 2500tours/min.

Packaging and constitution of batches of mice

The experimental animals used to evaluate the acute toxicity of the aqueous extracts of *Ruta chalepensis L* were Swiss-type white mice (males) approximately 6-8 weeks old with an average weight of 28 ± 0.7 grams. The solutions administered were prepared the day before. The mice of the control group (batch 1) received 0.5 ml of physiological NaCl solution (0.9%). The mice of the other batches received different concentrations of aqueous extracts of *Ruta chalepensis L* according to the average weights of the different batches. These solutions were administered intraperitoneally using an insulin syringe previously sterilized with 90% alcohol. The animals thus treated were subjected to continuous observation for 24 hours in order to record the number of deaths and the clinical signs observed for each batch. (Bruneton, 2009.)

Observation of symptomatic disorders

After the injection of the extract, the animals are replaced in their metal cages where they could access the pellets again. They were observed immediately, every 30 minutes, for eight hours, the first day then once a day for 48 hours. During this period, symptomatic disorders (agitation, lack of appetite, motor difficulties and dyspnea) were noted, in the animals of the constituted batches.

Evaluation of acute toxicity

Concentrations of aqueous extract of *Ruta chalepensis L* were prepared on the basis of the principle that the concentrations to be administered should be reduced to the body weight of the mice since the injected doses are expressed in g / kg of body weight.

Determination of lethal doses; The 100 mice averaging 28 ± 0.7 grams divided into 10 batches of 10 mice received intra-peritoneal injections. The 10 batches were treated respectively with doses of aqueous extract of *Ruta chalepensis L* at the respective concentrations of (Table 1)

Table 1: Corresponding injected doses of aqueous extract of *Ruta chalepensis L* for the treatment of each batch.

Batch Number	Dose g/kg
1	0.6
2	1.5
3	4.5
4	7.5
5	15
6	22.5
7	30
8	45
9	60
10	75

Then the lethal dose 50 that caused 50% of death and the one that resulted in 100% of death were sought. The acute toxicity parameters to be determined in this study were:

- Tolerated Maximum Dose (DMT);
- Lethal dose for 50% effect (LD50);
- Lethal dose for 100% effect (DL100).

For the determination of toxicological parameters, a comparative study of three methods was carried out

Method 1: The 50% lethal dose (LD50) was obtained from the TREVAN curve (Trevan, 1927) given by the percentage mortality of the mice as a function of the decimal logarithm of the doses administered.

Method 2: calculation method from the formula of (Karber and Berhens., 1935). It is calculated as follows

DL50=DL100-somme (a*b)/n

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

Method 3: the method of calculating the LD50 and his confidence limits were described by (Miller and Tainter, 1944) and (Muhammad, 2009).It consists to report directly on Log Probit paper, the percentage of mortality according to the log of the doses. The calculations of the DL50/DL99 and DL1/DL50 ratios were used to validate the regression line.

RESULTS

Clinical signs noted after injection of the extract

A few moments after injection of the extract, at doses ranging from 0.6 to 75 g / kg, a lack of appetite, motor difficulties and dyspnea were noted. A brief stirring period of 5 minutes was followed by drowsiness and stretching. Twenty minutes later, the animals resumed their normal habit in batches: 1; 2; 3. Mortalities are noted in batches: 4; 5; 6; 7; 8; 9; And changes in the general appearance of mice (hair, skin, eye, ear and mouth conditions) were observed in batches: 5; 6; 7.

In summary, behavioral changes were observed compared to the control group. This experiment shows that the extract of the plant seems to exert, at different doses, a stressful effect on mice (Table 2).

Table 2: Clinical signs observed during the first 24 hours after injection of the aqueous extract of *Ruta chalepensis L*.

Batches / Clinicalsigns	Batch témoin	Batch1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10
Abdominal constrictions	-	-	-	*	*	*	*	*	*	*	*
Immobility	-	-	-	-	-	-	*	*	*	*	*
Feed	*	*	*	*	*	-	-	-	-	-	-
Accelerated breathing	-	*	*	*	*	*	*	*	*	*	*
Paralysis of hind limbs	-	-	-	-	-	-	-	*	*	*	*

- = absence of signs
* = Presence of signs

Injection effect of the extract on mice mortality

During these investigations on mice, various signs of toxicity were noted (Table 2). The extract of *Ruta chalepensis L*, injected at doses ranging from 0.6 to 75 g / kg, caused the death of the mice, according to the batches constituted. An increase in animal mortality is observed as the dose increases. There is therefore a dose-dependent effect (Table 3).

Table 3: Mortality versus time with aqueous extract of *Ruta chalepensis L.*

Batch	T	1	2	3	4	5	6	7	8	9	10
Number of dead animals	0	0	0	1	2	4	6	8	9	10	10
Observation time of batches of mice	24h	24h	24h	24h	12	11h	8h	1h	1h	1h	1h

Determination of toxicological parameters

The highest dose that kills all animals or 100% lethal dose (LD100) is 30g / kg / vi and the maximum tolerated dose (MTD) that does not kill any animals when the extract is administered, is 4.5g / kg / vi. In the numerical application of the formula (Karber and Berhens, 1935), calculating the LD50 of the extract, gives the value of 19.6g / kg / vi and also from the TREVAN curve (Trevan, 1927), and the value of the LD50 obtained by the method of (Miller and Tainter, 1944) is 19.8g/kg/vi.

According to the toxicity scale of (Hodge and Sterner, 1943), this LD50 value (19.6g / kg) over 48 hours of observation indicates that the aqueous extract of *Ruta chalepensis L*, administered by intraperitoneal injection, is relatively harmless in mice under the conditions of this study (Table 4 , 5 and Figure 1 , 2).

Table 4: Toxicity class, according to the toxicity scale of (Hodge and Sterner, 1943).

Index or class of toxicity	Commonly used term	Toxicological parameters (LD50)
1	Extremely Toxic	DL**50** ≤ 1mg/kg
2	Hotly Toxic	1mg/kg ≤ DL**50** ≤ 50mg/kg
3	Moderately Toxic	50mg/kg ≤ DL**50** ≤ 500mg/kg
4	Slightly Toxic	500mg/kg ≤ DL**50** ≤ 5g/kg
5	Almost Toxic	5g/kg ≤ DL**50** ≤ 15g/kg
6	Relatively Harmless	15g/kg ≥ DL**50**

Table 5: Evolution of the mortality of the mice according to the doses of the aqueous extract of *Ruta chalepensis L.*

Batch	1	2	3	4	5	6	7	8	9	10
Doses g/kg/vi	0.6	1.5	4.5	7.5	15	22.5	30	45	60	75
Mouse Mortality %	0	0	10	20	40	60	80	90	100	100
Mortality (Probit unit)	0	0	3.52	4.16	4.75	5.25	5.84	6.64	8.09	8.09
Logarithm doses of injected product	-0.22	0.176	0.653	0.875	1.170	1.352	1.477	1.653	1.778	1.875

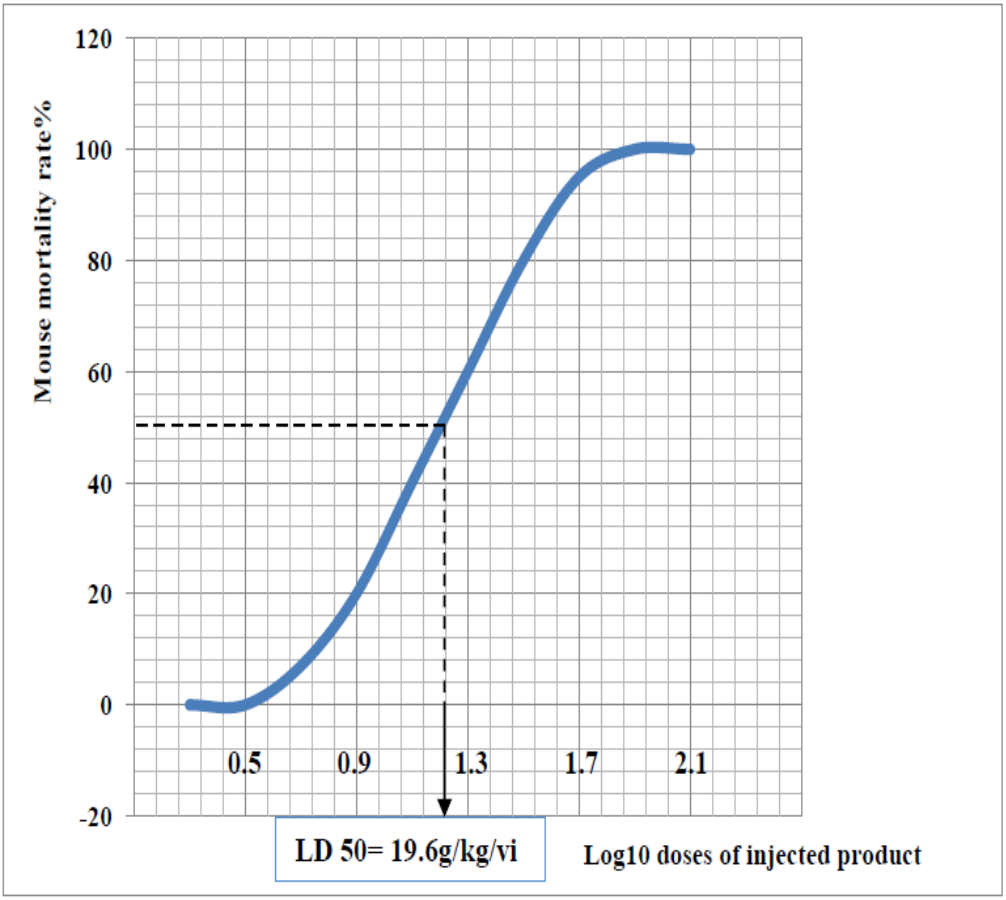

Figure 1: Evolution curve of mouse mortality as a function of the doses of the total aqueous extract of *Ruta chalepensis L.*

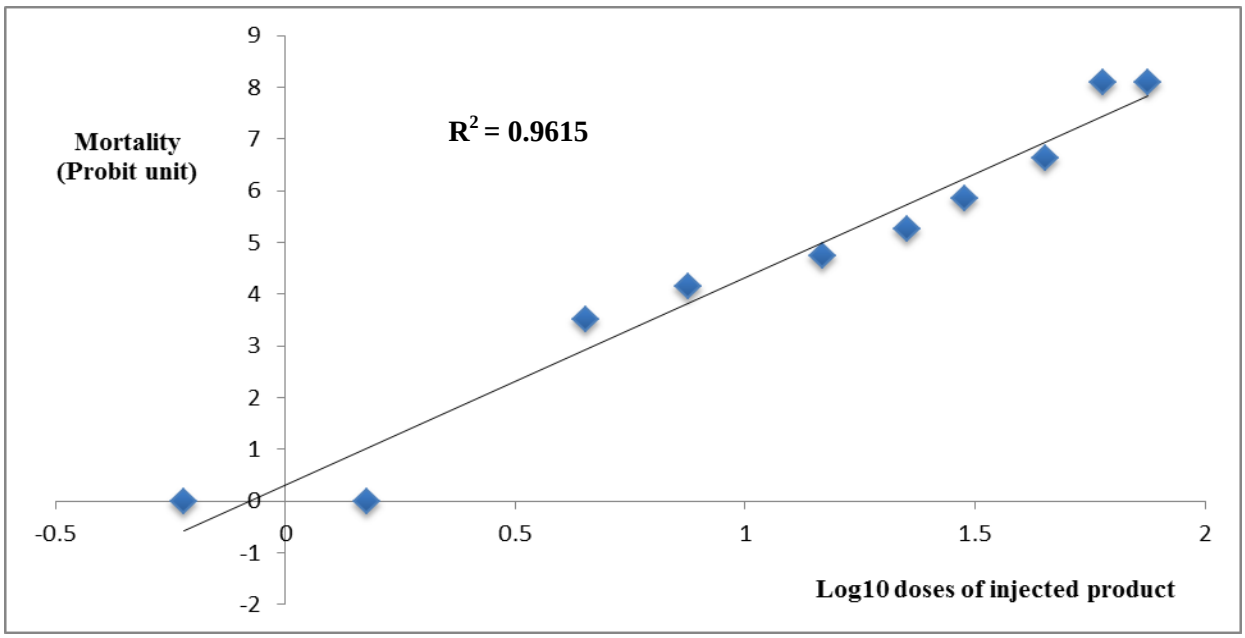

Figure 2: Regression line of mouse mortality (Probit unit) as a function of the doses (Log 10) of the total aqueous extract of *Ruta chalepensis L.*

DISCUSSION

The analysis of the results obtained indicates a growth in the mortality rate as the doses of the aqueous extract *Ruta chalepensis L* are increased. Indeed, the mortality rate increased by 20% of the dose from 7.5 g / kg / vi to 15g / kg / vi, and from 20% of the dose 15g / kg / vi to 22.5g / kg / vi, and 40% of the dose 22.5g / kg /vi at 30g / kg / vi. This allows to deduce a dose response effect (WHO, 2009) of the total aqueous extract of *Ruta*

chalepensis L in mice. The various tests of toxicity of the aqueous extract of *Ruta chalepensis L* allowed us to obtain the following results whose maximum tolerated dose is 4.5g / kg / vi of body weight; the lethal dose 50 of 19.6g / kg / vi and the lethal dose 100 of 30g / kg / vi. The acute toxicity parameters thus obtained show that there is no mortality in the mice at doses between 0 and 4.5 g / kg / vi body weight. However, at doses above 4.5g / kg / kg body weight, dead mice were found after a definite time. The resulting DMT appears to be that tolerated by the body, and could therefore potentially be used experimentally in a subacute or chronic toxicity study. Thus, the value of 19.6 g / kg / vi of body weight obtained for the LD50 in mice makes it possible to classify the aqueous extract of *Ruta chalepensis L* administered by intraperitoneal injection, as relatively harmless, in mice under the conditions of this study on the classification scale of Hodge and Sterner (OECD, 2004), developed by Cotonat (Cotonat, 1996).In addition, for the value of the LD50, a 70 kg person should receive 19.6 × 70, or 1372g of product in a single dose to run the same risks. This dose of 1372 g of aqueous extract of *Ruta chalepensis L* on the classification scale of Gosselin Smith and Hodge (Gosselin, 1984) could be classified as a substance that is harmless to humans.

CONCLUSION

At the end of this work, it appears that the aqueous extract of *Ruta chalepensis L* is a relatively harmless substance according to the classification scale of Hodge and Sterner (OECD, 2004). The harmless nature of its toxicity would confer on this phytomedicine the possibility of a therapeutic use which should be safe at doses below 4.5g / kg / vi of body weight. This makes this plant a real hope in the treatment of certain diseases indicated in the literature, and in particular in the pathologies of rheumatism (Perry, 1980, El-Tawil, 1983, Ageel et al., 1987). In perspective, it would be interesting initially to perform biochemical, hematological and anatomo-histopathological investigations to identify the organs affected by the product, understand its mechanisms of action and the nature of toxic effects induced at high doses. Then, studies of purification and chronic toxicity of extracts of the aerial part of *Ruta chalepensis L* could make it possible to apprehend its long-term effects in order to constitute a complete toxicological file on this phytomedicine.

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