World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

TOXICITY STUDY OF THE AQUEOUS EXTRACT OF TEUCRIUM CAPITATUM L AND SILENE VULGARIS OF THE EL HAJEB REGION (CENTRAL MOROCCO)

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Article Received on 08/08/2017 **Article Revised on 29/08/2017** **Article Accepted on 20/09/2017**

ABSTRACT

In this study, we studied the acute toxicity of the aqueous extract of two plants *Teucrium capitatum L* and *Silene vulgaris*, selected from an ethnobotanical study of the EL HAJEB region (Morocco), followed by a phytochemical, antimycobacterial and toxicity study. Administration of the aqueous extract at different doses taken orally on male and female mice to severe symptoms of toxicity. These symptoms are caused by respiratory problems, tremors, paralysis of the hind legs and comas causing death. According to the results obtained, the highest dose killing all animals or 100% lethal dose (LD100) is 9600 mg / kg / vo for *Teucrium capitatum L* and 4800 mg / kg / vo for *Silene vulgaris*. Whereas for the maximum tolerated dose (DMT) it is 1200mg / kg / vo for *Teucrium capitatum L* and 600mg / kg / vo for Silene vulgars. . The LD50 value (5880 mg / kg / vo) over 48 hours of observation indicates that the aqueous extract of the *Teucrium capitatum L* plant administered orally (VO) is almost non-toxic, for mice. The calculation of the LD50 value of the extract of *Silene vulgaris* gives the value of 2430 mg / kg / vo, the plant is considered to be slightly toxic.

KEYWORDS: *Teucrium capitatum L*, *Silene vulgaris*, toxicity, lethal dose, tolerated dose.

INTRODUCTION

Any biologically active substance is susceptible, at high doses or at low doses, and for prolonged administration to produce adverse or even harmful effects, this is the particular case of plant products rich in secondary metabolites, the main properties of which are effects antifungals and antimycotics demonstrated by the phytochemical screening already carried out in *Teucrium capitatum L* and *Silene vulgaris*. Since these chemical compounds can give the plant toxic properties at high doses by the general route, it is essential to determine their toxic potential for a rational adaptation of tradithapy, especially for the modes of administration and the precautions to be observed in cases of non-integrity in the digestive mucous membranes (mouth, stomach, intestine, etc.). Similarly, the evaluation of the acute general toxicity of the extract is necessary to situate the tolerance limits of the studied plants.

MATERIALS AND METHODS

Preparation of the aqueous crude extract.

The plants of *Teucrium capitatum L* and *Silene vulgaris* (980 g), harvested and rinsed, were then subjected to a decoction for 45 minutes with 6 liters of distilled water. The mixture was drained in a square of clean cloth, filtered successively twice on hydrophilic cotton and then on 3 mm Wattman paper. The volume of the filtrate (approximately 3 l) was evaporated in a rotavapor and then in an oven at 60 ° C. After two days, the crystals obtained were sprayed, using a porcelain mortar and pestle. The collected fine powder (34.5 g) constituted the total dry extract which we stored in a sterile glass jar in the refrigerator.

Evaluation of doses

The maximum concentration, which corresponds to a concentration at the limit of the solubility of the extract, was sought. 6.4 g of total extract was dissolved in 20 ml of distilled water, corresponding to a maximum concentration of 320 mg / ml. This concentration led to the study of acute toxicity. From this stock solution, successive dilutions are prepared at 1/2,, 1/4, 1/8 and 1/16. This made it possible to obtain, from the stock solution, a range of concentrations of 160; 80; 40 and 20 mg / ml. After subjecting the animals to a 12-hour fast, the stock solution (320 mg / ml) was administered by gavage using an intubation cannula with a slightly curved tip. Gavage was done with a volume of 0.6 ml per 20 grams of body weight. The dose of extract to be

administered is then expressed in mg / kg / vol of body weight. Overall, volumes of 0.39 to 0.75 ml were administered to the animals, depending on their body weight. The different concentrations obtained: 20; 40; 80; 160 and 320 mg / ml) correspond to the respective doses of 300; 600; 1200; 2400; 4800; 9600mg / kg / vol of body weight

Animals used for the assessment of acute toxicity
To conduct this acute toxicity study, sixty (70) mice for each plant. The animals (mice) were kept at the animal facility. The animals were 4 to 6 weeks old and weighed between 20 grams of body weight. They were placed in aerated metal cages containing regularly renewed wood chip litter and then acclimatized to the conditions of the animal facility for three days before treatment.

Prior to treatment, the animals were subjected to a 12-hour fast. They were divided into seven lots of 10 as follows.
-lot 01: control mice receiving Nacl (control group).
-lot 02: mice treated with the extract at 320 mg / ml.
-lot 03: mice treated with the extract at 160 mg / ml.
-lot 04: mice treated with the extract at 80 mg / ml.
-lot 05: mice treated with the extract at 40 mg / ml.
-lot 06: mice treated with the extract at 20 mg / ml.
-lot 07: mice treated with the 10 mg / ml extract.

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

Evaluation of toxicological parameters
Determination of DMT and DL100
After administration of the extract, at the various concentrations (320, 160, 80, 40, 20 and 10 mg / ml) corresponding to the mg / kg / p.c. / vo doses, the dead animals were counted in each batch for 48 hours. This acute toxicity trial was conducted to determine the toxicological parameters of the 50% lethal dose (LD50), a dose that kills 50% of the animals, the 100% lethal dose (LD100), a dose that kills all animals and the maximum tolerated dose (MTD) which represents the maximum dose that does not kill any animals when the extract is administered.

The lethal dose 50 (LD50) is the dose of a chemical that, when administered to experimental animals, causes the death of half of them; it can be determined by several methods which are: Dragstedt and Lang method, Karberet Behrens method (Stowtchiva, 1988) and Miller and Tainter method. In our study, the Karber and Behrens method is used to determine the LD50 of the two plant extracts *Teucrium capitatum L* and *Silene vulgaris*. The principle of this technique consists in administering increasing doses of substance to batches of rats of uniform mass, the dose administered is expressed in mg / kg of body mass of the animals and the difference between the neighboring doses must be constant. For each lot, the percentage of mortality is recorded within one hour or at the end of the time allowed.

The LD50 is obtained by the formula: LD50 = DL100 -Σ (a.b) / nLD50: Lethal Dose 50%; DL100: 100% lethal dose; a: mean of the sum of deaths between two successive doses; b: difference between two successive doses; n: number of animals used per batch.

RESULTS AND DISCUSSIONS

Determination of Acute General Toxicity Clinical signs noted after gavage of the extract
After gavage of the plant extract of *Teucrium capitatum L* and *Silene vulgaris* at doses ranging from 300 to 9600 mg / kg / v, several symptoms were observed and marked in the following table.

Table 1: Acute toxicity symptoms of *Teucrium capitatum L* and *Silene vulgaris* extracts based on severity.

Plants	Concentration (mg/ml)	Dose (mg/kg)	Signs et Symptoms
Teucrium capitatum L	0	(NaCl 9‰)	Normal
	10	300	Stitched hair
	20	600	Stitched hair, stressed rats
	40	1200	Tired breathing
	80	2400	Accelerated heart rate
	120	4800	Convulsion, immobilization of rats,
	320	9600	State of drowsiness, paralysis, mortality
Silene vulgaris	0	(NaCl 9‰)	Normal
	10	300	Stitched hair
	20	600	Stitched hair, stressed rats
	40	1200	Tired breathing
	80	2400	Accelerated heart rate
	120	4800	Convulsion, immobilization of rats,
			State of drowsiness, paralysis, mortality

Mortality according to dose

An increase in animal mortality was observed as the dose increased, thus allowing a dose response to be deduced, the results are shown in Table 2.

Table 2: Mortality rate of mice after gavage of the extract of *Teucrium capitatum L.*

	Nomber of batch						
	1	2	3	4	5	6	7
Concentration (mg/ml)	0	10	20	40	80	160	320
corresponding Dose (mg/kg/p.c./vo)	0	300	600	1200	2400	4800	9600
Number of mice per batch	10	10	10	10	10	10	10
Number of death	0	0	0	0	1	3	10
Percentage of death	0	0	0	0	10	30	100

The first dead mouse was observed in batch 5, whereas in batch 6 the death of three mice was noted. At the 24th hour, all the mice of batches 7 were found dead for a dose of 9600mg / Kg of the extract of *Teucrium capiatum L*.

Table3: Mortality rate of mice after gavage of the extract of *Silene vulgaris.*

	Number of batch						
	1	2	3	4	5	6	7
Concentration (mg/ml)	0	10	20	40	80	160	320
corresponding Dose (mg/kg/p.c./vo)	0	300	600	1200	2400	4800	9600
Number of mice per batch	10	10	10	10	10	10	10
Number of death	0	0	0	3	5	10	10
Percentage of death (%)	0	0	0	30	50	100	100

For the aqueous extract of *Silene vulgaris*, the total mortality of the mice was discovered in lot 6 for a dose of 4800 (mg / kg / p.c. / vo).

Determination of toxicological parameters

According to the results, the highest dose killing all animals or 100% lethal dose (LD100) is 9600 mg / kg / vo for *Teucrium capitatum L* and 4800 mg / kg / vo for *Silene vulgaris*. And the maximum tolerated dose (DMT) is 1200mg / kg / vo for *Teucrium capitatum* and 600mg / kg / vo L for *Silene vulgaris*. In the numerical application of the formula of Karber and Berhens (1935), the calculation of the LD 50 of the extract gives the value 5880 mg / kg / vo and 2430 mg / kg / vo for *Teucrium capitatum L* and *Silene vulgaris* respectively.

According to the Hodge and Sterner (1943) toxicity scale, applied to our plants allowed us to measure the toxicity of our extracts and to establish toxicity classes (Table 46). In general, the lower the LD50, the more toxic the substance is. On the contrary, the higher the LD50, the lower the toxicity (Stowtchiva, 1988; Oduola et al., 2007).

Table 4: Mortality rate of mice after gavage of the extract of *Silene vulgaris.*

(Ulanova, 1975 ; Frank, 1992)

LD50 Oral Toxicity Index	LD50 Oral Toxicity Index
Up to 1 mg / kg	1 = extremely toxic
1 to 50 mg / kg	2 = highly toxic
50 to 500 mg/kg	3 = moderately toxic
500 to 5 000 mg/kg	4 = slightly toxic
5 000 to 15 000 mg/kg	5 = almost non-toxic
Plus de 15 000 mg/kg	6 = relatively harmless

Under the conditions of this study, the LD50 value (5880 mg / kg / vo) over 48 hours of observation indicated that the aqueous extract of the plant of *Teucrium capitatum L*, administered orally (VO) is almost non-toxic, for mice, calculation of the LD50 of the extract of Silene vulgaris gives the value of 2430 mg / kg / vo, the plant is considered slightly toxic.

DISCUSSIONS

The analysis of the results obtained indicates an increase in the mortality rate as the doses of the total aqueous extract of two plants increase. Indeed, this makes it

possible to deduce a dose response effect (Who, 2009; Djyh, 2010) of the total aqueous extract on the mice.

The acute toxicity parameters thus obtained show that there is no mortality in mice for doses between 300 and 1200 mg / kg of body weight for the *Teucrium capitatum L* plant and for the plant *Silene vulgaris* this range is located between 300 and 600 mg / kg. The obtained DMT of 1200mg / kg / vo for *Teucrium capitatum L* and 600mg / kg / vo L for *Silene vulgaris* appears to be that tolerated by the organism, and could therefore potentially be used experimentally in a subacute toxicity study or chronic.

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