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An Acute Oral Toxicity Study of Seeds of *Spermacoce Hispida* Linn, in Albino Wistar Mice as Per OECD Guidelines 425

Dhevi R*[1], Elango V[2], Gayathri K[3], Mohamed Shabi M[4]
1. Research Scholar, Department of Siddha medicine, Tamil University, TN, India.
2. Assistant Professor, Department of Siddha medicine, Tamil University, TN, India.
3. Assistant Professor, Department of Biochemistry, Vels University, TN, India.
4. Lecturer, Department of Pharmacology, Asia Metropoliton University, Malaysia

ABSTRACT

Acute toxicity refers to those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours. Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use and are useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. The present study has been undertaken to study the adverse or hazardous effects of hydro alcoholic extract of seeds of *Spermacoce hispida* Linn, in Albino wistar mice as per OECD guidelines 425. Single dose of *spermacoce hispida* seed extract at the dose of 500, 1000 and 2000mg/kg b.wt were administered to albino wistar mice. No mortality or any clinical sign change was observed during the entire study period. The LD_{50} and maximum tolerable dose value of the extract was greater than 2000mg/kg body weight.

Keywords: *Spermacoce hispida*, Acute Toxicity, OECD Guidelines 425, Lethality (LD_{50}).

*Corresponding Author Email rdhevi23@gmail.com
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INTRODUCTION

OECD guidelines for the Testing of Chemicals are periodically reviewed for scientific progress. The concept of the up-and-down testing approach was first described by Dixon and Mood[1,2]. In 1985, Bruce proposed to use an up-and-down procedure (UDP) for the determination of acute toxicity of chemicals[3]. For estimating LD_{50} several variations exist in up-and-down procedure. This guideline is based on the procedure of Bruce and revised in 1990[4]. The test procedure described in this Guideline is of value in minimizing the number of animals required to estimate the acute oral toxicity of a chemical. In addition to the estimation of LD50 and confidence intervals, the test allows the observation of signs of toxicity. Revision of Test Guideline 425 was undertaken concurrently with revisions to the Test Guidelines 420 and 423.

Acute toxicity testing is to measure the adverse effects that occur within a short period, after the administration of single dose of herbal extract. The studies performed principally in rodents, provide information on the health hazards likely to arise from short-term exposure and are usually an initial step in the evaluation of toxic characteristics of a substance of both health and environmental effects. Acute testing can be used to identify doses associated with target organ toxicity and lethality that may be preferable to humans. Acute toxicity data may also provide information about the mode of toxic action of substances, which can aid in the diagnosis and treatment of toxic reaction. It is used to standardize biological products and can serve to establish dosing levels for repeated dose studies. Acute oral toxicity in mice is also used to determine the level of lethality to terrestrial mammals[5]. In recent times there is an increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicines[6]. The major hindrance to the use of traditional herbal preparations is the lack of scientific and clinical data in support of better understanding of the efficacy and safety of the drugs. Many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds[7]. The present study has been undertaken to estimate the toxic effects of hydroalcoholic extract of seeds of Spermacoce hispid in Albino Wistar mice at the dosage of 500, 1000 and 2000 mg/kg body weight of an animal for a period of 14 days using OECD 425, results and observation were recorded accordingly and are texted here to present publicly.

MATERIALS AND METHODS

Plant material

The seeds of *Spermacoce hispida* were collected from Kollimalai, Salem District. After identification and authentication, the hydro alcoholic (70% ethanol:30%water) extract of the seeds were made by cold percolation method for the study.

Selection of animal's species:

The preferred rodent species is mice. In the normal procedure female mices are used, because literature surveys of conventional LD_{50} tests show that, there is little difference in sensitivity between sexes, in those cases where differences were observed, females were in general slightly more sensitive[8]. When there is adequate information to infer that males are more sensitive, they should replace females in the test. Healthy young adult mice (16-25 gm both sex) were kept separately in individual polypropylene cages with stainless steel hopper. The females were nulliparous and non-pregnant. At the commencement of the study, the weight variations of the animals were minimal and not exceed ± 20 % of the mean weight for each sex. The test animals should be characterized by species, strain, source, sex, weight and/or age.

Housing and feeding conditions

The temperature in the experimental animal room was 22˚C ± 3˚C. Although the relative humidity was 30 % and preferably not exceeding 70 % other than during room cleaning, the aim was 50-60 %. Lighting used artificially, the sequence being 12 hours light and 12 hours dark. The animals were housed individually. For feeding, conventional laboratory diets was used with an unlimited supply of drinking water.

Preparation of animals

The animals were uniquely identified and kept in their cages for five days prior to dosing for acclimatized to the laboratory conditions. During acclimatization the animals were observed for ill health. Animals demonstrating signs of spontaneous disease or abnormality prior to the start of the study were eliminated from the study.

Grouping of animals for acute toxicity study:

Groups	No of Animals	Sex	Dose of *Spermacoce hispida* seed extract (mg/Kg b.wt)
Group I	1	F	500
Group II	1	F	1000
Group III	3	F	2000
Group IV	3	M	2000

Preparation of doses

The test substance was dissolved in distilled water. For vehicles other than water, the toxicity of the vehicle must be known. The test substance is administered in a single dose by oral gavage.

Fasting

The animals were fasted prior to dosing by withholding food overnight. Fasted body weight of mice is determined and the dose is calculated according to their body weight. After dosing, food was withheld for a further 3-4 hours.

Principle of the test

Animals are dosed, one at a time, at 24 hour intervals. The first animal receives a dose at the level of the best estimate of the LD_{50}. Depending on the outcome for the previous animal, the dose for the next animal is adjusted up or down. If an animal survives, the dose for the next animal is increased; if it dies, the dose for the next animal is decreased. After reaching the reversal of the initial outcome, i.e. the point where an increasing (or decreasing) dose pattern is reversed by giving a smaller (or a higher) dose, four additional animals are dosed following the same UDP. The LD_{50} is calculated using the method of maximum likelihood[2,8].

RESULT AND DISCUSSION

The acute oral toxicity of seed extract of *Spermacoce hispida* is studied in both sexes of albino wistar mice at different concentrations as follows:

LD $_{50}$& Maximum Tolerable Dose (MTD) Determination:

At the dose levels tested, no mortality was observed in the different groups of mice that received *Spermacoce hispida* seed extract at the dose of 500, 1000 and 2000mg/kg, b.wt orally after 72 h. Similarly, 500, 1000 and 2000 mg/kg of this extract was well tolerated in mice even after 72 h. Hence the LD_{50} and Maximum tolerable dose values were estimated to be as follows: > 500, >1000 &>2000mg/kg b.wt (p.o) (Table 1)

Table 1 Observation of LD_{50} and Maximum tolerable dose determination

Batch	Group	Sex	LD_{50} and Maximum tolerable					
			LD_{50} Values (mg/kg)			Maximum Tolerable Dose Values (mg/kg)		
Group I	1	F	> 500	> 500	> 500	> 500	> 500	> 500
Group II	1	F	>1000	>1000	>1000	>1000	>1000	>1000
Group III	3	F	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000
Group IV	3	M	> 2000	> 2000	> 2000	> 2000	> 2000	> 2000

Mortality and Clinical sign

After administration of single dose of *Spermacoce hispida* seed extract at the dose of 500, 1000 and 2000mg/kg b.wt, there were no mortalities & Clinical sign changes during the entire study period and there is no changes in skin and fur, eyes and mucous membranes, but no other adverse clinical manifestations (e.g. respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attentions have directed to

observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.) were seen in the experimental animals.(Table 2 & 3)

Table 2Clinical Observation of animals

Batch	Group	Sex	Clinical observation											
			Ocular sign	Mucous	Muscle Tone	Respiratory	Circulatory	Automonic	CNS-Motor	CVS	GIT	Somotomotor	Behavior	Reflexes
I	1	F	N	N	N	N	N	N	N	N	N	N	N	N
II	1	F	N	N	N	N	N	N	N	N	N	N	N	N
III	3	F	N	N	N	N	N	N	•	N	N	N	N	N
		F	N	N	N	N	N	N	•	N	N	N	N	N
		F	N	N	N	N	N	N	•	N	N	N	N	N
IV	3	M	N	N	N	N	N	N	•	N	N	N	N	N
		M	N	N	N	N	N	N	•	N	N	N	N	N
		M	N	N	N	N	N	N	•	N	N	N	N	N

• = Mild sedative; N = Normal

Table 3Direct Observation of Animals

Batch	Group	Sex	Direct Observation								
			Tremor	Convulsion	Salivation	Pilo erection	Diarrhea	Lethergy	Sleep	Coma	Urine
I	1	F	Nil	Nil	Nil	Nil	Nil	Nil	PT•	Nil	N
II	1	F	Nil	Nil	Nil	Nil	Nil	Nil	PT♣	Nil	N
III	1	F	Nil	Nil	Nil	Nil	Nil	Nil	PT♥	Nil	PT•
	2	F	Nil	Nil	Nil	Nil	Nil	Nil	PT♥	Nil	PT•
	3	F	Nil	Nil	Nil	Nil	Nil	Nil	PT♥	Nil	PT•
IV	1	M	Nil	Nil	Nil	Nil	Nil	Nil	PT♥	Nil	PT•
	2	M	Nil	Nil	Nil	Nil	Nil	Nil	PT♥	Nil	PT•
	3	M	Nil	Nil	Nil	Nil	Nil	Nil	PT♥	Nil	PT•

PT = Present; **N** = Normal; • = Moderate; ♣ = Mild; ♥ = Very Mild

Hematological Studies

On 14th day, the heamoglobin, RBC, WBC were significantly increased and decreased within the normal range when compared to initial day at 500, 1000 and 2000 mg/kg b.wt but it did not suggest any kind of Haemalological abnormal changes when comparable with normal animals (Table 4).

Table 4 Observation of Haematological Parameters on Pre And Post Treatment

Batch	Group	Sex	Initial (0 hour)			Final (14th day)		
			Hb (g/dl)	RBC (m/cu mm)	*WBC* (Cells/cu mm	Hb (g/dl)	RBC (m/cu mm)	WBC (Cells/cu mm
I	1	F	9.6 ± 0.2	9.6 ±0.2	7223.0 ± 282.0	11.2 ± 0.7 *	9.2 ± 0.2	6943.0 ± 235.0
II	1	F	9.8	8.0	9120	10.6	5.0	8030
III	1	F	8.8	11.4	8100	12.6	11.5	5510
	2	F	10.4 ± 0.5	10.9 ± 0.2	10023 ± 313.2	12.3 ± 0.6	10.7 ± 0.6	8450.0 ± 396.6*
	3	F	10.2 ± 0.6	9.2 ± 0.5	6670.0 ± 500.0	12.4 ± 0.4*	8.5 ± 0.5	5593.3 ± 118.7
IV	1	M	9.0±0.2	8.9 ± 0.2	10230 ± 313.2	10.3 ± 0.2	8.7 ± 0.6	8450.0 ± 396.6*
	2	M	10.5±0.3	10.1 ± 0.2	7670.0 ± 500.0	11.4 ± 0.4*	9.5 ± 0.5	7593.3 ± 118.7
	3	M	9.9±0.2	11.5±0.2	7050±252	10.10±0.2	10.3±0.2	70112±235

Note - * -p<0.1, ** - p<0.05, *** - 0.001, Values are Mean ± SEM

Body weight, feed & water intake

From Day 0 to Day 14, there was no abnormal change in the body weight of mices in all groups. The mice gained weight throughout the study period (Table 5). The rate of food and water intake of animals given different levels of *Spermacoce hispida* seed extract was thus comparable with normal mice.

Table 5 Body weight of animals on 0, 7 and 14th day

Batch	Group	Sex	Body weight (g)		
			Day 0	Day 7	Day 14
I	1	F	25.57	27.83	27.67
II	1	F	24.12	26.17	27.28
III	1	F	30.14	23.54	23.87
	2	F	26.14	27.56	28.25
	3	F	27.28	28.81	29.02
IV	1	M	27.92	28.35	28.55
	2	M	27.53	29.17	29.11
	3	M	30.59	31.21	29.47

Gross Pathology

No treatment-related gross pathological changes were found in the heart, liver, lungs, spleen, kidneys and testes of the mice at the dose levels tested. The extract *Spermacoce hispida* seed extract has been evaluated by using either sex of animals. Animals were given extract dissolved with distilled water showed no ill effects. All animals tolerated the maximum test doses of the extract. Since there were no clinical signs of toxicity or mortality of animals at the dose of 500, 1000 and 2000mg/kg body weight, the LD_{50} and maximum tolerable dose values were greater than 2000mg/kg body weight. The growth pattern food and water intake of animals were comparable with those of the normal animals. Absence of clinical signs of toxicity, gross

pathological observations in vital organs and hematological alteration in drug treated animals suggest that the extract offers no potential ill effects to animals.

CONCLUSION

Hydroalcoholic seed extract of *Spermacoce hispida*shows no toxic effects when given orally at the concentration of 2000 mg/kg body weight. This reveals the safety of the herb for further preclinical and clinical evaluation.

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