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Acute Oral Toxicity Studies of Ethanol Leaf Extracts of Derris *Scandens & Pulicaria Wightiana* in Albino Rats

Vidya Sabbani[1*], Alluri Ramesh[1] and Satla Shobharani[2]

[1]*Department of Pharmacology, Vishnu Institute of Pharmaceutical Education & Research, Narsapur, Medak, Telangana. India.*
[1]*Department of Pharmaceutical Chemistry, IST, CPS, JNTUH, Hyderabad, Telangana, India.*

Corresponding author*
Vidya Sabbani,
Research Scholar,
Department of Pharmacology,
VIPER, Narsapur, Medak, Telangana. India.
E-mail: vidyasabbani@yahoo.com

Abstract
Objective: The present study was designed to find out LD_{50} and to ascertain the safety of ethanol extracts of leaves of *Derris scan dens and Pulicaria wightiana* by acute oral toxicity study in female rats as per OECD guideline 425.
Methods: Rats were sequentially administered with ethanol leaf extracts of *Derris scandens (Ds) & Pulicaria wightiana(Pw)* in single dosages of 175, 550, and 2000 mg/kg of body weight. All the animals were individually studied for mortality, wellness parameters and body weight for 14 days.
Results: No mortality and no significant changes were observed in body weight and wellness parameters at 175, 550 and 2000 mg/kg body wt. doses of both *Derris scandens and Pulicaria wightiana* , which reveal the safety of these plants in the doses up to 2000 mg/kg body weight.
Conclusion: Conclusively, LD_{50} value of ethanol extracts of leaves of *Derris scandens and Pulicaria wightiana* were found to be more than 2000 mg/kg body weight.
Keywords: Acute oral toxicity, *Derris scandens, Pulicaria wightiana***,** OECD guideline 425.

1. Introduction
Traditionally the 'science of poisons' known as Toxicology which began with early cave dwellers who recognized poisonous plants and animals and used their extracts for hunting or warfare. Later, with time, it integrated the practice of determining the safety of a particular compound. Broadly, toxicology may be defined as the study of harmful poisonous effects of drugs and other chemicals with emphasis on detection, prevention and treatment of poisonings. After gaining appropriate information on the harmful effects of a compound, the levels for its safe usage or the degree of its safety is recognized, this is known as its (compound) Biosafety level [1]

Prevention, diagnosis, and treatment of various illnesses, is extensively practiced by the traditional and alternative medicine. Over the past 20 years this type of medicine has attracted public attention as it is easily accessible in some regions [2]. As human diet consists of plant-derived foods, particularly vegetables and fruits are generally considered to be highly beneficial components, they contribute great importance in daily life by providing wide range of nutrients, vitamins and other compounds which widen the therapeutic arsenal. Treatment of prevention of diseases by natural products plays a dominant role in the development of novel drug leads. [3]

Derris scandens(Ds) Benth is a spreading, climbing shrub and is widely distributed throughout theplains of Southeast Asia. Its dried stems are used for the treatment of muscle ache and pain, as wellas arthritis, as an expectorant, anti-tussive and a diuretic [4]. A hydro-alcoholic extract of the stem was reported to have both antimicrobial [5] and immune-stimulating activities [6]. In a pharmacological study, the polar fractions of *Derris scandens* when applied, resulted in a marked decrease in blood pressure and heart rate [7].Comorians, isoflavones and their glycosides have been previously reported as chemical constituents from various parts of *Derris scandens* .[8-18].

Pulicaria wightiana (Pw) (Sontiki) palnt belonging to the family Asteraceae. Leaves are smaller towards the top. Flower-heads are yellow, 2-4 cm across, arising singly on leafy stalks, 10-20 cm long, at the end of branches. The stalks are hollow, enlarged upwards. Ray florets are 1.5-2 cm long, 2-3 mm wide, 3 -toothed at the tip. Flowering season August-september.Sesquiterpenoids[19] , diterpenoids [20,21] and flavonoids[22,23]. From Pulicariawightianaan isopimarane was previously reported. Recently five new clerodane diterpenoids, 1–5, together with 30, 5, 6-trihydroxy-3, 40, 7-

trimethoxyflavone and 2-methyl-5-hydroxy-chroman-4-one from the aerial part of the plant were isolated. This plant having the analgesic, antibacterial activity and antiulcer activities. [24]

The present study was designed to find out LD_{50} and to ascertain the safety of ethanol extracts of leaves of *Derris scan dens and Pulicaria wightiana* by acute oral toxicity study in female rats as per Organization for Economic Cooperation and Development (OECD) guideline 425. The test procedure described in this guideline uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonized System for the classification of chemicals that cause acute toxicity. Also, this method 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 LD_{50} and confidence intervals, the test allows the observation of signs of toxicity.

2. Materials and Methods

2.1 Collection of Plant Material

The plant, *Derris scandens (Ds)* & *Pulicaria wightiana(Pw)* were collected from narsapur forest in Medak district in the month of September. The plant was authenticated and voucher number is 5467 by Dr.MadhavaShetty, Department of Botany, Sri Venkateshwara University, and Tirupathi.

2.2 Preparation of extraction [25]

The plant material first washed with water thoroughly to remove dirt and soil deposits and dried under shade until complete removal of moisture content, such dried plants were powdered by mechanically and passed through sieve no 80. Approximately 250 g of this dried powder of both *Derris scandens* & *Pulicaria wightiana* were extracted with 90% ethanol respectively by continuous hot percolation, using soxhlet apparatus, (before that the crude powders were extracted with various solvents in increasing polarity and the antioxidant potential principle like flavonoids were identified in ethanol extract). The resultant dark brown extract was then concentrated up to 100 mL on Rota evaporator under reduced pressure. The concentrated crude extracts were then lyophilized before being used for the study. The different dose levels of plant extracts were prepared in 1% gum acacia solution and were used for studies. The yield was found to be 10%.

2.3 Acute oral toxicity study

2.3.1 Target animal

Healthy young adult nulli-parous and non- pregnant Swiss albino female rats, weighing 150-180 g at the start of the experiment, were procured NIN Hyderabad, Telangana Dist. The present study was approved by Institutional Animal Ethics Committee of VIPER, Narsapur, Medak Dist ,Regd.No: 1358/ac/10/CPCSEA -14.07.2016. Female rats were selected because literature surveys of conventional LD_{50} tests show that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally slightly more sensitive [26]. A total of five rats were systematically selected out of a population of 40 rats by systematic randomization techniques and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed individually in clean polypropylene cages. Room temperature and humidity were maintained at 25°C (± 30°C) and 45-55% respectively with a light-dark cycle of 12 h (light from 06:00 AM to 06:00 PM). Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard pellet chow and unlimited supply of filtered drinking water.

2.3.2 Methodology: Procedure for main test:

Acute toxicity study was carried out *in vivo*. The Acute oral toxicity study was conducted using the limit dose test of up and down procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme-AOT425statPgm, version 1.0 (Acute Oral Toxicity (OECD Test Guideline 425) (AOT), 2001), at a limit dose of 2000 mg/kg b.w/p.o .Prior to dosing, animals were fasted overnight before being weighed, and all the extracts were orally administered in a single dose using gastric tube (Table 1). The volume given was not more than 2 ml/100 gm body weight body wt.). Following the period of fasting, the fasted body weight of each animal was determined and the dose was calculated according to the body weight. After the extract was administered, food was with held for a further 3-4 hours. Control animals were administered with calculated amount of 1% gum acacia suspension. Single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses were selected from the sequence 1.75, .5, 17.5, 55, 175, 550, and 2000 (or 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000 for specific regulatory needs). Because no estimate of the substance's lethality was available, dosing was initiated at 175 mg/kg till 2000 mg/kg as recommended in OECD Guidelines 425 [27-29].

2.3.3 Wellness parameters

Animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were

systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Changes in wellness parameters were compared with that of control animals. Body weight Individual weights of animals were recorded before the administration of drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment. Changes in the weight of individual animals were calculated and compared with that of the control animals

2.3.4 Statistical Analysis

Changes in body weights were expressed as Mean (M) ± Standard Deviation (SD) and their statistical significance was calculated using t-test.

3. Result

3.1 Body Weight

The body weights of the animals were calculated and are recorded in Table 3. There were no significant changes in body weight. However, all animals exhibited a normal increment in body weight without drastic difference between both control and treated groups. Although, the body weights of the entire rat s were increased after the oral administration of Ds &Pw. But, the changes of the body weights were found to be statistically insignificant in Table 1. Insignificant increase in body weight of test animals indicates that the administration of the both plants does not affect the growth of the animals.

Table 1: Effect of ethanol leaf extracts of Derris scandens and Pulicaria wightiania on the body weight of rats at 2,000 mg/kg dose after 14 days.

Group	Treatment	Body weights(Gm)	
		Before treatment (Mean±SD)	After treatment (Mean ±SD)
Control	1% Gum acacia	152±1.67	167±2.78
Treated	2000mg/kg EDs	182±1.43	198±1.89
	2000mg/kg EPw	167±2.89	187±1.67

3.2 Wellness parameters analysis

No significant changes were observed in wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation, sleep of the treated as well as the control animals were found to be normal. Tremors, lethargy, diarrhea and coma did not occur in any of the animals in Table 2.

Table 2: Observations for the main test at 2,000 mg/kg body wt of ethanolic leaf extract of Derris scandens & pulicaria wightiania

Observations	30min			4hrs			24 hrs			48 hrs			7 days			14 days		
	C	EDs	EPw	C	EDs	EPw	C	EDs	EPw	C	EDs	EPw	C	EDs	EPw	C	EDs	EPw
Skin fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Alertness	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Grooming	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Torch response	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Torch response	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
pain	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremors	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Gripping strength	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pinna reflex	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Corneal reflex	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pupils	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin color	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hyper activity	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Mortality	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

C-Control: 1% Gum Accaia Suspension, EDs- Ethanol leaf extract of *Derris scandens*

EPw- Ethanol leaf extract of *Pulicaria Wightiana*, N-Normal, AB-Absent

3.3 Mortality

No mortality was observed at 175, 550 and 2000 mg/kg body wt. doses of *Derris scandens and Pulicaria wightiana* ethanolic leaf exrtracts and resulted were tabulated in Table-3 .

Table-3: Mortality record for ethanol leaf extract of *Derris sacdens & Pulicaria wightiana* for 14 days*:*

Group	Control	EthanolLeaf Extract *Derris scandens*	Ethanol Leaf Extract *Pulicaria Wightiana*
Hour .1	NIL	NIL	NIL
Hour .2	NIL	NIL	NIL
Hour .3	NIL	NIL	NIL
Hour .4	NIL	NIL	NIL
Hour .24	NIL	NIL	NIL
Day2	NIL	NIL	NIL
Day3	NIL	NIL	NIL
Day4	NIL	NIL	NIL
Day5	NIL	NIL	NIL
Day6	NIL	NIL	NIL
Day7	NIL	NIL	NIL
Day8	NIL	NIL	NIL
Day9	NIL	NIL	NIL
Day10	NIL	NIL	NIL
Day11	NIL	NIL	NIL
Day12	NIL	NIL	NIL
Day13	NIL	NIL	NIL
Day14	NIL	NIL	NIL
Mortality	NIL	NIL	NIL

3.3.1 LD_{50} Value

As per calculations from Acute Oral Toxicity (Guideline 425) the LD_{50} value of *Derris scandens and Pulicaria wightiana* ethanolic leaf exrtract was found to be more than 2000 mg/kg body weight

4. Discussion

In primary healthcare, the phyto-theraputic products from medicinal plants have become universally popular, particularly in developing countries, and some have been wrongly regarded as safe for the reason that they are a natural source. However, these bioactive products from medicinal plants are recognized to be safe without any compromising health effect, and thus extensively used as self medication [30]. However, there is a need to establish the studies scientifically on the toxicity and adverse effect of these remedies. Therefore, additional acute oral toxicity study is crucially needed not only to identify the range of doses that could be used subsequently, but also to reveal the possible clinical signs elicited by the substances under investigation. It is also a useful parameter to investigating the therapeutic index of drugs and xenobiotics [31].As use of medicinal plants increases, experimental screening of the toxicity of these plants is essential to assure the safety and efficacy of those natural sources. However, acute toxicity studies do not detect effects on vital functions like the cardiovascular, central nervous, and respiratory systems which are not usually assessed during the study and these should be evaluated earlier to human exposure [16]. Hence, the present study was mainly designed to investigate toxicity of ethanol leaf extract of *Derris scandens and Pulicaria wightiana* using acute oral toxicity investigation. In this oral acute toxicity study, the Swiss albino rats were used to observe the toxicity effects of ethanol leaf extract of *Derris scandens and Pulicaria wightiana*. The route of administration depends on the dosage form in which the compound is available. Based on historical research, the oral route administration is the most convenient and commonly used one when studying acute toxicity. The absorption might be slow, but this method costs less and is painless to the animals. Since the crude extracts are administered orally, the animals should be fasted prior to taking the dose because food and other chemicals in the digestive tracts may affect the reaction(s) of the compound. All the procedures were performed based on the appropriate OECD guideline [27].

In the present study, the rats in the control and treated groups were administrated with vehicles and crude leaf

extracts, respectively. The rats were monitored daily until day fourteen for any toxic signs and mortality. The clinical symptom is one of the major important observations to indicate the toxicity effects on organs in the treated groups. During the 14 days of period acute toxicity evaluation, rats which are orally administrated with single dose 2000 mg/kg of ethanolic leaf extract of *Derris scandens and Pulicaria wightiana* showed no overt signs of distress, and there were no observable symptoms of neither toxicity nor deaths. All of the rats gained weight and displayed no significant changes in behavior. Apart from that, the physical appearance features such as skin, fur and eyes were found to be normal and even as the body weight of the rats showed as increase ,this indicates that the administration of the crude extract has negligible level of toxicity on the growth of the animals. Furthermore, determination of food intake and water consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the Accomplishment of the proper response to the drugs tested [32].

In this study, the food intake and water consumption also not affected by the administration of ethanol leaf extract of *Derris scandens and Pulicaria wightiana* and it did not induce appetite suppression and had no deleterious effects. Thus, this indicates there was no disturbance in carbohydrate, protein or fat metabolism.This study reckoned that the ethanol leaf extract of these two plant extracts do not cause acute toxicity effects and an LD_{50}value greater than 2000 mg/kg In principle, the limit test method is not intended for determining a precise LD_{50}value, but it serves as a suggestion for classifying the crude extracts based on the expectation at which dose level the animals are expected to survive [18]. According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, the ethanol leaf extract of *Derris scandens and Pulicaria wightiana* were assigned (LD_{50}> 2000 mg/kg) which was the lowest toxicity class.

5. Conclusions

The present study confirms that the ethanol leaf extract of *Derris scandens and Pulicaria wightiana* does not cause any apparent toxicity in an animal model. No death or signs of toxicity were observed in rats treated with the 2000 mg/kg of ethanol leaf extract of Ds & Pw, thus establishing their safety in use. Hence, these two plants can be used as medicinal agents in known dosage for various activities as mentioned in the literature.

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