Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

ACUTE ORAL TOXICITY STUDIES OF *ANACYCLUS PYRETHRUM* DC ROOT IN ALBINO RATS

V. KISHOR KUMAR[1], K.G. LALITHA[2,*]

[1]Department of Phytopharmacy and Phytomedicine, J K K Munirajah Medical Research Foundation's Annai J K K Sampoorani Ammal College of Pharmacy, B. Komarapalayam 638183, Namakkal District, Tamil Nadu, India, [2]Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai 625020, Tamil Nadu, India. Email: kg.lalitha@gmail.com, kishorkpm2006@gmail.com

Received: 18 Aug 2013, Revised and Accepted: 12 Sep 2013

ABSTRACT

Objective: 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. The present study was aimed to determine LD_{50} and to establish the safety of different solvents likewise petroleum ether, chloroform, ethyl acetate, acetone, ethanol, water extracts of *Anacyclus pyrethrum* DC (Asteraceae) root by acute oral toxicity study in female rats as per OECD guideline 425.

Methods: Rats were sequentially administered all the extracts 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, which reveal the safety of these extracts in the doses up to 2000 mg/kg body weight.

Conclusion: Conclusively, LD_{50} value of *A. pyrethrum* DC root extracts was found to be more than 2000 mg/kg body weight.

Keywords: Acute oral toxicity, *A. pyrethrum* DC, Root, OECD guideline 425.

INTRODUCTION

'Toxicology' traditionally known as the 'science of poisons' began with early cave dwellers who recognized poisonous plants and animals and used their extracts for hunting or warfare. Later, with time, it included the practice of determining the safety of a particular compound. Comprehensively, in its present form, 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 relevant information on the harmful effects of a compound, the levels for its safe usage or the degree of its safety is established, this is known as its (compound) Biosafety level [1].

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the past 20 years as this type of medicine is easily accessible in some regions [2]. Plant-derived foods, particularly vegetables and fruits, are generally considered to be highly beneficial components of the human diet. They contribute great importance in daily life by providing wide range of nutrients, vitamins and other compounds which widen the therapeutic arsenal. In general, natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases [3].

Anacyclus pyrethrum DC is a highly medicinal plant, belongs to family Asteraceae. It is a perennial, procumbent herb, widely distriputed in North Africa, elsewhere in the Mediterranean region, in the Himalayas, in North India, and in Arabian countries [4].

The plant roots are stimulant, cordial, rubifacient. Use of the drug in patient with insulin-depended diabetes mellitus reduces the dose of insulin. It decreases the plasma glucose and serum cholesterol level after administration for 3-6 weeks. (The plant is mixed with *Hellebores niger* in a ratio of 1:3). The plant extract inhibited tobacco-induced mutagenesis by 47.5% at a concentration of 1mg/plate. Roots along with the root of *Withania somnifera* and *Vitis vinifera* are used for epilepsy [5].

In large dose the powdered root is an irritant to the mucous membrane of the intestine causing blood stools, tetanus-like spasms and profound stupor [4]. An infusion of the roots is used as a cordial and stimulant and also in certain stages of types fever. A decoction of the roots is useful for pharyngitis & tonsillitis. It is used in the treatment of hemi-plegia and chronic opthalmia [6].

The present study was aimed to determine LD_{50} and to establish the safety of different solvent likewise petroleum ether, chloroform, ethyl acetate, acetone, ethanol, water extracts of *A. pyrethrum* DC (Asteraceae) roots 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.

MATERIALS AND METHODS

Plant Materials

The species for the proposed study that is *A. pyrethrum* DC root were purchased in the month of January 2011 from the M.A.S. Stores, Country drugs wholesale and retail in Erode and authenticated as A. pyrethrum DC by Prof.P.Jayaraman, Director, National Institute of Herbal Science, Chennai-45, (Ref. no: PARC/2011/896).

Processing of plant samples

The roots of *A. pyrethrum* DC are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at a temperature of 35-40°C for 3 days. The dried roots of plant are pulverized, using a sterile electric blender, to obtain a powered form. The powdered form of these plants is stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation of extracts of *A. pyrethrum* DC root

The powder root 600gm was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with petroleum ether, chloroform, ethyl acetate, acetone, ethanol, and aqueous using hot percolation method (72 Hours). The extract obtained was filtered, concentrated and dried in a hot air oven [7-8].

Acute oral toxicity study

Target animal

Healthy young adult nulliparous and nonpregnant Swiss albino female rats, weighing 150-180 g at the start of the experiment, were

procured from College of Vetnary & Animal Science, Mannuthy, Thrissur District. The present study was approved by Institutional Animal Ethics Committee of JKKMMRF's Annai JKK Sampoorani Ammal College of Pharmacy, B.Komarapalayam (JKKMMEFCP/IAEC/2012/007). 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 [9]. The animals were randomly selected 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 (Amrut Laboratory) and unlimited supply of filtered drinking water.

Methodology

Paragraph 22 of OECD Guideline 425 suggests two types of acute oral toxicity tests i.e. limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, only the main test should be performed. Because the literature survey of this herb indicates about its potential toxicity, therefore, the main test was performed.

Procedure for main test

Prior to dosing, animals were fasted overnight before being weighed, and all the extracts were orally administered in a single dose (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 water for injection. Single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses were selected from the sequence 1.75, 5.5, 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 [10-12].

Table 1: Dose and frequency of administration of different extracts for main test

Agent	Diluent	Route of Administration	Frequency of Administration
Root extracts	Gum acacia suspension	Oral route	Single dose

Observations

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

Statistical Analysis

Changes in body weights were expressed as Mean (M) ± Standard Deviation (SD) and their statistical significance was calculated using t-test. LD50 was determined by using Acute Oral Toxicity (Guideline 425) Statistical Program (Version: 1.0) [13].

RESULTS

Percentage yield of *A. pyrethrum* DC root

The powdered root (600gm) was successively extracted with different solvents and the percentage yield of the all extracts of *A. pyrethrum* DC was calculated and recorded in Table 2.

Table 2: Percentage yield of different extracts of *A. pyrethrum* DC root

S. No.	Extracts	%Yield (w/w)
1	Petroleum ether	0.36
2	Chloroform	0.76
3	Ethyl acetate	1.18
4	Acetone	0.80
5	Ethanol	6.10
6	Aqueous	12.75

Body Weight Statistical Analysis

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 all the rats were increased after the oral administration of extracts. But, the changes of the body weights were found to be statistically insignificant in Table 3. Insignificant increase in body weight of test animals indicates that the administration of the extracts does not affect the growth of the animals.

Table 3: Effect of all extracts of *A. pyrethrum* DC root on the body weight of rats at 2,000 mg/kg dose after 14 days

Group	Treatment	Body weights (g)		Calculated 't' value	remarks
		Before treatment M1±SD1	After treatment M2±SD2		
Control	Gum acacia suspension	156.33 ± 2.51	164.33 ± 3.78	t=3.464	NS
Treated	2,000 mg/kg of pet.ether extract of root	173.00 ± 2.64	177.33 ± 4.61	t=3.606	NS
	2,000 mg/kg of chloroform extract of root	163.33 ± 3.05	167.33 ± 1.15	t=3.464	NS
	2,000 mg/kg of ethyl acetate extract of root	162.66 ± 2.08	168.00 ± 1.00	t=3.617	NS
	2,000 mg/kg of acetone extract of root	172.66 ± 2.30	177.33 ± 1.52	t=3.212	NS
	2,000 mg/kg of ethanol extract of root	158.33 ± 4.04	164.00 ± 1.00	t=2.795	NS
	2,000 mg/kg of aqueous extract of root	173.00 ± 2.64	176.33 ± 3.51	t=3.780	NS

N = 3; M1, SD1 and M2, SD2 are mean weights and standard deviations before and after treatment respectively; NS = Not significant

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 4.

Mortality

No mortality was observed at 175, 550 and 2000 mg/kg body wt. doses of *A. pyrethrum* DC root.

LD_{50} Value

As per calculations from Acute Oral Toxicity (Guideline 425) Statistical Program (Version: 1.0), the LD_{50} value of *A. pyrethrum* DC root extracts was found to be more than 2000 mg/kg body weight (Figure 1).

Table 4: Observations for the main test at 2,000 mg/kg body wt of *A. pyrethrum* DC root

Observations	30 min		4 hrs		24 hrs		48 hrs		1 wk		2 wks	
	C	ARE	C	ARE	C	ARE	C	ARE	C	ARE	C	ARE
Skin & Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	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
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Convulsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

C – Control, ARE – All Root extracts, N – Normal

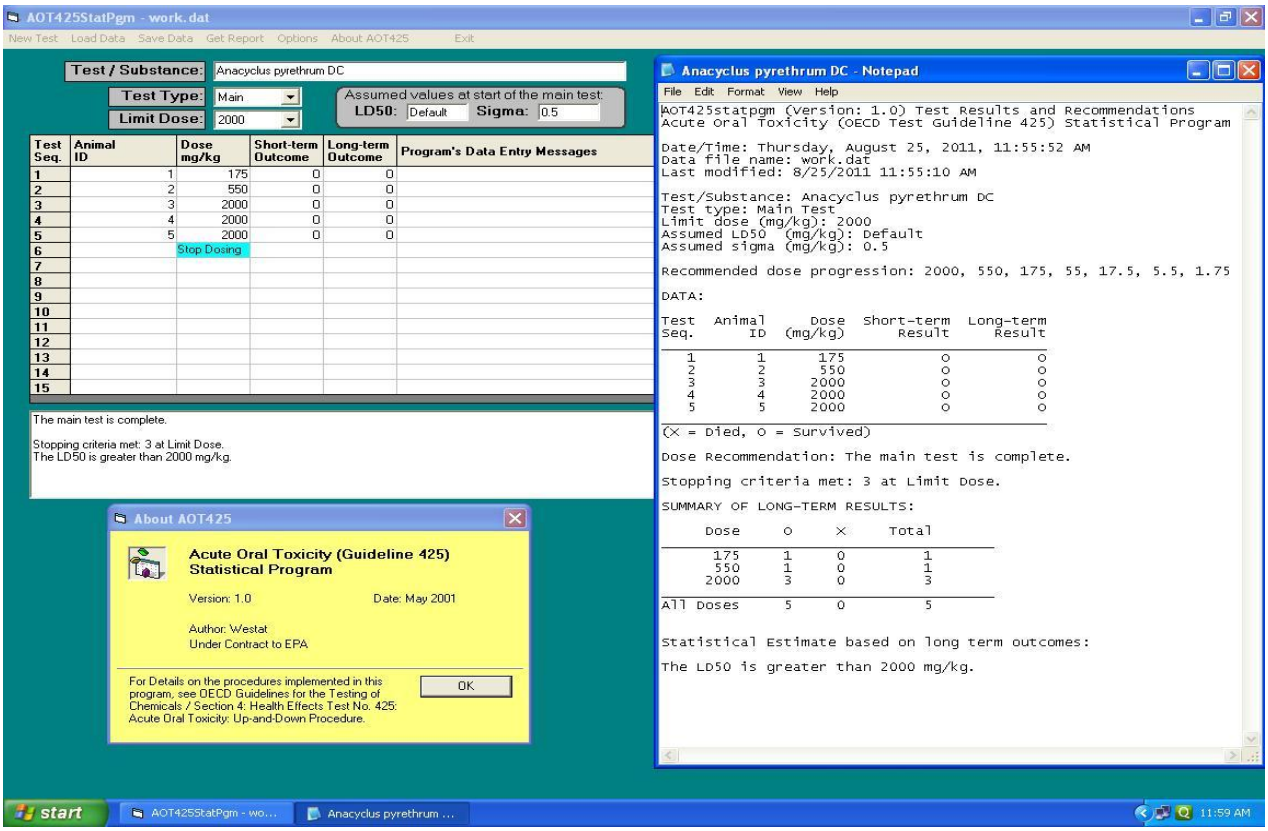

Fig. 1: Acute Oral Toxicity (Guideline 425) Statistical Program (Version: 1.0) of *A. pyrethrum* DC root

DISCUSSION

Phytotheraputic products from medicinal plants have become universally popular in primary healthcare, particularly in developing countries, and some have been mistakenly regarded as safe just because they are a natural source. Nevertheless, these bioactive products from medicinal plants are presumed to be safe without any compromising health effect, and thus widely used as self medication [14]. However, there is a lack of proven scientific studies on the toxicity and adverse effect of these remedies. Therefore, further acute oral toxicity study is vitally 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 [15].

As use of medicinal plants increases, experimental screening of the toxicity of these plants is crucial to assure the safety and effectiveness 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 prior to human exposure [16].

Hence, the present study was particularly designed to investigate toxicity of different roots extracts of *A. pyrethrum* DC by using acute oral toxicity analysis. In this oral acute toxicity study, the Swiss albino rats were employed to observe the toxicity effects of the different crude extracts of *A. pyrethrum* DC roots. 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 before 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 [10].

In this study, the rats in the control and treated groups were administrated with vehicles and crude 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 different root extracts at single dose 2000 mg/kg 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 whilst the body weight of the rats showed as increase (Table 3 and 4), this indicates that the administration of the crude extracts 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 [17]. In this study, the food intake and water consumption also was not affected by the administration of different root extracts of *A. pyrethrum* DC 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 *A. pyrethrum* DC root 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 crude extracts of *A. pyrethrum* DC root was assigned (LD_{50} > 2000 mg/kg) which was the lowest toxicity class. Similar results were found for a single dose at 2000 mg/kg oral administration of *C. spectabilis* leaf extracts that was shown to be nontoxic to the tested mice [19].

CONCLUSIONS

The present results show that different root extracts of *A. pyrethrum* DC does not cause any apparent in toxicity of an animal model. No death or signs of toxicity were observed in rate treated with extracts at dose 2000 mg/kg thus establishing its safety in use. Hence, *A. pyrethrum* DC can be used as a medicinal agent in known dosages, especially in rural communities where conventional drugs are unaffordable because of their high cost. A detailed experimental analysis of its chronic toxicity is essential for further support of this drug.

REFERENCE

1. Tripathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2008.
2. Humber JM. The role of complementary and alternative medicine: Accomodating pluralism. J Am Med Assoc 2002; 288:1655-1656.
3. Newman DJ, Cragg GM, Snader KM. Natural Products as Sources of New Drugs. J Nat Prod 2003; 66: 1022-1103.
4. Sastri BN. The Wealth of India: Raw Materials Vol I, New Delhi: CSIR; 1985. p.248.
5. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New Delhi: Published by Springer; 2007. p. 46-47.
6. Joshi SG. Medicinal Plants, New Delhi: Published by Oxford and IBH co Pvt. Ltd; 2000. p. 73-74.
7. Vinod D Rangari. Pharmacognosy and phytochemistry: Part 1. 1st ed. Pune: Published by Career Publication; 2002. p. 129-139.
8. Pulok K Mokherjee. Quality control of crude drugs. 1st ed. New Delhi: Published by Business Horizones Pharmaceutical Publishers; 2002. p. 403-405.
9. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, Chu I, Goddard M, Segal L, Springer JA, Myers RC. Comparison of the Up-and- Down, Conventional LD_{50} and Fixed Dose Acute Toxicity Procedures. Fd Chem Toxicol 1995; 33: 223-231.
10. Organization for Economic Cooperation and Development (OECD) guidelines for acute toxicity of chemicals. No. 425 (Adopted: 3 October 2008).
11. Joshi CS, Priya ES, Venkataraman S. Acute and subacute toxicity studies on the polyherbal antidiabetic formulation diakyur in experimental animal models. J Health Sci 2007; 53: 245-249.
12. Yee K Ming, Noraisyah Bt Zulkawi, Vandana Kotak C, Yogendra K Choudhary, Acute and sub-acute oral toxicity of *Polygonum minus* aqueous extract (Biotropics®pm101) in wistar rats. Int J Pharm Pharm Sci; 5(2): 120-124.
13. Acute Oral Toxicity (OECD Test Guideline 425) Statistical Programme (AOT 425 StatPgm). Version: 1.0, 2001. [http://www.oecd.org/ oecd/pages/home/displaygeneral/0, 3380, ENdocument-524-nodirectorate-no-24-6775-8, FF.html].
14. Vaghasiya YK, Shukla VJ, Chanda SV. Acute oral toxicity study of Pluchea arguta boiss extract in mice. J Pharmacol Toxicol 2011; 6: 113-123.
15. Rang HP, Dale M, Ritter J. *Pharmacology*. Volume 13. 4th ed. New York, NY, USA; Churchill Livingstone; 2001.
16. Syahmi ARM, Vijayarathna S, Sasidharan S, Yoga Latha L, Kwan YP, Lau YL, Shin LN, Chen Y. Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis* Jacq., (Oil Palm leaf) methanol extract. Molecules 2010; 15: 8111-8121.
17. Iversen PO, Nicolaysen G. Water for life. J Norw Med Assoc 2003; 123: 3402-3405.
18. Roopashree TS, Raman D, Rani RHS, Narendra C. Acute oral toxicity studies of antipsoriatic herbal mixture comprising of aqueous extracts of *Calendula officinalis*, *Momordica charantia*, *Cassia tora* and *Azadirachta indica* seed oil. Thai J Pharm Sci 2009; 33: 74-83.
19. Sangetha S, Zuraini Z, Sasidharan S, Suryani S. Fungicidal effect and oral acute toxicity of *Cassia spectabilis* leaf extract. Jpn J Med Mycol 2008; 49: 299-304.