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An Acute Toxicity Study of *Tylophora Indica* Leaves Extract in Wistar Rats

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Abstract

The present investigation was carried out to evaluate the safety of hydroalcoholic extract of *Tylophora indica* leaves after single dose administration to determine its potential toxicity individually as per Organisation for Economic Co-operation and Development [OECD] 423 guideline. General behaviour, adverse effects and mortality parameters were studied for up to 14 days. Extract was found safe at the oral doses of 2000 mg/kg b. wt. There were no apparent toxicity signs and death observed during experimental trial. Gross necropsy examination revealed no abnormal lesions. In view of the dose of *Tylophora indica* leaves consumed in traditional medicine, it is observed that there is a wide margin of safety for the therapeutic use.

Keywords: Acute Toxicity, OECD Guideline 423, *Tylophora indica*, Wistar Rats

1. Introduction

Liver is an important organ which is actively involved in many metabolic functions and is the major target organ for a number of toxicants[1]. Liver damage or injury is associated with distortion of these metabolic functions[2]. Hepatic diseases are still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects on health of animals and humans[3]. In the absence of a reliable hepatoprotective drug in modern medicine there are a number of herbal preparations in Ayurveda recommended for the treatment of hepatic disorders in view of severe undesirable side effects of synthetic agents. *Tylophora indica* family *Apocynaceae*, herb very commonly used as a folk medicine in various parts of India to control the asthma and liver diseases. Raina V. and Raina S authors explore the study on a brief exposure of human peripheral leukocytes from asthmatic children to tylophorine (an alkaloid occurring in *Tylophora* asthmatica) caused the stimulation of adenyl cyclase. Limitation of this effect was not observed in the leukocytes from the non asthmatic children or adults[7]. The methanolic extract of *Tylophora indica* leaves was screened for hepatoprotective activity in carbon tetrachloride induced hepatotoxicity in albino rats. The degree of protection was measured by estimating biochemical parameters like Serum glutamate,

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oxalo acetate transaminase, serum glutamate, pyruvate transaminase, total protein and level of serum bilirubin (both total and direct). Hepatoprotective activity of methanolic extract at a dose of 200 mg/kg and 300 mg/kg body weight, i.p. was compared with Silymarin (25 mg/kg, i.p.) treated animals. *Tylophora indica* leaves (200 and 300 mg/kg) exhibited significant reduction in serum hepatic enzymes when compared to rats treated with carbon tetrachloride alone[8]. In spite of the use of this plant in traditional medicine, their potential for toxicity and systematic evaluation of hazardous effects is lacking. The present investigation was planned to establish the Maximum Tolerated Dose (MTD) and No Observed Effect Level (NOEL) of plant extract *Tylophora indica* under screening as per OECD guidelines No. 423 of hydroalcoholic extract *Tylophora indica* extracts when fed individually and in combination to rats.

2. Materials and Methods

2.1 Plant Material

Tylophora indica plant leaves were collected from the ICAR research institute of "Directorate of Medicinal and Aromatic Plants Research, Anand, Gujrat". Plant material thus collected were washed thoroughly and dried under shade, segregated, pulverized by a mechanical grinder and passed through mesh sieves to obtain powder. The plant under study was identified and authenticated by a Botanist at "Blatter Herbarium" from the Department of Botany, Saint Xaviers College, Fort, Mumbai.

2.2 Preparation of Extracts

For hydroalcoholic extract, powered herbs were subjected to Soxhlet's Extraction Apparatus as per standard procedures described by[4] using 70% ethanol. The flask was placed on heating mantle till the colourless solvent started returning back to reservoir. Content thus obtained was transferred to a dry and clean Petridis and kept on water bath till complete evaporation of the solvent resulting into semi-solid material. The extract prepared was kept in airtight screw cap vials and stored in refrigerator for use in experimental investigations.

2.3 Experimental Animals

The experimental protocol was approved by Institutional Animal Ethical Committee (approval No. MVC/IAEC/04/2019) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy, young, Wistar female rats of 8–12 wks and weighing 180-200 g were selected as per the recommendations of OECD 423 guidelines for the study and maintained following ethical and standard managemental practices.

2.4 Housing and Feeding

The animals were housed in groups of three in solid bottom polypropylene cages with stainless steel grill tops having facilities for feed and water bottle with bedding of clean paddy husk. The cages were suspended on stainless steel racks in air- conditioned rooms with temperature between 22°C (±3°C), relative humidity 30-70% and illumination cycle set to 12 hours light and 12 hours dark. The animals were given balanced pelleted standard laboratory rodent feed. Wholesome potable filtered water was provided ad libitum in glass bottles with stainless steel sipper tubes.

2.5 Preparation of Animals

Before the start of an experiment, rats were acclimatized to the laboratory conditions for seven days. After acclimatization and clinical examination, the rats were randomly assigned to control and treatment groups with approximately same average weights within the different groups. Individual weights of rats were considered while grouping and it was in an interval within ±20% of the mean weight of animals under the group. After grouping, each animal was assigned a unique Identification (ID) number. The animals were identified by individual colour body markings, also specified on individual cage tag.

3. Methodology

The procedure was followed by using OECD 423 (Acute Toxic Class Method) guideline. Paragraph 22 of OECD Guideline 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 researcher has information indicating that the test material is likely to be non-toxic, 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 is performed. Paragraph 23 of OECD Guidelines suggests a limit test at one dose level of 2000 mg/kg b. wt. should be carried out with six animals (three animals per step). Exceptionally a limit test at one dose level of 5000 mg/kg may be carried out with three animals. If test substance related mortality is produced, further testing at the next lower level should be carried out.

3.1 Test Procedure Followed

Based on literature reviewed, test compounds being herbal drug considered non-toxic hence limit test procedure starting at one dose level of 2000 mg/kg body weight was followed as stated in ANNEX 2d of OECD 423 guideline as mentioned below:

3.2 Dosing

The dose was freshly prepared using distilled water. Dose volume of 2 ml/100 g of b. wt. was administered in a single dose by oral gavage using stainless steel intubation canula. Prior to the dosing, the animals were fasted overnight for food and water was provided ad-lib. The fasting body weight of each animal was determined and quantity of dose was calculated according to their body weights.

3.3 Experimental Design

Experiment was conducted as per OECD 423 limit test at one dose level of 2000 mg/kg body weight on six animals (three animals per step) per group (Table 1). In first step, three animals were dosed with plant extract and food was withheld for 3-4 hours. The animals were keenly observed for 48 hours for clinical signs, morbidity and mortality. Based on mortality record, the second step was carried out on additional three animals per group. For each step, healthy control of three rats per group was maintained. Experimental design followed for the study is tabulated in Table 1.

The animals were keenly observed daily for 14 days for clinical signs, morbidity and mortality pattern. The body weight of each rat was assessed using sensitive balance at weekly interval. At the end of trial period, all the animals were sacrificed by exsanguinations under overdose of CO_2 exposure and then cutting an abdominal aorta to ooze out the complete blood from the body and detailed necropsy examination was conducted for gross pathological changes, if any.

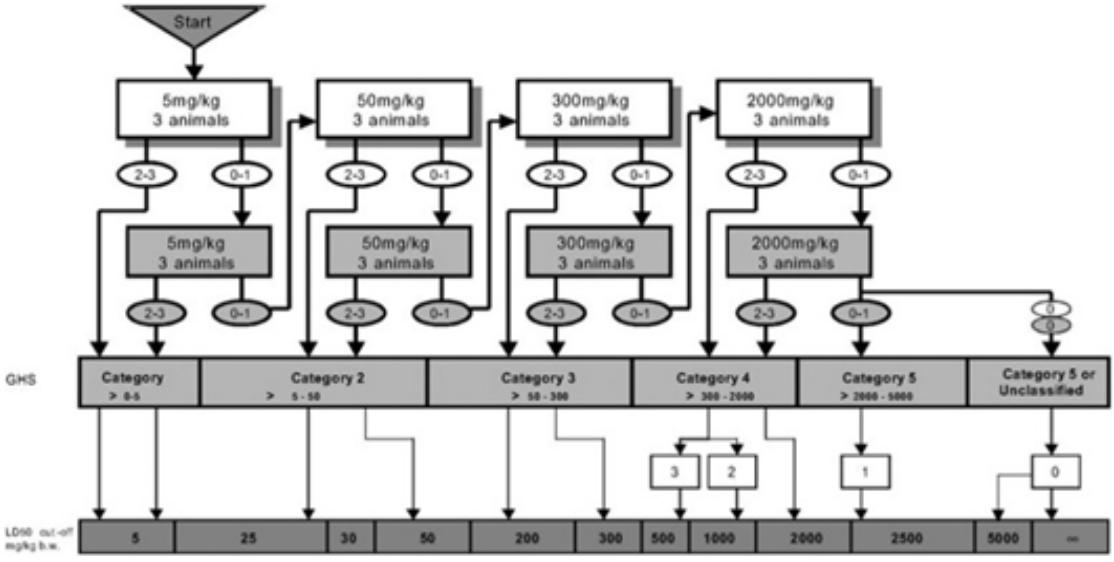


Table 1. Experimental design

Gr. No.	Group Description	Dose Per kg BW	Sex	No. of Animals	
				Step I	Step II
1.	Healthy Control	0 mg	Female	3	3
2.	Extract of leaves of *Tylophora indica.*	2000 mg	Female	3	3
Total No. of Animals Required				6	6
				12	

4. Results and Discussion

4.1 Clinical Signs

Wellness parameters of animals fed with plant extract individually was observed at different time intervals (Table 2). Prime importance was given for first 30 min considering the peak period of anticipated effects of extracts after dosing and observed periodically (special attention was 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 behavioural pattern. Attention was also given for observations of tremors, convulsions, salivation, diarrhoea, lethargy and sleep. No significant abnormal ill health was recorded in any of the rats from treatment group, except hyperactivity and piloerection during first 30 min dosing of hydroalcoholic extracts of all group animals. Hyperactivity and piloerection recorded could be due to alcoholic effect for instance on the rats for short duration. Based on the results of clinical signs observed, No Observed Effect Level (NOEL) of plant extract under screening was considered to be more than 2000 mg/kg b. wt.

4.2 Observations Recorded

Observation for the *Tylophora indica* extract at 2000 mg/kg bw of an animal recorded in Table 2.

4.3 Morbidity and Mortality

Throughout the study period, all cages were checked early at morning, afternoon and in evening to look for any clinical sign, morbidity and mortality in rats. Morbidity and mortality was not seen in any of the rats of all groups during entire study period indicating Maximum Tolerated Dose (MTD) of hydroalcoholic extract individually is more than 2000 mg/kg b. wt.

Individual body weights of overnight fasted animals were recorded before administration of extract on 1st day of the study and thereafter on the 7th and 14th day of experiment (Tables 3 and 4). Changes in the weight of individual animals were calculated and compared with control animals. There were no any significant changes recorded in rats of any of the groups of step I and step II study. The body weights were at par in all the treated groups as compared to control animals.

4.4 Gross Pathology

On completion of observation period, all surviving rats were sacrificed and detailed post-mortem examination was carried out. Necropsy examination revealed no any apparent gross lesions in any of the rats from different groups except mild degree of multifocal congestion and haemorrhages in the lungs from all groups. Changes recorded in lungs were considered to be within the range of normal background lesions which may be seen in rats of this strain and age of this study type and were supposed incidental with carbon dioxide inhalation and terminal changes at sacrifice; representing an adaptive metabolic

Table 2. Observation for the *Tylophora indica* extract at 2000 mg/kg bw of an animal

Observations Recorded	Recorded time and duration											
	30 min		4 hours		24 hours		48 hours		1 week		2 week	
	C	HA	C	HA	C	HA	C	HA	C	HA	C	HA
Skin and fur	N	P	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Hyperactive	N	All	N	N	N	N	N	N	N	N	N	N
MM	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	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhoea	N	N	N	N	N	N	N	N	N	N	N	N
C = Control, HA = Hydrolcoholic extract, N = Normal, P = Piloerections												

Table 3. Step I study animals mean body weights with standard error

Group	Treatment	Day 0	Fasting	Day 7	Day 14
1	Healthy Control	182.33 ±2.80	178.67 ±1.90	191.33 ±2.48	198.33 ±2.4
2	*Tylophora indica* hydroalcoholic extract	182.33 ±2.80	179.67 ±2.10	192.33 ±3.20	197.33 ±1.80

Table 4. Step II study animals mean body weights with standard error

Group	Treatment	Day 0	Fasting	Day 7	Day 14
1	Healthy Control	191.33 ±2.80	187.67 ±1.90	197.33 ±2.48	205.33 ±2.4
2	*Tylophora indica* Hydroalcoholic extract	191.33 ±2.80	189.67 ±2.10	198.33 ±3.20	207.33 ±1.80

change associated with the treatment and sacrifice procedure reflecting an individual variability.

5. Conclusions

From the experiment performed as per the OECD Guidelines 423, the results reveal that hydroalcoholic extract of *Tylophora indica* individually did not cause any apparent toxicity in rat model. No death or signs of toxicity were observed in rate treated with extract at dose 2000 mg/kg. Thus LD_{50} cut of value are more than 2000 mg/kg body weights establishing its safety in use. Hence, *Tylophora indica* can be used as a medicinal agent in known dosage, especially in rural communities, where conventional drugs are unaffordable because of their high cost. A detailed experimental analysis of its sub-acute and chronic toxicity is essential for further support of these plant extracts.

6. Acknowledgments

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7. Conflict of Interest

There are no conflicts of interest.

8. References

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