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An acute oral toxicity study of methanolic extract from *Tridex procumbens* in Sprague Dawley's Rats as per OECD guidelines 423.

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ABSTRACT

Toxicology may be defined as the study of harmful, poisonous and adverse effects of drugs and other chemicals constituents found in plants, which may increase the chances of mortality or weakness in the general health, physically as well as mentally. The present study has been under taken to study the adverse or hazardous effects of methanolic extract from Tridex procumbens, dissolved in Dimethyl sulphoxide (DMSO) & accordingly to determine the LD_{50}, to establish the safety of methanolic extract of Tridex procumbens in SD Rats as per OECD guidelines 423. All the Rats were sequentially administered orally the methanolic extract first in a single dosage of 2000 mg/kg body weight. All the animals were observed for mortality, wellness parameters and body weight for 14 days and due to some morbidity and mortality the experiment was again performed at same dosage and same results were observed. Then decrease in the dosage to 300 mg/kg body weight was performed and accordingly observed as per OECD Guidelines 423. No mortality or any significant change was observed at 300 mg/kg body weight, however at 2000 mg/kg body weight dose the mortality rate was 2/3. Conclusively indicates the LD_{50} value of Tridex procumbens methanolic extract to be less than 2000 mg/kg body weight and more than 300 mg/kg body weight ($LD_{50} \geq$ 300 mg/kg body weight, but < 2000 mg/kg body weight).

Key Words: *Tridex procumbens*, Acute Oral Toxicity, OECD Guidelines 423, Lethality (LD_{50}).

INTRODUCTION

A thousand years ago an extensive use of plants as medicines have been reported and were initially taken in the form of crude drugs such as tinctures, elixirs, poultices, powders, and other herbal formulations[1]. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. Furthermore, the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs. A typical example for a toxic herbal product are the leaves of *Atropa Belladonna*[2] and *Digitalis purpurea*[3] , which show severe systemic toxicity if taken orally.

Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms prior to the use as drug or chemical in clinical use [4]. As per the OECD guidelines, in order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like mice, rat, guinea pig, dog, rabbit, monkey etc under various conditions of drug. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. OECD 401, 423 & 425 does not allows the use of drug

clinically without its clinical trial as well as toxicity studies. Depending on the duration of drug exposure to animals toxicological studies may be three types such acute, sub-acute and chronic toxicological studies.

In **acute toxicity studies**, single dose of drug given in large quantity to determine immediate toxic effect. Acute toxicity studies are commonly used to determine LD_{50} of drug or chemicals and natural products.

In **sub-acute toxicity studies**, repeated doses of drug are given in sub-lethal quantity for a period of 15 to 20 days. Sub acute toxicity studies are used to determine effect of drug on biochemical parameters of tissues.

In **chronic toxicity studies**, drug is given in different doses for a period of 90 days to over a year to determine carcinogenic and mutagenic Potential of drug [5].

The present study has been undertaken to estimate the toxic effects of methanolic extract from *Tridex procumbens* in Spargue Dawley's Rats (female) at the dosage of 2000, 300 mg/kg body weight of an animal for a period of 14 days using OECD 423, results and observation were recorded accordingly and are texted here to present publicly.

MATERIALS AND METHODS

Plant Material: After the identification and authentication of plant specimen of *Tridex procumbens*, the whole Plant was dried under shade at room temperature for 07 days.

Preparation of Extract: The dried plant material was grinded to a coarse powdered form, and was kept for defatting with Petroleum ether and extracted exhaustively with methanol at 45 degree temperature, in a soxhlet extractor. The extract was concentrated in Vaccum Rotary Evaporator and residue was dried in petridish till the crystalline form was available.

Experimental Animals: Animals were selected as per the OECD guidelines. Healthy young and nulliporous, non pregnant Sprague Dawleys female Rats weighing from 160-180 mg of 8 – 12 weeks old were selected, because literature survey of LD_{50} Test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive [6].

To acclimatize with the laboratory conditions, randomly selected animals marked to permit individual identification were kept in clean polypropylene cages for 5 days prior to start an experiment.

Table 1: Following Laboratory Conditions were maintained As Per OECD 423

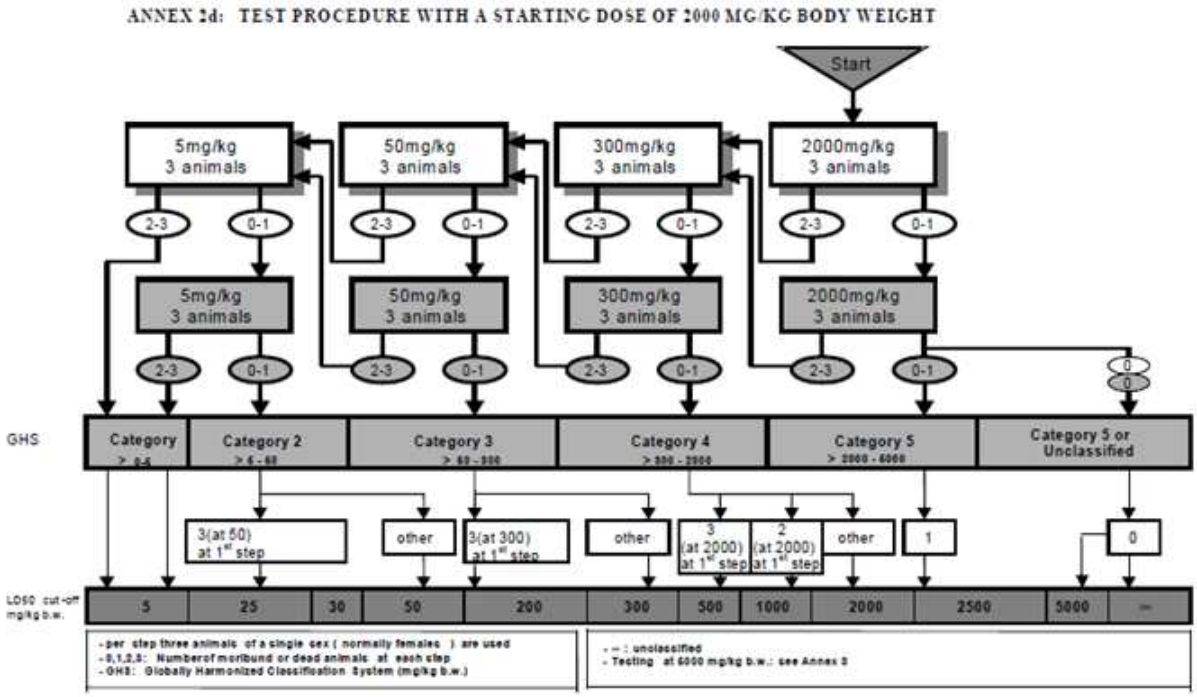
S. No.	Condition	Requirement
01.	Room Temperature	22^0C ($\pm 3^0C$)
02.	Humidity	50 to 60%
03.	Light and Dark Period	12/12 Hours
04.	Bedding	Clean Sterilized Husk
05.	Oral Feed	Conventional Laboratory Diets, Like Standard Pellet Chow.
06.	Distilled Drinking Water	Unlimited Supply

Methodology:
Paragraph 22 of OECD Guideline 423 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.

Paragraph 23 of OECD Guidelines suggests a limit test at one dose level of 2000 mg/kg body weight may 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 (see Annex 3). If test substance-related mortality is produced, further testing at the next lower level may need to be carried out.

Test Procedure followed:

Prior to the dosing, the animals were fasted overnight for 24 hours. Following the period of fasting, the fasted body weight of each animal was determined as stated in paragraph 26 of OECD Guidelines 423 and the dose was calculated according to the body weight as per the Annex 2d of OECD Guidelines 423 and as stated in Paragraph 23 of OECD Guidelines 423. Annex 2d as under: [6].



As per above Annex 2d the starting dose of 2000 mg/kg body weight of an animal was used and prepared at 500mg/ml of DMSO as diluents, the details are annexed in Table 2.

Table-2 First Group (3-Animals) and Their acute oral Toxicity Study detailed initials

S. No.	Experimental Animals	Body Weight	Extract Used at 2000mg/kg body wt.	Dose Prepared In DMSO at 500mg/ml.
01.	SD R-I ^{2000}	163 mg	326 mg	326mg/0.652ml DMSO
02.	SD R-II ^{2000}	162 mg	324 mg	324mg/0.648ml DMSO
03.	SD R-III ^{2000}	160 mg	320 mg	320mg/0.64ml DMSO

After the extract was orally administered, animals were observed keenly for about 48 hours, within the first 30 minutes time period morbidity of 2 animals was observed and in the next 4 hours all the 2 impending deaths were found, Only 1 survived out of the three and food was withheld for a further 3-4 hours for the same single animal. Control animals were administered with calculated amount of water for injection.

As mortality was indexed at the dosage of 2000mg/kg body weight of an animal, the same procedure was again followed with the next three animals, and the results were found same. Since then the dose was brought up to the lower limit and a dose of 300 mg/kg body weight of an animal was introduced into the second selected group under same laboratory conditions and were fasted for overnight and the dose was prepared as in Table 3.

Table 3 Second Group (3-Animals) and Their acute oral Toxicity Study detailed initials

S. No.	Experimental Animals	Body Weight	Extract Used at 300mg/kg body weight.	Dose Prepared In DMSO at 500mg/ml.
01.	SD R-I ^{300}	185 mg	0.0555 mg	0.0555mg / 0.000111 ml DMSO
02.	SD R-II ^{300}	193 mg	0.0579 mg	0.0579 mg / 0.0001158 ml DMSO
03.	SD R-III ^{300}	187 mg	0.0561 mg	0.0561 mg / 0.0001122 ml DMSO

Dose, route and frequency of administration of extract for the Toxicity Test is given in Table 4.

Table 4

S. No.	Agent	Diluent	Route of Administration	Frequency of Administration
01.	Plant Extract	Dimethyl sulphoxide (DMSO)	Oral Route	Single Dose at 2000 mg/kg Body weight of animal.
02.	Plant Extract	Dimethyl sulphoxide (DMSO)	Oral Route	Double Dose at 300 mg/kg Body Weight of animal every after 48 hours gap.

Observations:

As per the Paragraph 24 and 25 of OECD Guidelines 423, Wellness parameters of 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 (Table 5 for 2000 mg/kg body weight and Table 6 for 300 mg/kg body weight).

Table 5

Observation for the Test at 2000 mg/kg Body weight of an animal.

Observation	30 Min.		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Weeks	
	C	E	C	E	C	E	C	E	C	E	C	E
Skin and Fur	N	N	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Eyes	N	N	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Mucous Membrane	N	N	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Salivation	N	All	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Lethargy	N	All	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Sleep	N	2/3	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Coma	N	2/3	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Convulsion	N	All	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Tremors	N	All	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Diarrhea	N	Nil	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Morbidity	N	2/3	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n
Mortality	N	Nil	N	2x1n	N	2x1n	N	2x1n	N	2x1n	N	2x1n

C= Control, E= Extract, N= Normal, 2x1n= 2 Expired and 1 Normal, 2/3= observation ratio

Table 6

Observation for the Test at 300 mg/kg Body Weight of an Animal

Observations	30 min.		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Weeks	
	C	E	C	E	C	E	C	E	C	E	C	E
Skin and 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
Convulsions	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
Morbidity	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, E= Extract, N= Normal

Further Individual body 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 as stated in the Paragraph 26 of OECD Guidelines 423.

Effect of Extract on S. D. Rats at 2000 mg/kg body weight and 300 mg/kg body weight.					
GROUP	TREATMENT	BODY WEIGHT		Calculated Value	Remarks
		BeforeTreatment M1 ± SD1	After Treatment M2 ± SD2		
Control	Water for injection	175.22 ± 13.23	169.21 ± 13.00	t = 3.013	NS
Group-I	2000mg/kg of extract	161.66 ± 12.71	158.34 ± 12.58	t = 2.013	NS
Group-II	300 mg/kg of extract	188.33 ± 13.72	178.74 ± 13.36	t = 2. 897	NS

NS= Not Significant M1, SD1 and M2, SD2 are Mean weight and standard deviation respectively for 1 (Before Treatment) and for 2 (After Treatment)

RESULTS AND DISCUSSION

From the experiment performed as per the OECD Guidelines 423, the results reveal that the methanolic extract of *Tridex procumbens* have been found toxic at 2000 mg/kg body weight of experimental animals as in the first 4 hours of observation 2/3 morbidity was observed and in the next 24 hours of observation mortality in the ratio 2/3 were found. At lower limit dose of 300 mg/kg body weight, No significant changes were observed in body weight and 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 animal. Although, the body weights of all the rats were decreased after the oral administration of extracts, but, the changes of the body weights were found to be statistically insignificant (Table 7). Insignificant decrease in body weight of test animals indicates that the administration of the extracts does not affect the growth of the animals.

LD_{50} Value: As per observations and calculations from Acute Oral Toxicity (OECD Guidelines 423), the LD_{50} value of Methanolic Extract of *Tridex procumbens* was found to be more than 300 mg/kg body weight but less than 2000 mg/kg body weight.

CONCLUSION

Methanolic extract of Tridex procumbens exhibit some toxic effects when given orally at concentration of 2000 mg/kg body weight. However the normalcy and insignificant changes in wellness parameters and body weights reveals the safety of methanolic extract at a dose of 300 mg/kg body weight.

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