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An Acute Oral Toxicity Study of Aqueous Extract of Polyherbal Formulation in Albino Rats as per OECD Guideline 423

Geetanjali*[1], Shakti Bhardwaj[2] and Rakesh Bhargava[3]

[1] Department of Zoology, S. M. S. Government Model Science College, Gwalior - 474 009, Madhya Pradesh, India
[2] Department of Zoology, Government KRG PG (Autonomous) College, Gwalior - 474 006, Madhya Pradesh, India
[3] Division of Microbiology, DRDE, Gwalior - 474 002, Madhya Pradesh, India

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Abstract

Toxicology is defined as the study of harmful and toxic components and the adverse effects of toxic components found in plants. The present study has been undertaken to study the adverse or hazardous effects of aqueous extract of polyherbal formulation, dissolve it in distilled water and determine the LD_{50} to establish the safety of aqueous extract of polyherbal formulation in albino rats as per OECD guideline 423. All rats were administered orally the aqueous extract of the polyherbal formulation in single dosage of 2000mg/kg body weight. All animal were observed for toxic signs at 24 hours and for the next 14 days. Conclusively indicates the LD50 value of the aqueous extract of the polyherbal formulation classified under category 5 as per OECD guideline 423. No mortality or any significant changes was observed at 2000 mg/kg body weight, behavior pattern, and wellness parameter. The present study promotes that an acute oral study of Polyherbal formulation was found to be non-toxic and safe drug in the tested experimental conditions.

***Key words*:** Polyherbal formulation, Toxicity, OECD 423, Acute oral toxicity, Lethality (LD_{50})

Toxicology is an important aspect of pharmacology that deals with the adverse effects of bioactive substances on living organisms prior to their use as drugs or chemicals in clinical settings [1]. 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, rats, guinea pigs, dogs, rabbits, monkeys, etc. under various conditions of drug use. Toxicological studies help us make decisions about whether a new drug should be adopted for clinical use or not. OECD 401, 423, and 425 do not allow the use of a drug clinically without its clinical trial as well as toxicity studies. In the modern era, herbal formulations have gained greater importance than ever before, mainly due to their efficacy, easy availability, and fewer side effects as compared to synthetic drugs [2]. Plant medicines are in wide use around the world [3]. A World Health Organization survey indicated that 70 to 80% of the global population depends on alternative medicine, predominantly herbal in nature, in their primary health care [4]. The use of medicinal plants as a source of drugs in primary health care has become popular universally, particularly in developing countries, because of their natural sources [5]. A thousand years ago, an extensive use of plants as medicines was reported and was initially taken in the form of crude drugs such as tinctures, elixirs, poultices, powders, and other herbal formulations [6]. Individual herbs are safe, but the combined effects of the herbs are not known. Thus, the main objective of the present study has been to estimate the toxic effects of aqueous extract from polyherbal formulation in Albino Rats (female) at a dosage of 2000 mg/kg body weight of an animal for a period of 14 days using OECD 423. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. OECD 401, 423 and 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 3 type such acute, sub-acute and chronic toxicological studies.

In acute toxicity studies- a single dose of drug is given in large quantity to determine the immediate toxic effect. Acute toxicity studies are commonly used to determine LD50 of drug or chemicals and natural products.

In sub-acute 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 studies- drug is given in different doses for a period of 90 days to 12 months to determine carcinogenic and mutagenic potential of drug. [7]

MATERIALS AND METHODS

Plant material

After the identification and authentication of plants, the plant part was shaded dried at room temperature for 1 week. Plants were authenticated at NISCAIR, Delhi. [*Trigonella foenum-graecum* (Methi)- NISCAIR / RHMD / Consult / 2019

***Correspondence to:** Geetanjali, E-mail: geet07pinki@gmail.com; Tel: +91 9792514291

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/ 3412-13-1, *Withania somnifera* (Ashwagandha)- NISCAIR/RHMD/Consult/2019/3412-13-3, *Butea monsperma* (Palash)- NISCAIR/RHMD/Consult/2019/3412-13-2].

Plants	Common name of plants	Parts uses of plants
Trigonella foenum-graecum	Methi	Seed
Withania somnifera	Ashwagandha	Leaves
Butea monsperma	Palash	Leaves

Preparation of extract

The dried plant material was grinded to a coarse powdered form and the extract was made by the maceration process. This process for concentration preparations which includes "Triple Maceration". The extract was concentrated in a vacuum rotary evaporator, and the residue was dried in Petridis until the crystalline form was available. A polyherbal formulation was made in the three different ratios given in the table.

Polyherbal formulation	Plants	Ratio
Formulation 1 (F_1)	*Trigonella foenum-graecum, Withania somnifera, Butea monsperma*	1:1:1
Formulation 2 (F_2)	*Trigonella foenum-graecum, Withania somnifera, Butea monsperma*	2:1:1
Formulation 3 (F_3)	*Trigonella foenum-graecum, Withania somnifera, Butea monsperma*	1:2:2

Experiment animal

Animals were selected as per OECD guidelines. Healthy, young, and nulliporous, non-pregnant Albino female rats weighing between 150 and 180 g were selected because the literature on the LD50 test shows that usually there is little difference in sensitivity between sexes, but females were found to be slightly sensitive [8]. All animals were maintained in laboratory conditions as per OECD 423, shown in (Table 1). Experimental animals were divided into 4 groups of 3 animals. The dose, route, and frequency of administration of the extract for the toxicity test are given in (Table 2). The research study protocol was approved by the Institutional Animal Ethics Committee (IAEC), DRDE, and Gwalior with approval number 37/Go/Rbi/S/99/CPCSEA.

Table 1 Following laboratory conditions were maintained as per OECD 423

Condition	Requirement
Room Temperature	22°C(±3°C)
Humidity	50-60%
Light and Dark Period	12/12 hours
Bedding	Clean Sterilized Husk
Oral Feed	Pelleted Diet-Amrut Feed
Distilled Drinking Water	Unlimited Supply

Test procedure followed

All animals were fasted for 12 hours, after the period of fasted each animal weighted and dose calculated according to the body weight as per Annex 2d of OECD Guideline 423.

As per above Annex 2d the starting dose of 2000mg/kg body weight of an animal was used and prepares at 100mg/ml of Aqueous Polyherbal formulation.

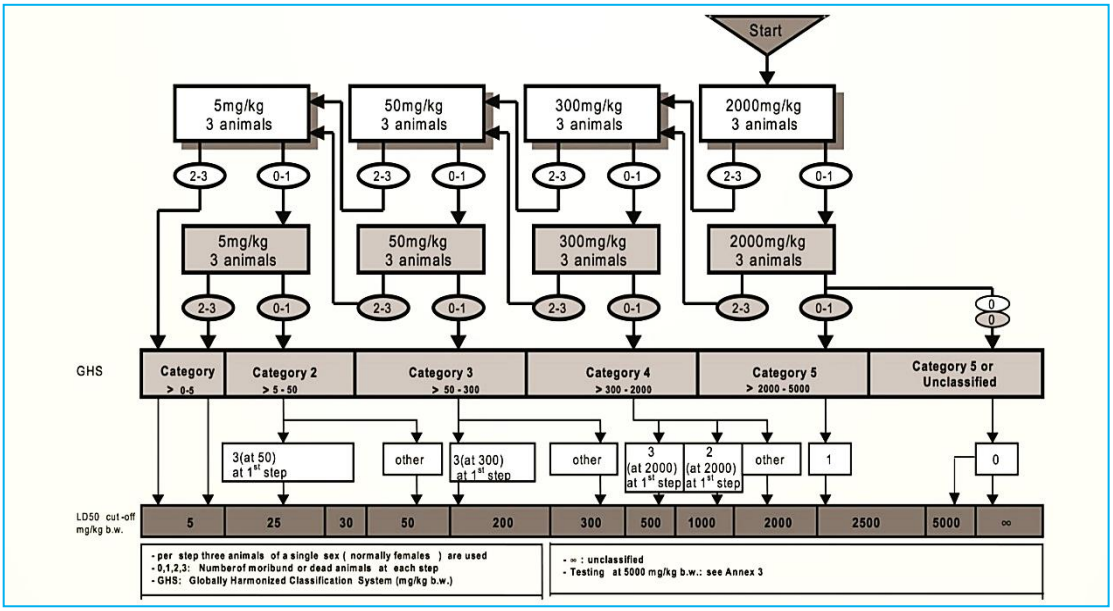

Fig 1 ANNEX 2d: Test procedure with a starting dose of 2000 mg/kg body weight

Table 2 Dose, route and frequency of administration of control and polyherbal formulation

S. No.	Groups	Diluent	Route of administration	Frequency of administration
1	Control	-	-	Given normal diet only
2	Polyherbal Formulation 1 (PHF1)	Distilled water	Oral Route	Single dose at 2000mg/kg body weight of animal.
3	Polyherbal Formulation 2 (PHF2)	Distilled water	Oral Route	Single dose at 2000mg/kg body weight of animal.
4	Polyherbal Formulation 3 (PHF3)	Distilled water	Oral Route	Single dose at 2000mg/kg body weight of animal.

Table 3 Observation for the test 2000mg/kg body weight of an animal Polyherbal Formulation 1 (PHF1)

Observation for 2000mg/kg body weight of an Animal												
Observation	30 Min.		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Week	
	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
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
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

Table 4 Observation for the test 2000mg/kg body weight of an animal Polyherbal Formulation 2 (PHF2)

Observation for 2000mg/kg body weight of an Animal												
Observation	30 Min.		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Week	
	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
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
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

Table 5 Observation for the test 2000mg/kg body weight of an animal Polyherbal Formulation 3 (PHF3)

Observation for 2000mg/kg body weight of an Animal												
Observation	30 Min.		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Week	
	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
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
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

RESULTS AND DISCUSSION

In acute oral toxicity study, polyherbal formulations (PHF1, PHF2, PHF3) treated animals did not show any change in their behavioral pattern. There was no significant difference in body weights. Thus, it was concluded that polyerbal formulations (PHF1, PHF2, PHF3) was safe at 2000mg/kg b.wt.

Behavioral parameters

All the animals were observed after dosing for the first 30 minutes at various times (4 hours, 24 hours, and 48 hours) and daily thereafter after 2 weeks. During the study, no changes were found in the eyes, skin, fur, mucous membrane, salivation, sleeping, or behavioral pattern. The observation showed that there was no lethargy, coma, convulsion, tremors, or diarrhea in the polyherbal formulation (PHF1, PHF2, PHF3) groups at

2000 mg/kg body weight compared to the control. There were no significant changes in behavioral patterns or wellness parameters in the 2000 mg/kg bodyweight group as compared to the control group shown in (Tables 3-5).

Body weight

The PHF-treated groups did not exhibit any appreciable changes in body weight in the acute oral toxicity trial. As seen in (Table 6), the study found that they had no negative impact on body weight after 14 days.

The present study provides information on the toxicological outline of polyherbal formulations obtained by acute oral toxicology in rats. According to OECD 423, using the starting dose of 2000 mg/kg body weight, there were no mortality or toxicological signs revealed. All the animals survive by the end of the study, so they can be classified in category 5 as per the OECD 423 guidelines.

LD_{50} value

An LD50 value is the dose at which 50 percent of the test animals can be expected to die. As per observation and calculation from acute oral toxicity (OECD Guidelines 423), the LD50 value of polyherbal formulations (PHF1, PHF2, and PHF3) was found to be in Category 5 based on a 14-day study.

Table 6 Effect on S.D. rats 2000mg/kg body weight and 300 mg/kg body weight

Group	Treatment	Body weight	
		1 Day	14 Day
Control	Received vehicle only	162.6 ± 1.453	164.66 ± 1.453
F_1	2000 mg/kg of extract	167 ± 1.732	168.33 ± 0.8819
F_2	2000 mg/kg of extract	159.66 ± 1.764	163.33 ± 2.404
F_3	2000 mg/kg of extract	164 ± 1.856	169.33 ± 2.028

Value are expressed as Mean ± SEM,
P>0.05, Compared with 1 day and 14 day

CONCLUSION

The aqueous extract of the polyherbal formulations (PHF1, PHF2, and PHF3) does not exhibit any toxic effect when given orally at a dose of 2000 mg/kg body weight, revealing a safe and nontoxic drug for further experiments. This finding suggests that the extract is safe and non-toxic for further experimentation or potential use as a drug.

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