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Acute Toxicity Study in Rat for Hydroalcoholic Extract of Combination Containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera*

Shital Devlekar[1]*, Jignyasha Raval[2] and Anubha Khale[3]
[1]Pacific Academy of Higher Education and Research University, Udaipur, India
[2]Pacific Academy of Higher Education and Research University, Udaipur, India
[3]Dean, Faculty of Sciences and Technology, SNDT Women's University, Mumbai, India

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***Corresponding Author Email:** spednekaring@gmail.com

Abstract
The general perception that herbal remedies or drugs are safe and devoid of adverse effects is not only untrue, but also misleading. Herbs have been shown to be capable of producing a wide range of side effects or adverse reactions, undesirable drug interaction causing serious injuries, life-threatening conditions and even death [6]. This has created a major concern about safety of plant extracts to both national health authorities and the general public [11,30] which calls for a need to further investigate the safety profile of plants and their extracts [28]. Acute toxicity study provides an estimate of the intrinsic toxicity of the substance, expressed as an appropriate lethal dose (e.g., LD50). Acute oral toxicity study is thus 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 [22]. In the present research work, the acute oral toxicity study was carried out on female rats, to check the safety of the standardized hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* as per OECD guideline No. 420 [18].

Keywords
Butea monosperma, Flemingia strobilifera, Moringa oleifera, Acute toxicity.

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INTRODUCTION
The general perception that herbal remedies or drugs are safe and devoid of adverse effects is not only untrue, but also misleading. Herbs have been shown to be capable of producing a wide range of side effects or adverse reactions, undesirable drug interaction causing serious injuries, life-threatening conditions and even death [5, 6, 25]. This has created a major concern about safety of plant extracts to both national health authorities and the public [11, 30] which calls for a need to further investigate the safety profile of plants and their extracts [28].
There are different types of in vivo tests involved for the assessment of the toxicity based on the length of the exposure i.e., acute, sub-acute and chronic toxicity tests. Out of these, acute toxicity tests are generally the first tests conducted and are commonly used for the initial assessment of the toxic compounds. Acute toxicity study provides an

estimate of the intrinsic toxicity of the substance, expressed as an appropriate lethal dose (e.g., LD50). Acute toxicity study provides information about doses that should be used in subsequent studies. These studies provide another prospect to identify compound-induced effects as observed by morphology, clinical chemistry, or other evaluations. Acute studies are also an indication of the possible target organ(s) [32].

Acute toxicity studies deliver the data on.

- The probable for acute toxicity in humans.
- An estimation of safe doses for humans.
- The possible target organs of toxicity.
- Time-course of drug-induced clinical observations.
- The proper dosage for multiple-dose toxicity studies; and
- Species variances in toxicity.

Mortality should not be a proposed endpoint in studies assessing acute toxicity [12]. Evidence on the drugs acute toxicity is useful to forecast the significances of overdose conditions in human and can be available to support Phase III. An earlier evaluation of acute toxicity might be important for therapeutic signs that patient populations are at higher risk for overdosing [33]. In this study we have used *Butea monosperma, Flemingia strobilifera and Moringa oleifera* leaf hydroalcoholic extract for studying acute toxicity. The present study is undertaken to evaluate the safety of the standardized hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* as per OECD guideline No. 420.

MATERIALS AND METHODS

Experimental protocol:

Dose of the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* was calculated with reference to the body weight of each animal, weighed individually in different tubes using analytical weighing balance and suspended in water just prior to administration. Animals were fasted overnight for 10 h prior to dosing (food but not water was withheld). Following the period of fasting, the rats were examined for health and weighed (initial). Individual doses were calculated based on their (initial) fasting body weight at the time of dosing and the test substance was administered orally only once by gavage attached to a graduated syringe. After the samples were administered, food was withheld for further 2 h whereas water was provided ad libitum. Individual body weights, food and water intake were recorded throughout the study period.

The experimental procedure and protocol for this study was reviewed and approved by Institutional Animal Ethics Committee (IAEC). The animals were randomly divided into two groups of five animals each. The animals were fasted overnight for 10 h and thereafter administered with the standardized hydroalcoholic extracts of *Butea monosperma, Flemingia strobilifera and Moringa oleifera* 1:1:1 (2000 mg/kg body weight). One other group of animals was kept as normal control and administered with distilled water (NMT 1 mL/100 g body weight).

Parameters Observed:

The parameters observed during the study period are as follows.

Mortality:

Animals were observed for mortality and mortality latency (time of death) during the study period, cage side observations, Body weight and Food and Water intake.

Cage side observations:

Assessment of the behavior of animals was carried out by general observations of each animal on a daily basis from the stage of dosing to the end of the study. Cage side observations included daily recordings of condition of fur, damaged areas of skin, subcutaneous swellings or lumps (size, shape and consistency), abdominal distension, eye dullness, eye opacity, pupil diameter, ptosis (drooping of upper eyelid), the colour and consistency of faeces, wetness or soiling of the perineum, condition of teeth, breathing abnormalities, gait, etc. Changes in the parameters were compared with that of the control group.

Body weight:

Individual weights of animals were recorded before the administration of the drug and daily thereafter till 14th day. Changes in the weight of individual animals were calculated and compared with the animals of control group.

Food and water intake:

The food intake and water consumption were estimated daily from the amount of food remaining in the feed hopper and water remaining in the feeding bottle. Results were compared with that of control animals.

Statistical Analysis

Excel 2007 (Microsoft, New York, USA) and GraphPad Prism 5 software (GraphPad Software, Inc., California, USA) were to determine arithmetic mean, standard error of mean (SE) and % mean difference.

RESULTS AND DISCUSSION

Herbs have a variety of complex chemical constituents which act on the body as a whole or on specific organ and systems. Some of the chemical constituents are mild and safe even in large doses while, other are toxic [16]. Therefore, the standardization methods and quality control data on safety are required for proper understanding of use of herbal extracts. The data of the toxicity studies on plant extracts or preparations should be obtained in order to increase the confidence in their safety to humans [29]. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects [21]. Out of the various in vivo toxicity assays available for testing of compounds, acute toxicity testing is the most ideal one as it avoids use of death of animals as an endpoint and relies instead on the observation of clear signs of toxicity at fixed dose level. The procedure is reproducible, uses fewer animals and causes less suffering than the traditional methods [15,18]. In this study, female rats were used to observe the toxicity effects of the plant extracts. Use of single sex was employed to reduce variability and as a means of minimizing the number of animals; females are slightly more sensitive than males [18, 20].

So far, there is no report suggesting adequate scientific evidence about the safety profile of the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* (1:1:1). Thus, safety of the standardized hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* was established in female Sprague dawley rats as per the OECD test guidelines no. 420 before the commencement of Efficacy study.

Mortality:

Mortality is the main criteria in assessing the acute toxicity (LD50) of any drug. In this study, the highest dose as recommended by OECD guidelines, 2001 was used for the study. All the animals showed no overt signs of distress, appeared active throughout the study and there was no mortality recorded even at the highest dose level; 2000 mg/kg body weight. Table 1 represents the data on mortality record.

Table. 1: Data on mortality record, mortality latency during acute oral toxicity study

Group	Sample details	D/T	Mortality latency*	Symptoms of toxicity**
I	Administered with distilled water	0/5	--	No toxic symptoms during the observation period
II	Administered with the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* at 2000 mg/kg body weight	0/5	--	

Cage side observations: Cage side observations are one of the major observations which indicate the toxic effect in the treated groups. The hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* did not provoke any gross behavioral changes or manifestations of toxic symptoms in the animals over an observation period of 14 days. Table 2 represents the data on cage side observations.

Table 2: Data on cage side observations during acute oral toxicity study

Observation	30 min		4 h		24 h		48 h		7 days		14 days	
	I	II	I	II	I	II	I	II	I	II	I	II
Condition of the fur	N	N	N	N	N	N	N	N	N	N	N	N
Skin	N	N	N	N	N	N	N	N	N	N	N	N
Subcutaneous swellings or lumps	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Eye dullness	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

N= Normal, b) Nil= No abnormality

Effect on body weight changes: The body weight changes serve as a sensitive indication of the general health status of animals [8]. Loss of body weight is the indicator of onset of an adverse effect. A dose that causes 10% or more reduction in the body weight is considered to be a toxic dose [24]. All the animals from treated groups did not show any significant decrease in body weights for all the fourteen days as compared with normal control indicating no signs of toxicity. However, a normal

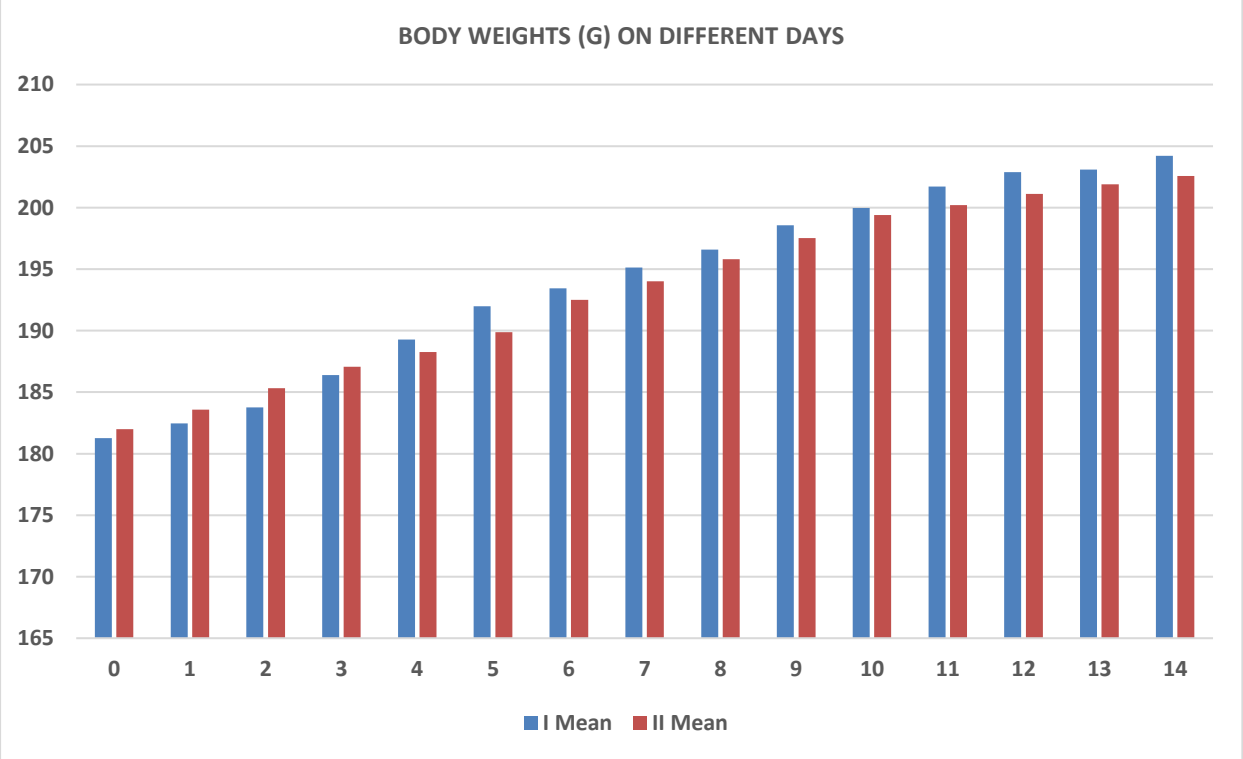
increment in body weight was observed in all animals administered with the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* and the control group. The mean difference in the body weight of the animals administered with plant extract or distilled water was found to be within ± 5% between consecutive days. From the results, it can be stated that these extracts did not interfere with the normal metabolism of animals as corroborated by the nonsignificant difference from animals in the normal control group; this indicates that the administration of the crude plant extracts has negligible level of toxicity on the growth of the animals. Body weight changes for animals treated with control, hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* are mentioned in Table 3 and demonstrated in Figure 1.

Table 3: Daily body weight (g) record of the animals showing the percent mean difference between consecutive days during acute oral toxicity study.

Groups	Parameters	Body weight (g) on different days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	Mean (n=5)	18	18	18	18	18	19	19	19	19	19	19	20	20	20	20
		1.25	2.45	3.76	6.38	9.27	1.97	3.43	5.14	6.59	8.55	9.97	1.72	2.87	3.09	4.22
	SE	0.03	0.25	0.03	0.16	0.11	0.11	0.16	0.47	0.06	0.09	0.14	0.02	0.02	0.25	0.03
II	Mean (n=5)	18	18	18	18	18	18	19	19	19	19	19	20	20	20	20
		2.00	3.58	5.31	7.06	8.25	9.87	2.51	4.00	5.80	7.52	9.40	0.20	1.10	1.90	2.58
	SE	0.24	0.18	0.06	0.02	0.03	0.16	0.14	0.04	0.19	0.05	0.08	0.11	0.02	0.12	0.23

Group I: Normal control; Group II: Administered with the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* at 2000 mg/kg body weight.

Figure 1: Body weight records chart



Effect on food and water consumption: 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 [10]. In this study, the food intake and water consumption also were not affected by the administration of hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* 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 [12]. There were no significant changes in food and water intake of the test animals at all dose levels as compared to the control group. The percent difference of food and water intake of all the animals treated with control, hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* were found to be within±5% between consecutive days (Table 4 and Table 5) and demonstrated in Figure 2.

Table 4: Daily food intake (g) record of the animals showing the percent mean difference between consecutive days during acute oral toxicity study

Group	Parameters	Days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	Mean (n=5)	73.73	72.71	75.26	72.94	73.15	75.35	75.10	74.69	72.86	75.82	73.99	75.40	73.02	74.06	73.89
	SE	0.80	1.59	1.20	1.30	1.60	1.07	1.53	1.08	0.95	1.14	1.10	0.75	1.12	0.58	0.45
II	Mean (n=5)	74.56	73.82	75.03	73.95	75.74	74.95	73.99	75.58	74.04	75.31	74.49	75.79	75.25	76.01	74.53
	SE	1.32	0.95	0.63	1.24	1.83	1.71	0.92	1.69	1.11	1.29	1.03	0.84	1.46	1.74	1.29

a) n=5.; b) Group I: Normal control,; c)Group II: Administered with the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* at 2000 mg/kg body weight

Figure 2: Food Intake Chart

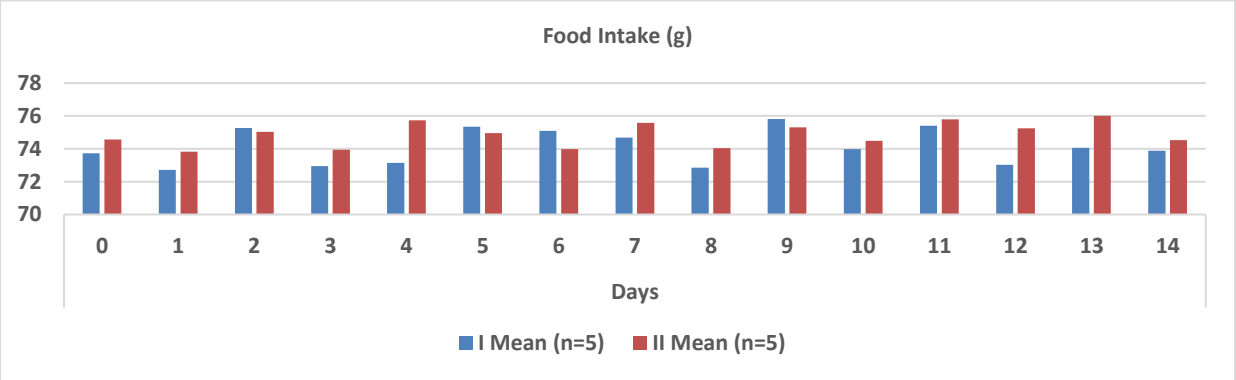
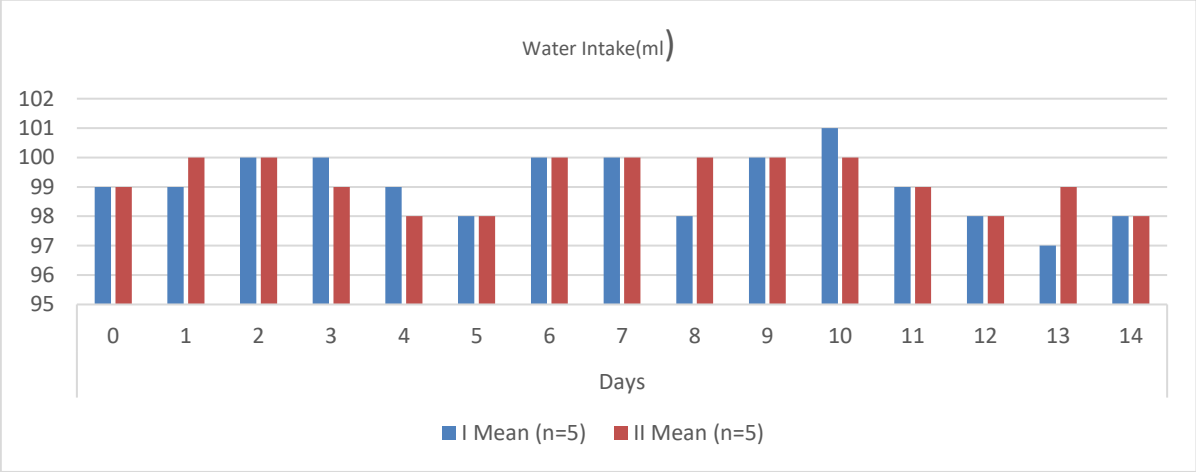


Table 5.: Daily water intake (mL) record of the animals showing the percent mean difference between consecutive days during acute oral toxicity study.

Group	Parameters	Different days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	Mean (n=5)	99.00	99.00	100.0	100.00	99.00	98.00	100.00	100.00	98.00	100.00	101.00	99.00	98.00	97.00	98.00
	SE	1.46	1.84	1.71	1.13	1.36	1.36	0.94	1.10	1.64	0.64	1.12	1.64	1.26	0.71	1.31
II	Mean (n=5)	99.00	100.00	100.00	99.00	98.00	98.00	100.00	100.00	100.00	100.00	100.00	99.00	98.00	99.00	98.00
	SE	1.47	1.54	0.81	1.56	1.25	1.39	1.48	1.19	1.56	1.15	1.33	1.55	1.74	1.63	0.95

n=5.Group b) I: Normal control; Group II: Administered with the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* at 2000 mg/kg body weight.

Figure 3: Water Intake Chart



CONCLUSION

In the present study, there was no mortality recorded even at the highest dose level of 2000 mg/kg body weight, which proves that the hydroalcoholic extracts of combination containing *Butea monosperma, Flemingia strobilifera and Moringa oleifera* are well tolerated by rats. From the observations recorded for behavioral changes, clinical observations, body weight changes and mortality, the study reckoned that the hydroalcoholic extracts of all three plants did not cause acute toxicity effects when administered orally in rats and the LD50 values is greater than 2000 mg/kg. The results of the study thus indicate that there was neither a change in body weight, food and water consumption nor any signs of behavioral changes or toxicity by the animals administered orally for all the dose groups.

Thus, the safety study provided firsthand information on the selection of appropriate safer concentrations/doses of the plant extracts for further pharmacological studies.

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