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High dosage toxicity testing of medicinal herbs *Momordica charantica, Emblica officinalis, Tribulus terrestris* and *Trigonella foenum graecium* in albino mice: a pilot Study

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Abstract
The aim of the present study was to investigate the toxicological effects of medicinal herb extract of *Momordica charantica*, Emblica officinalis, *Tribulus terrestris* and *Trigonella foenum graecium* in Albino mice. The herbal extract was prepared by maceration of the fruits of these plant, procured locally. The extracts were dried and store separately. These were reconstituted separately with carboxy methyl cellulose and administered by oral route as per study design (OECD guidelines). The dose was administered and both food and water were withheld for a period of 2 hours post dosing. The animals were observed for 24 hours and no toxic effects were observed. There as zero percent mortality in the study animals. Hence the LD50 is above this high dose for these four medicinal herbal extracts. It can be concluded that maximum non-lethal dose for these four herbs is beyond 5000 mg/kg in the albino mice model. The four medicinal herbs can be administered at higher dose without encountering any toxic effect in albino mice model. Larger study with emphasis on chronic toxicity testing must be conducted in order to evaluate the effect of chronic exposure to these medicinal plants. The limitations of the present study were that no chronic toxicity, reproductive toxicity and geno-toxicity testing were done, however these have been planned to follow. However despite these limitations, the present pilot study is an attempt to assess the acute toxicity of high dosage of these four medicinal plants for the first time in albino mice model.

Keywords: *Momordica charantia*, Emblica officinalis, *Tribulus terrestris*, *Trigonella foenum graecium*, Albino mice, acute toxicity study

1. Introduction
Indian herbs are used for various medicinal purposes since the ancient time period and its efficacy has been established in wide area of therapeutic usage. Amongst the different medicinal herbs; commonly used four herbs are *Momordica charantica, Emblica officinalis, Tribulus terrestris* and *Trigonella foenum graecium*. Bitter Melon or Bitter Gourd or Karela as known in India is scientifically known as *Momordica charantica*; it is commonly harvested in tropical countries like India, South East Asia, South America and Africa. It belongs to the family of Cucurbitaceae. Amongst many other established beneficial effects on human population, it is known for its efficacy against diabetes mellitus [1]. *Momordica charantica* improves the glucose metabolism and thus imparts a hypoglycemic effect in the diabetic patients. Apart from its anti-diabetic effect this medicinal plant is found to be effective in cholesterol and lipid lowering property with added anti-cancer and cyto-toxic effects [2, 3].
Emblica officinalis which is popularly known as Indian Gooseberry or Amla in local language is known for its numerous therapeutic effects including those of usage in cardiovascular disorders, liver dysfunction, and for inflammation [4]. Amla has been considered as an anti-obesity preparation. No evidence base approach has yet been established but it show excellent efficacious result in various animal trials [5, 6].
Another important herb is *Tribulus terrestris* belonging to the family Zygophyllaceae. It is recognized for its hypotensive effect and its efficacy against angina pectoris and cardiac depressant effect has been established. It has been shown to increase the free serum testosterone and to be effective in the treatment of sexual and erectile dysfunction by conversion of its phyto-chemical derivative, protodioscin to De Hydro Epi Androsterone (DHEA) [7, 8].
Fenugreek (*Trigonella foenum graecum*) is one of the oldest medicinal plants originating in India and North Africa regions [9]. The Uses of fenugreek dates backed to ancient Egyptians, Greeks and Romans. Fenugreek seeds are commonly used as condiment and seasoning in food

preparation and are assumed to possess nutritive properties [10]. Several studies indicated the hypoglycemic and hypo-lipidemic properties of fenugreek seeds suggesting that fenugreek may help to control diabetes [11].

Toxicity studies are concerned with assessing the damaging effects of chemical, biological and physical substances on biological systems of the living organisms. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Most of these studies are conducted to assess the degree to which substances are toxic (poisonous) for humans, animals or the environment, to investigate the mechanism of toxic chemicals, or to develop new or improved tests for specific types of chemically induced effects [12]. Although, these four medicinal herbs have been in use for many years in the human population very scarce literature is available on the toxicity profile of these herbs. There are no published studies on the toxicity profile of large dosage of these medicinal plants in animal models. Hence, the main aim of this pilot study is to observe the toxicity effect of large dosage of four medicinal plant extracts in albino mice models.

2. Materials and Methods

The plants *Emblica officinalis*, *Tribulus terrstris*, *Trigonella foenumgracum* and *Momordica charantia* were obtained from local market and a sample of each was stored in the Museum of Department of Pharmacology, Dayan and Medical College & Hospital, Ludhiana, Punjab.

The doses were selected for the toxicity studies (Table 1).

Table 1: Dose distribution of herbal extracts as per study design

S. No	Name of Extract	Doses
1a	*Emblica Officinalis*	5000mg/kg (Hydro-alcoholic extract)
1b		2000mg/kg (Hydro-alcoholic extract)
2a	*Tribulus terrstris*	5000mg/kg (Hydro-alcoholic extract)
2b		2000mg/kg (Hydro-alcoholic extract)
3a	*Trigonella foenum gracum*	5000mg/kg (Hydro-alcoholic extract)
3b		2000mg/kg (Hydro-alcoholic extract)
4a	*Momordica charantia*	5000mg/kg (Hydro-alcoholic extract)
4b		2000mg/kg (Hydro-alcoholic extract)

2.1 Administration of dose

All herbal extract were separately dissolved in Carboxy Methyl Cellulose (CMC, 0.5%) and administered by per oral route 5 ml/100 gm and 1 ml/200 gm.

The acute oral toxicity studies were performed as per revised OECD guideline No. 423 in the albino mice (OECD, 2000). The animal used in this study were female albino mice (n = 3).

The animals were fasted overnight with water ad-libitum and administered with single dose of 2000 or 5000 mg/kg test drugs as per study design randomly. The test substance was administered in a single dose by cannula. Following the period of fasting, the animals were weighed and the test substance administered. After the substance was administered, food was withheld for a further 1-2 hours. The study animals were observed individually at predetermined time intervals during the first 24 hrs, with special attention given during the first 4 hrs. The animals were observed for toxic symptoms such as behavioral changes, locomotion and mortality.

3. Results

The results obtained after administration of large dosage (2000 mg/kg or 5000 mg/kg) were quite surprising. None of the animals in any of the group (Table no 1) revealed any signs or features of toxicity. Zero percent mortality occurred in the study animals (all groups). The results are summarized in Table 2a, 2b, 2c and 2d

Table 2 a: Acute toxicity effects of *Emblica Officinalis*

S. No.	Animal (Mice)	Animal Weight (Gm)	Dose (Mg/Kg)	Observations Sedation/Writing Reflex/Licking/	Mortality
1a	1	25	5000	No Toxicity	No
	2	30			No
	3	30			No
1b	1	35	2000	No Toxicity	No
	2	30			No
	3	28			No

Table 2 b: Acute toxicity effects of *Tribulus terrstris*

S. No.	Animal (Mice)	Animal Weight (Gm)	Dose (Mg/Kg)	Observations Sedation/Writing Reflex/Licking/	Mortality
2a	1	25	5000	No Toxicity	No
	2	30			No
	3	30			No
2b	1	30	2000	No Toxicity	No
	2	35			No
	3	30			No

Table 2 c: Acute toxicity effects of *Trigonella foenumgracum*

S. No.	Animal (Mice)	Animal Weight (Gm)	Dose (Mg/Kg)	Observations Sedation/Writing Reflex/Licking/	Mortality
3a	1	30	5000	No Toxicity	No
	2	35			No
	3	35			No
3b	1	30	2000	No Toxicity	No
	2	30			No
	3	35			No

Table 2 d: Acute toxicity effects of *Momordica charantia*

S. No.	Animal (Mice)	Animal Weight (Gm)	Dose (Mg/Kg)	Observations Sedation/Writing Reflex/Licking/	Mortality
4a	1	30	5000	No Toxicity	No
	2	29			No
	3	32			No
4b	1	30	2000	No Toxicity	No
	2	30			No
	3	35			No

4. Discussion

No serious detrimental effects were observed during the acute toxicity study of these four medicinal plants even at very high dosage of 5000 mg/kg. Hence the LD_{50} (Lethal dose 50) is above 5000 mg/kg for all of these plants and cannot be precisely calculated. The acute toxicity studies provide a great impact to design the pharmacological profile of these medicinal plants and to set $LD_{50.}$

Information from toxicity tests is used to classify a chemical, for example to assign appropriate warning labels for containers, and, where necessary, for selecting measures, such as protective equipment, during manufacture, exposure and use. Data from tests help exemplify the relationship between dose and toxicological response to be further integrated with information on human exposure to produce a risk assessment, and to identify control measures necessary to manage and reduce any identified risk (if any) [13]. For pharmaceuticals, results from animal tests are used in combination with data on the efficacy of a potential medicine to decide whether the beneficial effects of the treatment would outweigh the risks of adverse side effects. These results are also used to establish a safe dose for use in clinical trials (First in man use - in Phase 1 trials). They may also indicate potential adverse effects that must be monitored carefully. Acute systemic toxicity is assessed by the administration of a single dose of compound, typically to rats and mice, orally, through dermal application or by inhalation. For pharmaceuticals, the main aims of these studies are to generate information useful in determining the nature (including delayed toxicity) and duration of any acute toxic response. These acute toxicity studies also determine the maximum non-lethal dose and provide preliminary information relevant to single exposure or over-dosage in humans [14, 15].

Every study has some limitations. The limitations of the present study were that the chronic toxicity, reproductive toxicity and geno-toxicity testing were not been performed but these studies have been planned to be undertaken soon. However inspite of these limitations the present study is a pilot study attempting to assess the toxicity effect of high dosage of these four medicinal plants for the first time in albino mice model.

5. Conclusion

To conclude the four medicinal herbs can be administered at higher dose without encountering any toxic effect in albino mice model. Larger study with emphasis on chronic toxicity testing must be conducted in order to assess the chronic exposure to these medicinal plants.

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