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Research Article

Study of Acute Toxicity of *Tribulus Terristris*

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ABSTRACT
In present work acute toxicity of aqueous slurry of leaves powder of *Tribulus Terristris* was studied. The plant of *Tribulus Terristris* were collected from Ambegaon Tehsil, Pune, Maharashtra, India. 2.00 gm/Kg to 10.00 gm/Kg body weight of sample powder was administered to Swiss mice orally. The treated animals were observed continuously for 14 days for the behavioral changes. Mortality was not observed. No any changes observed in the behavior, food and water intake of the mice. These observations indicate the non toxic nature of plant *Tribulus terristris*.

KEYWORDS
Acute Toxicity, mortality. *Tribulus terristris*,

1. INTRODUCTION

Medicinal plants are known as backbone of traditional medicine which is widely used to treat acute and chronic diseases. The medicinal plants were used for treating diseases, which are also beneficial and without any side effects. Traditional medicines are also used to protect, restore and improve health. Toxicity is the ability of substance to cause adverse effect in an organism [3]. The neurobehavioral and immunological effect of toxicants on the cellular and biochemical functions were assessed. Due to natural origin Medicinal plants are considered to be nontoxic and safe. Medicinal plants are used to treat various diseases like infections, cold, inflammation, GIT disorders, insomnia depression, heart disease, diabetics, cancer, acquired immunodeficiency syndrome and liver disease. Reports prove that some of the plants have cytotoxic, geno toxic and carcinogenic effects when used chronically [4]. Safety dose level should be administrated to the animals, the dose considered into acute toxicity, sub-acute toxicity, sub-chronic toxicity and chronic toxicity [5].

Proper dose of drug should be monitored by study of acute toxicity. Skin, lungs and gastrointestinal tract are three major ways for the absorption of toxic compounds. Digestive system and lungs is an important route for toxic compounds. LD_{50}The lethal dose (LD_{50}) is dosage of a toxicant which kills 50% of the test animals. Acute Toxicity is the toxic effect produced by single exposure of toxicants.

With high dose animal may leads to the gastrointestinal symptoms and dysfunction. By acute dose, the plant material should be nonlethal and nontoxic which is called the test limit. Test limit for oral toxicity is generally considered to be 5.0g/kg body weight.

2. MATERIALS AND METHODS

2.1 Acute toxicity study of *Tribulus Terrestris*

We have carried out acute toxicity study as per CPCSEA guideline with CPCSEA reference number 25/1/99-AWD dated 15th December 2000 at Mumbai. An acute toxicity study was carried out for *Tribulus Terrestris* by using Swiss mice. The study was carried out to assess the acute toxicity of *Tribulus Terrestris* on oral administration (Table 1).

Table 1. Toxicity study of *Tribulus Terrestris* on oral administration.

Name of the study	**Acute toxicity study**
Test material	**Plant powder (in the form of aqueous Slurry)**
Animal model	**Albino Swiss Mice**
Animals procured from	**Raj Biotech (INDIA) Ltd., Pune**
Sex	**Male and Female**
Weight range of animals	**Between 35 to 55 g**
No. of dose groups	**Three groups**
Animals per group	**2 males and 2 females**
Route of administration	**Intragastric administration with the help of gavage No. 16**
Dose volume	**2.0 ml per animal**
Vehicle for administration	**Distilled water**

No. of administrations	**Single**
Concentration of dose	**2, 4, 6, 8 and 10 gm/Kg body weight**
Study duration	**Acclimatization for 14 days,** **One day drug administration ,** **Continuous 14 days observation period.**
Parameters observed	**Cage side observations,** **Daily food and water intake,** **Daily body weight and mortality record etc.**

Study protocol

2.2 Maintenance of Animals

The animals were housed in polyurethane cages, with rice husk bedding and daily cleaning. The drinking water provided with ad libitum and simultaneously fed with commercially available mice feed.

Following table 2 shows the specifications of the feed:

Table 2. Specifications of the feed.

Name	Percentage
Crude Protein	**20 - 21 % minimum**
Ether Extractive	**04 - 05 % minimum**
Crude Fiber	**04 % maximum**
Ash	**08 % maximum**
Calcium	**1.2%**
Phosphorus	**0.6 % minimum**
NFE	**54 %**
ME Kcal/Kg	**3600**
Pallet Size	**12 mm**

The feed provided to mice was rich in stabilized content viz. Vit. A, D_3, B_{12}, Thiamine, Riboflavin, Folic acid etc. Food was also supplemented with essential minerals and microelements. Animals were fed with controlled quantities of water and food. The consumption of water and food by animal was estimated.

3 RESULTS AND DISCUSSION

3.1 Cage Side Observations

Assessment of animal's behavior was carried out daily with general observations from the stage of dosing to the end of the study. Observations include daily recording offer condition, skin damage, swellings or lumps (with consistency, size, and shape), abdominal distension, tenderness. Then eyes were observed for dullness, discharges, opacities, pupil diameter, drooping of upper eyelid. The colour and consistency of the feces, condition of teeth, gait wetness or soiling of the perineum, breathing abnormalities, etc. Such changes or symptoms are toxicity indicators, which were not observed in feed treated animals at all dose levels. Animals showed no significant behavioral changes before and after the oral administration of plant powder slurry

Table 3. Doses regime.

Sr. No.	Sex	Dose gm/Kg Body Wt.	No. of animals used	Total Vol. given in cm3
1	Male	2.00	02	**2.00**
2	Female	2.00	02	**2.00**
3	Male	4.00	02	**2.00**
4	Female	4.00	02	**2.00**
5	Male	6.00	02	**2.00**
6	Female	6.00	02	**2.00**
7	Male	8.00	02	**2.00**
8	Female	8.00	02	**2.00**
9	Male	10.00	02	**2.00**
10	**Female**	**10.00**	**02**	**2.00**

Table 4. Cage side observations for all animals.

Sr. No.	Parameters	Cage Side Observations
1	Condition of the fur	**Normal**
2	Skin	**Normal**
3	Subcutaneous swellings	**Nil**
4	Abdominal distension	**Nil**
5	Eyes –dullness	**Nil**
6	Eyes – opacities	**Nil**
7	Pupil diameter	**Normal**
8	Ptosis	**Nil**
9	Colour and consistency of the faeces	**Normal**
10	Wetness or soiling of the perimenum	**Nil**
11	Condition of teeth	**Normal**
12	Breathing abnormalities	**Nil**
13	**Gait**	**Normal**

Table 5. Mortality record.

Group	**2**	**2**	**4**	**4**	**6**	**6**	**8**	**8**	**10**	**10**
Gm/Kg										
Sex	-	-	-	-	-	-	-	-	-	-
Hr. 1	-	-	-	-	-	-	-	-	-	-
Hr. 2	-	-	-	-	-	-	-	-	-	-
Hr. 3	-	-	-	-	-	-	-	-	-	-
Hr. 4	-	-	-	-	-	-	-	-	-	-
Day 1	-	-	-	-	-	-	-	-	-	-
Day 2	-	-	-	-	-	-	-	-	-	-

Day 3	-	-	-	-	-	-	-	-	-	-
Day 4	-	-	-	-	-	-	-	-	-	-
Day 5	-	-	-	-	-	-	-	-	-	-
Day 6	-	-	-	-	-	-	-	-	-	-
Day 7	-	-	-	-	-	-	-	-	-	-
Day 8	-	-	-	-	-	-	-	-	-	-
Day 9	-	-	-	-	-	-	-	-	-	-
Day 10	-	-	-	-	-	-	-	-	-	-
Day 11	-	-	-	-	-	-	-	-	-	-
Day 12	-	-	-	-	-	-	-	-	-	-
Day 13	-	-	-	-	-	-	-	-	-	-
Day 14	-	-	-	-	-	-	-	-	-	-
Mortality	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**	**0/2**

3.2 Observations of changes in Body Weight

To monitor the health of an animal, body weight is an important factor. All the animals from treated groups did not show any significant decrease in body weights. A dose which reduces 10 % or more body weight is considered to be a toxic dose.

Table 6. Daily body weight record in grams.

Group gm/Kg	**2**	**2**	**4**	**4**	**6**	**6**	**8**	**8**	**10**	**10**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 0	41	36	50	33	42	32	51	40	38	**40**
Day 1	41	37	50	32	43	33	51	41	37	**41**
Day 2	42	38	51	33	42	34	52	41	36	**41**
Day 3	42	38	52	34	43	35	52	42	37	**42**
Day 4	43	39	51	33	43	35	53	42	38	**41**
Day 5	44	39	53	35	44	36	53	43	39	**43**
Day 6	44	40	53	34	44	36	54	43	39	**42**
Day 7	45	41	54	35	45	37	55	44	40	**44**
Day 8	46	42	54	35	46	37	56	44	41	**43**
Day 9	46	42	55	34	45	38	55	45	41	**44**
Day 10	47	43	54	35	46	38	56	44	42	**44**
Day 11	48	43	55	34	45	38	57	45	42	**45**
Day 12	48	44	55	36	47	37	57	46	41	**45**
Day 13	49	45	56	37	45	38	58	46	42	**46**
Day 14	**50**	**45**	**55**	**36**	**46**	**38**	**58**	**47**	**43**	**45**

(all the values expressed as mean of two animals in each group)

3.3 Consumption of Food and Water by mice

Observations shown on significant changes in food and water intake by mice at all dose levels. Tables **7** and 8 indicates the data of food and water consumption.

Table 7. Daily food intake record in grams.

Group Gm/Kg	**2**	**2**	**4**	**4**	**6**	**6**	**8**	**8**	**10**	**10**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 0	11	14	14	13	20	14	20	14	14	**16**
Day 1	12	16	14	13	20	14	20	15	14	**17**
Day 2	11	15	15	14	19	15	19	14	15	**16**
Day 3	11	16	13	14	20	15	20	15	16	**17**
Day 4	10	16	15	12	20	14	20	15	15	**17**
Day 5	11	17	15	12	21	14	21	14	16	**17**
Day 6	12	17	16	13	21	14	21	14	17	**18**
Day 7	12	18	15	13	20	14	21	14	17	**17**
Day 8	11	18	16	13	20	14	22	14	18	**19**
Day 9	11	19	17	14	19	14	21	15	18	**18**
Day 10	12	20	17	13	20	15	22	14	17	**17**
Day 11	12	20	17	14	20	14	22	14	19	**18**
Day 12	12	21	18	14	21	14	22	15	19	**18**
Day 13	11	20	18	15	21	14	22	15	18	**17**
Day 14	**11**	**22**	**19**	**15**	**22**	**14**	**23**	**13**	**19**	**19**

(all the values expressed as mean of three animals in each group)

Table 8. Daily water intake record in ml.

Group gm/Kg	**2**	**2**	**4**	**4**	**6**	**6**	**8**	**8**	**10**	**10**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 0	12	15	15	11	12	14	23	13	22	**14**
Day 1	12	15	15	11	12	14	21	13	21	**13**
Day 2	12	14	14	12	13	13	23	14	22	**11**
Day 3	12	14	14	12	12	13	22	14	23	**12**
Day 4	13	15	15	12	12	15	23	14	21	**12**
Day 5	12	16	16	13	12	15	23	14	23	**14**
Day 6	12	14	15	12	12	14	21	13	22	**13**
Day 7	12	15	15	12	13	14	23	11	23	**11**
Day 8	12	16	14	12	12	14	22	12	22	**12**
Day 9	13	14	14	11	12	14	23	12	22	**12**
Day 10	12	15	15	12	12	13	23	12	21	**12**
Day 11	12	16	16	11	13	13	23	12	23	**14**

Day 12	12	15	18	12	12	14	21	13	22	**13**
Day 13	12	14	19	11	12	13	23	13	22	**11**
Day 14	**13**	**14**	**19**	**11**	**12**	**14**	**22**	**12**	**23**	**12**

(all the values expressed as mean of three animals in each group)

3.4 Rate of Mortality

There was no significant mortality observed even at the highest dose level i.e.10 gm / Kg. body weight.

4. CONCLUSION

The result analysis showed that there was no significant changes in body weight, food and water consumption by the mice with all doses (2.00 gm/Kg body weight to 10.0 gm/Kg body weight), Even at the highest dose level i.e. 10.0 gm / Kg body weight mortality was not observed. All the observations prove that the *Tribulus terrestris* plant powder having no significant toxic effect in mice.

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