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## Abstract

Although in Homoeopathy different potencies of Tribulus terrestris (Ikshugandha) have been commonly prescribed in urinary affections, especially dysuria and in debilitated status of the sexual organs, no scientific experimental data has been documented to establish their safe use. The present study was, therefore, undertaken to generate preliminary data on the biochemical and haematological parameters with homoeopathic drug Tribulus terrestris. The four potencies (3x, 6x, 12x and 30c) of this drug were administered orally in daily doses of 0.1ml, 0.2ml and 0.5ml/rat for 14 days and their effects on biochemica/ and haematological parameters were studied on 21" and 28" day during post-treatment period. Preliminary findings on biochemical (serum glucose, serum total cholesterol, serum triglycerides, serum total protein, serum albumin, serum urea and SGOT and SGPT levels) and haemotological (haemaglobin content, total R.B.C. and total W.B.C. and differential leucocyte counts) parameters showed variable effects of different potencies of T. terrestris, but all the observed values for both biochemical and haematological profiles were found to be within the normal range of healthy animals. There was no apparent effect on the behaviour of animals during the period of study. This homoeopathic drug did not have any toxic effect in the four potencies studied. However, in order to arrive definite conclusion on the complete safety profiles of this drug, further research on acute and chronic toxicity studies are needed in different species of experimental animals.



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COLLABORATIVE RESEARCH

# Biochemical and haematological evaluation of homoeopathic drug *Tribulus terrestris* in rats

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Although in Homoeopathy different potencies of *Tribulus terrestris* (Ikshugandha) have been commonly prescribed in urinary affections, especially dysuria and in debilitated status of the sexual organs, no scientific experimental data has been documented to establish their safe use. The present study was, therefore, undertaken to generate preliminary data on the biochemical and haematological parameters with homoeopathic drug *Tribulus terrestris*. The four potencies (3x, 6x, 12x and 30c) of this drug were administered orally in daily doses of 0.1ml, 0.2ml and 0.5ml /rat for 14 days and their effects on biochemical and haematological parameters were studied on 21st and 28th day during post- treatment period. Preliminary findings on biochemical (serum glucose, serum total cholesterol, serum triglycerides, serum total protein, serum albumin, serum urea and SGOT and SGPT levels) and haemotological (haemaglobin content, total R.B.C. and total W.B.C. and differential leucocyte counts) parameters showed variable effects of different potencies of *T. terrestris*, but all the observed values for both biochemical and haematological profiles were found to be within the normal range of healthy animals. There was no apparent effect on the behaviour of animals during the period of study. This homoeopathic drug did not have any toxic effect in the four potencies studied. However, in order to arrive definite conclusion on the complete safety profiles of this drug, further research on acute and chronic toxicity studies are needed in different species of experimental animals.

**Key words:** *Tribulus terrestris*, homoeopathic potencies, biochemical and haematological profiles, albino rats.

## INTRODUCTION

The plant *Tribulus terrestris* L. (Sans: Ikshugandha, Gokshura; Hindi: Chota-gokhru) has long been known in folk medicine of Eastern countries and Bulgaria and has been used in the treatment of sexual deficiency.[1] Plant and dried spiny fruits are used in decoction or infusion in cases of spermatorrhoea, phosphaturia, diseases of the genitourinary system such as dysuria, gonorrhoea, chronic cystitis, calculus affections, urinary disorders, incontinence of urine, gout and impotence, also in uterine disorders after parturition and to ensure fecundity; and is used in Northern India in cough, diseases of the heart and suppression of urine.[2] Alcoholic extract of fruit showed antibacterial activity against *E. coli*, *Staph. aureus*, antifungal effect against *T. montagrophytes*, *M. tonsurans*, *T. rubrum* and *C. albicans*, while, aqueous alcoholic extract of seeds exhibited hypotensive effects in anaesthetized dogs and produced cardiac depressant effect in isolated rabbit heart as well as on Straub's frog heart.[3,4] Toxicity studies of active ingredient (Tribestan) of T. *terrestris* have been found to be safe in rats[5].

In Homoeopathy, different potencies prepared from the whole plant of *Tribulus terrestris* are prescribed commonly by the physicians for patients suffering from urinary tract infections, especially dysuria and in debilitated status of the sexual organs. It meets the auto-traumatism of masturbation, correcting the emissions and spermatorrhoea and partial impotence caused by over indulgence of advancing age, when

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prescribed in mother tincture form.[6] Homoeopathic preparation of *T. terrestris* is assumed to be safe for clinical use, but it lacks documentary evidence on scientific basis. In order to validate its safe use in clinical practice and to carry out its safety evaluation in experimental animals, it was decided to have some preliminary ideas on the biochemical and haematological parameters of this drug in experimental animals.

The present preliminary study was therefore undertaken to evaluate the effects of 3x, 6x, 12x and 30c potencies of *T. terrestris* on the biochemical and haematological parameters, in addition to their effects on the behaviour of animals during the period of 28 days.

## MATERIALS AND METHODS

### Drug

Different potencies (3x, 6x, 12x and 30c) of *T. terrestris* were prepared by M/S. Bahola Laboratory, Puducherry, India from a single batch of whole plant supplied by Survey of Medicinal Plants and Collection Unit, Udagamandalam, Tamil Nadu.

### Animals

Healthy albino rats of both sexes, weighing between 140 - 175 gm were procured from National Centre for Laboratory Animals Sciences, National Institute of Nutrition, Hyderabad and housed (12/12hrs, light/dark cycles, room temp. 22 -24 $^{0}$C) in polypropylene cages (47 x 34 x 20 cm) lined with husk which was renewed on every alternate days. Animals were acclimatized to standard laboratory conditions for 15 days prior to the initiation of drug treatment and fed balanced diet and water *ad libitum*.

### Reagents and chemicals

Readymade kits/ reagents for estimation of serum glucose, serum total cholesterol, serum triglycerides and urea (M/S. Excel Diagnostic Pvt. Ltd, Hyderabad), SGOT and SGPT (M/S Medsource Ozone Biochemicals), serum total protein and albumin (M/S Span Diagnostic Pvt. Ltd, Surat) and for haematological parameters (M/S. Nice Chemical Pvt. Ltd., Cochin) were used. Alcohol was procured from M/S.Venkateswara Winery & Distillery Pvt. Ltd., Nagole, Hyderabad and was distilled before use. All other chemicals used in this study were of analytical grade.

### Experimental design

The experimental protocol was approved by Institutional Animals Ethics Committee (383/01/a/ CPCSEA) of Department of Zoology, Osmania University, Hyderabad. The animals were weighed and marked on ear pinna for identification. A total of 108 rats were grouped into 6 batches of 18 each. Each batch was further divided into 3 subgroups of 6 each. The different potencies (3x, 6x, 12x and 30c) of *T. terrestris* were orally administered in doses of 0.1 ml, 0.2 ml and 0.5 ml per rat per day for 14 days. Thereafter, these rats were left for another 14 days without any drug treatment. The test potencies of *T. terrestris* and vehicle (91.5% v/v alcohol) were diluted with distilled water in a ratio of 1:10 or 1:4 so that each rat should not receive total volume of more than 2 ml per day. Two parallel controls were run. One received equivalent volume of diluted vehicle (91.5% v/v alcohol used to prepare homoeopathic potencies) and other normal saline.

### Collection of blood sample

In order to examine the influence of different potencies of *T. terrestris* on hematological and biochemical parameters, the blood was collected from 50% of the animals of each group on 21$^{st}$ day and remaining 50% animals on 28$^{th}$ day of the initiation of drug treatment from the corneal plexus of the eye through heparinized coated glass capillaries into the non- heparinized test tubes. After processing for hematological parameters, the blood was allowed to clot and serum was separated by centrifuging at 5000 rpm for 10 min.

### Effect on biochemical parameters

Sugar (GOD-POD Method)[7,8], total protein (Modified biuret method)[9,10], albumin (Bromocresol green method)[11,12], total cholesterol (CHOD-PAP with LCF, enzymatic method)[13,14], triglycerides (GPO-PAP method)[15], serum glutamate oxaloacetate transminase ( SGOT) and serum glutamate pyruvate transminase (SGPT)[16] and urea (Berthelot method)[17] were measured from the serum of rats. The absorbance of the serum samples and the standard sample were measured on spectrophotometer (Systronic) at specified wave lengths for sugar (505 nm), total protein (578 nm), albumin (630 nm), cholesterol (500 nm), triglycerides (546 nm) and urea (570 nm) after calibrating it against their respective blank samples, where as for SGOT and SGPT estimation, absorbance of serum samples, standard, calibrator, and blank were measured at 505 nm against distilled water.

**Table-1** Effect of different potencies of *Tribulus terrestris* (0.1ml/rat/day) on rats' biochemical profiles

| Groups | No. of rats used | Dose/ rat/ day | ON 21st DAY$ | | | | | | | | ON 28th DAY$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pro-tein g/dl | Albu-min g/dl | Sugar mg/dl | Chole sterol mg/dl | Tri-glyce-rides mg/dl | Urea mg/dl | SGOT IU/l | SGPT IU/l | Pro-tein g/dl | Albu-min g/dl | Sugar mg/dl | Chole sterol mg/dl | Tri-glyce-rides mg/dl | Urea mg/dl | SGOT IU/l | SGPT IU/l |
| Control (Normal saline) | 6 | 1.0ml | 6.7 ± 0.19 | 3.7 ± 0.14 | 72.5 ± 3.42 | 90.3 ± 2.97 | 94.6 ± 3.85 | 41.4 ± 2.36 | 24.0 ± 4.93 | 36.0 ± 6.35 | 6.7 ± 0.32 | 3.8 ± 0.27 | 76.8 ± 2.65 | 89.7 ± 3.19 | 102.1 ± 2.00 | 40.4 ± 2.08 | 22.0 ± 3.46 | 31.0 ± 5.20 |
| Vehicle (91.5%v/v alcohol) | 6 | 0.1ml | 6.8 ± 0.22 | 3.8 ± 0.23 | 74.9 ± 3.76 | 86.0 ± 3.06 | 83.2 ± 5.39 | 45.2 ± 3.49 | 27.3 ± 3.72 | 40.3 ± 6.23 | 6.7 ± 0.33 | 3.7 ± 0.26 | 75.9 ± 4.10 | 85.3 ± 4.06 | 89.4* ± 4.06 | 42.3 ± 3.46 | 22.0 ± 3.06 | 30.3 ± 6.35 |
| *T.* terrestris 3x | 6 | 0.1ml | 6.5 ± 0.18 | 4.1 ± 0.18 | 76.2 ± 2.31 | 92.0 ± 2.31 | 79.0 ± 7.77 | 48.0 ± 2.08 | 24.7 ± 3.54 | 39.0 ± 6.24 | 6.7 ± 0.15 | 3.8 ± 0.12 | 75.4 ± 4.08 | 88.7 ± 2.42 | 76.7 ** ± 2.42 | 44.9 ± 3.06 | 25.3 ± 2.61 | 34.0 ± 4.16 |
| *T.* terrestris 6x | 6 | 0.1ml | 6.7 ± 0.41 | 3.9 ± 0.18 | 77.6 ± 5.83 | 94.3 ± 2.35 | 88.1 ± 3.79 | 42.2 ± 3.06 | 24.0 ± 5.29 | 35.0 ± 7.37 | 6.5 ± 0.18 | 3.8 ± 0.23 | 78.2 ± 2.08 | 94.0 ± 5.03 | 88.4 ± 5.37 | 41.8 ± 2.61 | 23.3 ± 4.38 | 36.3 ± 7.97 |
| *T.* terrestris 12x | 6 | 0.1ml | 6.8 ± 0.20 | 3.9 ± 0.20 | 75.1 ± 3.46 | 90.7 ± 2.97 | 85.3 ± 3.19 | 47.9 ± 4.58 | 22.0 ± 6.43 | 33.7 ± 6.81 | 6.9 ± 0.24 | 3.9 ± 0.17 | 76.0 ± 4.67 | 90.7 ± 2.42 | 83.8* ± 5.13 | 41.3 ± 2.65 | 20.0 ± 5.29 | 36.0 ± 8.14 |
| *T.* terrestris 30c | 6 | 0.1ml | 6.7 ± 0.26 | 3.7 ± 0.18 | 70.6 ± 1.47 | 90.0 ± 3.49 | 76.8 ± 5.79 | 41.6 ± 2.08 | 21.7 ± 6.89 | 36.0 ± 6.93 | 6.6 ± 0.26 | 3.7 ± 0.12 | 76.6 ± 3.49 | 89.7 ± 2.35 | 86.8 ± 5.46 | 41.4 ± 2.34 | 24.3 ± 4.98 | 35.7 ± 6.18 |

Values differ significantly ($p$–value* < 0.05, **< 0.01) between drug or vehicle/ normal saline administered rats
$ Represents Mean ± S.E.M. of 3 rats
Potencies of test drug and vehicle (91.5% v/v alcohol) were diluted in a ratio of 1:10 with distilled water in order to make the volume one ml.

**Table-2** Effect of different potencies of *Tribulus terrestris* (0.2ml/rat/day) on rats' biochemical profiles

| Groups | No. of rats used | Dose/ rat/ day | ON 21st DAY$ | | | | | | | | ON 28th DAY$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Protein g/dl | Albumin g/dl | Sugar mg/dl | Cholesterol mg/dl | Triglycerides mg/dl | Urea mg/dl | SGOT IU/l | SGPT IU/l | Protein g/dl | Albumin g/dl | Sugar mg/dl | Cholesterol mg/dl | Triglycerides mg/dl | Urea mg/dl | SGOT IU/l | SGPT IU/l |
| Control (Normal saline) | 6 | 2.0ml | 6.8 ± 0.15 | 3.9 ± 0.15 | 74.2 ± 2.61 | 89.3 ± 1.78 | 89.8 ± 3.94 | 40.5 ± 3.39 | 25.0 ± 2.65 | 38.3 ± 3.76 | 6.8 ± 0.17 | 3.9 ± 0.15 | 77.9 ± 3.21 | 86.0 ± 5.13 | 97.3 ± 2.04 | 35.9 ± 3.06 | 25.0 ± 3.46 | 36.3 ± 4.92 |
| Vehicle (91.5%v/v alcohol) | 6 | 0.2ml | 6.6 ± 0.17 | 3.4 ± 0.19 | 79.6 ± 4.08 | 85.3 ± 4.81 | 85.1 ± 3.46 | 38.4 ± 3.29 | 26.0 ± 4.16 | 39.0 ± 4.04 | 6.7 ± 0.23 | 3.7 ± 0.15 | 79.5 ± 4.08 | 84.3 ± 3.49 | 87.5 ± 3.56 | 43.6 ± 3.19 | 25.7 ± 3.29 | 40.3 ± 3.85 |
| *T.* terrestris 3x | 6 | 0.2ml | 6.9 ± 0.32 | 4.5 ± 0.18 | 82.5 ± 5.61 | 91.3 ± 2.42 | 87.3 ± 4.93 | 42.4 ± 3.56 | 22.3 ± 3.85 | 32.0 ± 8.08 | 6.9 ± 0.21 | 3.8 ± 0.12 | 77.4 ± 2.77 | 92.0 ± 2.31 | 86.3* ± 2.61 | 22.7 ± 4.67 | 36.7 ± 6.57 | 45.2 ± 3.39 |
| *T.* terrestris 6x | 6 | 0.2ml | 7.0 ± 0.42 | 3.9 ± 0.18 | 72.3 ± 2.31 | 92.7 ± 2.92 | 81.8 ± 4.92 | 45.1 ± 3.76 | 24.7 ± 4.38 | 37.7 ± 5.90 | 6.9 ± 0.07 | 4.1 ± 0.24 | 76.9 ± 1.78 | 94.0 ± 2.31 | 75.9 ** ± 3.21 | 43.0 ± 3.79 | 25.3 ± 7.06 | 36.7 ± 6.96 |
| *T.* terrestris 12x | 6 | 0.2ml | 6.7 ± 0.27 | 3.7 ± 0.24 | 74.8 ± 4.44 | 89.7 ± 3.19 | 78.5 ± 3.29 | 42.2 ± 2.04 | 21.3 ± 6.87 | 30.7 ± 6.87 | 6.70 ± 0.15 | 3.8 ± 0.12 | 74.6 ± 4.26 | 89.0 ± 2.52 | 87.5 ± 3.76 | 41.5 ± 3.29 | 22.3 ± 5.55 | 36.7 ± 7.54 |
| *T.* terrestris 30c | 6 | 0.2ml | 6.9 ± 0.23 | 3.7 ± 0.18 | 72.3 ± 2.89 | 83.7 ± 2.97 | 83.8 ± 4.10 | 43.0 ± 3.46 | 25.7 ± 3.19 | 37.0 ± 5.51 | 6.5 ± 0.18 | 3.6 ± 0.15 | 75.3 ± 4.06 | 84.7 ± 4.06 | 79.6 ± 6.23 | 44.2 ± 3.21 | 21.0 ± 4.73 | 33.3 ± 5.79 |

Values differ significantly ($p$–value* < 0.05, **< 0.01) between drug or vehicle/ normal saline administered rats.
$ Represents Mean ± S.E.M. of 3 rats
Potencies of test drug and vehicle (91.5% v/v alcohol) were diluted in a ratio of 1:10 with distilled water in order to make the volume 2 ml

**Table-3** Effect of different potencies of *Tribulus terrestris* (0.5ml/rat/day) on rats' biochemical profiles

| Groups | No. of rats used | Dose/ rat/ day | ON 21st DAY$ | | | | | | | | ON 28th DAY$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pro-tein g/dl | Albu-min g/dl | Sugar mg/dl | Chole sterol mg/dl | Tri-glyce-rides mg/dl | Urea mg/dl | S G O T IU/l | S G P T IU/l | Pro-tein g/dl | Albu-min g/dl | Sugar mg/dl | Chole sterol mg/dl | Tri-glyce-rides mg/dl | Urea mg/dl | S G O T IU/l | S G P T IU/l |
| Control (Normal saline) | 6 | 2.0ml | 6.8 ± 0.29 | 3.9 ± 0.21 | 73.5 ± 4.08 | 92.3 ± 2.04 | 88.2 ± 2.42 | 41.4 ± 2.68 | 25.7 ± 5.05 | 41.0 ± 4.36 | 6.8 ± 0.17 | 3.8 ± 0.12 | 74.1 ± 1.53 | 88.0 ± 3.61 | 97.8 ± 3.61 | 42.4 ± 2.61 | 25.7 ± 5.05 | 35.0 ± 7.23 |
| Vehicle (91.5%v/v alcohol) | 6 | 0.5ml | 6.8 ± 0.26 | 3.8 ± 0.21 | 78.6 ± 2.35 | 84.3 ± 3.76 | 84.9 ± 4.36 | 41.1 ± 2.52 | 26.3 ± 7.22 | 38.0 ± 8.62 | 6.6 ± 0.21 | 3.6 ± 0.24 | 78.3 ± 3.19 | 83.7 ± 6.18 | 84.4* ± 3.29 | 40.0 ± 4.98 | 24.7 ± 3.54 | 38.3 ± 3.49 |
| *T.* terrestris 3x | 6 | 0.5ml | 6.5 ± 0.35 | 3.2 ± 0.29 | 83.9 ± 5.80 | 98.3 ± 3.76 | 114.7 ** ± 4.38 | 43.3 ± 2.61 | 25.3 ± 3.72 | 35.3 ± 5.21 | 6.8 ± 0.23 | 3.3 ± 0.24 | 83.9 ± 4.04 | 116.7 ** ± 4.12 | 118.4 * ± 5.55 | 25.3* ± 4.67 | 36.3 ± 6.65 | 45.7 ± 1.47 |
| *T.* terrestris 6x | 6 | 0.5ml | 6.8 ± 0.23 | 3.8 ± 0.36 | 76.4 ± 4.10 | 101.6 ± 3.56 | 93.2 ± 6.24 | 41.2 ± 1.47 | 20.3 ± 3.49 | 33.3 ± 5.21 | 6.9 ± 0.29 | 3.7 ± 0.26 | 82.6 ± 4.10 | 100.7 * ± 2.42 | 104.4 ± 4.98 | 43.1 ± 2.65 | 24.3 ± 5.37 | 36.0 ± 6.93 |
| *T.* terrestris 12x | 6 | 0.5ml | 6.7 ± 0.26 | 3.9 ± 0.24 | 73.5 ± 3.32 | 93.0 ± 2.65 | 79.2 ± 3.79 | 43.9 ± 2.08 | 21.0 ± 4.73 | 32.0 ± 5.57 | 6.8 ± 0.15 | 3.7 ± 0.09 | 78.3 ± 3.29 | 88.5 ± 3.19 | 90.2 ± 2.74 | 41.0 ± 3.21 | 17.3 ± 4.67 | 30.0 ± 9.07 |
| *T.* terrestris 30c | 6 | 0.5ml | 6.7 ± 0.26 | 3.6 ± 0.20 | 76.1 ± 4.36 | 91.3 ± 3.72 | 92.3 ± 2.31 | 42.1 ± 2.31 | 22.0 ± 3.06 | 34.3 ± 5.82 | 6.8 ± 012 | 3.9 ± 0.12 | 73.1 ± 1.73 | 87.0 ± 5.86 | 85.7 ± 6.70 | 42.3 ± 2.34 | 21.3 ± 4.81 | 30.7 ± 7.52 |

Values differ significantly ($p$–value* < 0.05, **< 0.01) between drug or vehicle/ normal saline administered rats
$ Represents Mean ± S.E.M. of 3 rats
Potencies of test drug and vehicle (91.5% v/v alcohol) were diluted in a ratio of 1:4 with distilled water in order to make the volume 2 ml.

### Effect on hematological parameters

Hemoglobin content (gm %), total R.B.C., total W.B.C. and differential leukocyte counts were measured immediately after the withdrawal of blood.[18]

### Effect on gross behaviour

The influence of 3x, 6x, 12x and 30c potencies of *T. terrestris* was observed between 10.30 a.m. to 4.00 p.m. every day during the period of drug administration and for two weeks thereafter on the behaviour (alertness, passivity, sedation, biting, fighting, facial movements), depth and rate of respiration, gross perception of heart rate and force of contractions and on mortality, if any, of the rats during the period of study as described earlier.[19]

## STATISTICAL ANALYSIS

The data were expressed as Mean ± S.E.M. The difference between mean values of groups were statistically analysed by student's 't' test. A difference at *p*- value < 0.05 was considered to be statistically significant.[20]

## RESULTS

### Effect of different potencies of *Tribulus terrestris* on the biochemical parameters

Tables 1 - 3 show the effects of 3x, 6x, 12x and 30c potencies of *T. terrestris* administered orally in doses of 0.1 ml, 0.2 ml and 0.5 ml/ rat/day for 14 days on sugar, total protein, albumin, total cholesterol, triglycerides, urea, SGOT and SGPT levels in the serum on 21$^{st}$ and 28$^{th}$ day of experiment as compared to normal saline and vehicle (91. 5% v/v alcohol) administered rats.

The results showed that there was a significant decrease in serum triglycerides concentration with 0.1 ml of vehicle, 3x and 12x potencies on 28$^{th}$ day of study (Table - 1) as compared to that of normal saline treated rats. Similarly, significant decrease in serum triglycerides concentration (3x and 6x potencies) with 0.2 ml dose (Table - 2); and decrease in serum urea (3x potency), triglycerides (vehicle) and increase in total cholesterol (3x and 6x potencies), serum triglycerides (3x potency) with 0.5 ml dose (Table - 3) were observed but all the observed values for biochemical parameters were found to be falling within the normal range.

### Effect of different potencies of *Tribulus terrestris* on the haematological parameters

The effect of 3x, 6x, 12x and 30c potencies of *T. terrestris* on haemoglobin content (gm%), total RBC, total WBC and differential leucocyte counts in daily oral doses of 0.1 ml, 0.2 ml and 0.5 ml/rat/day for 14 days are presented in Tables 4 to 6.

The results showed that all the four potencies of *T. terrestris* in doses upto 0.5 ml/rat/day for 14 days did not have any apparent effect on haematological parameters, as compared to vehicle (91.5% v/v alcohol) and normal saline administered rats, when estimated on 21$^{st}$ and 28$^{th}$ day of experiments (Tables 4 - 6).

### Effect of different potencies of *Tribulus terrestris* on the gross behaviour

Neither any of the four potencies (3x, 6x, 12x and 30c) of T. *terrestris* nor vehicle (91.5% v/v alcohol) produced any perceptible change in the behaviour of rats when administered in doses of 0.1 ml and 0.2 ml/ rat/ day for 14 days. On the other hand, rats when given 0.5 ml/rat /day of all the four potencies of *T. terrestris* or vehicle (91.5% v/v alcohol), started scratching their face with fore paws within 2-3 min and later by both fore and hind paws. Thereafter, some rats showed laboured breathing and a few of them became calm and sat in one corner of cage for 8-10 min but responded immediately to the tapping of cage. However, these effects were more prominent in the beginning of the study and subsided slowly on the continuation of treatment. Normal saline administered rats remained active throughout the period of observations.

## DISCUSSION

Although homeopathic medicine *T. terrestris* in different potencies have been used for patients suffering from urinary tract infection and impotence[6], but it lacks documentary evidence for a safe use in clinical practice. In order to generate the same preliminary data for safety evaluation and to validate their use in clinical practice on scientific basis, the present study was undertaken to evaluate the effect of four potencies (3x, 6x, 12x and 30c) of *T. terrestris* on biochemical and haematological profiles. In addition, the effect of these potencies on the behaviour of rats were also studied.

**Table-4** Effect of different potencies of *Tribulus terrestris* (0.1ml/rat/day) on rats' haematological profiles

| Groups | No. of rats used | Dose/ rat/ day | 21st Day *$ | | | | | | | 28th Day *$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hb (gm) % | Total R.B.C. (mill /cubic mm) | Total W.B.C (number/ cubic mm) | Differential counts (%) | | | | Hb (gm) % | Total R.B.C. (mill /cubic mm) | Total W.B.C (number/ cubic mm) | Differential counts (%) | | | |
| | | | | | | Polymorps | Lympocytes | Eosinophils | Monocytes | | | | Polymorps | Lympocytes | Eosinophils | Monocytes |
| Control (Normal saline) | 6 | 1.0ml | 14.4 ± 0.52 | 4.8 ± 0.27 | 9733 ± 291 | 28.7 ± 2.74 | 66.3 ± 3.29 | 3.3 ± 0.33 | 1.7 ± 0.33 | 14.7 ± 0.17 | 4.8 ± 0.12 | 9533 ± 406 | 25.3 ± 0.91 | 69.7 ± 0.33 | 3.3 ± 0.33 | 1.7 ± 0.33 |
| Vehicle (91.5%v/v alcohol) | 6 | 0.1ml | 14.9 ± 0.21 | 4.9 ± 0.18 | 9800 ± 503 | 27.4 ± 1.22 | 66.0 ± 1.87 | 4.3 ± 0.33 | 2.3 ± 0.33 | 14.4 ± 0.30 | 4.7 ± 0.18 | 9533 ± 406 | 24.0 ± 1.00 | 70.3 ± 1.47 | 4.0 ± 0.58 | 1.7 ± 0.33 |
| *T. terrestris* 3x | 6 | 0.1ml | 14.8 ± 0.15 | 5.0 ± 0.15 | 9733 ± 240 | 30.7 ± 2.52 | 63.0 ± 1.15 | 4.0 ± 0.58 | 2.3 ± 0.33 | 14.5 ± 0.29 | 4.8 ± 0.12 | 9333 ± 291 | 28.7 ± 2.52 | 65.0 ± 2.08 | 4.0 ± 0.58 | 2.3 ± 0.33 |
| *T. terrestris* 6x | 6 | 0.1ml | 14.9 ± 0.18 | 4.9 ± 0.14 | 10067 ± 267 | 27.7 ± 1.47 | 66.7 ± 1.78 | 3.3 ± 0.33 | 2.3 ± 0.33 | 14.9 ± 0.18 | 4.9 ± 0.07 | 9800 ± 346 | 30.3 ± 1.35 | 64.0 ± 1.00 | 3.7 ± 0.33 | 2.0 ± 0.58 |
| *T. terrestris* 12x | 6 | 0.1ml | 14.7 ± 0.15 | 4.8 ± 0.12 | 9667 ± 291 | 24.3 ± 2.35 | 69.0 ± 1.53 | 4.7 ± 0.91 | 2.0 ± 0.58 | 14.4 ± 0.30 | 4.5 ± 0.18 | 8333 ± 240 | 26.3 ± 1.87 | 68.0 ± 1.53 | 4.0 ± 0.58 | 1.7 ± 0.33 |
| *T. terrestris* 30c | 6 | 0.1ml | 14.6 ± 0.20 | 4.7 ± 0.18 | 8533 ± 546 | 26.7 ± 1.78 | 66.7 ± 0.91 | 4.3 ± 0.88 | 2.3 ± 0.33 | 14.9 ± 0.18 | 4.9 ± 0.07 | 8133 ± 657 | 25.7 ± 3.19 | 69.3 ± 2.92 | 3.3 ± 0.33 | 1.7 ± 0.33 |

*The values for haematological parameters did not differ between drug, alcohol and normal saline administered rats.

$ Represents Mean ± S.E.M. of 3 rats

Potencies of test drug and vehicle (91.5% v/v alcohol) were diluted in a ratio of 1:10 with distilled water in order to make the volume one ml.

**Table-5** Effect of different potencies of *Tribulus terrestris* (0.2 ml/rat/day) on rats' haematological profiles

| Groups | No. of rats used | Dose/ rat/ day | 21st Day *$ | | | | | | | 28th Day *$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hb (gm) % | Total R.B.C. (mill /cubicmm) | Total W.B.C (number/ cubic mm) | Differential counts (%) | | | | Hb (gm) % | Total R.B.C. (mill /cubicmm) | Total W.B.C (number/ cubic mm) | Differential counts (%) | | | |
| | | | | | | Poly morps | Lympo cytes | Eosino phils | Mono cytes | | | | Poly morps | Lympo cytes | Eosino phils | Mono cytes |
| Control (Normal saline) | 6 | 2.0ml | 14.9 ± 0.18 | 4.7 ± 0.12 | 9067 ± 352 | 27.3 ± 1.35 | 67.3 ± 1.78 | 3.7 ± 0.33 | 1.7 ± 0.33 | 14.5 ± 0.58 | 4.7 ± 0.29 | 9533 ± 348 | 25.0 ± 3.79 | 68.7 ± 4.06 | 4.0 ± 0.58 | 2.03 ± 0.33 |
| Vehicle (91.5%v/v alcohol) | 6 | 0.2ml | 14.8 ± 0.38 | 4.8 ± 0.26 | 9667 ± 291 | 29.3 ± 2.35 | 64.7 ± 2.92 | 4.3 ± 0.68 | 1.7 ± 0.33 | 14.9 ± 0.38 | 4.7 ± 0.29 | 9467 ± 467 | 27.0 ± 4.04 | 66.7 ± 4.38 | 4.0 ± 0.58 | 2.3 ± 0.33 |
| *T. terrestris* 3x | 6 | 0.2ml | 14.7 ± 0.35 | 4.8 ± 0.15 | 9600 ± 462 | 32.0 ± 2.31 | 63.0 ± 2.08 | 3.3 ± 0.62 | 1.7 ± 0.33 | 14.7 ± 0.35 | 4.7 ± 0.12 | 9667 ± 291 | 31.3 ± 2.42 | 63.0 ± 2.83 | 4.0 ± 0.58 | 1.7 ± 0.33 |
| *T. terrestris* 6x | 6 | 0.2ml | 14.8 ± 0.12 | 4.9 ± 0.06 | 9133 ± 176 | 27.7 ± 0.91 | 66.3 ± 1.22 | 3.3 ± 0.33 | 2.7 ± 0.33 | 14.4 ± 0.35 | 4.8 ± 0.12 | 8667 ± 406 | 28.0 ± 2.08 | 66.3 ± 2.04 | 3.7 ± 0.33 | 2.0 ± 0.58 |
| *T. terrestris* 12x | 6 | 0.2ml | 14.9 ± 0.33 | 4.9 ± 0.24 | 10133 ± 291 | 26.7 ± 2.42 | 68.0 ± 2.52 | 3.6 ± 0.34 | 1.7 ± 0.33 | 14.8 ± 0.49 | 4.8 ± 0.27 | 8733 ± 437 | 24.7 ± 2.92 | 69.3 ± 2.35 | 3.7 ± 0.67 | 2.3 ± 0.33 |
| *T. terrestris* 30c | 6 | 0.2ml | 14.5 ± 0.24 | 4.5 ± 0.17 | 8133 ± 291 | 26.7 ± 1.78 | 66.7 ± 1.35 | 4.3 ± 0.88 | 2.3 ± 0.33 | 14.3 ± 0.29 | 4.5 ± 0.18 | 7867 ± 481 | 26.4 ± 3.29 | 68.0 ± 3.51 | 3.3 ± 0.33 | 2.3 ± 0.33 |

*The values for haematological parameters did not differ between drug, alcohol and normal saline administered rats.

$ Represents Mean ± S.E.M. of 3 rats

Potencies of test drug and vehicle (91.5% v/v alcohol) were diluted in a ratio of 1:10 with distilled water in order to make the volume 2 ml.

**Table-6** Effect of different potencies of *Tribulus terrestres* (0.5 ml/rat/day) on rats' haematological profiles

| Groups | No. of rats used | Dose/ rat/ day | 21st Day *$ | | | | | | | 28th Day *$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hb (gm) % | Total R.B.C. (mill /cubicmm) | Total W.B.C (number/ cubic mm) | Differential counts (%) | | | | Hb (gm) % | Total R.B.C. (mill /cubicmm) | Total W.B.C (number/ cubic mm) | Differential counts (%) | | | |
| | | | | | | Poly morps | Lympo cytes | Eosino phils | Mono cytes | | | | Poly morps | Lympo cytes | Eosino phils | Mono cytes |
| Control (Normal saline) | 6 | 2.0ml | 14.6 ± 0.23 | 4.6 ± 0.23 | 9067 ± 352 | 27.6 ± 1.47 | 67.0 ± 1.53 | 3.7 ± 0.33 | 1.7 ± 0.33 | 14.4 ± 0.31 | 4.5 ± 0.17 | 9200 ± 346 | 20.0 ± 1.73 | 74.0 ± 1.15 | 4.3 ± 0.33 | 1.7 ± 0.33 |
| Vehicle (91.5%v/v alcohol) | 6 | 0.5ml | 14.6 ± 0.23 | 4.5 ± 0.18 | 8733 ± 521 | 29.0 ± 2.08 | 64.7 ± 2.67 | 4.0 ± 0.58 | 2.3 ± 0.33 | 14.6 ± 0.23 | 4.7 ± 0.29 | 8867 ± 406 | 23.0 ± 1.73 | 71.7 ± 2.74 | 3.3 ± 0.88 | 2.0 ± 0.58 |
| *T. terrestris* 3x | 6 | 0.5ml | 14.6 ± 0.09 | 4.7 ± 0.15 | 9533 ± 240 | 30.7 ± 2.52 | 63.7 ± 1.87 | 3.3 ± 0.33 | 2.3 ± 0.33 | 14.7 ± 0.29 | 4.9 ± 0.10 | 9700 ± 208 | 31.3 ± 1.35 | 63.7 ± 1.35 | 3.3 ± 0.33 | 1.7 ± 0.33 |
| *T. terrestris* 6x | 6 | 0.5ml | 14.5 ± 0.18 | 4.6 ± 0.15 | 10067 ± 593 | 24.3 ± 2.35 | 70.3 ± 2.35 | 3.0 ± 0.58 | 2.4 ± 0.34 | 14.5 ± 0.18 | 4.5 ± 0.18 | 9200 ± 346 | 23.3 ± 2.42 | 69.7 ± 1.47 | 4.7 ± 0.91 | 2.3 ± 0.33 |
| *T. terrestris* 12x | 6 | 0.5ml | 14.9 ± 0.18 | 4.9 ± 0.15 | 8733 ± 437 | 28.0 ± 1.15 | 66.7 ± 0.91 | 3.7 ± 0.33 | 1.7 ± 0.33 | 14.5 ± 0.47 | 4.7 ± 0.26 | 8733 581 | 26.3 ± 1.22 | 69.4 ± 1.78 | 3.0 ± 0.58 | 1.3 ± 0.33 |
| *T. terrestris* 30c | 6 | 0.5ml | 14.8 ± 0.37 | 4.8 ± 0.26 | 7800 ± 346 | 26.7 ± 1.87 | 67.3 ± 1.47 | 4.3 ± 0.33 | 1.7 ± 0.33 | 14.4 ± 0.30 | 4.6 ± 0.19 | 8467 ± 657 | 24.0 ± 1.73 | 67.4 ± 2.92 | 6.3 ± 0.91 | 2.3 ± 0.33 |

*The values for haematological parameters did not differ between drug, alcohol and normal saline administered rats.
$ Represents Mean ± S.E.M. of 3 rats
Potencies of test drug and vehicle (91.5% v/v alcohol) were diluted in a ratio of 1:4 with distilled water in order to make the volume 2 ml.

The results of present studies showed a variable effect of different potencies (3x, 6x, 12x and 30c) of *T. terrestris* on biochemical (serum sugar, total protein, albumin, total cholesterol, triglycerides, urea, SGOT and SGPT levels) and haematological (haemoglobin content, total RBC and total WBC and differential leucocyte counts) profiles, as compared to vehicle (91.5% v/v alcohol) and normal saline administered rats when estimated on 21st and 28th day of the experimental study, but all the values were found to be within the normal range of healthy animals.

The present results showed that all the four potencies (3x, 6x, 12x and 30c) of *T. terrestris* and vehicle (91.5% v/v alcohol) did not produce any significant change in the behaviour of animals when administered in doses up to 0.2ml/rat/day for 14 days but when administered in doses upto 0.5ml/rat/day,rats started scratching their faces initially with fore and hind paws followed by laboured breathing and finally they became calm and sat in one corner of the cage. The effect lasted for about 12-15min. These effects were prominent in the beginning of the study but tapered off slowly during the period of 14 days of drug treatment. On the contrary, rats administered with normal saline remained active throughout the period of study.

## CONCLUSION

The results of the present study showed that *T. terrestris* did not have any toxic effect in four potencies studied. However, based on the present preliminary study we can not conclude the complete safety profiles of various potencies of this drug, unless more detailed study can be carried out on different species of experimental animals for acute and chronic toxicity studies.

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