EFFECTS OF TRIBULUS TERRESTERIS;
TO STUDY ON URINE OUTPUT AND ELECTROLYTES IN RABBITS.

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ABSTRACT... Objective: The objective of this study was to find the effects of herbal extract of tribulus terristris on the urine output and electrolytes in rabbits and to find a herbal remedy for fluid and electrolyte abnormalities. **Study design:** Descriptive. **Setting:** Department of Pharmacology and Therapeutics, Post Graduate Medical Institute, Lahore. **Period:** The duration of study was for two weeks (in 2003). **Material and methods:** Sixteen rabbits of mixed breed were purchased locally and kept in the animal house of Postgraduate Medical Institute, Lahore for a week for acclimatization before starting the experiment. Twelve hours light and dark cycle was maintained. They were fed on grass, grain, seasonal vegetables and water adlibitum. Animals were weighed for calculation of dosage of herb.

Key words: Ct=Control group, Tt=Tribulus terrestris group, D/W=Distilled water, ml=milliliter Electrolytes=Sodium (Na+) & Potassium (K+), R. No.=Rabbit No.

INTRODUCTION

Tribulus terrestris (Tt) Vernacular: English; small caltrop, Urdu; Gokhro, Arabic; Al-Gutab (Zygophyllaceae). It is an aunual plant growing throughout India, Iraq, Kashmir, Ceylon and also in Africa[1,2].

T. terrestris is a tropical plant distributed throughout India and Sri Lanka. The entire plant and particularly, the fruits are extensively used in indigenous medicine. The roots and fruits are useful in improving appetite, urinary output, vesicular calculi and pruritus ani, alleviate burning sensation, reduce inflammation, cough, asthma, and cure renal diseases[3]. T. terristris is useful in the treatment of urolithiasis, dysurea, impotence or erectile dysfunction and kidney dysfunction, and has also shown antibacterial & antifungal activity and anti-inflammatory Activity[4].

There is no much literature or data available considering its diuretic effect therefore, we conducted this study to asses or observe diuretic effect.

Study Duration

Study duration was two weeks.

Drug used

Tribulus terrestris extract (Aqueous).

This work was conducted in Department of Pharmacology & Therapeutics, Post Graduate Medical Institute, Lahore

Experimental animals

Experimental animals were divided into two groups (Control & Tribulus Terresteris). Urine volume was measured on day 1, day 8 and day 15. There was a significant increase in the urine volume on day 1 and day 8 and insignificant on day 15 and decreased serum sodium, serum potassium significantly on day 1, day 8 and day 15.

Preparation of extract

Tribulus terrestris was purchased from local market with the help of an expert Botanist & Dr. Khalid Mahmood janjua, Chief Scientific Officer PCSIR, Lahore. The herb was made free of particulate impurities manually and spread in a stainless steel tray for drying[5].

The extract was prepared by Maceration method (5gm in 100ml water). 100 gram air dried Tribulus terrestris was soaked in 2 liter (2000ml) of distilled water in a flask for 24 hours, shaking frequently during 6 hours and allowed to stand for 18 hours. Than filterate was taken and fiber waste material was discarded. After that concentrated dry powder extract was obtained by evaporating the

filtrate at 70C^0 in a scientific instrument/oven mod Eminex 854 SCHWABACH, Germany Din 12880 Kim Nem Tempt. 220[11].

The dry powder extract thus obtained was weighed with electronic balance which came out to be 8.5 gram/100 gram air dried tribulus terrestris and this dry powder of herb was dissolved in 1000ml D/W to get herbal preparation as 100 mg/ml for oral use. Herbal preparation was kept in refrigerator[12].

Methodology
Sixteen rabbits of mixed breed were divided into two groups, control (CT) and Tribulus terrestris (Tt).

Group-I (Ct)
Control groups (No medicine). They were kept under the same condition and handled like drug group animals.

Group-II (Tt)
They received tribulus terrestris.

Dose
100mg / kg body weight and administered orally twice daily (Said 1996).

Collection of sample
For urine collection rabbits were kept in special cages for twenty four hours on day 0, day 7 and day 14 completed on day 1, day 8, day 15.

Urine
Twenty four hours urine sample was collected in plastic bottles attached below the cages. The urine samples were taken three times during the study period i.e. on day 1, day 8 and day 15.

Blood
Blood was collected three times during study on day 1, day 8 and day 15, from the marginal vessels of the ear. For this purpose hairs were shaved from the ear margin. It was then disinfected with 70% alcohol. Xylene was applied for vasodilatation and 5ml of blood was taken in a disposable syringe and then kept in centrifuge tube, the bleeding vessel was pressed with a sterilized cotton swab till stoppage of bleeding. Xylene was removed first by alcohol and then by soap and water. The collected blood was allowed to clot at room temperature and then centrifuged a 3000 rpm for ten minutes. Serum was separated with the help of an automatic micropipette and stored in a clean and dry serum storage vial in a deep freezer for further analysis.

RESULTS
(CT Vs Tt), the change in 24 hours urine volume was found to be statistically significant (P-Value<0.05)on day 1, day 8 and insignificant on day 15.

Statistical Analysis
Data is analyzed by SPSS program in computer and value of significance (P-Value) kept (<0.05).

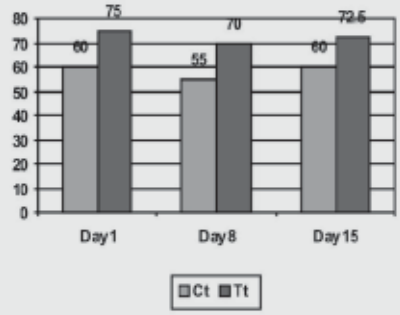
DISCUSSION
It produced diuresis, increased (24 hours urine output) and our result is in agreement with the study conducted by various researchers[2,3,6]. The result of our study are consistent with the results of study conducted in Punjab University 1972 and same results were seen in other studies as well[8,9,10]. Study conducted in university Jaffna reviewed that urine volume increased after administration of Tt. and the result is consistent with our study conducted on rabbits[7].Decreased serum sodium level (Hyponatremia) produced by Tt compared with control, the effect on serum sodium is in agreement with[10]. Decreased serum potassium level (Hypokalemia) produced by Tt compared with control, is in agreement with[10].

We conclude that our findings have demonstrated that (Tt) has significant diuretic effect which can make it useful in the treatment of hypertension and various other diseases where diuresis is required. But further clinical studies may be done to elaborate its use. At a moment, only scanty data is available for comparison.

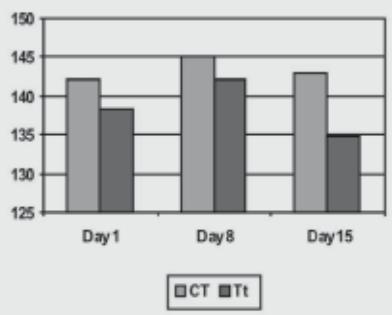
CONCLUSIONS
Tribulus terestris is a natural herb commonly known as the puncture vine, tradionally it has been used for centurils (in Europe and other countries) as a demulcent, astringent, diuretic, aphrodisiac and other many medical uses[1]. We conducted study for two weeks and reached to

Results are shows in following tables and presented in graph.																	
Urine volume (ml) on day 1		Urine volume (ml) on day 8		Urine volume (ml) on day 15		Serum sodium level (mmol/L) on day 1		Serum sodium level (mmol/L) on day 8		Serum sodium level (mmol/L) on day 15		Serum potassium level (mmol/L) on day 1		Serum potassium level (mmol/L) on day 8		Serum potassium level (mmol/L) on day 15	
CT	Tt	CT	Tt	CT	Tt	CT	Tt	CT	Tt	CT	Tt	CT	Tt	CT	Tt	CT	Tt
55	80	55	70	60	75	141	138.33	145	142	143.5	134	4.0	4.03	4.6	3.36	4.3	3.16
65	60	75	65	40	90	143	138.30	144	141	144	135.32	5.0	4.23	4.2	3.16	4.7	3.0
60	80	35	80	50	60	142	138.36	143	143	142.5	135	4.5	4.10	5.0	3.56	4.5	3.32
50	75	60	75	80	80	141.5	138.20	147	141.5	144.5	134.32	4.5	4.16	4.3	3.06	4.0	3.06
70	75	50	60	70	70	142.5	138.46	144	142.5	141.5	134.16	4.6	4.05	4.9	3.66	5.0	3.26
60	80	45	50	60	75	142	138.33	147	142	143	135.16	4.4	4.21	4.5	3.20	4.5	3.10
55	65	65	90	50	55	143.5	138.40	145	143	142	133.66	4.8	4.26	4.7	3.52	4.2	3.22
65	85	55	70	70	75	141.5	138	145	141	143	135.66	4.9	4.0	4.6	3.36	4.8	3.16

Effect of Tf on urine volume



Effect of Tf on serum sodium level mmol/L



Effect of Tf on serum potassium level mmol/L

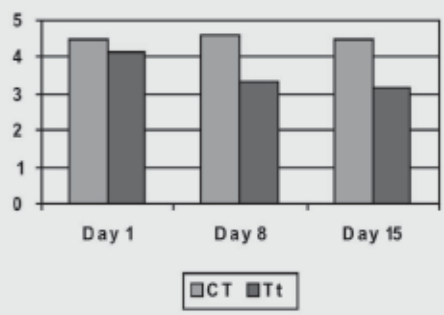


Table-I. Urine volume level control & tribulus terrestris

Groups	Day 1	Day 8	Day 15	Comparison of levels		
				Day 1-8	Day 8-15	Day 1-15
		Mean ± SEM			Significance	
Control	60.00 ± 2.31	55.00 ± 4.31	60.00 ± 4.63	.359	.499	1.00
Tribulus terrestris	75.00 ± 2.99	70.00 ± 4.33	72.50 ± 3.90	.419	.743	.649

the conclusion that Tt has definite effect on urine volume. Herb was given 100mg / kg body weight and noted significant increase in urine volume over a period of study. It also significantly effected (decreased serum sodium level and serum potassium level through out the study period). Keeping in view, the result of our study, we recommend that the use of this herb may be promoted as diuretic agent will be helpful in hypertensive and renal

Table-I. Sodium serum level control & tribulus terrestris

Groups	Day 1	Day 8	Day 15	Comparison of levels		
				Day 1-8	Day 8-15	Day 1-15
		Mean ± SEM			Significance	
Control	142.13 ± .30	145.00 ± .50	143.00 ± .35	.003	.003	.155
Tribulus terrestris	138.33 ± .07	142.00 ± .28	134.66 ± .25	0.00	0.00	0.00

Table-I. Potassium serum level control & tribulus terrestris

Groups	Day 1	Day 8	Day 15	Comparison of levels		
				Day 1-8	Day 8-15	Day 1-15
		Mean ± SEM			Significance	
Control	4.50 ± .11	4.60 ± .096	4.50 ± .12	.558	.456	1.00
Tribulus terrestris	4.13 ± .03	3.36 ± .07	3.16 ± .04	0.00	.002	0.00

Table-II. Urine volume level control vs tribulus terrestris

Groups	Comparison of levels		
	Day 1	Day 8	Day 15
	Significance		
Control Vs Tribulus terrestris	.005	.031	.066

Table-II. Sodium serum level control vs tribulus terrestris

Groups	Comparison of levels		
	Day 1	Day 8	Day 15
	Significance		
Control Vs Tribulus terrestris	.000	.002	.000

Table-II. Potassium serum level control vs tribulus terrestris

Groups	Comparison of levels		
	Day 1	Day 8	Day 15
	Significance		
Control Vs Tribulus terrestris	.004	.000	.000

diseased patients after evaluating the parameters of the herb.

Tribulus terrestris currently being studied as a diuretic agent regardless of knowing its exact mechanism. The study demonstrated that it increased the 24 hours urine output.

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"The world will not be destroyed by those who do evil, but by those who watch them without doing anything."

Albert Einstein