TO COMPARE THE EFFECTS OF TRIBULUS TERRISTRIS WITH HYDROCHLOROTHIAZIDE ON URINE VOLUME AND ELECTROLYTES

Abdul Jabbar,1 Abira Nazir,2 Javed Khalil,3 Naveed Iqbal Ansari,4 Khalid Mahmood Janjua5 Farooq Javed6

Abstract

Study was conducted on rabbits after administration of herbal extract of Tt for two weeks. Experimental animals were divided into three groups: Control (CT), Tribulus terristris (Tt) and Hydrochlorothiazide (H-CT). Urine volume and serum Na+, K+ levels were measured on day 01 and day 15. There was significant increase in urine volume and reduction in serum Na+, K+ levels regarding comparison of Tt vs HCT. Tribulus terristris has diuretic effect as compared to control group and less diuretic effect as compared to Hydrochlorothiazide.

Background: Tribulus terristris belongs to Zygophyllaceae family. It is suggested to increase the urine output, although the plant was recognized as a diuretic and aphrodisiac but its diuretic activity is not much evaluated and compared.

Material and Methods: The study was conducted in the department of Pharmacology and Therapeutics, Post-graduate Medical Institute, Lahore. The duration of study was two weeks. Twenty Four 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 ad libitum. Animals were weighed for calculation of dosage of herb and HCT. They were divided into three equal groups randomly, group CT; (no medicine was given), group Tt; (tribulus terristris was given), group HCT; (Hydrochlorothiazide was given).

Results: Tt VS HCT, the change in 24 hours urine volume was found statistically significant P < 0.05 on day 01 and day 15. Serum Na+ level was also found statistically significant P < 0.05 throughout the study period. But serum potassium level was statistically
significant < 0.05 on day 01 and insignificant on day 15.

**Conclusion:** The results of our study reveal that Tt contains diuretic properties but its magnitude of response is less than HCT. On the basis of our study we suggest its further evaluation and then recommend it for the treatment of mild to moderate essential hypertension like HCT.

**Key words:** Control = CT, Tribulus terrestris = Tt, Hydrochlorothiazide = HCT, Versus = VS, Sodium = Na+, Potassium = K+, Distilled water = D/W.

**Introduction**

Tribulus terrestris is a perennial creeping herb¹ and tropical plant distributed throughout India, belongs to the family Zygophyllaceae.² Tribulus is a genus of plants found in many warm regions and T. terrestris (puncture vine) is best – known member.³ Tribulus terrestris vernacular (English = Caltrops, Urdu = Gokhru, Punjabi = Bhakra) and in Sanskrit Gokshura signifies cow’s hoof (cloven hoof).⁴

Fruits contain traces (0.001%) of alkaloid, a fixed oil, a small quantity of essential oil, resins and nitrates.⁵ Particularly, the fruits are extensively used since ancient times as aphrodisiac. It has also shown antibacterial, antifungal and anti-inflammatory activity.¹²

Extracts from this plant have been used traditionally in treating a variety of diseases including hypertension and coronary heart disease, ocular inflammation and infertility in both sexes. The extracts have also been used as diuretics. Recent pharmacological studies tend to support these uses and diuretic activity in rats have been demonstrated.⁶ Crude extract of Tribulus terrestris enhanced electrically – and nitroglycerine – induced relaxation of the rabbit corpus cavernosum consistent with a pro-erectile function.⁷ Tribulrid terssrs has a stimulating effect on Leydig cells which may have led to increased testosterone levels producing precocious development of the testes.⁸

T. terrestris possesses antihypertensive activity.⁹ The biological properties of Tribulus extracts include diuretic properties, increased release of nitric oxide from endothelium and nerve endings; it relaxes smooth muscles and increases angiotensin converting enzyme (ACE) inhibition. Hence reduces the hypertension.¹⁰,¹¹ Thiazide diuretic (HCT) is clinically used to treat mild to moderate essential hypertension because it has diuretic action and vasodilatory effect. Hydrochlorothiazide inhibit the Na⁺Cl⁻ transport at distal convoluted tubule of nephron and cause NaCl, water excretion. Because in the distal convoluted tubule 5-10% of the filtered load is absorbed via Na⁺Cl⁻ transport in the luminal Na+, K+ ATPase.¹²

**Material and Method**

**Setting**

In the department of Pharmacology and Therapeutics, Post Graduate Medical Institute, Lahore.

**Duration**

Study duration was two weeks.

**Sample Size**

Twenty four rabbits of mixed breed were purchased locally and kept in the animal house of Postgraduate Medial 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 ad libitum. Animals were weighed for calculation of dosage of herb. They were divided into three equal groups, 8 rabbits in each group.

**Drug Used**

Tt extract and HCT.

**Preparation of Extract**

Tribulus terrestris was purchased from local market with the help of experts of PCSIR, Lahore. The herb was made free of particulate impurities manually and spread in a stainless steel tray for drying.¹³

The extract was prepared by Maceration method (5 gm in 100 ml water). 100 gram air dried Tribulus terrestris was soaked in 2 liter (2000 ml) of distilled water in a flask for 24 hours, shaking frequently during 6 hours and allowed to stand for 18 hours. Then filtrate was taken and fiber waste material was discarded. After that concentrated dry powder extract was obtained by evaporating the filtrate at 70°C in a scientific instrument / oven mod Exinex 854 SCHWA-BACH, Germany Din 12880 Kim Nem Tempt. 220.¹⁴

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 1000 ml D/W to get herbal preparation as 100 mg/ml for oral use. Herbal preparation was kept in refrigerator.¹⁵

Methodology

Twenty four rabbits of mixed breed were divided into three groups; control (CT), Tribulus terrestris (Tt) and hydrochlorothiazide (HCT).

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, 100 mg / kg body weight and administered orally twice daily.¹⁶

Group – III (HCT)
They received hydrochlorothiazide, 5 mg / kg body weight and administered orally twice daily.¹⁷

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

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

Blood
Blood was collected two times during study on day 1 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 5 ml 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 at 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.

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

Results
Tt VS HCT, the change in 24 hours urine volume was found statistically significant, P < 0.05 on day 01 and day 15.

Serum Sodium level was also found statistically significant P < 0.05 throughout the study period.

But potassium level was statistically significant < 0.05 on day 01 and insignificant on day 15. Results are shown in tables and presented in graphs.

Discussion
The results of our study reveal changes in 24 hours urine volume (Increased) and Na+, K+ serum levels (decreased) in comparison with the control group and the results are consistent with the study conducted⁵. Tribulus terrestris produced diuresis and our results are consistent with the different studies conducted on this herb.¹⁸-²²

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 15</th>
<th>Comparison of Levels Day 1 – 15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Significance</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>60.00 ± 2.31</td>
<td>60.00 ± 4.63</td>
<td>1.00</td>
</tr>
<tr>
<td>Tribulus terrestris</td>
<td>75.00 ± 2.99</td>
<td>72.00 ± 3.90</td>
<td>.649</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>105.00 ± 4.22</td>
<td>105.00 ± 5.82</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Table 2: Comparison of effect of drugs on Urine Volume (ml).

<table>
<thead>
<tr>
<th>Group</th>
<th>Comparison of Levels</th>
<th>Day 1</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Vs Tribulus terrestris</td>
<td>.005</td>
<td>.066</td>
<td></td>
</tr>
<tr>
<td>Tribulus terrestris Vs Hydrochlorothiazide</td>
<td>.000</td>
<td>.009</td>
<td></td>
</tr>
</tbody>
</table>

Graph 1: Effect of Drugs on Urine Volume (ml).

Table 3: Sodium Serum Level (mmol/L)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 15</th>
<th>Comparison of Levels Day 1 – 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>142.13 ± .30</td>
<td>143.00 ± .35</td>
<td>.155</td>
</tr>
<tr>
<td>Tribulus terrestris</td>
<td>138.33 ± .07</td>
<td>134.66 ± .25</td>
<td>0.00</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>134.60 ± .07</td>
<td>126.00 ± .35</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Graph 2: Sodium Serum Level (mmol/L)

Table 4: Comparison of effects of drugs on Sodium Serum Level (mmol/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Comparison of levels</th>
<th>Day 1</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Vs Tribulus terrestris</td>
<td>.000</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>Tribulus terrestris Vs Hydrochlorothiazide</td>
<td>.000</td>
<td>.000</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Effect of drugs on Serum Potassium Level (mmol/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 15</th>
<th>Comparison of Levels Day 1 – 15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td></td>
<td>Significance</td>
</tr>
<tr>
<td>Control</td>
<td>4.50 ± .11</td>
<td>4.50 ± .12</td>
<td>1.00</td>
</tr>
<tr>
<td>Tribulus terrestris</td>
<td>4.13 ± .03</td>
<td>3.16 ± .04</td>
<td>.000</td>
</tr>
<tr>
<td>Hydro chlorothiazide</td>
<td>3.70 ± .10</td>
<td>3.20 ± .06</td>
<td>.004</td>
</tr>
</tbody>
</table>

Table 6: Comparison of effect of drugs on Serum Potassium Level (mmol/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Comparison of levels Day 1</th>
<th>Day 15</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Vs Tribulus terrestris</td>
<td>.004</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>Tribulus terrestris Vs Hydro chlorothiazide</td>
<td>.001</td>
<td>.189</td>
<td></td>
</tr>
</tbody>
</table>

Graph 3: Effect of drugs on Serum Potassium Level ((mmol/L))

It significantly increased the 24 hours urine volume and reduction in serum sodium level as compared to Tt group but the effect on serum on potassium level were not similar. We conclude that our finding have demonstrated that Tt has diuretic properties but less effective than conventionally used HCT.

Study conducted and results revealed that the crude aqueous and methanolic extract produced dose dependent reduction in blood pressure of spontaneously hypertensive rats. This effect was by virtue of its vasodilator properties.23

Extracts of Tribulus terrestris displayed significant antihypertensive properties in spontaneously hypertensive rats, which could involve smooth muscle relaxation via NO release and membrane hyperpolarization. Further studies are in progress to identify the active principle(s) in the plant extract.24

Tt is a herbal diuretic agent that also contains vasodilatory effect and it is an excellent addition to the list of diuretics. By virtue of these properties it would serve as a good herbal medicine in the treatment of mild to moderate hypertension that also contain antihypertensive effect so it may be suggested / recommended in mild to moderate essential hypertension patients after the evaluation of pharmacological aspects of this herb and drug interactions.

**Conclusion**

Tribulus terrestris L., have great significance in the Traditional System of Medicine (Ayurveda, Unani and Chinese) for the treatment of various ailments such as aphrodisiac, diuretic, anthelmintic, antimicrobial, antihypertensive, spermatogenesis and effects on cardiovascular diseases.

The results of our study reveal that tribulus terrestris has diuretic properties but less in magnitude than hydrochlorothiazide without producing much disturbance in urine volume and electrolyte.

Keeping in view, the result of our study, we recommend that the use of this herb may be suggested as a diuretic agent and also as an antihypertensive agent for the treatment of mild to moderate essential hypertension after proper evaluation of pharmacological parameters of this herb.
TO COMPARE THE EFFECTS OF TRIBULUS TERRISTRIS WITH HYDROCHLOROTHIAZIDE ON URINE VOLUME AND ELECTROLYTES

References

4. Lubna Fatima MS (U), Arshiya Sultana MD (U), Saad Ahmad, MD (U) and Shabia Sultana MD (U). Pharmacological activities of tribulus terrestris. Linn: A systemic review, world journal of Pharmacy and Pharmaceutical Sciences, 2015; Vol. 4, Issue 02: Page 136 – 150.
9. Aruna Bashir¹, Muhammad Zubair², Abdul Jabbar³, effects of puncturvine (TT) on leydig cells of prepubertal Albino rats. Pakistan Journal of Medical and health sciences, Jan – Mar, 2014; Vol. 8: Page 8 – 11.
24. Oludotun A. Philips*, Koyippalli T Mathewb, Mabayoje A. Oriowo*, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kuwait University, P.O Box 24923, 13110 Safat, Kuwait.