EFFECT OF *TRIBULUS TERRESTRIS* FRUIT EXTRACTS ON CHLORIDE AND CREATININE RENAL CLEARANCES IN DOGS*

R. C. P. SINGH AND C. S. SISODIA**

Department of Pharmacology,
College of Veterinary Medicine, Haryana Agricultural University, Hissar

Summary In the anaesthetized dogs the ether extract of the fruits of *T. terrestris* produced diuresis and increased the creatinine renal clearance; which suggest increase in the glomerular filtration rate. However, the ether extract did not significantly increase the chloride clearance which excludes inhibition of tubular chloride reabsorption. The aqueous extract of the fruits produced no significant effect on the urine volume or on the creatinine or the chloride renal clearance.

Increased glomerular filtration rate induced by the ether extract may explain its diuretic effect.

INTRODUCTION

T. terrestris Linn. (Hindi-Gokhru) is an annual plant which grows in India and other warm countries. Small yellow flowers and thorny fruits are its characteristic features (3). In the ancient literature on Ayurvedic Medicine, the fruits of this plant have been recommended for treating various urinary disorders. The plant is one of the constituents of some important Ayurvedic preparations such as "Dashamula-Kvatha", "Gokshuradi Leha" and "Gokshuradi Guggula" which are frequently used for treating "mutrakruchchra" (denoting dysuria and oliguria), for dissolving stones in the urinary tract and for other related urinary troubles in men and animals (11).

The fruits contain a fair amount of potassium nitrate and their diuretic property may be partly due to the nitrate (3). The later work (1, 2, 5) supports this finding; recently Singh and Sisodia (7) also observed that most of the toxicity of the fruits may be due to the high nitrate content. The unwashed fruit powder of *T. terrestris* when orally fed (1 *g/kg*) to dogs produced severe diarrhoea and dehydration (9); on the other hand, the water-washed fruit powder in the same dose produced no apparent physical signs of toxicity (8). Further it was observed that the water-washed powder of the fruits did manifest good diuretic activity (8). Work undertaken to explore the mechanism of the diuretic action of the water-washed *T. terrestris* fruit powder is reported here.

MATERIALS AND METHODS

Mongrel dogs of both sexes were used in the experiments. They were maintained on a

*This work was conducted in the Department of Pharmacology, U.P. College of Veterinary Sciences & A.H., Mathura, and was submitted as M.V.Sc. Thesis to the Agra University in 1967.

**Present address : Department of Pharmacology, University of Saskatoon, Canada.

ration containing meat meal, skimmed milk and crushed wheat (dalia) which was boiled in water.

Fruits of *T. terrestris* of unspecified age were obtained from the local market, Their identification was kindly carried out by the Botanical Survey of India, Southern Circle, R.S. Puram, Coimbatore.

Hundred *g* of the fruit powder was thoroughly macerated for abont 5 min by hands in about 1000 *ml* of distilled water and then decanted and filtered. The washed powder was completely dried at about 40-50°C. Ten *g* of washed powder was placed in the thimble of the Soxhlet extraction apparatus with about 100 *ml* of solvent ether in the flask and then extracted for about 10-12 hr. The extraction was considered to be complete when the solvent siphoning down from the thimble became colourless. The ether extract was poured into a weighed beaker which was then kept at 30-40°C till ether evaporated completely. The yield of the ether extract was about 300-400 *mg*/10 *g* of the dried water-washed powder. It was thick semi-solid greasy and of light yellow colour. Propylene glycol (propane-1:2 diol, B.D.H.) was employed as a vehicle for its parenteral administration. The extract remained in the form of a fine suspension in this solvent.

Water extract was prepared by boiling of 10 *g* water-washed powder in 100 *ml* of distilled water for 30 min. It was then filtered and used as such.

Experimental Technique: Adult dogs (10-15 *kg*) of either sex were deprived of food (but not of water) for 24 hr and anaesthetized by pentobarbitone sodium (35 *mg/kg* i. v.). The anaesthetized dog was secured on the operation table and was administered intragastrically 20 *ml/kg* of 0·9% NaCl solution (normal saline) containing 400-500 *mg/kg* creatinine (Merck). Femoral vein was cannulated for the intravenous administration. The ureters were exposed near the urinary bladder by a ventral midline incision and catheterized with polyethylene cannulae. To obtain a regular and high urine flow, about 100 *ml* of normal saline was injected intravenously at the beginning of the dissection.

Diuretic Studies: The experiment was started after ascertaining a steady urine flow over 3-4 consecutive 15 min periods; then urine samples were collected for 2 consecutive periods of 15 min and the total urine volume thus collected in 30 min was taken as control urine flow. Blood samples for the determination of creatinine and chloride contents were collected from the jugular vein at O, 15 and 30 min of the urine collection. The test extract (300-400 *mg* of the ether extract or 100 *ml* of the aqueous extract) was injected intravenously and urine samples during 2 consecutive 15 min periods were again collected. Blood samples were also collected at O, 15 and 30 min after the administration of the individual extracts. For control study, propylene glycol (10 *ml*) and normal saline (100 *ml*) were injected intravenously.

The urine and blood samples were stored in the freezing chamber of the refrigerator and were analysed within 24 hr for creatinine and chloride contents by the methods described by

Folin (4) and Schales and Schales (6). The formula used for calculating renal clearance was—

$$\text{Renal Clearance} = \frac{UV}{P}$$

U = concentration of the substance as *mg*/100 *ml* of urine.
V = urine volume *ml*/min.
P = concentration of the substance as *mg*/100 *ml* of plasma.

Student's 't' test and analysis of variance (10) were applied for testing the significance of difference of 2 correlated series of urine volumes (collected before and after the administration of the extracts) and their respective creatinine and chloride levels.

RESULTS

Ether and aqueous extracts of *T. terrestris* fruits (about 350 *mg* ether extract or 100 *ml* decoction per dog) did not produce any significant ($P>0.05$) change in the renal chloride clearance (Table I).

TABLE I : *Effect of Tribulus terrestris fruit extracts on creatinine and chloride renal clearance (average ± standard error) in six dogs of mean body weight 12.8 ± 4 kg*

	Control	*After propylene glycol*	*After ether extract*	*After normal saline*	*After aqueous extract*
Creatinine clearance *ml/min*	45.7 ± 22.9	42.7 ± 22.3 $P>0.05$	66.2 ± 22.8 $P<0.02$	40.4 ± 13.2	49.6 ± 11.9 $P>0.05$
Chloride clearance *ml/min*	1.58 ± 0.67	1.56 ± 0.86 $P>0.05$	1.86 ± 0.90 $P>0.05$	1.42 ± 0.87	1.6 ± 0.81 $P>0.05$

The urine flow rate significantly ($P< 0.05$) increased after the injection of the ether extract (from 37 *ml* in 30 min to 52 *ml* in 30 min). On the other hand, the aqueous extract did not significantly ($P>0.05$) increase the urine flow (from 38 *ml* in 30 min to 42 *ml* in 30 min; 8).

The ether extract produced a significant ($P<0.02$) increase in the renal creatinine clearance whereas the aqueous extract did not seem to have such activity (Table I).

DISCUSSION

In the dog, creatinine is excreted in the glomerular filtrate and is neither absorbed nor excreted by the tubules. Creatinine clearance in the dog, therefore, is a direct measure of the glomerular filtration rate. Significant increase in glomerular filtration produced by the ether extract can be the mechanism of its diuretic action.

We have earlier reported (8) that the ether extract produced a marked fall in blood pressure for about 30 min and atropine and mepyramine faild to prevent it. As such, hypotension would tend to reduce the renal blood flow and consequently glomerular filtration rate; but the ether extract was found to increase the glomerular filtration rate. Such an effect, therefore, can be compared to that of another diuretic theophylline which increases glomerular filtration rate though reduces blood pressure.

The results of the present study indicate that neither the ether extract nor the aqueous one produced any significant increase in the renal chloride clearance. Therefore, the possibility that the ether extract produced diuresis by interfering with the tubular reabsorption of chloride can be ruled out.

ACKNOWLEDGEMENT

The authors are thankful to Dr. R.P. Singh, Associate Professor of Medicine, College of Veterinary Medicine, H.A.U. , Hissar, for his valuable suggestions.

REFERENCES

1. Bhatnagar, J.N. (1958). Contribution of Phytochemical and Pharmacological Studies on *N. jatamansi* and *T. terrestris*, and their Use as Indigenous Diuretic Agents. M.D. Thesis, Vikram University (Cited from Shrivastava, P.N., M.V.Sc. Thesis, Vikram University, 1965).
2. Bhide, N.K., W.W. Altekar, J.C. Trivedi and U.K. Sheth. Potassium diuretics in the Ayurvedic System of Medicine. *J. Postrgraduate Med.* (Bombay), **4** : 21, 1958.
3. Chopra, R.N. and S. Ghosh. Observations on certain medicinal plants used in the indigenous medicine, *Ind. Jour. Med. Res.*, **17** : 377, 1929.
4. Folin, O. On the determination of creatinine and creatine in urine. *J. Biol. Chem.*, **17** : 469, 1914.
5. Gujral, M.L., P.N. Saxena and S.S. Mishra. An experimental study of the comparative activity of indigenous diuretics. *J. Ind. Med. Ass.*, **25** : 1, 1955.
6. Schales, O. and S.S. Schales. Determination of chloride in blood or serum. *J. Biol. Chem.*, **140** : 879, 1941.
7. Singh, R.C.P. and C.S. Sisodia. A preliminary experimental study of diuretic activity of some indigenous plants, unpublished work, 1967.
8. Singh, R.C.P. and C.S. Sisodia. Pharmacological studies on some indigenous diuretics, unpublished work, 1967.
9. Singh, R.C.P. and C.S. Sisodia. Toxicological studies on some indigenous diuretic plants, unpublished work, 1967.
10. Snedecor, G.W. and G.C. William. Statistical Methods Ed. VI (Indian). Oxford and I.B.H. Publishing Company 1968, p. 419.
11. Vidyalankar, J.D., Charak Samhita. Varanasi, Moti Lal Banarsi Dass, 1963. p. 410.