

RESEARCH ARTICLE

## A study of *Tribulus terrestris* extract on risk factors for urinary stone in normal subjects and urolithic patients

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**Abstract:** This study was conducted to determine the effect of *Tribulus terrestris* extract on urinary risk factors in normal subjects and urolithic patients. Twenty five urolithic patients and 18 normal subjects were selected and administered once daily with 10 mL of *T. terrestris* extract for one week. Serum, urine collected between 10 pm and 6 am, and 24 h urine samples were analysed for urinary risk factors for stone formation on day 0 and day 8 after administration of the extract. In urolithic patients the mean serum calcium level increased significantly and the mean urinary calcium level decreased significantly after treatment for one week with *T. terrestris* extract. The mean uric acid level of the urolithic patients decreased significantly in serum and urine after treatment with *T. terrestris* extract. The mean citrate, oxalate, proteins and glycosaminoglycan levels decreased significantly in urine collected between 10 pm and 6 am, and 24 h urine samples of the urolithic patients after treatment with the extract. Urine volume and phosphate level in serum were not altered significantly in the urolithic patients. Hence *T. terrestris* extract may be useful in the treatment of urolithiasis.

**Keywords:** Calcium, citrate, glycosaminoglycans, inorganic phosphate, oxalate, *Tribulus terrestris* extract, uric acid.

### INTRODUCTION

Urolithiasis or urinary stone formation is a common health problem in the northern region of Sri Lanka. All forms of calculi are found in the kidney and urinary tract of patients. Urolithiasis is more prevalent in males than in females with the ratio of 4:1<sup>1</sup>. Renal calculi are found mainly in adults while children tend to get vesicle calculi in situations where they are malnourished and also suffer from diarrhoea<sup>1</sup>. There are multiple factors involved in the stone formation. High intake of oxalate, carbohydrate, fat, purine, dairy products, proteins

and sodium chloride increases the incidence of stone formation<sup>1</sup>. Individuals who work in warm environments and experience strenuous physical exertion are more prone to urolithiasis<sup>1,2</sup>. Further, *Staphylococci* and *Proteus vulgaris* infection facilitates the precipitation of crystals by splitting urea<sup>1</sup>. Most of the stones are developed by the deposition of glycosaminoglycans on an organic matrix<sup>3</sup>.

Allopurinol<sup>4,5</sup>, thiazide and several non-thiazide diuretics<sup>6</sup> and percutaneous nephrostomy and hemiacidrin<sup>7</sup> are used to treat urolithiasis. High energy shock waves were used to disintegrate kidney stones<sup>8</sup>. Laser and pneumatic lithotripsy are safe and effective means of treatment<sup>9</sup>. Extra corporeal shock wave lithotripsy (ESWL) is a safer, more effective means in many cases. Some native extracts such as banana stem extract has also been tested in rats<sup>10,11</sup>. Rice bran therapy has been tested to prevent the recurrence of urinary stone disease<sup>12,13</sup>.

This paper describes a preliminary study carried out to reveal the effect of *Tribulus terrestris* extract in the urolithic patients. *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<sup>14</sup>. *T. terrestris* is useful in the treatment of urolithiasis, dysuria, impotence or erectile dysfunction and kidney dysfunction, and has also shown antibacterial & antifungal activity and anti-inflammatory activity<sup>15</sup>. The principal aim of this study is to assess the variations in the levels of uric acid, calcium,

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magnesium, citrate, oxalate, protein, glycosaminoglycans and urine volume before and after the administration of *T. terrestris* extract on the urolithic patients and the normal subjects.

## METHODS AND MATERIALS

**Selection of subjects:** Twenty five renal stone patients and 18 normal individuals (volunteers) were selected. Their age ranged between 22–60 years. The patients were selected from the surgical ward, Teaching Hospital, Jaffna, who had been discharged without having any medication. Patients with urinary infection were excluded from the study. The patients selected were non-pregnant, without severe renal dysfunction and without signs of primary hyperparathyroidism. The approval of the Ethical Clearance Committee of the Faculty of Medicine, University of Jaffna was obtained for this study.

**Preparation of *T. terrestris* extract:** *T. terrestris* (Sinhala-'Hokaddu'; Tamil-'Nerunchi'; family-Zygophyllaceae) extract was prepared as follows. The leaves, flowers and fruits (10 g) of *T. terrestris* plant were washed in distilled water, mixed with 450 mL of double distilled water and boiled until the volume was reduced to about 225 mL.

**Administration of the extract and collection of samples:** *T. terrestris* extract (10 mL) was orally administered daily (Table 1) in the morning for 7 d to the patients and normal subjects who fasted overnight. Serum and urine were collected between 10 pm and 6 am. Twenty four hour urine samples were collected from patients and normal individuals before and after the administration of *T. terrestris* extract, i.e. on day 0 and day 8. Urine samples were collected in a container with 10 mL of conc. HCl between 10 pm and 6 am. The urine collection was continued for 24 h until the following day.

**Analytical methods:** *T. terrestris* extract was analysed for uric acid<sup>16</sup>, calcium<sup>17</sup>, magnesium<sup>18</sup>, inorganic phosphate<sup>19</sup>, citric acid<sup>20</sup>, oxalate<sup>21</sup> and glycosaminoglycan<sup>22</sup>. Serum was analysed for uric acid<sup>16</sup>, calcium<sup>17</sup>, magnesium<sup>18</sup>, and inorganic phosphate<sup>19</sup>. Urine collected between 10 pm and 6 am, and 24 h urine samples were analysed for uric acid<sup>16</sup>, calcium<sup>17</sup>, citric acid<sup>20</sup>, oxalate<sup>21</sup> and inorganic phosphate<sup>19</sup>. Twenty four hour urine samples were also analysed for glycosaminoglycan<sup>22</sup> and proteins<sup>23</sup>, and the urine volume was determined. Similar estimations were made with urine samples collected between 10 pm and 6 am.

The mean and the standard deviations were calculated and the results were analysed with Students' t' test.

**Table 1:** Composition of *T. terrestris* extract

Constituents	Amount (g/g <i>T. terrestris</i> )
urate	5.5 x 10 <sup>-5</sup>
calcium	3.452 x 10 <sup>-3</sup>
magnesium	2.0 x 10 <sup>-5</sup>
inorganic phosphate	9.0 x 10 <sup>-5</sup>
citrate	1.08 x 10 <sup>-2</sup>
oxalate	1.38 x 10 <sup>-2</sup>
glycosaminoglycans	4.09 mol

## RESULTS

After the administration of 10 mL of *T. terrestris* extract for a week, serum uric acid level in normal individuals and patients decreased significantly (p<0.05) (Table 2). Uric acid levels in urine samples collected between 10 pm and 6 am, and 24 h also decreased significantly in the normal subjects and the urolithic patients after the treatment (p<0.05) (Tables 3 and 4).

**Table 2:** Changes in the serum calcium, uric acid, magnesium and inorganic phosphate of urolithic patients (25 nos.) and normal subjects (18 Nos.) before and after the administration of *T. terrestris* extract for one week

Parameters	Patients			Normal subjects		
	Before	After	Statistical analysis	Before	After	Statistical analysis
uric acid (mg L <sup>-1</sup> )	77.30 (± 16.1)	38.50 (± 8.1)	p<0.05	47.0 (±19.4)	39.8 (±19.9)	p<0.05
calcium (mmol/L)	2.47 (± 0.42)	2.68 (± 0.29)	p<0.05	2.38 (± 0.008)	2.50 (± 0.021)	p<0.05
magnesium (mmol/L)	0.64 (± 0.16)	0.87 (± 0.24)	p<0.05	1.16 (± 0.25)	1.22 (± 0.46)	p<0.05
inorganic phosphate (mmol/L)	0.58 (± 0.03)	0.70 (± 0.12)	p<0.05	2.009 (± 1.063)	2.041 (± 0.98)	p<0.05
calcium/ inorganic phosphate ratio	4.26	3.83		1.18	1.22	

Values are indicated as the mean (±SD)

**Table 3:** Changes in the urinary calcium, uric acid, citric acid, oxalate and inorganic phosphate of urolithic patients (25 nos.) and normal subjects (18 nos.) before and after the administration of *T. terrestris* extract for one week

Parameters	Patients			Normal subjects		
	Before	After	Statistical analysis	Before	After	Statistical analysis
uric acid (mg L <sup>-1</sup> )	421.2 (± 125.0)	212.0 (± 80.4)	p<0.05	389.3 (± 214.2)	334.6 (± 65.3)	p<0.05
calcium (mmol/L)	4.10 (± 1.30)	3.53 (± 1.49)	p<0.05	2.82 (± 1.67)	2.34 (± 0.65)	p<0.05
citric acid (mg/L)	550.0 (± 350.0)	570.0 (± 460.0)	p>0.05	740.0 (± 360.0)	750.0 (± 560.0)	p>0.05
oxalate (mg/L)	24.16 (± 7.13)	31.06 (± 8.03)	p<0.05	25.02 (± 9.39)	ND	
inorganic phosphate (mmol/L)	6.10 (± 2.01)	8.06 (± 2.96)	p<0.05	9.09 (± 0.3)	9.80 (± 0.45)	p<0.05
calcium/ inorganic phosphate ratio	0.672	0.437		0.310	0.238	

ND- Not determined; values are indicated as the mean (±SD)

**Table 4:** Changes in the 24 h urinary calcium, uric acid, citric acid, oxalate, inorganic phosphate, glycosaminoglycan, protein and urine volume of urolithic patients (25 nos.) and normal subjects (18 nos.) before and after the administration of *T. terrestris* extract for one week

Parameters	Patients			Normal subjects		
	Before	After	Statistical analysis	Before	After	Statistical analysis
uric acid (mg/24 h)	413.91 (± 140.22)	271.75 (± 99.89)	p<0.05	389.35 (± 93.98)	347.97 (± 78.02)	p<0.05
calcium (mmol/24 h)	7.07 (± 1.63)	5.96 (± 0.70)	p<0.05	3.61 (± 0.56)	2.65 (± 0.56)	p<0.05
protein (mg/24 h)	944.24 (± 623.60)	640.05 (± 391.51)	p<0.05	126.57 (± 6.542)	123.71 (± 7.23)	p<0.05
glycosaminoglycans (µmol/24 h)	95.13 (± 57.99)	73.08 (± 55.66)	p<0.05	124.83 (± 11.18)	121.42 (± 17.10)	p<0.05
urine volume (mL/24 h)	1350 (± 619.35)	1418 (± 578.49)	p>0.05	1118 (± 397.57)	1125 (± 406.00)	p<0.05
citric acid (mg/L)	661.30 (± 661.30)	865.22 (± 213.86)	p<0.05	748.39 (± 92.31)	1004.50 (± 146.16)	p>0.05
oxalate (mg/24 h)	46.35 (± 6.76)	71.31 (± 8.87)	p<0.05	40.56 (± 3.22)	ND	
inorganic phosphate (mmol/24 h)	5.02 (± 3.71)	5.44 (± 7.21)	p<0.05	9.502 (± 3.53)	11.02 (± 3.73)	p<0.05
calcium/ inorganic phosphate ratio	1.408	1.096		0.3799	0.2405	

ND – Not Determined; Values are indicated as the mean (±SD).

Twenty four hour urinary output in both the urolithic patients and normal individuals increased after treatment with the extract (Table 4).

After treatment with *T. terrestris* extract, the serum Ca<sup>2+</sup> levels in the normal individuals and the patients increased significantly while the excretion of Ca<sup>2+</sup> in the urine samples collected between 10 pm and 6 am, and 24 h significantly decreased (p<0.05) (Tables 2, 3 and 4).

The mean 24 h urine total proteins and glycosaminoglycan levels reduced significantly in the urolithic patients and the normal subjects after the treatment with *T. terrestris* extract (p<0.05) (Table 4).

The serum Mg<sup>2+</sup> levels in the patients and the normal subjects increased significantly after the administration of *T. terrestris* extract (p<0.05) (Table 2).

In the patients, the levels of citrate excretion in the 10pm and 6 am samples became elevated but the

increase was not statistically significant. However, citrate excretion in 24 h urine samples of the patients increased significantly, while that of the normal subjects also increased although not statistically significant. Oxalate excretion in urine collected between 10 pm and 6 am, and after 24 h increased significantly (p<0.05) (Table 3) in the patients after the treatment with *T. terrestris* extract.

The inorganic phosphate level in the serum of the patients and the normal individuals increased significantly after the administration of the extract (Table 2). The excretion of inorganic phosphate in the urine sample collected between 10 pm and 6 am, and after 24 h also increased significantly in both the patients and normal individuals (Tables 3 and 4).

## DISCUSSION

The significant decrease of serum uric acid level in the patients who received treatment could be explained by

allopurinol-like action of *T. terrestris* extract. Allopurinol is a drug used in the treatment of hyperuricemia<sup>24</sup>. Allopurinol inhibits xanthine oxidase leading to a decrease in the formation of uric acid. Urate stones are formed in 15 – 20% of patients with hyperuricosuria<sup>4,5,25,26</sup>. The reduction in urinary uric acid level could be due to the consequence of the decrease in serum uric acid level. The increase in urinary uric acid level is a cause for urinary stone formation<sup>1</sup> and therefore the *T. terrestris* extract has a positive effect on the reduction of urinary stone formation. While reducing the uric acid level in urine, the extract also increased the urine volume. This diuretic effect of the extract would help in the dilution of uric acid and thereby reducing urate stone formation. Stone formers must maintain a high urine volume<sup>6,27</sup> to keep solutes well diluted<sup>1</sup>.

In both, the patients and normal individuals, the extract increased the serum calcium level and decreased urinary calcium excretion. Increased urinary calcium excretion is a high risk factor in renal stone formation<sup>28,29</sup>. The action of *T. terrestris* extract could be related to thiazide. Thiazide prevents recurrent calcium urolithiasis by reducing the urinary output of calcium<sup>29</sup>. Glycosaminoglycans are the major constituents of *T. terrestris* extract<sup>4</sup>, which are macromolecules and may reduce Ca<sup>2+</sup> excretion by complexing with Ca<sup>2+</sup>. There is evidence that *T. terrestris* extract alters the tubular maximum for Ca<sup>2+</sup> and increases its reabsorption in tubules<sup>27</sup>. Recent studies suggest that chondroitin sulphate and hyaluronic acid promote both growth rate and suspension density of the crystals<sup>3,25,31</sup>. *T. terrestris* could be altering the permeability of membranes and reducing the urinary excretion of glycosaminoglycans. Reduction in glycosaminoglycan urinary excretion (Table 4) might be due to the alteration in the membrane permeability of the Bowman's capsule and the stimulation of glycosyltransferase leading to formation of large molecules of glycosaminoglycans or due to the increased degradation of glycosaminoglycans. *T. terrestris* extract may reduce the degradation process and there may be larger glycosaminoglycans which cannot be filtered through the glomerular membrane. This may also reduce the urinary glycosaminoglycan concentration in urine after the *T. terrestris* extract administration<sup>1</sup>. Glycosaminoglycans of human urine have an influence on crystal aggregation. The diuretic effect of *T. terrestris* has been reported in Guinea pigs<sup>30</sup>.

*T. terrestris* extract contains high concentration of Mg<sup>2+</sup> (Table 1) and also increases serum Mg<sup>2+</sup> (Table 2). Magnesium inhibits calcium oxalate and calcium phosphate stone formation by inhibiting the formation and aggregation of calcium with oxalate and phosphate to form crystals in the urinary tract<sup>31</sup>.

Binding of magnesium with oxalate in the gut reduces its absorption and in urine prevents insoluble calcium oxalate formation. Reduction in urinary citrate output has been demonstrated in calcium oxalate stone secondary malabsorption syndromes<sup>2</sup>. Urinary citrate has an inhibitory effect on the lithogenesis of calcium salts at crystallisation stages. They inhibit the precipitation of calcium oxalate and calcium phosphate and prevent the growth and aggregation of both crystal types<sup>2</sup>. Thus, intake of *T. terrestris* extract could increase the citrate excretion and give a positive effect on the reduction of calcium stone formation.

In urine collected between 10 pm and 6 am, the citrate excretion in both the patients and subjects was elevated, but the elevation was statistically not significant. However the excretion of citrate in 24 h urine was significant both in the patients and normal subjects. This elevated level could also be due to the direct contribution by the extract. Citrate chelates calcium and hence considered to be an inhibitor of urinary stone formation<sup>2</sup>.

There was an increase in the serum calcium level with an increase in inorganic phosphate level (Table 2). The effects on the patients and the normal subjects were different because the serum calcium to phosphate ratio was lowered in the patients while it was increased in the normal subjects. The urine samples collected between 10 pm and 6 am, and 24 h from the patients and the normal subjects contained reduced calcium to inorganic phosphate ratio (Tables 3 and 4). Neutral phosphates decrease renal calcium excretion and increase renal pyrophosphate excretion. Inorganic phosphate therapy increases the serum phosphorous concentration and in turn it decreases the serum calcium concentration<sup>6</sup>.

## CONCLUSION

This preliminary study shows that administration of *T. terrestris* extract brings about changes in the levels of the risk factors, which cause or activate the urinary stone formation. After the administration of *T. terrestris* extract, the levels of uric acid, oxalate, calcium, proteins and glycosaminoglycans in serum and urine changed significantly, while the citrate, inorganic phosphate and urine volume were not significantly affected. Therefore the *T. terrestris* extract may be useful in the treatment of urolithiasis.

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