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# Effect of ethanolic extracts of *Tribulus terrestris, Phyllanthus niruri* and combination on calcium oxalate urolithiasis in rats

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**Abstract**

In the present study the effect of calcium oxalate urolithiasis urinary risk factor of ethanolic extract of *Tribulus terrestris, Phyllanthus niruri* and their combination have been studied in albino rats. From this study it is deduced that the possible effect of the ethanolic extract of *Tribulus terrestris, Phyllanthus niruri* and their combination can be assigned to be positive effect on the main urolithiasis risk factors.

**Key words** :*Tribulus terrestris, Phyllanthus niruri*, Urolithiasis, Ethylene glycol (EG)

## Introduction

Urinary stone disease continues to reside in an important place in daily urological practice. The average life time risk of stone formation has been reported in the range of 5-10 %. A predominance of men over women can be observed with an incidence peak between the fourth and fifth decade of life. (Yadav R. D., 2011). Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70–81% in males, and 47–60% in females . Occurrence of urolithiasis requires formation of a nidus, its retention and growth in the urinary tract which may cause obstruction of the ureter. ( Souccar C., 1999 ).

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of various ailments. Urolithiasis is the condition where urinary calculi are formed in the urinary tract. It is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females. It causes serious health problems such as severe pain, urinary-tract obstruction and infection that adversely affect well-being of individuals.

The main objectives of present study are extraction of crude drugs in hydroalcoholic solvent and perform it Antiurolithiatic activity

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## Material and Methods

### Description of plant

*Tribulus terrestris* is commonly known as puncture vine, caltrop, yellow vine, goat head and devil's horn. It is is a member of the Zygophyllaceae family and is widely distributed in both tropical and mild temperate regions *T. terrestris* is native to warm temperate and tropical regions of southern Europe, southern and western Asia, throughout Africa, and Australia. *T. terrestris* grows well in light textured soils however it grows over a wide range of soil types. Generally, it can be found in cultivated crops, overgrazed pastures, roadsides, lawns and neglected areas. *T. terrestris* is a small silky, hairy and prostrate herb with stems up to 2m long. Leaves are pinnate, short (~1.25 cm length), opposite and each consisting of 4-8 pairs of spear shaped leaflets. *T. terrestris* is characterized by small (8-15 mm diameter) yellow petal.

*Phyllanthus niruri* originated in India, usually occurring as a winter weed throughout the hotter parts. The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas. *Phyllanthus niruri* is a herb of Euphorbiaceae family that grows upto 60 cm. *Phyllanthus niruri* is an annual herb which grows in the wild after first showers of monsoon in Jharkhand, Bihar, Chhattisgarh, etc. states of India. However, it has also been reported to grow commonly in coastal areas. In Indian states it usually grows during second week of June and starts bearing fruits up to mid July or August. It remains in the wild up to the end of the rainy season. However, under safe conditions it can grow and survive upto mid-winter. The annual herb is 30-60 cm high, quite glabrous, stem often branched at the base. Leaves: Numerous, sbsessile distichous often imbricating, elliptic oblong obtuse. Stipules present, very acute. Flowers: Yellowish, very numerous, axillary.

### Pharmacological studies

**Anti-urolithiatic activity** (Mousa al Reza et al 2007, F. Atmani et al 2004, R. Vargas S. 1999)

### Experimental design

Animal 6 groups and each group having 5 albino rats weighing 120-180 gm. were selected and housed under standard laboratory condition for a period of 14 days prior to the experiment. Experimental protocols were approved by our Institutional animal ethical committee, which follows guidelines of CPCSEA/ IAEC (Committee for the purpose of Control and Supervision of Experiments on Animals/Institutional Animal Ethics Committee).

Model Ethylene glycol induced model

Standard drug Cystone tablet 500 mg/kg body weight

Method 0. 75% ethylene glycol induced kidney stone

3.2.1.2 Experimental group

Six groups contain 5 animals in each group were subjected to 0.75% ethylene glycol into drinking water for four weeks.

GROUP I : Control group received only drinking water.

GROUP II : Model control group received drinking water + 0.75% ethylene glycol

GROUP III : Received drinking water +0.75% ethylene glycol + *Tribulus terrestris* extract 200 mg/kg

GROUP IV : Received drinking water + 0.75 % ethylene glycol + *Phyllanthus niruri* extract 200mg/kg

GROUP V : Received drinking water +0.75 % ethylene glycol + Combination of both extract

GROUP VI : Received drinking water +0.75 % ethylene glycol + Standard drug 500 mg/kg

### Statistical analysis

Standard evaluation was done using one-way analysis of variance (ANOVA) Statistical significance was set at $P< 0.0001$. Results are presented as mean ± standard errors (S .E.). (Mousa al reza Hadizadeh et al 2007)

326.1.3 Parameter

### Total urinary volume

Animals were placed in separate metabolic cages 24 hours before the surgery. Total urinary volume was measured, by using measuring cylinder, and reported in ml (C.Barbas, A.Garcia 2002).

### Test for acidity

Uric acid crystals were found to deposit most frequently in the concentrated acid urine. Thus the acidity of the urine was tested using pH meter (C.Barbas, A.Garcia 2002)

### Biochemical parameter of urine

Urinary concentration of calcium, oxalate and cretinine were measured. Urinary oxalate was estimated according to the method described by

Hodgkinson et al (Hodgkinson 1970). 1 ml of urine was acidified by concentrated $HNO_3$ to solubelize crystals and then adjusted to pH 7 by NaOH in the presence of color indicator, the bromothymol blue. About 2 ml of saturated $CaSO_4$ and 14 ml of pure ethanol were added to precipitate oxalate overnight. The sample were centrifuged at 450 X g for 10 min and then filtered on filter paper. The precipitate obtained was solubelize in 10 ml of water acidified by 2 ml concentrated sulfuric acid. The samples were titrated by a solution of $KMnO_4$.

Calcium analysis was performed by using a merck thermo spectronic U.V. double beam spectrophotometer equipped with a Varian hollow cathode and a deuterium background corrector. Creatinine was estimated based on principle, the production of an orange colour by the interaction of Creatinine with alkaline sodium pirate. The colour produced was compared in a colorimeter, and the Creatinine content of the urine estimated by comparison with a Creatinine solution of known concentration. (F. Atmani et al 2004, C.Barbas, A.Garcia 2002)

## Results and Discussion

Percentage yield (% w/w) of ethanolic extract of *Tribulus terrestris, Phyllanthus niruri* were found to be 14% w/w and 10 %w/w respectively.

**Qualitative chemical evaluation**

Ethanolic extract of *Tribulus terrestris* showed the presence of alkaloids, glycoside, flavonoid, Saponin.

Ethanolic extract of *Phyllanthus niruri* showed the presence of alkaloids, tannins, flavonoid, Saponin.

**Anti-urolithiatic activity**

The changes in the urine parameters in the experiment animals during the study are presented .The urine concentration of oxalate, calcium and Creatinine were increased significantly in animals administered with 0.75 percentage ethylene glycol. The concentration of oxalate (3.13± 0.05), calcium (2.73±0.13**)** and creatinine (4.09 ± 0.07) of group III treated with ethanolic extract of *Tribulus terrestris* were found to be significant as compared to model control (Group II ). The concentration of oxalate (3.34± 0.04), calcium (2.96±0.11**)** and creatinine (4.11 ± 0.07) of group IV treated with ethanolic extract of *Phyllanthus niruri* were found to be significant as compared to model control (Group II ). The concentration of oxalate (3.37 ± 0.13), calcium (2.85±0.04**)** and creatinine (4.15 ± 0.05) of group V treated with combination of ethanolic extracts of *Tribulus terrestris* and *Phyllanthus niruri* were found to be more significant as compared to group II,group III,group IV,group VI**.**. The percentage reduction of all parameters of urine was found more in group V and in group VI (standard). Urinary volume significantly decreased in the animals treated with the 0.75 % of ethylene glycol. Urinary volume were increased by 248(Group III), 254 (group IV), 274 (group V), 281(group VI) percentage compared to model control group. Urinary pH significantly increased in the animals treated with the 0.75 % of ethylene glycol. Urinary pH were decreased by 24.46(Group III), 22.72 (group IV), 25.58 (group V), 24.97 (group VI) percentage with compared to model control group.

From the above results it was noted that the combination (ethanolic From the above results it was noted that the combination (ethanolic extract *Tribulus terrestris* and ethanolic extract of *Phyllanthus niruri* ) were most significant.

In the present study of kidney stone, after the administration of ethanolic extract of *Tribulus terrestris* and ethanolic extract of *Phyllanthus niruri* and their combination to the group of rats urine analysis shows that the occurrence of stone was decreased when compared to the kidney stone control group and combination (ethanolic extract of *Tribulus terrestris* and ethanolic extract of *Phyllanthus niruri)* as effective as the standard group.

Under formulation development the components were examined for incompatibility, with dried extracts being as therapeutically active ingredient.

## Conclusion

The developed formulation was evaluated for various pharmaceutical parameters including stability studies at different environmental conditions. There was no significant physical change observed out in three month's storage. Formulation showed marked antiurolithiatic activity on albino rats, it was concluded that the developed formulation show significant reduction in urine concentration oxalate, calcium and Creatinine.

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