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Research Article

Evaluation of Ethanolic seed extract of *Tribulus terrestris*. Linn in serum electrolytes and urine oxalte of Urolithiatic male Albino Wistar rats

Shamina S*, Abilash P S, Jishamol G.

Dept.of Biochemistry , Rathnavel Subramanium College of Arts & Science,

Sulur. Coimbatore. Tamilnadu, India.

ABSTRACT

In the Indian subcontinent the medicinal plants are source of great economic value. Nature has been bestowed on us a very rich botanical wealth and also large number of diverse types of plants. *T.terrestris* is an annual plant belonging to the family *Zygophllaceae,* found widely distributed in the region of Asia, Europe, America and Australia. In folk medicine, the plant is used as tonic, aphrodisiac, diuretic, lithontriptic and urinary tract anti-infections. The current study focused on the effect of the plant in the urolithiatic rats (induced Ethylene glycol) by considering the level of Urine Oxalate and serum electrolytes (Sodium, Potassium & Chloride). The study lights up on the effect of ethanolic seed extract of *Tribulus terrestris* in those parameters in urolithiatic male albino Wistar rats. Urolithiasis was induced in rats by administrating 0.75 % of ethylene glycol orally for 30 days and after that the Urine sample was collected by using metabolic cage. This analysis helps to find out whether the groups having the urolithiasis or not. The Group III (Treated Group) rats were treated with the ethanolic seed extract of *Tribulus terrestris* was administered in 250 mg/kg body weight of rats orally for 30 days. This showed the increased level of serum electrolytes in urolithiatic Group II and a gradual declining to the near normal level of Group III. The administration of the plant extract in the Preventive, Group IV results there is no side effect of the plant. Hence, the current study concluded that this plant is regulating the level of electrolytes and oxalate level in urolithiatic condition.

Key Words: Urolithiasis, *Tribulus terrestris*, Ethylene Glycol.

INTRODUCTION

In history, most of the tribal economies have been engaged in subsistence agriculture or hunting and gathering at initial stage. With the course of time, they have developed a great deal of knowledge on the use of plant and products in for the treatment of kidney stone. Plant used in traditional medicine may constitute an important source of new biologically active compounds. Utilization of plants for medicinal purposes in India has been documented long back in ancient literature. [1]

Many medicinal plants have been used since ages to treat urinary stones though the rationale behind their use is not well established through systematic and pharmacological studies, except for some composite herbal drugs and plant medicines are which great demand both in developed as well as in developing countries for the health care because of their wide biological and medicinal activities, higher safety margin and costs [2].Variety of herbal drugs and alternative treatments has been tried for prevention and treatment of renal calculi but none of them got the commercial importance. [3]

In humans, the urinary stones are one of the oldest and the most common afflictions. This is the third most common condition of the urinary tract infection and pathologic condition of the prostate. Urinary calculi are formed or located anywhere in the urinary system or the process of forming stones in the kidney, bladder, and ureters. The formation of these calculi involving several physiochemical aggregations and retention within the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate stones representing up to 80% of the analyzed stones. Currently no allopathic medicines are available for urolithiasis. Surgery, lithotripsy, and local calculus disruption using a high power laser are used to treat calculi. However, these procedures are expensive and recurrence is quite common. [4]

In most cases, the management of urolithiasis involves both surgical and medical approaches. However these treatments are relatively costly, painful and require expert hands with availability of appropriate equipments. This has stimulated research on traditional remedies showing anti-urolithiatic activity. Many remedies have been employed through the ages to treat urolithiasis.[5]
T.terrestris is an annual plant belonging to the family *Zygophllaceae*, found widely distributed in warm region of Asia, Europe, America and Australia. It is used in folk medicine as tonic, aphrodisiac, diuretic, lithontriptic and urinary tract anti-infections.

Figure No : 1 Seed of *Tribulus terrestris*

T. terrestris has long been a constituent in tonics in Indian Ayurveda practice, where it is known by its Sanskrit name, *"gokshura/ sarrata"*It is also used in Unani, another medical system of India. *Tribulus terrestris* is known in Sanskrit as Goksura. The word, Gokshura, comes from two Sanskrit words: Go (cow) and Kshura (hoof). This is because the small fruits look very much like the hooves of cows, with several big thorns and lots of little spines on them. It is believed to contribute to overall physical, as well as sexual, strength by building all the tissues, especially shukra dhatu (reproductive tissue).
Animal studies in rats, rabbits and primates have demonstrated that administration of *Tribulus terrestris* extract can produce statistically significant increases in levels of testosterone, dihydrotestosterone and dehydroepiandrosterone, and produces effects suggestive of aphrodisiac activity. On the other hand, one recent study found that *T. terrestris* caused no increase in testosterone or LH in young men, and another found that a commercial supplement containing androstenedione and herbal extracts, including *T. terrestris*, was no more effective at raising testosterone levels than androstenedione alone.The current study focuses on serum electrolytes and urine oxalates in rats induced with ethylene glycol.

MATERIALS AND METHOD

Animal Model Used

Adult Albino Wistar male rats (130-150 g) were chosen for study. Animals were housed in plastic cages in an animal colony room 12/12 hours light/dark cycle at 21 ± 2 °C with food and water All animal experiments were carried out in accordance with Ethical Committee Acts.

Antiurolithiatic Experimental Study

Ethylene glycol induced urolithiasis was used to assess the antiurolithiatic activity in albino rats. The acclimatized animals were divided into 4 groups, from Group I to Group IV, each with 6 rats. The urolithiasis (stone) was induced in all the groups except Group-I as it was reserved as .Normal Control and Group IV (Preventive). The Group II, animals received 0.75% ethylene glycol in drinking water for 28 days and served as the urolithiatic control. The Group III group animals received 0.75% ethylene glycol in drinking water for 28 days followed by the ethanolic extract of *Tribulus teerrestris* as 1.0 ml/rat/day in accordance to 250mg/kg body weight and Group IV group animals received ethanolic seed extract (1.0 ml/rat/day) of the *Tribulus teerrestris*, orally for 28 days.

Plant Material

Seeds of *Tribulus terrestris* were collected from the local areas of Idukki in Kerala. The seeds was shade dried and coarsely powdered finely. The dried powder was soxhlet- extracted with 70% ethyl Alcohol, in 1:5 w/v ratios for 48 hrs. The extract was dried in vacuum and suspended in water before use.

Collection of Biological Samples

At the end of the experimental period, the animals were kept in the metabolic cage to collect the Urine sample and sacrificed by cervical decapitation, under mild anesthesia. The blood was carefully collected by pumping the heart after the rat was killed. From the collected blood, the serum was separated by centrifugation at 3000 rpm for 20 minutes.

Serum Analysis

After the experimental period, blood was collected by heart puncture under anesthetic condition. Serum was separated by centrifugation at 3000 rpm for 20 min and analyzed for the electrolytes such as Sodium, Potassium and Chloride.

Statistical Analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANO-VA) followed by Dunnett's Multiple Comparison Test using Graph Pad Prism software (GraphPad software Inc., Version 4.0.0.255). The mean values ± SEM were calculated for each parameter. The differences in biochemical parameters between the calculi induced group and standard drug treated group

were considered as 100% and the changes in biochemical parameters by the plant extracts treated groups against the calculi induced group were analyzed accordingly. Level of significance was kept at $P<0.05$.[6]

RESULT AND DISCUSSION

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 nidus, its reaction and growth in the urinary tract which may cause obstruction of the ureter [7]

The worldwide incidence of urolithiasis is quite high and there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid of this painful disease. The main initiating factor for Urolithiasis is Hyperoxaluria. Recurrent stone formation is a common part of the medical care of patients with the stone disease. Calcium containing stones, especially calcium oxalate monohydrate, calcium oxalate dehydrate and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1% . [8]

The size and nature of crystals governs overall clinical manifestations of this complaint whereas urinary chemistry is one of the important factors in determining the type of crystals formed and the nature of macromolecules included on the surface of the crystals. Calcium oxalate stones make up the majority as they account for 70-80% of all kidney stones [9]

Urine is always supersaturated with common stone forming minerals. The crystallization inhibiting capacity of urine does not allow urolithiasis to happen in most individuals, whereas this natural inhibition is in deficit in stone formers.[10]

ESTIMATION OF URINE OXALATE IN NORMAL AND UROLITHIATIC RATS

	GROUP I	GROUP II	GROUP III	GROUP IV
Oxalate (mg/day)	3.29± 0.02	5.96± 0.10 a*	4.57± 0.02 b*	3.01± 0.02c^{ns}

Values are expressed as mean ± standard division of six animals each

COMPARISON

'a' represents comparison between Group II and Group I

'b' represents comparison between Group III and Group II

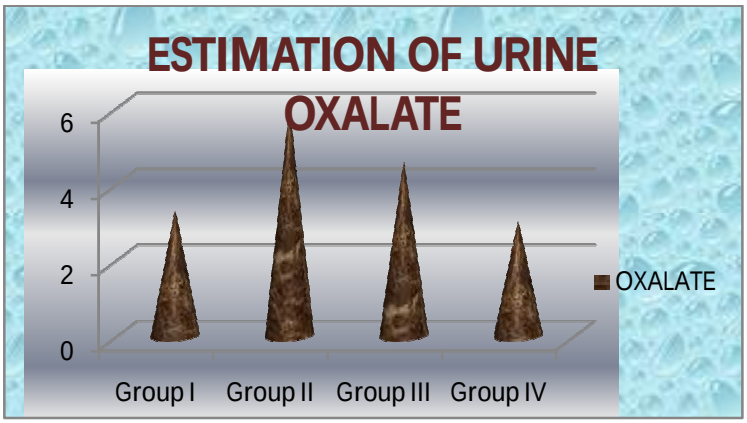
'c' represents comparison between Group IV and Group I

SYMBOL: * This symbol represents the statistical represent p* < 0.05; ns – not significant

The serum electrolytes are highly increased in the toxic group by the administration of the ethylene glycol. The Level of Sodium, Potassium & Chloride in group III regain its level by treating with the plant extract. The Group IV rat doesn't show a drastic change in these levels and it indicates that the plant has no significant side effects.

The ethanolic seed extract of *Tribulus terrestris* can able to regulate the level of Urine oxalate and Serum electrolytes in the Urolithiatic rats. The experimental animals showed the abnormal level of the Urine Oxalate and the Serum Electrolytes is because of the initiation of the Urolithiatic condition and the plant extract regain the normal level within the experimental period. This preliminary study reports leads us to the detailed analysis of antiurolithiatic effect of the ethanolic seed extract of *Tribulus terrestris* as a scope of this research.

Graph.1: Estimation of Urine Oxalate



ESTIMATION OF SERUM ELECTROLYTES IN NORMAL AND UROLITHIATIC RATS

PARAMETERS	GROUP I	GROUP II	GROUP III	GROUP IV
SODIUM	138.43±0.02	197.95±0.02a*	148.01±0.03b*	138.47±0.04 ns
POTASSIUM	8.12±0.03	7.15±0.01a*	8.13±0.06b*	8.16±0.01 ns
CHLORIDE	100.54±0.02	152.13±0.04a*	99.00±0.04b*	100.43±0.02 ns

Values are expressed as mean ± standard division of six animals each

COMPARISON

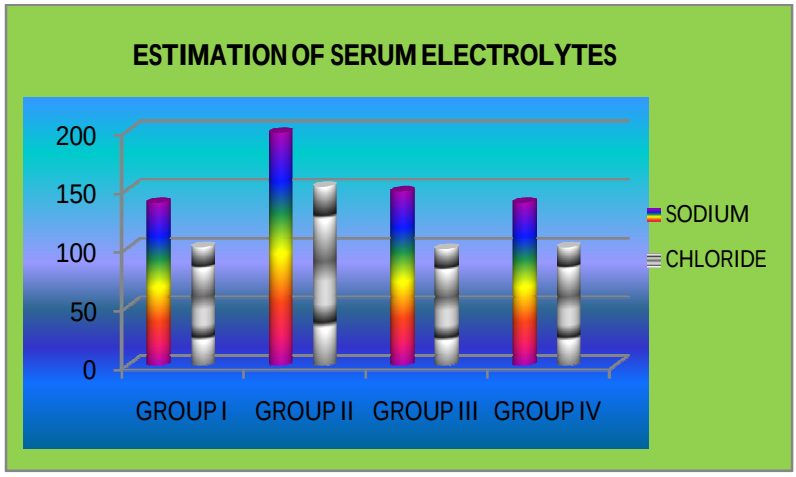
'a' represents comparison between Group II and Group I

'b' represents comparison between Group III and Group II

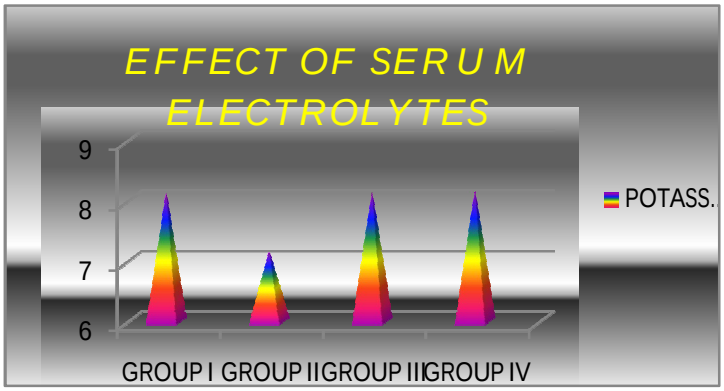
SYMBOL: * This symbol represents the statistical represent p* < 0.05; ns – not significant

UNITS: The given serum electrolytes are expressed in mg/dl

GRAPH No: 2 Effect of Serum Electrolytes in Normal & Urolithiatic Rats



Graph No:3 Effect of Serum Electrolytes in Normal & Urolithiatic Rats



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