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ANTIUROLITHIATIC ACTIVITY OF *TRIBULUS TERRESTRIS* FRUITS AND *PUNICA GRANATUM* SEEDS IN ETHYLENE GLYCOL INDUCED RAT MODELS.

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ABSTRACT

Objective: Herbal therapies had become an integral part of health care sciences. Urolithiasis is a condition in which crystals in the urine combine to form stones. In our work we underwent research on antiurolithiatic activity of herbal medicine.

Method: Antiurolithiatic activity of Tribulus terrestris (TT) fruits and Punica granatum (PG) seeds by using Ethylene glycol induced rat model.

Result: Treatment of TT fruits and PG seeds (100mg+ 100mg) extracts showed to prevent the elevation of serum and urine levels of urinary markers (BUN, Urea, Uric acid and Creatinine).

Conclusion: Further study is needed to explore the exact active constituent of plant and mechanism of action responsible for Antiurolithiatic activity.

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INTRODUCTION

Kidney is the main excretory organs for filtration of blood. Nephron is the main functional unit of kidney [1]. The function of Nephron is to regulate the concentration of water and soluble substances like sodium salts by filtering the blood. Urolithiasis is a condition in which crystals in the urine combine to form stones, also called calculi or urolithiasis. These can be found any where in the urinary tract, where they cause irritation and secondary infection. Several different types have been identified, with struvite stone (magnesium ammonium phosphate) being by far the most common. Any one can develop urolithiasis, but a genetic predisposition to producing crystals makes the development of stones more likely. Patients have a defect in the pathway that normally leads to the breakdown of urates, which are a by-product of protein digestion. This results in increased urate excretion in the urine and this predisposes them to the formation of urate crystals and eventually stones. In some other cases, an inherited defect in a different pathway causes excessive urinary excretion of the amino acid cystine, resulting in cystine crystals and potentially stones in the urine [2, 3]. Tribulus terrestris fruits and Punica granatum seeds are presently used in Ayurvedic medicine for various medicinal purposes. In our work we underwent research on antiurolithiatic activity of Tribulus terrestris fruits and Punica granatum seeds by using ethylene glycol induced rat model.

MATERIALS AND METHODS

Instruments

Calorimeter (Systronics), weighing balance (Cyberlab, Infra), centrifuge (Remi), distillation unit (Borosil), microscope (weswox, Olympus), refrigerator (Godrej), metabolic cage (Dolphin), test tubes, slides, beakers (Borosil, Jsw)

Commercial kits

Commercial kits for the determination of Creatinine, Urea and Uric acid M/s Excel Diagnostics Pvt. Ltd, Kukatpally, Hyderabad.

Preparation of the extract [5]

The powders of Tribulus terrestris fruits and Punica granatum seeds were collected from local ayurvedic shop (Figure 1&2). The aqueous extract for Tribulus terrestris fruits and Punica granatum seeds were prepared by using distilled water: ethanol (1:1) (50-60ºc) (4 litre×4times) for 4 days in a water bath with shaking. After the extraction it was filtered, the ethanol was evaporated under low temperature and pressure. Then using a rotary evaporator, then the water in the extract was lyophilized and stored at 5ºc. 1gm from each extract was weighed and makeup with 0.3% CMC upto 10ml.

Figure1: Pomegranate seeds

Figure 2**:** Tribulus terrestris fruits

Experimental animals

Healthy adult male Wistar rats weighing between 200-250 g were used in the study. Animals were housed in a laboratory maintained at 12 hrs light – dark cycle, controlled room temperature (23±2ºc) and

relative humidity (50±10%). Animals were given standard diet and drinking water ad libitum. The protocol of the study got approval from CPCSEA

Acute toxic studies

Acute toxic studies were performed for both T.T and P.G extracts at a dose of 2000 mg/kg body weight in 2 groups of 6 rats respectively. The CNS activity of both group of rats were observed for its behavioral change. No death is reported for this selected dose and $1/10^{th}$ of the dose selected for the anti urolithiastic activity (100mg and 200mg).

Induction of kidney stones in rats

Ethylene glycol is used to induce urolithiasis in rats. They were exposed to 0.3% of ethylene glycol with 1%Ammonium chloride in their drinking water for 3 days. Later on 0.3% ethylene glycol in drinking water was continued for 28 days [6].

Treatment of animals

Rats were divided into 9groups having 6 rats each. Group-I served as negative control rats (without urolithiasis), Group-II served as positive control rats (with urolithiasis but without treatment), Group-III serves as standard (which receives the drug cystone 750 mg/kg body weight), Group-IV receives 200 mg of sample T.T (Tribulus terrestris), Group-V receives 200 mg of sample P.G. (Punica granatum), Group-VI receives 100 mg of sample T.T and 100 mg of sample P.G in combination for 28 days.Group-IV, group-V, group-VI receives the concerned drugs for 28 days, and these three group's acts as preventive doses, Group-VII and group-VIII rats were given with a dose of 200mg of sample T.T and sample P.G respectively for 14 days. Group- IX receive 100 mg of sample T.T and 100 mg of sample P.G in combination for 14 days. These three groups (VII, VIII, and IX) serve as curative doses.

Collection of urine and serum

After the end of the treatment period, the rats were placed in a metabolic cages and urine were collected for 24 hours period having 20 µl of sodium azide as a preservative. After measuring urine volume an aliquot of urine was acidified by addition of 3N Hcl for the determination of urinary Creatinine, urea and uric acid. The rats were anaesthetized with diethyl ether and the blood is taken from orbital sinus into centrifuge tube without anti coagulant, allowed to clot at room temperature and centrifuged to collect serum. After collecting the serum it is subjected for analysis of serum urea, uric acid, creatinine and blood urea nitrogen (BUN) [7, 8].

RESULTS

Serum BUN, Creatinine, and Uric acid were decreased significantly ($P<0.01$) in Group-IV, V and VII, VIII rats as compared with positive control group-II rats. Co-treatment of T.T and P.G extract (100mg+100mg) in both preventive and curative dose regimen (group VI and IX) were showed significant ($P<0.001$) result when compared with the standard (group III). The values are given in the Table-1.

Urine Urea, creatinine and Uric acid were decreased significantly ($P<0.01$) in Group-IV,V and VII, VIII rats as compared with positive control group-II rats Co treatment of TT and PG extract (100mg+100mg) in both preventive and curative dose regimen (group VI and IX) were showed significant ($P<0.001$) result when compared with the standard (group III). The values are given in the Table-2.

Table 1**:** Serum BUN, Creatinine, Urea and Uric acid levels

Groups		Treatment	Serum parameters			
			BUN	Creatinine	Urea	Uric acid
I	Negative control	No Urolithiasis	37.37 ±0.80	0.66 ±0.30	32.12 ±0.36	1.89 ±0.12
II	Positive control	Urolithiasis without treatment	71.48 ±0.35	1.22 ±0.36	70.23 ±0.38	4.13 ±0.14
III	Standard	Cystone 750mg/kg	40.20 ±0.36	0.72 ±0.40	28.02 ±0.55	2.0 ±0.12
IV	Preventive dose for 28 days	200mg TT	46.70* ±0.35	0.808* ±0.31	29.0 ±0.60*	3.0 ±0.16*
V		200mg PG	49.2 ±0.36*	0.86 ±0.35*	32.0 ±0.30*	3.20 ±0.18*
VI		100mg TT + 100mg PG	47.60 ±0.76**	0.90 ±0.32**	28.0 ±0.26**	2.20 ±0.20**
VII	Curative dose for 14 th day to 28 th day	200mg TT	38.4 ±0.82*	0.70 ±0.37*	30.0 ±0.28*	3.20 ±0.12*
VIII		200mg PG	41.30 ±0.84*	0.80 ±0.40*	37.0 ±0.24*	3.10 ±0.13*
XI		100mg TT + 100mg PG	35.0 ±0.88**	0.85 ±0.42**	22.0 ±0.26**	1.80 ±0.19**

All values are expressed as mean ± SEM; (n=6) animals in each group*p<0.01, **p<0.001, Urolithiatic control was compared with group IV, V, VII, VIII and Cystone treated and group VI, IX were compared.

Table: 2: **Urine urea, creatinine, uric acid levels.**

Groups		Treatment	Urine parameters		
			Urea	Creatinine	Uric acid
I	Negative control	No Urolithiasis	110.12 ±1.10	14.23 ±0.78	10.21 ±0.55
II	Positive control	Urolithiasis	501.0 ±1.40	38.0 ±0.89	25.0 ±0.52
III	Standard	Cystone 750mg/kg	112.12 ±1.12	16.12 ±0.92	12.11 ±0.34
IV	Preventive dose for 28 days	200mg TT	349.70 ±1.34*	26.0 ±0.78*	28.0 ±0.65*
V		200mg PG	222.0 ±1.21*	20.0 ±0.76*	17.0 ±0.26*
VI		100mg TT + 100mg PG	124.50 ±1.24**	150.50 ±0.84**	14.0 ±0.38**
VII	Curative dose for 14 th day to 28 th day	200mg TT	478.4 ±1.47*	24.0 ±0.88*	31.0 ±0.49*
VIII		200mg PG	380.0 ±1.35*	19.6 ±0.92*	21.0 ±0.39*
IX		100mg TT + 100mg PG	167.5 ±1.15**	14.0 ±0.95**	9.0 ±0.19**

All values are expressed as mean ± SEM., (n=6) animals an each group *p<0.01, **p<0.001; Urolithiatic control was compared with group IV, V, VII, VIII and Cystone treated and groups VI, IX were compared

DISCUSSION

A number of models using rats have been used to induce calcium oxalate Urolithiasis. The most commonly employed method is providing Ethylene glycol and Ammonium chloride in drinking water. Ethylene glycol is readily absorbed in the intestine and is metabolized in the liver to oxalate leading to hyperoxaluria.

The oxalate precipitate in the urine as calcium oxalate as a result of it's poor solubility ammonium chloride has been reported to accelerate lithiasis. [9] and hence this model was selected for evaluating antiurolithiatic potential of P.G, T.T plant seed and fruit extract respectively. Calcium oxalate stone deposition decreases glomerular filtration rate due to obstruction to the flow of urine. This leads to accumulation of waste products mainly nitrogenous substances such as BUN, Creatinine and Uric acid [9] in both blood and urine. Treatment of TT and PG extracts showed to prevent the elevation of serum levels of these markers. This shows the extracts act by minimizing the extent of tubular dysfunction. The urinary oxalate cause renal tissue damage by reacting with poly saturated fatty acids in all membranes .The treatment of TT and PG extracts prevents these damage due to its anti oxidant effects.

CONCLUSION

The present study supports the use of these plants in Ayurvedic medicine against urolithiasis. Further study is needed to explore the exact active constituents of the plant and mechanism of action responsible for antiurolithiatic activity.

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